Comprehensive Gynecology and Obstetrics

Ikuo Konishi
Editor

Precision Medicine in Gynecology and Obstetrics

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Comprehensive Gynecology and Obstetrics

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This series presents the current and future perspectives of medical science in gynecology and obstetrics. The authors fully describe the current understanding of a disease including clinical features, imaging, pathology, and molecular biology, and also include the historical aspects and theories for exploring the etiology of the disease. Also, recent developments in diagnostic strategy, medical treatment, surgery, radiotherapy, prevention, and better health-care methods are clearly shown. Thus, each volume in the series focuses on the scientific basis for the pathogenesis of a disease and provides clinical applications that make it possible to offer personalized treatment for each patient. Over the past 20 years, physicians have been working to develop a standard treatment and publish clinical guidelines for a disease based on epidemiological evidence, mainly through the use of randomized clinical trials and meta-analyses. Recently, however, comprehensive genomic and genetic analyses have revealed the differences and variations in biological characteristics even among patients with the same diagnosis and have been focusing on personalized therapy. Now all physicians and patients are entering a new world of "precision medicine" through the use of genomic evidence. We are confident that readers will greatly benefit from the contents of the series with its purview of the exciting and promising future of gynecology and obstetrics.

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Preface

With recent significant advances in gynecology and obstetrics, physicians now face a new era of clinical medicine and decision making in daily practice. Beginning in the 1980s, the idea of evidence-based medicine (EBM) was introduced in clinical practice and medical education, the appropriate treatment modality for patients being based on clinical epidemiology. Various treatment guidelines have been published according to such epidemiological evidence along with the consensus of experts. Because the strongest evidence has been obtained from randomized clinical trials (RCTs) and meta-analyses, many physicians and patients have enthusiastically been involved in those RCTs. Thus, we have been living in the era of "epidemiology evidence-based medicine". Although the ideal treatment should be specialized for a patient after considering the specific nature of the disease and the desire of each patient, we first present to the patient the standard treatment in the guidelines, which may be appropriate for the patient but sometimes is not, due to the heterogeneity of the disease. For years, therefore, physicians have been looking intently for a strategy to personalize the treatment for each patient. In the twenty-first century, we have gradually been approaching an era of new EBM, which is "genome evidence-based medicine". The advance of comprehensive genomic analyses using next-generation sequencing (NGS) and gene expression profiling using DNA microarray along with bioinformatics has revealed the diversity of genome, epigenome, and expression profiles of disease. Development of novel drugs and technologies has made it possible to indicate the specific treatment according to the specific genomic nature of the disease in a patient. Thus, physicians are pleased to face this new era of "precision medicine" for clinical practice and decision making. This book presents the current perspective on precision medicine in the field of gynecology and obstetrics. The authors have made great efforts to update the scientific evidence in each field, and I would like to express my sincere thanks to all of them for the successful contribution of their chapters. I am also grateful to Ms. Yoko Arai at Springer Japan for her kind co-operation with me for the publication of this book.

Kyoto, Japan — Ikuo Konishi, M.D., Ph.D.

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Toward Precision Medicine in Gynecology and Obstetrics

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Ikuo Konishi

Abstract

This chapter is an introduction to the contents of this book on precision medicine in gynecology and obstetrics, describing "where are we now, and where should we go" with regard to evidence-based medicine (EBM). At the end of the twentieth century, we faced a drastic change in clinical medicine, i.e., a big wave of EBM which was the application of epidemiology to clinical decision making. Standard treatment under the guidelines based on epidemiologic evidence is very useful in our daily clinical practice. Such treatment is appropriate for more than half of patients, but it may not benefit the remaining patients owing to the heterogeneity of disease. However, recent advances in medical technologies is clearly disclosing the diversity of disease with regard to the differences in genome, epigenome, and expression profiling. Medical treatment has been personalized according to the specific, genomic nature of the patient. Thus, the second big wave of EBM, which is genome-based personalized medicine, started at the beginning of the twenty-first century and is now expanding as "precision medicine". Here we see the current and future perspectives on precision medicine in gynecology and obstetrics, namely, genome evidence-based personalized medicine, clinical practice, and decision making.

Keywords

Evidence-based medicine (EBM) • Clinical epidemiology • Personalized medicine • Genomics • Whole-genome sequencing • Gene-expression profiling Precision medicine

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1.1 Introduction

Physicians know a priori that there should be one best treatment for the patient who lies down in front of them, and have earnestly been seeking it among the various available modalities. Because physicians also are aware of the heterogeneity of disease among patients, even after the same clinical diagnosis is made, they try to shed light on the specific nature of the disease for a particular patient, using clinical history, physical examination, laboratory tests, histopathology, and imaging. To explore the right treatment strategy for the patient, it is also important to consider the pathophysiology of the patient's disease, study the principles and theories about the disease, and review the empirically employed treatment modalities and previous case reports. Advice from experienced professors and experts are very useful. Collecting all these data, we discuss the patient at a clinical conference, finally decide the most appropriate course for this specific patient, and then explain it to the patient and the family. Under such conditions, both physicians and patients reach a consensus. All of them seem to be happy under such an ideal doctor–patient relationship.

1.2 Evidence (Clinical Epidemiology)-Based Medicine Era Since the 1980s

Since the 1980s, however, the term "evidence-based" has been introduced in clinical decision making, guidelines and policies, and medical education [1]. As early as 1972, Archie Cochrane reported that many practices that had previously been assumed to be effective were not supported by controlled clinical trials [2]. In 1987, David Eddy first used the term "evidence-based" and expanded in his work on clinical practice guidelines and policies [3]. Alvin Feinstein, David Sackett, and others also claimed the importance of clinical epidemiology in decision making by physicians [4]. The term "evidence-based medicine (EBM)" has also been introduced in medical education. In 1990, Gordon Guyatt first used EBM at McMaster University for new medical students [1], and later published it as a new approach to teaching the practice of medicine. Such a big wave of EBM became popular in order to make individual clinical practice more objective by reflecting the evidence and required the application of population-based data to individual patient care. At that time, however, it was also emphasized that practitioners'clinical expertise should be reflected in efficient diagnosis and deep thought about the rights and preferences of individual patients [4]. Thus, during the 1990s, EBM gradually was established as a scientific approach for medical practice and decision making based on clinical epidemiology.

EBM further developed by classifying evidence levels by epidemiological strength, and now requires that only the strongest levels based on data obtained by randomized controlled trials (RCTs), meta-analyses, and systematic reviews can produce the strongest recommendations [5]. Opinions by experienced experts or case studies have been regarded as weaker levels [6]. Then EBM expanded to the design of clinical guidelines and policies that apply to patients and populations and

subsequently spread to decision making that is used at every level of health care. Thus, EBM advocates that decision making should not be based on a clinician's opinion or expert belief that may be limited by gaps in knowledge or by biases, but on the scientific evidence supplemented by all available data. Therefore, publication of clinical guidelines describing the standard treatment along with evidence levels has been greatly needed for daily practice, and for years many physicians have enthusiastically been involved in RCTs to seek the necessary scientific evidence. For the most part, such great efforts have resulted in success for establishment of novel treatments as standard ones. For example, in development of the standard chemotherapy for epithelial ovarian cancer, so many RCTs have been conducted and currently the combination chemotherapy with triweekly paclitaxel and carboplatin (TC) has been standard for first-line treatment [7]. Numerous patients with postoperative or recurrent ovarian cancer participated voluntarily in those RCTs not for themselves but for future patients. Thus, we have to continue our efforts to seek the scientific evidence that will be adopted in clinical guidelines and used for daily decision making in clinical practice.

Nevertheless, there have been many critical opinions of EBM expressed to date [8]. Before the era of EBM, the understanding of basic pathophysiologic mechanisms of disease coupled with clinical experience was of primary importance in medical teaching and clinical medicine. Because some of the original EBM proponents mistakenly touted EBM as a revolutionary new paradigm disregarding the philosophic basis for medicine, EBM was thought to be unscientific [9]. Although the strongest recommendations have been made by use of RCTs and meta-analyses in EBM, studies have failed to show that they are consistently more than "good quality". Similarly designed RCTs frequently disagree with one another, and cohort studies with better quality often disagree with those from RCTs. Actually, EBM may be able to answer clinical questions suited to the evidence but not in questions specific to small patient populations or subjective evaluations. Clinically important details may be hidden, because EBM does not integrate non-statistical forms of medical information such as professional experience and patient-specific factors. Also, EBM may reduce the autonomy of the doctor-patient relationship [10]. At the beginning of the era of EBM, it was clearly declared that EBM is not "cookbook medicine" and should not be applied to restrict options of the patient or doctor, which would be "misuse of EBM" [1]. However, EBM has been hijacked by accountants and managers to cut the cost of health care. Under the clinical guidelines, EBM has been used to prevent physicians from being held hostage and unable to treat a willing patient while waiting for statistical evidence.

Most importantly, it has been recognized that the usefulness of applying EBM to individual patients is limited [8, 11]. Patients are individuals, not groups. Because EBM is based on applying principles of clinical epidemiology to individual patient care, it carries with it many of the assumptions of epidemiological strategy. Individual circumstances and values are varied, and there are a great many uncommon diseases and variants. There is often a lack of studies relevant to the specific patient and intervention under consideration. Although medical research has focused on common clinical situations, there are many rare diseases

and conditions where EBM does not work well. Furthermore, individual patients will respond in their own unique way to a therapy that was not predicted from data by RCTs. In epithelial ovarian cancer, for example, although triweekly TC chemotherapy has been established as standard, i.e., proven to be most effective, the overall rate of obtaining a response is approximately 70% with the remaining 30% being resistant [7]. Among the four histological types, clear cell carcinoma and mucinous carcinoma will usually not respond to TC chemotherapy. Even in patients with serous carcinoma, approximately 20% are resistant even at the first-line treatment. This is a limitation of clinical guidelines based on EBM. For individual patients, therefore, our clinical medicine must resolve disagreements between general rules, empirical data, theories, principles, and patient values. In this setting, recent development of personalized medicine using genome analyses appears to overcome the limitations of an EBM approach for clinical decision making.

1.3 Toward a New Era of Evidence (Genomics)-Based Medicine for Patients

Recent advances in clinical oncology and novel drug discoveries have been playing the major leadership roles in personalized medicine. The final goal of modern medicine is increasing patient specificity so that the right treatment is given to the right patient at the right time. While current cancer studies have largely focused on identification of genomic or epigenomic properties of tumor cells, emerging evidence has clearly demonstrated the heterogeneity between tumors among patients and even in the same patients. In the twenty-first century, the advance of comprehensive genomic analyses using next-generation sequencing (NGS) and gene expression profiling using DNA microarray along with bioinformatics is clearly revealing the diversity of genome, epigenome, and expression profiles of cancer. If the driver oncogene and the main signaling pathway for cancer growth and survival is identified, the specific, molecular-targeted drug is shown to be greatly effective due to the "oncogene addiction" of tumor cells. One representative example is EML4-ALK lung cancer. In 2007, Hiroyuki Mano and his colleagues identified the fusion oncogene *EML4-ALK* in a subset of non-small-cell lung cancer with poor prognosis, and then clearly showed that an ALK kinase inhibitor such as crizotinib was quite effective and dramatically improved the survival of patients with EML4-ALK lung cancer [12]. A RCT was not necessary for approval of the drug in a short period of time by the FDA in 2011. Thus, we are coming into an era where selection of anti-cancer drugs is determined by genomic analysis for the patient rather than by the standards in guidelines.

The natural history of the development of epithelial ovarian cancer was unclear because most patients visit us with advanced disease. Our clinicopathological approach using transvaginal ultrasound disclosed the diversity of natural history of ovarian cancer along with the respective genetic mutations [13]. Therefore, ovarian cancer is not a single disease entity but a heterogeneous group of diseases with

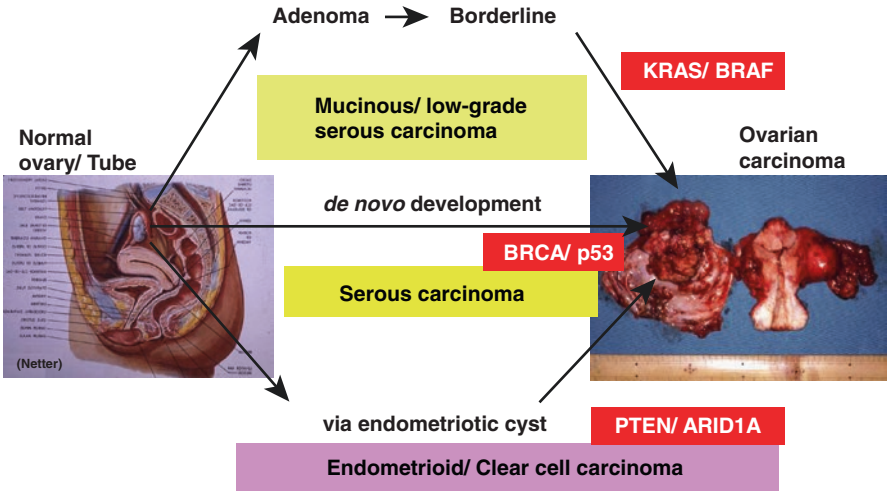

Fig. 1.1 Ovarian cancer is a heterogeneous disease with diverse scenarios

different clinical and molecular scenarios (Fig. 1.1). Regarding clear cell cancer that is resistant to standard chemotherapy, our comprehensive genomic analyses demonstrated that there is a specific gene-expression signature (OCCC signature) [14], in which many anti-oxidative stress genes are upregulated for cell survival via an epigenetic mechanism against the stressful microenvironment of an endometriotic cyst filled with the free iron of menstrual blood [15]. Our analyses also revealed that clear cell cancer is resistant to cisplatin but sensitive to multikinase inhibitors such as sorafenib [16], and the subsequent clinical trial for patients with recurrent clear cell cancer demonstrated its clinical efficacy. Another important step in clinical oncology is immunotherapy using antibodies against immune-checkpoint molecules. We have demonstrated that the immune-checkpoint PD-L1/PD-1 signaling plays an important role in the escape from the host immune system and in peritoneal dissemination in ovarian cancer cells. We then conducted a clinical trial on the safety and efficacy of the anti-PD-1 antibody nivolumab in patients with platinum-resistant, recurrent ovarian cancer, and some patients including those with clear cell cancer showed a remarkable and durable response [17]. Thus, genomic analyses with novel drug development will be able to overcome the resistance to standard chemotherapy.

The Cancer Genome Atlas (TCGA) Network published data from the whole genome sequencing and molecular profiling using NGS and microarray in 2011. For high-grade serous ovarian cancer (HGSC), which comprises the most common histological type in epithelial ovarian cancer and usually responds well to TC chemotherapy, it was shown that HGSC does not have the definitive driver oncogene. Interestingly, however, it was also revealed that there are four subtypes in the gene expression profile, i.e., differentiated, immunoreactive, mesenchymal, and proliferative, and that patients with HGSC with the mesenchymal subtype showed the worst prognosis [18]. Such novel classification is relevant with the difference in the microenvironment of cancer cells. Recent bioinformatics and clinicopathology

approaches have shown that the mesenchymal subtype accompanied by dense fibroblastic stroma is more sensitive to paclitaxel than to other drugs [19]. These findings suggest that the mesenchymal subtype may fit the weekly dose-dense TC regimen, in which a higher dose of paclitaxel than usual is given [20]. Anti-vascular endothelial growth factor (VEGF) antibody, bevacizumab, may also improve the survival of HGSC patients with the mesenchymal subtype. Thus, selection of chemotherapeutic and molecular-targeted drugs will be considered under genomic profiling analyses indicating the cancer microenvironment.

The most important factor for poor prognosis of epithelial ovarian cancer is peritoneal dissemination. Therefore, molecular and genomic analyses for the mechanisms in the special metastatic process are mandatory. Through our extensive analyses, we have demonstrated that the hypoxic microenvironment at the beginning of metastasis plays an essential role in downregulation of E-cadherin, upregulation of S100A4, followed by increased RhoA signaling, which is responsible for cancer cell metastasis, motility, and invasion [21]. RhoA inhibitors such as lovastatin have been effective in an animal model for experimental peritoneal dissemination. In addition, we also have observed the epigenetic change of the *S100A4* gene in ovarian cancer cells under a hypoxic environment, which suggests "evolution" of cancer cells during progression [22]. Upregulation of VEGF is also important in the disseminated lesions for angiogenesis and immunosuppression. Therefore, each anti-cancer drug will be directed to each microenvironment and signaling of cancer cells, which continuously evolve via changes in genomics and epigenomics and gene expressions. Accordingly, we must consider now the two-dimensional map model of the cancer genome, which shows both the diversity in carcinogenesis (*X*-axis) and the diversity of evolution in progression (*Y*-axis) (Fig. 1.2). The place

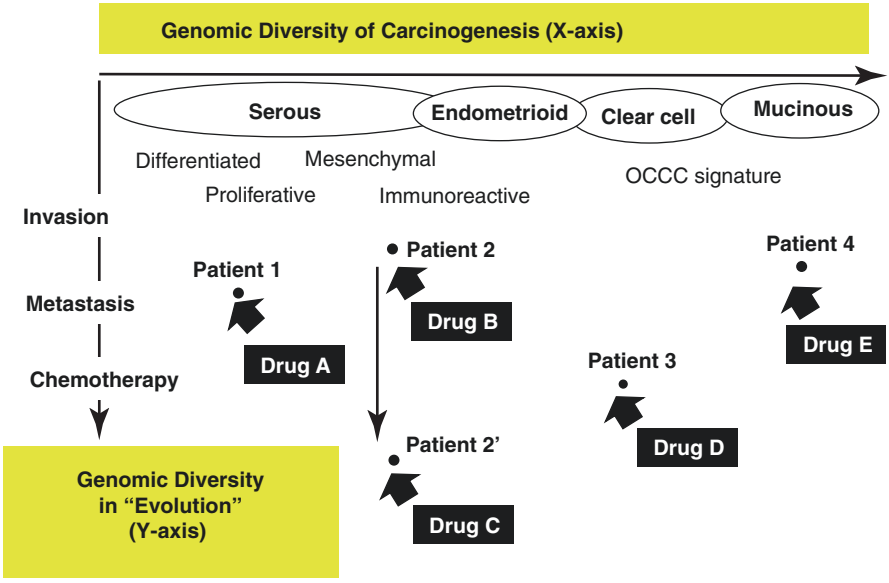

Fig. 1.2 Two-dimension model of cancer genome

of each patient will be identified on the map via genomic analyses, and the right treatment will be given at the right time in the near future.

1.4 Acceleration of "Precision Medicine" for Patients

More recently, the direction of personalized medicine is expanding to "precision medicine". The National Institutes of Health (NIH) in the United States defines precision medicine as an emerging approach for disease treatment and prevention that takes into account individual variability in genes, environment, and lifestyle for each person. This approach will allow doctors and researchers to predict more accurately which treatment and prevention strategies for a particular disease will work in which groups of people. It is in contrast to a "one-size-fits-all" approach, in which disease treatment and prevention strategies are developed for the average person, with less consideration for the differences between individuals. Thus, all of us are coming into an ideal world for healthcare and a better doctor-patient relationship. We now must accelerate such movement in clinical medicine for our patients.

References

1. Evidence-Based Medicine Working Group. Evidence-based medicine: a new approach to teaching the practice of medicine. JAMA. 1992;268:2420–5.
2. Cochrane AL. Archie Cochrane in his own words. Selections arranged from his 1972 introduction to "Effectiveness and Efficiency: Random Reflections on the Health Services" 1972. Control Clin Trials. 1989;10:428–33.
3. Eddy DM. Clinical decision making: from theory to practice. Practice policies - what are they? JAMA. 1990;263:877–8.
4. Sackett DL, Rosenberg WM, Gray JA, Haynes RB, Richardson WS. Evidence based medicine: What it is and what it isn't. BMJ. 1996;312:71–2.
5. Farmer A. Medical practice guidelines: Lessons from the United States. BMJ. 1993;307:313–7.
6. Tonelli MR. In defense of expert opinion. Acad Med. 1999;74:1187–92.
7. Sugiyama T, Konishi I. Emerging drugs for ovarian cancer. Expert Opin Emerging Drugs. 2008;13:523–36.
8. Cohen AM, Starvi PZ, Hersh WR. A categorization and analysis of the criticisms of Evidence-Based Medicine. Int J Med Inform. 2004;73:35–43.
9. Charlton BG, Miles A. The rise and fall of EBM. QJM. 1998;91:371–4.
10. Graham-Smith D. Evidence-Based Medicine: Socratic dissent. BMJ. 1995;310:1126–7.
11. Kenny NP. Does good science make good medicine? Incorporating evidence into practice is complicated by the fact that clinical practice is as much as art as science. CMAJ. 1997;157:33–6.
12. Soda M, Choi YL, Enomoto M, Takada S, Yamashita Y, Ishikawa S, Fujiwara S, Watanabe H, Kurashina K, Hatanaka H, Bando M, Ohno S, Ishikawa Y, Aburatani H, Niki T, Sohara Y, Sugiyama Y, Mano H. Identification of the transforming EML4-ALK fusion gene in non-small-cell lung cancer. Nature. 2007;448:561–6.
13. Horiuchi A, Itoh K, Shimizu M, Nakai I, Yamazaki T, Kimura K, Suzuki A, Shiozawa I, Ueda N, Konishi I. Toward understanding the natural history of ovarian carcinoma development: a clinicopathological approach. Gynecol Oncol. 2003;88:309–17.

14. Yamaguchi K, Mandai M, Oura T, Matsumura N, Hamanishi J, Baba T, Matsui S, Murphy SK, Konishi I. Identification of an ovarian clear cell carcinoma gene signature that reflects inherent disease biology and the carcinogenic processes. Oncogene. 2010;29:1741–52.
15. Mandai M, Yamaguchi K, Matsumura N, Baba T, Konishi I. Ovarian cancer in endometriosis: molecular biology, pathology, and clinical management. Int J Clin Oncol. 2009;14:383–91.
16. Matsumura N, Mandai M, Okamoto T, Yamaguchi K, Yamamura S, Oura T, Baba T, Hamanishi J, Kang HS, Matsui S, Mori S, Murphy SK, Konishi I. Sorafenib efficacy in ovarian clear cell carcinoma revealed by transcriptome profiling. Cancer Sci. 2010;101:2563– 658.
17. Hamanishi J, Mandai M, Ikeda T, Minami M, Kawaguchi A, Murayama T, Kanai M, Mori Y, Matsumoto S, Chikuma S, Matsumura N, Abiko K, Baba T, Yamaguchi K, Ueda A, Hosoe Y, Morita S, Yokode M, Shimizu A, Honjo T, Konishi I. Safety and Antitumor Activity of Anti-PD-1 Antibody, Nivolumab, in Patients With Platinum-Resistant Ovarian Cancer. J Clin Oncol. 2015;33:4015–22.
18. Cancer Genome Atlas Research Network. Integrated genomic analyses of ovarian carcinoma. Nature. 2011;474:609–15.
19. Murakami R, Matsumura N, Brown JB, Wang Z, Yamaguchi K, Abiko K, Yoshioka Y, Hamanishi J, Baba T, Koshiyama M, Mandai M, Yamada R, Konishi I. Prediction of taxane and platinum sensitivity in ovarian cancer based on gene expression profiles. Gynecol Oncol. 2016;141:49–56.
20. Katsumata N, Yasuda M, Isonishi S, Takahashi F, Michimae H, Kimura E, Aoki D, Jobo T, Kodama S, Terauchi F, Sugiyama T, Ochiai K, Japanese Gynecologic Oncology Group. Long-term results of dose-dense paclitaxel and carboplatin versus conventional paclitaxel and carboplatin for treatment of advanced epithelial ovarian, fallopian tube, or primary peritoneal cancer (JGOG 3016): a randomised, controlled, open-label trial. Lancet Oncol. 2013;14:1020–6.
21. Imai T, Horiuchi A, Wang C, Oka K, Ohira S, Nikaido T, Konishi I. Hypoxia attenuates the expression of E-cadherin via up-regulation of SNAIL in ovarian carcinoma cells. Am J Pathol. 2003;163:1437–47.
22. Horiuchi A, Hayashi T, Kikuchi N, Hayashi A, Fuseya C, Shiozawa T, Konishi I. Hypoxia upregulates ovarian cancer invasiveness via the binding of HIF-1α to a hypoxia-induced, methylation-free hypoxia response element of S100A4 gene. Int J Cancer. 2012;131:1755–67.

Genomics in Gynecological Cancer: Future Perspective

2

Takeshi Motohara and Hidetaka Katabuchi

Abstract

All cancers arise as a result of dynamic changes in the cancer genome. Cancer cells show diverse biological capabilities that are conferred by numerous genetic and epigenetic changes. Over the past years, comprehensive genomic studies using next-generation sequencing technology have resulted in an increasing wealth of the understanding of molecular mechanisms with respect to the genomic features of gynecological malignancies, including ovarian, endometrial, and cervical cancers. These studies can be exploited to develop and improve cancer classification, new diagnostic methods, and novel therapeutic strategies.

In this chapter, we review the principles of our current understanding of cancer genomes in gynecological malignancies, particularly ovarian, endometrial, and cervical cancers. Furthermore, a vision for the future of genomics in gynecological cancer has been discussed. We hope that cancer genomic research will ultimately guide clinical decision-making in association with the development of novel therapeutic strategies and biomarker-based clinical trials, affecting the clinical outcome of cancer patients.

Keywords

Cancer genome • Gynecologic cancer • Ovarian cancer • Endometrial cancer Cervical cancer

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2.1 Introduction

After a quarter of a century of rapid advances, comprehensive genomic studies have generated a complex body of knowledge, demonstrating cancer to be a disease involving dynamic changes in the "cancer genome" [1]. A cancer genome harbors numerous alterations at the level of the nucleotides, chromatin, and chromosomes [2, 3]. These alterations comprise irreversible aberrations in the DNA structure and in the number of particular sequences, genes, or chromosomes. Additionally, they include reversible changes, such as epigenetic modifications in the DNA and histone proteins. These reversible and irreversible changes collectively induce the activation or inhibition of various biological molecular pathways, affecting cancer pathophysiology, including invasion, metastasis, immune evasion, angiogenesis, or cell death [4].

Integrated genome-wide sequencing has recently demonstrated the genomic landscapes of common forms of human cancer (Fig. 2.1) [5, 6]. The valuable

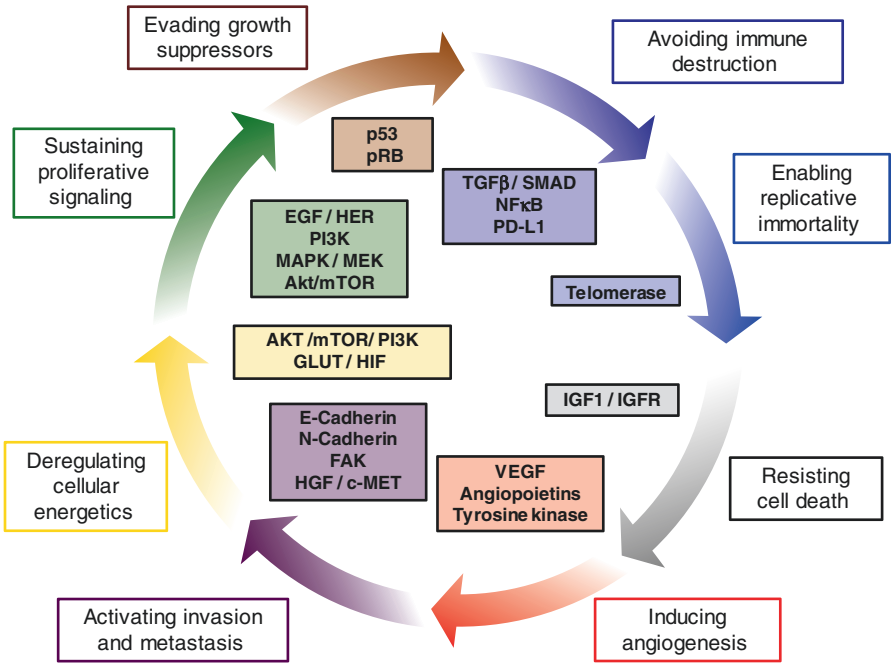

Fig. 2.1 The hallmarks of cancer. The hallmarks of cancer comprehend several capabilities acquired during the multistep development of cancers. These hallmarks constitute an organizing principle for rationalizing the complexities of cancer and also become major targets for cancer research and therapeutic strategies

information from cancer genome studies can be exploited to develop methods for prevention and early detection of cancer, which will be essential to reduce cancer morbidity and mortality [2]. Furthermore, these studies can identify the underlying molecular mechanisms that can be targeted for cancer therapy and the prediction of response to cancer therapies, affecting the clinical outcome of cancer patients [7–9].

This chapter aims to demonstrate the impact of comprehensive genomic research on gynecological cancer, including ovarian, endometrial, and cervical cancers. We review their implications for better understanding of the cancer genome, leading to improved cancer classification and development of new diagnostic methods and therapeutic approaches in gynecological malignancies. Furthermore, a vision for the future of genomic research in gynecological cancer is discussed.

2.2 The Cancer Genome Atlas Project

The latest development in the technological advances of genome-wide sequencing and bioinformatics has shed new light on the cancer genome [3, 7]. In 2005, The Cancer Genome Atlas (TCGA) was launched as the main project accelerating the comprehensive understanding of cancer genomics using innovative genomic technologies [7]. TCGA has profiled and analyzed major molecular alterations at the DNA, RNA, protein, and epigenetic levels in large cohorts of over 30 human tumors through large-scale genome-wide sequencing and integrated multidimensional analyses [8, 9]. The large amount of available data provides a crucial opportunity to develop an integrated picture of commonalities, differences, and emergent themes across tumor lineages. Evaluation of the molecular aberrations and their functional roles across tumor types will guide us in how to extend effective cancer therapies in one cancer type to others with a similar genomic profile [8].

Phase I of TCGA project aimed to test the research infrastructure based on the characterization of chosen tumors having poor prognosis: brain, lung, and ovarian cancers. Since then, phase II analyses have expanded to more than 30 different tumor types, including endometrial and cervical cancers [8]. By January 2015, TCGA announced that it had successfully collected the necessary quality and quantity of samples for all 33 selected tumor types. Table 2.1 shows a summary of the available TCGA genomic data as of May 2016. In the field of gynecological malignancies, these recent advances in innovative genome analysis technologies have resulted in an increasing understanding of molecular mechanisms with respect to the genomic features of ovarian, endometrial, and cervical cancer [10].

Table 2.1 Summary of the Cancer Genome Atlas cases with data as of May 2016

Selected cancer	No. of cases with data	Selected cancer	No. of cases with data
Breast invasive carcinoma	1097	Kidney renal papillary cell carcinoma	291
Ovarian serous cystadenocarcinoma	586	Sarcoma	261
Uterine corpus endometrial carcinoma	548	Acute myeloid leukemia	200
Kidney renal clear cell carcinoma	536	Esophageal carcinoma	185
Glioblastoma multiforme	528	Pancreatic adenocarcinoma	185
Head and neck squamous cell carcinoma	528	Pheochromocytoma and paraganglioma	179
Lung adenocarcinoma	521	Rectum adenocarcinoma	171
Brain lower grade glioma	516	Testicular germ cell tumors	150
Thyroid carcinoma	507	Thymoma	124
Lung squamous cell carcinoma	504	Mesothelioma	87
Prostate adenocarcinoma	498	Adrenocortical carcinoma	80
Skin cutaneous melanoma	470	Uveal melanoma	80
Colon adenocarcinoma	461	Kidney chromophobe	66
Stomach adenocarcinoma	443	Uterine carcinosarcoma	57
Bladder urothelial carcinoma	412	Lymphoid neoplasm diffuse large B-celll lymphoma	48
Liver hepatocellular carcinoma	377	Cholangiocarcinoma	36
Cervical squamous cell carcinoma and endocervical adenocarcinoma	307		

2.3 The Genomics of Ovarian Cancer

2.3.1 Molecular Pathogenesis of Ovarian Cancer

Epithelial ovarian cancer has the highest case fatality rate of any gynecological cancer, and it is the leading cause of death among female genital tract malignancies [11, 12]. Because most patients with ovarian cancer are diagnosed at an advanced stage, the clinical outcome for ovarian cancer is poor even after treatment with extirpative surgery and chemotherapy [13]. Despite a high response rate to initial chemotherapy, most patients will suffer relapse and the development of drug-resistant disease [14, 15].

Currently, based on histopathology, ovarian cancers are divided into five main histological types: high-grade serous carcinoma, low-grade serous carcinoma, endometrioid carcinoma, clear cell carcinoma, and mucinous carcinoma [16]. These tumors account for 98% of all ovarian cancers and can be reproducibly diagnosed by light microscopy [17]. These histological types are essentially distinct diseases, as indicated by differences in precursor lesions, patterns of spread, response to chemotherapy, and prognosis [16, 18].

Table 2.2 Dualistic model of ovarian carcinogenesis based on morphological and molecular genetic analysis

	Histological type	Precursors	Molecular genetic alterations
Type I tumors	Low-grade serous carcinoma	Serous cystadenoma/adenofibroma Atypical proliferative serous tumor Noninvasive micropapillary serous carcinoma	*BRAF* and *KRAS* mutations
	Mucinous carcinoma	Mucinous cystadenoma Atypical proliferative mucinous tumor	*KRAS* mutations
	Endometrioid carcinoma	Endometriosis Endometrioid adenofibroma Atypical proliferative endometrioid tumor	LOH or *PTEN* mutations *KRAS* mutations Microsatellite instability
	Clear cell carcinoma	Endometriosis Clear cell adenofibroma Atypical proliferative clear cell tumor	*KRAS* mutations Microsatellite instability TGF-β RII mutations
Type II tumors	High-grade serous carcinoma	Not yet identified	*p53* mutations Amplification and overexpression of *HER2/neu* gene Inactivation of *p16* gene
	Undifferentiated carcinoma	Not yet identified	Not yet identified

Recent research into molecular biology of ovarian cancers demonstrated that ovarian cancers comprise both clinically diverse and molecularly heterogeneous malignancies, encompassing subtypes with distinct gene expression patterns that are correlated with different clinical outcomes [11, 19]. In the early twenty-first century, morphologic, immunohistochemical, and molecular studies led to a new paradigm for the pathogenesis of ovarian cancer, which divided ovarian cancer into two groups designated as type I and type II (Table 2.2) [18, 20]. Type I tumors include low-grade serous carcinoma, endometrioid carcinoma, clear cell carcinoma, and mucinous carcinoma, which develop in a stepwise fashion from well-recognized precursor lesions, such as borderline tumors or endometriosis [20]. They present as large masses that are confined to the ovary; they are generally indolent and have a favorable prognosis. These tumors are genetically stable and are typically characterized by a variety of somatic sequence mutations, including *KRAS*, *BRAF*, *ERBB2*, *CTNNB1*, *PTEN*, *PIK3CA*, and *ARID1A* [16, 18, 19]. On the other hand, type II tumors comprise of high-grade serous carcinoma and undifferentiated carcinoma, which develop de novo, and are highly aggressive, and have a poor prognosis [19, 20]. These tumors are chromosomally highly unstable and harbor *TP53* mutations, and BRCA inactivation occurs in up to 40%–50% of high-grade serous carcinoma [21].

Recognition of the dualistic model of ovarian carcinogenesis provided a new opportunity for better management of ovarian cancer patients, and knowledge of molecular mechanisms and the pathogenesis of various types of ovarian cancer could lead to more targeted therapeutic interventions [14, 22].

2.3.2 Comprehensive Genomic Characterization of High-Grade Serous Ovarian Carcinoma

In 2011, TCGA project reported the results of a wide-range analysis of the genomic and epigenetic changes that occur in 489 high-grade serous ovarian carcinomas and demonstrated several potential therapeutic molecular targets [23]. TCGA scientists determined the presence of *TP53* mutation in almost all tumor specimens of high-grade serous carcinoma and a low prevalence but statistically significant frequency of somatic mutations in nine further genes, including *BRCA1*, *BRCA2*, *NF1*, *RB1*, and *CDK12*. Identification of these molecular pathways is likely to provide novel therapeutic approaches [23, 24]. Furthermore, the four molecular subtypes were validated in high-grade serous carcinoma cases using approximately 1500 intrinsically variable genes and were termed (a) immunoreactive, (b) differentiated, (c) proliferative, and (d) mesenchymal on the basis of gene expression in the clusters [23].

Understanding the molecular classification of ovarian cancer using comprehensive genomic analysis could lead to the development of prediction of response to therapies and improved prognostic indicators [22, 25]. In fact, these four molecular subtypes have been independently validated and have been shown to be of independent prognostic relevance [25, 26]. Moreover, TCGA data have helped to clarify the effect of *BRCA1/2* mutations on survival outcomes in patients with ovarian cancer [27]. These evolving subgroups in ovarian cancer have distinct biologic characteristics that can translate into different therapeutic implications, which will allow gynecologists to identify women likely to benefit from a given cancer therapy [6].

Taken together, ovarian cancer is a spectrum of diseases and not a single disease entity. Nevertheless, current clinical management fails to incorporate these facts into treatment strategies for ovarian cancer patients because of the lack of insight into distinct molecular mechanisms for these cancers. Improvements in ovarian cancer survival should be achieved by translating recent biological insights at the molecular level into personalized individual treatment strategies [2, 7].

2.4 The Genomics of Endometrial Cancer

2.4.1 Pathological and Molecular Characteristics of Endometrial Cancer

Endometrial cancer is one of the most prevalent malignant tumors of the female genital tract, and its incidence rate is increasing rapidly in developed countries [28]. The majority of patients with endometrial cancer are diagnosed at an early stage,

resulting in overall favorable prognosis with high cancer-specific survival rates [29]. However, for patients with advanced-stage disease or for those with recurrent endometrial cancer, the prognosis remains poor and the optimal adjuvant therapy is yet to be established [30].

Endometrial cancer is divided into several histologic categories based on cell type. Endometrioid carcinoma is the most common cell type, accounting for 75–80% of cases, and subdivided into grade 1 to grade 3, according to degree of differentiation [31]. In addition, other aggressive pathologic variants include serous, clear cell, mixed, and undifferentiated types [32].

In 1983, Bokhman proposed that there are two different pathogenetic types of endometrial cancer that are primarily based on light microscopic appearance, clinical behavior, and epidemiology [33, 34]. Type I tumors are mostly composed of endometrioid carcinomas and are generally correlated with endometrial hyperplasia, express estrogen, and progesterone receptors [35]. These tumors arise in a background of unopposed estrogen stimulation, occur in premenopausal and perimenopausal women, and histologically show low-grade endometrioid differentiation. In contrast, type II tumors are more aggressive and mostly include high-grade endometrioid, serous, or clear cell histological types, and generally develop from atrophic endometrial tissues unrelated to estrogen stimulation in older women [35–37].

Previous molecular studies of endometrial cancer demonstrated that type I tumors are correlated with mutations in *PTEN*, *KRAS*, *PIK3CA*, and *CTNNB1* and frequently show microsatellite instability (MSI) [38, 39] but do not usually have mutations in the *TP53* tumor suppressor gene [35]. In contrast, a majority of type II tumors have *TP53* mutations, and loss of heterozygosity (LOH) on several chromosomes, as well as molecular alterations affecting *p16*, *STK15*, *E-cadherin*, and *c-erb-B2* [35, 36].

In the past decade, it has become more obvious that endometrial cancer comprises a clinically, histologically, and genetically heterogeneous group of tumors. However, Bokhman's dualistic classification model does not entirely take into account this heterogeneity. As a consequence, traditional classifications are insufficient overall for successful treatment and are limited in predicting response to specific therapies [36].

2.4.2 New Genomic Classification of Endometrial Cancer

In 2013, TCGA Research Network reported a comprehensive genomic and transcriptomic analysis of endometrial cancers, using next-generation sequencing technologies in combination with analysis of DNA methylation, reverse phase protein array, and MSI [40]. This study focused on common histological types, including endometrioid (n = 307), serous (n = 53), and mixed endometrioid and serous (n = 13) carcinomas. On the basis of integrated analysis, endometrial cancers were classified into four distinct molecular subgroups: (a) *POLE* ultramutated, (b) MSI hypermutated, (c) copy-number low, and (d) copy-number

Table 2.3 Genomic classification of endometrial cancer

	POLE ultramutated	MSI hypermutated	Copy-number low	Copy-number high
Copy-number aberrations	Low	Low	Low	High
Mutation rate	Very high	High	Low	Low
MSI/MLH1 methylation	Mixed MSI high, low, stable	MSI high	MSI stable	MSI stable
Genes mutated (%)	*POLE* (100%) *PTEN* (94%) *PIK3CA* (71%) *PIK3R1* (65%) *FBXW7* (82%) *ARID1A* (76%) *KRAS* (53%)	*PTEN* (88%) *RPL22* (37%) *KRAS* (35%) *PIK3CA* (54%) *PIK3R1* (40%) *ARID1A* (37%)	*PTEN* (77%) *CTNNB1* (52%) *PIK3CA* (53%) *PIK3R1* (33%) *ARID1A* (42%)	*TP53* (92%) *PPP2R1A* (22%) *PIK3CA* (47%)
Histological type	Endometrioid	Endometrioid	Endometrioid	Serous, endometrioid, and mixed serous and endometrioid
Tumor histological grade	Mixed (grade 1-3)	Mixed (grade 1-3)	Grade 1 and 2	Grade 3

high (Table 2.3). The *POLE* ultramutated group was characterized by extraordinarily high mutation rates and hotspot mutations in the exonuclease domain of *POLE*, which is a catalytic subunit of DNA polymerase epsilon and is involved in nuclear DNA replication and repair. The MSI hypermutated group had tumors showing increased MSI because of *MLH1* promoter methylation. The copy-number low group was microsatellite stable and had a lower mutation frequency. In this group, most of the tumors were grade 1 and 2 endometrioid carcinomas characterized by frequent *CTNNB1* mutations. The copy-number high group had a low mutation frequency but a high rate of somatic copy number alterations, and this group contained most of the serous and mixed histology tumors with frequent *TP53* mutations [40].

Comprehension of the genomic classification of endometrial cancer has an important role in developing improved prognostic indicators. When the progression-free survival (PFS) was analyzed in TCGA study, it was demonstrated that the *POLE* ultramutated group had a significantly favorable PFS, whereas the copy-number high group had the poorest survival outcome [40].

Overall, the TCGA genomic characterization of endometrial cancers has confirmed and expanded knowledge of molecular signaling pathways and permitted reclassification of endometrial cancers, which could directly affect prognostic assessment, prediction of response to therapies, and treatment decisions [34, 36, 40]. In order to achieve the ultimate goal of developing clinical measures that will improve the outcomes of patients with endometrial cancer, further studies of genomic abnormalities in endometrial cancer are needed to identify new therapeutic molecular targets, leading to personalized individual treatment strategies.

2.5 The Genomics of Cervical Cancer

2.5.1 Molecular Mechanisms of HPV-Induced Cervical Carcinogenesis

Cervical cancer is the second most common malignancy in women worldwide after breast cancer and the leading cause of cancer-related deaths in developing countries [41]. Unlike many other solid cancers, cervical cancer is currently more prevalent in younger women. Even though early-stage and locally advanced cervical cancers may be cured with radical surgery and chemoradiotherapy, patients with metastatic cancers or recurrent disease have limited therapy options.

The major histopathologic types of cervical cancer are squamous cell carcinoma and adenocarcinoma, which constitute approximately 80% and 20% of all cases of cervical cancer, respectively [42]. Cervical squamous cell carcinoma arises in the squamocolumnar junction and is preceded by a long phase of cervical intraepithelial neoplasia [43]. Cervical adenocarcinoma originates from glandular precursor lesions of the endocervical mucosa and comprises several histological subtypes, including mucinous, endometrioid, clear cell, and serous adenocarcinomas [44].

Human papillomavirus (HPV) infection is recognized as the main cause of cervical cancer [41]. Oncogenic HPVs, mainly HPV16 and 18 genotypes, have been closely associated with the risk of developing intraepithelial lesions, squamous cell carcinoma, and adenocarcinoma of the cervix [45]. The viral oncoproteins E6 and E7 of high-risk HPVs contribute to the transformation of infected epithelial cells mainly through the inactivation of the *TP53* and *RB* tumor suppressor genes and related pathway [42]. However, recent studies have shown that alterations of additional pathways are equally important for transformation of HPV-infected cells, and these additional factors are crucial regulators of cell cycle progression, apoptosis, and chromosomal stability [42]. As a consequence, the accumulation of genetic and epigenetic alterations over time may ultimately lead to cervical cancer.

The Nobel Prize-winning identification of a causative correlation between the viral infection HPV and cervical carcinogenesis served as a driving force behind the development of HPV vaccines in an effort to prevent HPV infection. Even though, in the past, cervical cancer was the most common cause of cancer-related mortality for women, major advancements in screening and prevention during the past half-century have significantly impacted this picture.

2.5.2 Genomic Alterations in Cervical Cancer

In an effort to develop more effective cancer therapies, the focus has shifted toward improving our understanding of the genetic foundations of cervical cancer. Thus far, relatively few reports on genomic alterations in oncogenes and tumor suppressor genes have been demonstrated for cervical cancer [46, 47]. In 2014, a comprehensive genomic analysis of cervical cancers was performed by whole-exome sequencing analysis in 79 squamous cell carcinomas and 24 adenocarcinomas [48]. *PIK3CA* is

Table 2.4 Significantly mutated genes in cervical cancer

Gene	Description	Relative frequency (%)
Squamous cell carcinoma		
PIK3CA	Phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha	14
FBXW7	F-box and WD repeat domain containing 7	15
MAPK1	Mitogen-activated protein kinase 1	8
HLA-B	Major histocompatibility complex class 1, B	9
Ep300	E1A binding protein p300	16
STK11	Serine/threonine kinase 11	4
ERBB2	Erb-b2 receptor tyrosine kinase 2	5
EGFR	Epidermal growth factor receptor	8
PTEN	Phosphatase and tensin homolog	6
Adenocarcinoma		
PIK3CA	Phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha	16
KRAS	KRAS proto-oncogene	8
ELF3	E74-like factor 3	13
CBFB	Core-binding factor, beta subunit	8

one of the most commonly mutated genes associated with cervical cancer in both squamous cell carcinoma and adenocarcinoma, indicating that the PIK3CA pathway could represent a promising therapeutic strategy. Because the gene products of *TP53* and *RB1* are inactivated by E6 and E7, they are rarely mutated in cervical cancer [46]. Intriguingly, a previous study also reported that *EGFR* mutations were identified in squamous cell carcinoma only, whereas *KRAS* mutations were detected in adenocarcinoma only, demonstrating that the genetic mutation pictures differed depending on tumor histology (Table 2.4) [49]. These data suggested that molecular targeted therapies should make a promising therapeutic avenue for cervical cancer.

2.6 Evolving Genomic Comprehension of HPV in Cervical Carcinogenesis

Following the initial discovery of HPV DNA in the human genome, various studies have evaluated its genomic role in cervical cancer development [46, 48]. A crucial mechanism in cervical carcinogenesis is represented by the integration of the HPV genome into human chromosomes [50]. Adding to the complex molecular background, whole-genome sequencing and high-throughput viral integration detection have newly begun to shed light on the central role of HPV in cervical carcinogenesis [51, 52]. A recent study has reported a genome-wide analysis of HPV integration in cervical intraepithelial neoplasias and cervical cancers, and the authors of this study identified HPV integration hotspots in the human genome [51]. The most frequently affected genes are *POU5F1B*, *FHIT*, *KLF12*, *KLF5*, *LRP1B*, *HMGA2*, and *SEMA3D*, supporting their oncogenic role in cervical cancer [51]. The relationship between HPV integration and increased expression of adjacent genes may be a widespread phenomenon in cervical carcinogenesis [48].

Therefore, elucidating the mechanisms of HPV integration will yield insight into HPV-induced cervical carcinogenesis [51]. A better comprehension of the molecular pathogenesis of cervical cancer is of critical importance to identify new therapeutic targets and should lead to the development of personalized individual treatment strategies for patients with cervical cancer [41].

2.7 Future Perspectives for Integrating Genomics in Gynecological Cancer

The overarching goal of TCGA is to improve our understanding of the molecular basis of cancer and advance our ability to diagnose, treat, and prevent cancer through the discoveries and insights enabled by comprehensive mapping of various types of cancer [8, 9]. Furthermore, it is expected that translation of cancer genomics into therapeutics and diagnostics will provide a great potential to develop personalized cancer medicine [53, 54].

To date, TCGA project has provided a strong foundation for genomic studies and has stimulated a diversity of gynecological cancer research [23, 24, 40, 48, 51]. Furthermore, the development of genomic research in gynecological malignancies has led to increased enthusiasm in relation to the promises of targeted therapies and has stimulated rapid advances in genomic technologies to identify the disease biomarkers for gynecological cancer [6]. Even though there has been tremendous success in the rapid accumulation of cancer genomic studies, most of these enormous data sets have not yet been translated into meaningful clinical end points.

We hope that cancer genomic research will ultimately guide clinical decision-making in association with the discovery of novel therapeutic agents and biomarker-based clinical trials that cross boundaries between tumor types. This process will require the amalgamation of expertise and insights from cancer biology, cancer genetics, as well as clinical experiences. It is clear that there is still a long way ahead of us, but the journey to find answers to eradicate gynecological cancer is sure to be an exciting one.

References

1. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell. 2000;100:57–70.
2. Chin L, Gray JW. Translating insights from the cancer genome into clinical practice. Nature. 2008;452:553–63.
3. Stratton MR, Campbell PJ, Futreal PA. The cancer genome. Nature. 2009;458:719–24.
4. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144:646–74.
5. Vogelstein B, Papadopoulos N, Velculescu VE, Zhou S, Diaz LA, Jr., Kinzler KW. Cancer genome landscapes. Science 2013;339:1546-1558.
6. Petrillo M, Nero C, Amadio G, Gallo D, Fagotti A, Scambia G. Targeting the hallmarks of ovarian cancer: the big picture. Gynecol Oncol. 2016;142(1):176–83.
7. Chin L, Andersen JN, Futreal PA. Cancer genomics: from discovery science to personalized medicine. Nat Med. 2011;17:297–303.
8. Tomczak K, Czerwinska P, Wiznerowicz M. The Cancer Genome Atlas (TCGA): an immeasurable source of knowledge. Contemp Oncol (Pozn). 2015;19(1A):A68–77.

9. Cancer Genome Atlas Research N, Weinstein JN, Collisson EA, et al. The Cancer Genome Atlas Pan-Cancer analysis project. Nat Genet. 2013;45:1113–20.
10. Liu J, Westin SN. Rational selection of biomarker driven therapies for gynecologic cancers: the more we know, the more we know we don't know. Gynecol Oncol. 2016;141:65–71.
11. Bast RC, Jr., Hennessy B, Mills GB. The biology of ovarian cancer: new opportunities for translation. Nat Rev Cancer. 2009;9:415-428.
12. Katabuchi H, Okamura H. Cell biology of human ovarian surface epithelial cells and ovarian carcinogenesis. Med Electron Microsc. 2003;36:74–86.
13. Okamura H, Katabuchi H. Pathophysiological dynamics of human ovarian surface epithelial cells in epithelial ovarian carcinogenesis. Int Rev Cytol. 2005;242:1–54.
14. Yap TA, Carden CP, Kaye SB. Beyond chemotherapy: targeted therapies in ovarian cancer. Nat Rev Cancer. 2009;9:167–81.
15. Tjhay F, Motohara T, Tayama S, Narantuya D, Fujimoto K, Guo J, et al. CD44 variant 6 is correlated with peritoneal dissemination and poor prognosis in patients with advanced epithelial ovarian cancer. Cancer Sci. 2015;106:1421–8.
16. Kurman RJ, Shih Ie M. Molecular pathogenesis and extraovarian origin of epithelial ovarian cancer-shifting the paradigm. Hum Pathol. 2011;42:918–31.
17. Prat J. Ovarian carcinomas: five distinct diseases with different origins, genetic alterations, and clinicopathological features. Virchows Arch. 2012;460:237–49.
18. Shih Ie M, Kurman RJ. Ovarian tumorigenesis: a proposed model based on morphological and molecular genetic analysis. Am J Pathol. 2004;164:1511–8.
19. Kurman RJ, Shih Ie M. Pathogenesis of ovarian cancer: lessons from morphology and molecular biology and their clinical implications. Int J Gynecol Pathol. 2008;27:151–60.
20. Singer G, Kurman RJ, Chang HW, Cho SK, Shih Ie M. Diverse tumorigenic pathways in ovarian serous carcinoma. Am J Pathol. 2002;160:1223–8.
21. Senturk E, Cohen S, Dottino PR, Martignetti JA. A critical re-appraisal of BRCA1 methylation studies in ovarian cancer. Gynecol Oncol. 2010;119:376–83.
22. Liu J, Matulonis UA. New strategies in ovarian cancer: translating the molecular complexity of ovarian cancer into treatment advances. Clin Cancer Res. 2014;20:5150–6.
23. Cancer Genome Atlas Research N. Integrated genomic analyses of ovarian carcinoma. Nature. 2011;474:609–15.
24. Patch AM, Christie EL, Etemadmoghadam D, Garsed DW, George J, Fereday S, et al. Whole-genome characterization of chemoresistant ovarian cancer. Nature. 2015;521:489–94.
25. Konecny GE, Wang C, Hamidi H, Winterhoff B, Kalli KR, Dering J, et al. Prognostic and therapeutic relevance of molecular subtypes in high-grade serous ovarian cancer. J Natl Cancer Inst. 2014;106
26. Winterhoff B, Hamidi H, Wang C, Kalli KR, Fridley BL, Dering J, et al. Molecular classification of high grade endometrioid and clear cell ovarian cancer using TCGA gene expression signatures. Gynecol Oncol. 2016;141:95–100.
27. Bolton KL, Chenevix-Trench G, Goh C, Sadetzki S, Ramus SJ, Karlan BY, et al. Association between BRCA1 and BRCA2 mutations and survival in women with invasive epithelial ovarian cancer. JAMA. 2012;307:382–90.
28. Siegel R, Ma J, Zou Z, Jemal A. Cancer statistics, 2014. CA Cancer J Clin. 2014;64:9–29.
29. Creutzberg CL, van Putten WL, Koper PC, Lybeert ML, Jobsen JJ, Wárlám-Rodenhuis CC, et al. Surgery and postoperative radiotherapy versus surgery alone for patients with stage-1 endometrial carcinoma: multicentre randomised trial. PORTEC study group. Post operative radiation therapy in endometrial carcinoma. Lancet. 2000;355:1404–11.
30. Rauh-Hain JA, Del Carmen MG. Treatment for advanced and recurrent endometrial carcinoma: combined modalities. Oncologist. 2010;15:852–61.
31. Group SGOCPECW, Burke WM, Orr J, Leitao M, Salom E, Gehrig P, et al. Endometrial cancer: a review and current management strategies: part II. Gynecol Oncol. 2014;134:393–402.
32. Creasman WT, Kohler MF, Odicino F, Maisonneuve P, Boyle P. Prognosis of papillary serous, clear cell, and grade 3 stage I carcinoma of the endometrium. Gynecol Oncol. 2004;95:593–6.

33. Bokhman JV. Two pathogenetic types of endometrial carcinoma. Gynecol Oncol. 1983;15:10–7.
34. Barlin JN, Zhou Q, St Clair CM, Iasonos A, Soslow RA, Alektiar KM, et al. Classification and regression tree (CART) analysis of endometrial carcinoma: seeing the forest for the trees. Gynecol Oncol. 2013;130:452–6.
35. Hecht JL, Mutter GL. Molecular and pathologic aspects of endometrial carcinogenesis. J Clin Oncol. 2006;24:4783–91.
36. Murali R, Soslow RA, Weigelt B. Classification of endometrial carcinoma: more than two types. Lancet Oncol. 2014;15:e268–78.
37. Lax SF. Molecular genetic pathways in various types of endometrial carcinoma: from a phenotypical to a molecular-based classification. Virchows Arch. 2004;444:213–23.
38. Tashiro H, Blazes MS, Wu R, Cho KR, Bose S, Wang SI, et al. Mutations in PTEN are frequent in endometrial carcinoma but rare in other common gynecological malignancies. Cancer Res. 1997;57:3935–40.
39. Katabuchi H, van Rees B, Lambers AR, Ronnett BM, Blazes MS, Leach FS, et al. Mutations in DNA mismatch repair genes are not responsible for microsatellite instability in most sporadic endometrial carcinomas. Cancer Res. 1995;55:5556–60.
40. Cancer Genome Atlas Research N, Kandoth C, Schultz N, Cherniack AD, Akbani R, Liu Y, et al. Integrated genomic characterization of endometrial carcinoma. Nature. 2013;497:67-73.
41. zur Hausen H. Papillomaviruses and cancer: from basic studies to clinical application. Nat Rev Cancer. 2002;2:342–50.
42. Moody CA, Laimins LA. Human papillomavirus oncoproteins: pathways to transformation. Nat Rev Cancer. 2010;10:550–60.
43. Spriggs AI, Boddington MM. Progression and regression of cervical lesions. Review of smears from women followed without initial biopsy or treatment. J Clin Pathol. 1980;33:517–22.
44. Christopherson WM, Nealon N, Gray LA, Sr. Noninvasive precursor lesions of adenocarcinoma and mixed adenosquamous carcinoma of the cervix uteri. Cancer. 1979;44:975–83.
45. Bouvard V, Baan R, Straif K, Grosse Y, Secretan B, El Ghissassi F, et al. A review of human carcinogens-part B: biological agents. Lancet Oncol. 2009;10:321–2.
46. Steenbergen RD, Snijders PJ, Heideman DA, Meijer CJ. Clinical implications of (epi)genetic changes in HPV-induced cervical precancerous lesions. Nat Rev Cancer. 2014;14:395–405.
47. Tewari KS, Monk BJ. New strategies in advanced cervical cancer: from angiogenesis blockade to immunotherapy. Clin Cancer Res. 2014;20:5349–58.
48. Ojesina AI, Lichtenstein L, Freeman SS, Pedamallu CS, Imaz-Rosshandler I, Pugh TJ, et al. Landscape of genomic alterations in cervical carcinomas. Nature. 2014;506:371–5.
49. Wright AA, Howitt BE, Myers AP, Dahlberg SE, Palescandolo E, Van Hummelen P, et al. Oncogenic mutations in cervical cancer: genomic differences between adenocarcinomas and squamous cell carcinomas of the cervix. Cancer. 2013;119:3776–83.
50. Annunziata C, Buonaguro L, Buonaguro FM, Tornesello ML. Characterization of the human papillomavirus (HPV) integration sites into genital cancers. Pathol Oncol Res. 2012;18:803–8.
51. Hu Z, Zhu D, Wang W, Li W, Jia W, Zeng X, et al. Genome-wide profiling of HPV integration in cervical cancer identifies clustered genomic hot spots and a potential microhomology-mediated integration mechanism. Nat Genet. 2015;47:158–63.
52. Liu CY, Li F, Zeng Y, Tang MZ, Huang Y, Li JT, et al. Infection and integration of high-risk human papillomavirus in HPV-associated cancer cells. Med Oncol. 2015;32:109.
53. Wong AH, Deng CX. Precision medicine for personalized cancer therapy. Int J Biol Sci. 2015;11:1410–2.
54. Aronson SJ, Rehm HL. Building the foundation for genomics in precision medicine. Nature. 2015;526:336–42.

Signal Transduction and Targeted Therapy for Gynecologic Cancer

3

Hiroaki Itamochi and Toru Sugiyama

Abstract

Numerous agents that target specific gynecologic cancer-related molecules have been developed and are now entering clinical trials. These agents target aberrant molecules/processes in tumor tissues, including angiogenesis, poly(ADP-ribose) polymerase (PARP), human epidermal growth factor receptor family, phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) signaling pathway, and a-folate receptor (a-FR). The anti-angiogenic compound bevacizumab is reportedly the most effective targeted agent for ovarian cancer. Bevacizumab plus chemotherapy prolonged progression-free survival (PFS) both for first-line treatment and recurrent ovarian cancer and may increase overall survival (OS) among high-risk patients. Bevacizumab with nonplatinum chemotherapy also prolonged OS in recurrent cervical cancer. Maintenance treatment with a PARP inhibitor, olaparib, improved PFS in platinum-sensitive relapsed ovarian cancer. Furthermore, mTOR inhibitor therapy, alone or with chemotherapy, is an attractive treatment strategy for endometrial cancer. An understanding of tumor molecular biology and identification of predictive biomarkers are essential steps in optimal treatment selection. This article reviews available clinical data of the most promising targeted agents for gynecologic cancer.

Keywords

Targeted therapy • Ovarian cancer • Endometrial cancer • Cervical cancer Clinical trials

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3.1 Introduction

Recent advances in our understanding of cancer molecular biology and carcinogenesis have led to the development of various targeted agents, mainly monoclonal antibodies and small-molecule protein-kinase inhibitors, which have been explored in the management of gynecologic cancers (e.g., ovarian, endometrial, and cervical cancer). Unlike most traditional cytotoxic anticancer drugs, which interfere indiscriminately with DNA synthesis, DNA repair systems, and mitosis, these novel targeted agents affect tumor cells, stroma, and vasculature and aberrant cellular signaling mechanisms in tumor tissues, thus effectively selecting tumor cells for growth inhibition and apoptosis induction with minimal toxicity to normal cells. However, the potential remains for serious toxicity, such as bleeding, thrombosis, and impaired hepatic or renal function. An understanding of tumor molecular biology and identification of predictive biomarkers are essential steps for optimal treatment selection. This chapter reviews the molecular mechanisms of the most promising targeted agents under clinical evaluation for gynecologic cancers.

3.2 Ovarian Cancer

An estimated 238,700 new cases of ovarian cancer and 151,900 deaths were expected worldwide in 2012, representing 4.3% of all cancer deaths in women [1]. Currently, standard primary therapy for advanced ovarian cancer combines maximum cytoreductive surgery and paclitaxel–carboplatin chemotherapy. Although this treatment initially yields a high response rate (>80%), platinum- and taxane-resistant recurrent disease is inevitable. Various targeted therapy agents for the management of ovarian cancer have recently been developed and tested.

3.2.1 Targeting Angiogenesis

Angiogenesis, the formation of new blood and lymphatic vessels from existing vasculature, is a crucial process involved in solid tumor growth, invasion, and metastasis [2]. This process is governed by a number of growth factor receptor pathways and cytokines, including vascular endothelial growth factors (VEGFs), fibroblast growth factors, angiopoietin, platelet-derived growth factors, tumor necrosis factor-α, and interleukin-6 and interleukin-8 (Fig. 3.1) [2]. The VEGF family and receptors (VEGFRs) comprise a major tumor angiogenic pathway. The mammalian VEGF family comprises five structurally related glycoproteins: VEGF-A, VEGF-B, VEGF-C, VEGF-D, and placental growth factor (PlGF) [3]. Of these ligands, VEGF-A primarily mediates tumor angiogenesis and is expressed as various mature isoforms comprising 121, 145, 165, 183, 189, or 206 amino acids via alternative splicing of the *VEGF-A* gene. $VEGF_{165}$ is the predominant isoform and is commonly overexpressed in various human tumors.

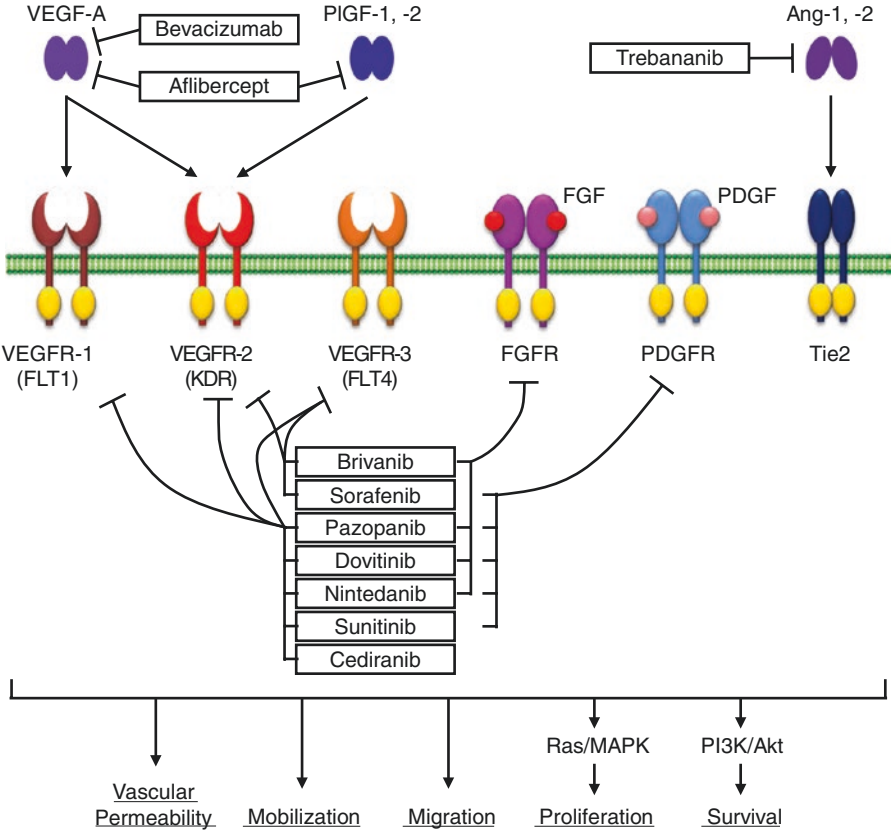

Fig. 3.1 Targeting the angiogenic cascades in gynecologic cancer. Angiogenesis is regulated by a number of growth factor receptor pathways. The specific ligands bind to their receptors, and each tyrosine kinase activates the intracellular signaling cascade, including mitogen-activated protein kinase (MAPK) and phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K)/Akt pathways. Subsequently, the pro-angiogenic signaling pathways are activated. *VEGF* vascular endothelium growth factor, *VEGFR* VEGF receptor, *PlGF* placental growth factor, *FLT* fms-related tyrosine kinase, *KDR* kinase insert domain receptor, *FGF* fibroblast growth factors, *FGFR* FGF receptor, *PDGF* platelet-derived growth factor, *PDGFR* PDGF receptor, *Ang* angiopoietin, *Tie* Tyrosine kinase with immunoglobulin-like and EGF-like domains

These VEGF ligands and PlGF uniquely bind to three structurally similar receptors: VEGFR1 [or fms-related tyrosine kinase 1 (FLT1)], VEGRF2 (or kinase insert domain receptor), and VEGFR3 (or FLT4). VEGF-A binds both VEGFR1 and VEGFR2, which are expressed mainly on vascular endothelial cells; VEFGR2 is predominant and mediates the angiogenic and vascular permeability effects of VEGF [4]. VEGF3 has been reported to play an important role in lymphangiogenesis through preferential binding to VEGF-C and VEGF-D. Neuropilin (NP)1 and NP2 (NRP1 and NRP2, respectively) act as VEGFR co-receptors, thus increasing the binding affinities of VEGFs to their receptors. Ligand binding activates multiple

intracellular signaling cascades downstream of VEGFRs, including mitogen-activated protein kinase (MAPK), phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K)/Akt, phospholipase Cγ, and small GTPase pathways [5] and induces proangiogenic effects such as endothelial cell proliferation, migration, survival, and differentiation. VEGF also increases vascular permeability and vasodilation, causing interstitial hypertension and leaky neovasculature.

VEGF and VEGFR overexpression is observed in many solid tumors, including ovarian cancer, and has been associated with an increased risk of metastatic disease and poor prognosis, [6–8]. In ovarian cancer, higher levels of VEGF-A expression were observed in tumors from patients with platinum-resistant disease vs. those with platinum-sensitive disease [9]. VEGF-A and VEGFR2 coexpression has been detected in both ovarian cancer cells and ovarian tumor tissues, suggesting excision of the autocrine VEGF-A–VEFGR2 loop in ovarian cancer [10, 11]. A recent study found that increased Zeste homolog 2 (EZH2) expression in ovarian tumor cells or tumor vasculature was predictive of a poor clinical outcome [12], and VEGF-A stimulation, which promotes angiogenesis by methylating and silencing vasohibin1, directly led to an increase in endothelial EZH2 expression. These observations indicate that VEGF signaling pathways are promising therapeutic targets in ovarian cancer.

3.2.1.1 Bevacizumab

Bevacizumab is an intravenously (i.v.) administered recombinant humanized monoclonal IgG1 antibody that targets VEGF-A, with clinical benefits in patients with metastatic colorectal cancer, non-small cell lung cancer, and breast cancer [13]. This drug binds and neutralizes all biologically active forms of VEGF-A (e.g., VEGF-A165), thus suppressing tumor growth and inhibiting metastatic disease progression by inhibiting neovascularization and inducing existing microvessel regression [14, 15]. Bevacizumab also normalizes tumor vessels that are structurally and functionally abnormal. These morphological changes lead to functional changes (e.g., decreased interstitial fluid pressure, increased tumor oxygenation, improved drug penetration in tumors) that may enhance the effects of chemotherapy [16].

The phase II trials Gynecologic Oncology Group (GOG)-0170D and AVF2949 evaluated bevacizumab as a monotherapy for recurrent ovarian cancer and yielded favorable results, with response rates of 16–21% [17, 18] and hypertension and proteinuria as the most common grade 3/4 adverse events. Although no gastrointestinal (GI) perforation was observed in patients of the GOG-0170D study who had received one or two previous regimens, the AVF2949 trial observed GI perforation in five patients (11.4%) previously subjected to heavy treatment (three or more prior regimens).

Two landmark phase III trials of bevacizumab for ovarian cancer, GOG-0218 and International Collaborative Ovarian Neoplasm (ICON) 7, were conducted in a first-line/adjuvant chemotherapy setting (Table 3.1) [19, 20]. In the GOG-0218 trial, patients who received combination chemotherapy (paclitaxel/carboplatin) plus bevacizumab (15 mg/kg) for six cycles and maintenance bevacizumab for 16 cycles had a significantly longer progression-free survival (PFS) than those who received first-line chemotherapy alone (median PFS: 10.3 vs. 14.1 months) [19]. However, no

Table 3.1 Phase III trials of targeted therapy in ovarian cancer

Trial	Patients	Treatment	Median PFS (M)	Median OS (M)	Selected Adverse Events [a]
Anti-angiogenic agents					
First-line treatment					
GOG-0218 [19]	1873				
Arm 1	625	CP + placebo → placebo	10.3	39.3	1.2% GI events (G ≥ 2), 7.2% HT (G ≥ 2), 5.8% VTE (any grade), 0.8% bleeding (G ≥ 3)
Arm 2	625	CP + Bevacizumab → placebo	11.2	38.7	2.8% GI events (G ≥ 2), 16.5% HT (G ≥ 2), 5.3% VTE (any grade), 1.3% bleeding (G ≥ 3)
Arm 3	623	CP + Bevacizumab → Bevacizumab	14.1 ***	39.7	2.6% GI events (G ≥ 2), 22.9% HT (G ≥ 2), 6.7% VTE (any grade), 2.1% bleeding (G ≥ 3)
ICON7 [20]	1528				
All patients					
Arm 1	764	CP	17.5	58.6	1.3% GI events, 0.3% HT, 1.7% VTE, 0.3% bleeding
Arm 2	764	CP + Bevacizumab → Bevacizumab	19.9	58.0	2.1% GI events, 6.2% HT, 4.3% VTE, 1.2% bleeding
High-risk patients					
Arm 1	254	CP	10.5	30.2	
Arm 2	248	CP + Bevacizumab → Bevacizumab	16.0 *	39.7 *	
GOG-0262 [22]	692				
All Patients					
Arm 1	346	CP ± Bevacizumab → Bevacizumab	14.0	39.0	15.7% anemia, 83.4% neutropenia
Arm 2	346	Weekly CP ± Bevacizumab → Bevacizumab	14.7	40.2	36.5% anemia, 72.4% neutropenia
Bevacizumab (+)					
Arm 1	289	CP + Bevacizumab → Bevacizumab	14.7	–	
Arm 2	291	Weekly CP + Bevacizumab → Bevacizumab	14.9	–	

(continued)

Table 3.1 (continued)

Trial	Patients	Treatment	Median PFS (M)	Median OS (M)	Selected Adverse Events [a]
Bevacizumab (−)					
Arm 1	57	CP	10.3	–	
Arm 2	55	Weekly CP	14.2 *	–	
AGO-OVAR12 [40]	1366				
Arm 1	455	CP + placebo → placebo	16.6	–	2.0% diarrhea, 0.4% HT, 6.9% anemia, 6.4% thrombocytopenia, 36.0% neutropenia
Arm 2	911	CP + Nintedanib → Nintedanib	17.2 *	–	21.5% diarrhea, 4.7% HT, 13.5% anemia, 17.7% thrombocytopenia, 42.1% neutropenia
Maintenance					
AGO-OVAR16 [37]	940			2nd interim OS analysis	
Arm 1	468	Pt CT → placebo	12.3	HR = 1.08 (0.87–1.33)	5.6% HT, 1.5% neutropenia, 0.7% liver-related toxicity, 1.1% diarrhea, 0.2% fatigue, 0.7% thrombocytopenia, 0.2% palmar-plantar erythrodysesthesia
Arm 2	472	Pt CT → Pazopanib	17.9 *		30.8% HT, 9.9% neutropenia, 9.4% liver-related toxicity, 8.2% diarrhea, 2.7% fatigue, 2.5% thrombocytopenia, 1.9% palmar-plantar erythrodysesthesia
Recurrent disease					
OCEANS [23, 25]	484				
Arm 1	242	CG + placebo	8.4	35.2	0.4% HT, 0.9% Proteinuria, 2.6% VTE
Arm 2	242	CG + Bevacizumab	12.4 ***	33.3	17.4% HT, 8.5% Proteinuria, 4.0 VTE
AURELIA [24]	361				
Arm 1	182	chemotherapy alone [b]	3.4	13.3	1.1% HT, 4.4% TEE

Table 3.1 (continued)

Trial	Patients	Treatment	Median PFS (M)	Median OS (M)	Selected Adverse Events [a]
Arm 2	179	chemotherapy + Bevacizumab	6.7 **	16.6	7.3% HT, 5.0% TEE, 1.7% proteinuria, 1.7% GI perforation
ICON6 [34]	456			OS data immature	
Arm 1	118	Pt CT + placebo → placebo	8.7	21.0	[n = 115] 7.8% fatigue, 3.5% HT, 1.7% diarrhea, 6.1% nausea/vomiting, 3.5% febrile neutropenia, 23.5% neutropenia, 2.6% thrombocytopenia
Arm 2	174	Pt CT + Cediranib → placebo	9.9	–	[n = 329] 16.4% fatigue, 11.6% HT, 10.3% diarrhea, 7.0% nausea/vomiting, 6.7% febrile neutropenia, 25.6% neutropenia, 7.6% thrombocytopenia (during chemotherapy phase: Arm 2/3)
Arm 3	164	Pt CT + Cediranib → Cediranib	11.0 *	26.3	
TRINOVA-1 [51]	919				
Arm 1	458	Weekly PTX + placebo	5.4	17.3	Any grade: 25.7% edema, 0.2% GI perforation, 3.5% HT, 3.8% VTE, 16.6% bleeding
Arm 2	461	Weekly PTX + Trebananib	7.2 ***	19.0	Any grade: 57.3% edema, 1.5% GI perforation, 6.1% HT, 6.3% VTE, 10.0% bleeding
EGFR inhibitors					
Maintenance					
EORTC 55041 [69]	835				
Arm 1	415	Pt CT → observation	12.4	59.1	
Arm 2	420	Pt CT → Erlotinib	12.8	50.8	12.8% rash, 4.8% diarrhea

(continued)

Table 3.1 (continued)

Trial	Patients	Treatment	Median PFS (M)	Median OS (M)	Selected Adverse Events [a]
FRα inhibitors					
Recurrent disease					
Farletuzumab [89]	1091				
Arm 1	352	PtTx CT + placebo → placebo	9.0	29.1	41.2% neutropenia, 8.0% thrombocytopenia, 13.6% leukopenia, 9.9% anemia
Arm 2	376	PtTx CT + Farletuzumab (1.25 mg/kg) → Farletuzumab	9.5	28.7	44.4% neutropenia, 13.0% thrombocytopenia, 11.7% leukopenia, 10.1% anemia
Arm 3	363	PtTx CT + Farletuzumab (2.5 mg/kg) → Farletuzumab	9.7	32.1	38.3% neutropenia, 11.6% thrombocytopenia, 9.9% leukopenia, 10.2% anemia
CA125 < 3 × ULN [c]					
Arm 1	118	PtTx CT + placebo → placebo	8.8	29.1	
Arm 2	174	PtTx CT + Farletuzumab (1.25 mg/kg) → Farletuzumab	9.5	NE	
Arm 3	164	PtTx CT + Farletuzumab (2.5 mg/kg) → Farletuzumab	13.6 *	NE *	

PFS progression-free survival, *M* months, *OS* overall survival, *GOG* Gynecologic Oncology Group, *ICON* International Co-operative Group for Ovarian Neoplasia, *AGO* Arbeitsgemeinschaft Gynäkologische Onkologie, *EORTC* European Organization for Research, *EGFR* epidermal growth factor receptor, *FR* folate receptor, *CP* carboplatin plus paclitaxel, *Weekly CP* weekly paclitaxel plus every 3-weeks carboplatin, *CG* carboplatin plus gemcitabine, *PTX* paclitaxel, *Pt CT* platinum-based chemotherapy, *PtTx* CT platinum- and taxane-based chemotherapy, *HR* hazard ratio, *NE* not estimated, *GI* gastrointestinal, *G* grade, *HT* hypertension, *VTE* venous thrombosis, *TEE* thromboembolic events

[a]Selected adverse events (grade ≥ 3), except for indicated

[b]Investigator selected chemotherapy (pegylated liposomal doxorubicin, topotecan, or weekly paclitaxel)

[c]Subgroup with CA125 levels not more than three times the upper limit of normal (ULN)

$^{*}P < 0.05$, $^{**}P < 0.001$, $^{***}P < 0.0001$ vs. control arm

statistically significant difference was observed in overall survival (OS). Similarly, patients in the ICON 7 trial were randomized to chemotherapy alone (carboplatin/paclitaxel) or plus bevacizumab (7.5 mg/kg) for six cycles, with 12 cycles of maintenance bevacizumab [20]. The latest report observed a significantly prolonged restricted mean survival time among poor-prognosis patients in the bevacizumab group vs. the chemotherapy group (39.3 vs. 34.5 months), although no OS benefit of bevacizumab was recorded [21].

A randomized trial, GOG-0262, evaluated the optimal combination of bevacizumab with dose-dense therapy (weekly paclitaxel plus carboplatin every 3 weeks) and conventional dose therapy (paclitaxel/carboplatin every 3 weeks; Table 3.1) [22]. Both groups of patients who opted to receive bevacizumab had a similar PFS, although among patients who did not receive bevacizumab, the medium PFS was 3.9 months longer with dose-dense therapy vs. conventional dose therapy (14.2 vs. 10.3 months).

Two phase III trials, OCEANS (Ovarian Cancer Study Comparing Efficacy and Safety of Chemotherapy and Anti- Angiogenic Therapy in Platinum-Sensitive Recurrent Disease) and AURELIA (Avastin Use in Platinum-Resistant Epithelial Ovarian Cancer), were conducted to evaluate recurrent disease (Table 3.1). Both trials evaluated the effect of bevacizumab in combination with chemotherapy and observed improvements in PFS [23, 24]. In the OCEANS study, platinum-sensitive recurrent ovarian cancer patients received six cycles of carboplatin/gemcitabine in combination with bevacizumab, followed by maintenance bevacizumab, and patients receiving bevacizumab had a significantly longer PFS vs. the control arm (median PFS: 12.4 vs. 8.4 months) [23]. However, the final OS analysis revealed no significant difference between the treatment arms [25]. In the AURELIA study, platinum-resistant recurrent ovarian cancer patients received single-agent chemotherapy [pegylated liposomal doxorubicin (PLD), weekly paclitaxel, or topotecan] alone or with bevacizumab. PFS was significantly improved in the chemotherapy plus bevacizumab arm vs. the chemotherapy arm (median PFS: 6.7 vs. 3.4 months). However, the trend toward improved OS (median: 16.6 vs. 13.3 months) was not statistically significant. GI perforation was observed only in the bevacizumab arm (2.2%), although the risk was lower than expected.

Several other ongoing phase III trials are investigating the optimal use of bevacizumab. The GOG-0252 study is evaluating the efficacy of bevacizumab in combination with intraperitoneal (i.p.) chemotherapy (i.v. paclitaxel and i.p. cisplatin or carboplatin) vs. i.v. chemotherapy (paclitaxel/carboplatin). The AGO-OVAR 17 (BOOST) trial is investigating the optimal bevacizumab treatment duration (15 vs. 30 months) with first-line chemotherapy (paclitaxel/carboplatin). GOG-0213, a study on platinum-sensitive recurrent disease, is comparing chemotherapy (paclitaxel/carboplatin) alone vs. with bevacizumab; surgical candidates in this cohort will undergo secondary randomization to surgery or no surgery.

3.2.1.2 Aflibercept

Aflibercept (VEGF Trap) is a fusion protein of the Fc region of immunoglobulin G1 with domain two of VEGFR1 and domain three of VEGFR2 ($VEGFR_{\delta 1R2}$). This decoy receptor binds with high affinity to VEGF-A, thus preventing VEGFR1 and VEGFR2 binding and subsequent stimulation [26]. Aflibercept also exhibits a strong binding affinity for VEGF-B and PlGF.

Two phase II studies of platinum-resistant disease and symptomatic malignant ascites have been conducted (Table 3.2) [27, 28], and both demonstrated effective control of malignant ascites with aflibercept, evidenced by a reduction in the interval between repeat paracenteses (e.g., 55.1 vs. 23.3 days) [28]. However, one study observed a higher frequency of fatal GI events in the aflibercept arm (3/29 patients) vs. the placebo arm (1/26 patients) [28]. In the other study, platinum-resistant ovarian cancer patients were randomized to receive aflibercept at different doses (2 or 4 mg/kg) (Table 3.2) [29]. Although aflibercept was generally well tolerated at both doses, the response rate was low.

Table 3.2 Randomized phase II trials of targeted therapy in ovarian cancer

Agents	Patients (n) [a]	Treatment	Response Rate (%)	Median PFS (M)	Median OS (M)	Selected Grade ≥ 3 Adverse Events
Anti-angiogenic agents						
Maintenance						
Nintedanib [39]	83			36-week PFS		
Arm 1	40	Chemotherapy → placebo	–	16.3%	–	8% hepatotoxicity
Arm 2	43	Chemotherapy → Nintedanib	–	5.0%	–	51% hepatotoxicity
Sorafenib [47]	246					
Arm 1	123	PtTx CT → placebo	–	15.7	–	0.8% hand–foot skin reaction
Arm 2	123	PtTx CT → Sorafenib	–	12.7	–	39.0% hand–foot skin reaction, 14.6% rash
Recurrent disease						
Aflibercept [27]	55		Time to repeat paracentesis			
Arm 1	26	placebo	23.3 days	–	–	8% dyspnea, 44% fatigue/asthenia, 4% GI fistula
Arm 2	29	Aflibercept: 4 mg/kg every 2 weeks	55.1 days *	–	–	20% dyspnea, 13% fatigue/asthenia, 10% GI perforation, 8% proteinuria, 7% HT, 7% VTE

Table 3.2 (continued)

Agents	Patients (n) [a]	Treatment	Response Rate (%)	Median PFS (M)	Median OS (M)	Selected Grade ≥ 3 Adverse Events
Aflibercept [28]	215					
Arm 1	106	Aflibercept: 2 mg/kg every 2 weeks	0.9	13.0 weeks	59.0 weeks	25.5% HT, 9.4% proteinuria, 5.7% fatigue
Arm 2	109	Aflibercept: 4 mg/kg every 2 weeks	4.6	13.3 weeks	49.3 weeks	27.5% HT, 7.3% proteinuria, 3.7% fatigue
Pazopanib [38]	73					
Arm 1	36	Weekly PTX	25	3.5	13.7	3% neutropenia, 6% fatigue, 3% leucopenia, 14% anemia
Arm 2	37	Weekly PTX + Pazopanib	56 *	6.5 **	19.1	30% neutropenia, 11% fatigue, 11% leucopenia, 8% hypertension, 8% raised aspartate aminotransferase or alanine aminotransferase, 5% anemia, 3% ileal perforation.
Sunitinib [43]	73					
Arm 1	36	Sunitinib: 50 mg daily for 4 weeks in a 6-week cycle	17	4.8	13.6	4.5% increased γ-glutamyl transferase (% of all reported adverse events)
Arm 2	37	Sunitinib: 37.5 mg daily continuously	5	2.9	13.7	6.1% increased γ-glutamyl transferase (% of all reported adverse events)
Trebananib [50]	161					
Arm 1	53	PTX + Trebananib 10 mg days 1, 8, 15	37	7.2	22.5	[$n = 52$] 12% hypokalemia, 10% peripheral neuropathy, 6% VTE.
Arm 2	53	PTX + Trebananib 3 mg days 1, 8, 15	19	5.7	20.4	11% hypokalemia, 9% dyspnea, 4% VTE
Arm 3	55	PTX + placebo days 1, 8, 15	27	4.6	20.9	4% hypokalemia, 9% VTE

(continued)

Table 3.2 (continued)

Agents	Patients (n) [a]	Treatment	Response Rate (%)	Median PFS (M)	Median OS (M)	Selected Grade ≥ 3 Adverse Events
PARP inhibitors						
Maintenance						
Olaparib [58, 59]	265					
Arm 1	129	Pt CT → observation	–	4.8	27.8	3.1% fatigue, 0.8% anemia
Arm 2	136	Pt CT → Olaparib	–	8.4 **	29.8	7.7% fatigue, 5.1% anemia
BRCA (+)						
Arm 1	62	Pt CT → observation		4.3	31.9	
Arm 2	74	Pt CT → Olaparib		11.2 ***	34.9	
BRCA (−)						
Arm 1	61	Pt CT → observation		5.5	26.2	
Arm 2	57	Pt CT → Olaparib		7.4 *	24.5	
Recurrent disease						
Olaparib [57]	97					
Arm 1	32	Olaparib: 200 mg twice per day	25	6.5	13.1	6% abdominal pain, 6% constipation, 6% anemia
Arm 2	32	Olaparib: 400 mg twice per day	31	8.8	13.0	13% anemia, 9% fatigue, 6% nausea
Arm 3	33	PLD	18	7.1	13.0	38% Palmar-plantar erythrodysesthesia syndrome, 9% fatigue, 9% rash
Olaparib [60]	90					
Arm 1	81	CP	58	9.6	37.6	4% fatigue 35% neutropenia, 7% anemia, 8% thrombocytopenia
Arm 2	81	CP + Olaparib	64 *	12.2 *	33.8	[$n = 75$] 7% fatigue 43% neutropenia, 9% anemia, 8% thrombocytopenia
Olaparib [61]	90					
Arm 1	46	Olaparib	48	9.0	–	11% fatigue
Arm 2	44	Olaparib + Cediranib	80 *	11.7 *	–	41% HT, 27% fatigue, 23% diarrhea

Table 3.2 (continued)

Agents	Patients (n) [a]	Treatment	Response Rate (%)	Median PFS (M)	Median OS (M)	Selected Grade ≥ 3 Adverse Events
Veliparib [63]	70					
Arm 1	36	Cyclophosphamide	19.4	2.3	–	8% lymphopenia
Arm 2	34	Cyclophosphamide + Veliparib	11.8	2.1	–	35% lymphopenia
HER2 inhibitors						
Recurrent disease						
Pertuzumab [76]	130					
Arm 1	65	GEM + placebo	5	2.6	13.1	22% neutropenia, 8% thrombocytopenia, 2% diarrhea, 2% back pain
Arm 2	65	GEM + Pertuzumab	14	2.9	13.0	35% neutropenia, 14% thrombocytopenia, 11% diarrhea, 9% back pain
Low HER3						
Arm 1	35	GEM + placebo		1.4	8.4	
Arm 2	26	GEM + Pertuzumab		5.3 **	12.5	
Recurrent disease						
Pertuzumab [77]	149					
Arm 1	75	CP or CG	59	40.0 weeks	NR	Adverse events during the first six cycles of treatment were similar in both arms
Arm 2	74	CP or CG + Pertuzumab	61	34.1 weeks	28.2	
Folate receptor						
Recurrent disease						
Vintafolide [91]	149					
Arm 1	49	PLD	12	2.7	–	[$n = 50$] 2% Palmar-plantar erythrodysesthesia syndrome, 6% fatigue, 2% abdominal pain, 4% stomatitis, 10% neutropenia, 8% anemia, 9% leukopenia

(continued)

Table 3.2 (continued)

Agents	Patients (n) [a]	Treatment	Response Rate (%)	Median PFS (M)	Median OS (M)	Selected Grade ≥ 3 Adverse Events
Arm 2	100	PLD + Vintafolide → Vintafolide	18	5.0 *	–	[*n* = 107] 11% Palmar-plantar erythrodysesthesia syndrome, 9% fatigue, 8% abdominal pain, 8% stomatitis, 23% neutropenia, 9% anemia
FT inhibitor						
First-line treatment						
Lonafarnib [92]	105					
Arm 1	52	CP	–	17.8	47.3	4% diarrhea
Arm 2	53	CP + Lonafernib → Lonafernib	–	14.2	33.4	23% diarrhea
IV, >1.0 cm [b]						
Arm 1	14	CP		16.4	43.4	
Arm 2	18	CP + Lonafernib → Lonafernib		11.5 *	20.6 *	
ET_A-receptor antagonist						
Recurrent disease						
Zibotentan [93]	120					
Arm 1	61 (58)	CP + placebo → placebo	59	10.0	–	[*n* = 58] 31% neutropenia, 9% anemia, 16% leukopenia, 9% thrombocytopenia
Arm 2	59 (55)	CP + Zibotentan → Zibotentan	38 *	7.6	–	[*n* = 58] 41% neutropenia, 12% anemia, 10% leukopenia, 5% thrombocytopenia
PKCβ inhibitor						
First-line treatment						
Enzastaurin [94]	142					
Arm 1	73 (18)	CP + placebo → placebo	39	15.2	47.3	[*n* = 72] 1% hypersensitivity, 3% constipation, 3% fatigue
Arm 2	69 (14)	CP + Enzastaurin → Enzastaurin	43	18.9	33.4	[*n* = 67] 1% constipation, 1% diarrhea, 3% dyspnea, 3% edema

Table 3.2 (continued)

Agents	Patients (n) [a]	Treatment	Response Rate (%)	Median PFS (M)	Median OS (M)	Selected Grade ≥ 3 Adverse Events
Methyltransferase inhibitor						
Recurrent disease						
Decitabine [95]	29					
Arm 1	14	Carboplatin	64	6.9	–	15% neutropenia
Arm 2	4	Carboplatin + Decitabine: 90 mg/m^2	20 [d] *	1.9	–	60% neutropenia [d]
Arm 3	11	Carboplatin + Decitabine: 45 mg/m^2		6.0	–	
Plk inhibitor						
Recurrent disease						
Volasertib [96]	109					
Arm 1	55	Non-Pt CT [c]	13	8.4 weeks	–	5% neutropenia, 2% anemia, 4% thrombocytopenia
Arm 2	54	Volasertib	15	13.1 weeks	–	44% neutropenia, 15% anemia, 17% thrombocytopenia, 17% leukopenia

PFS progression-free survival, *M* months, *OS* overall survival, *PARP* poly (ADP-ribose) polymerase, *HER2* human epidermal growth factor receptor, *BRCA (+)* mutated BRCA, *BRCA (−)* wild-type BRCA, *FT* farnesyltransferase, *ET* endothelin, *PKC* protein kinase C, *Plk* Polo-like kinase, *PtTx* CT platinum- and taxane-based chemotherapy, *PTX* paclitaxel, *Pt CT* platinum-based chemotherapy, *PLD* pegylated liposomal doxorubicin, *CP* carboplatin plus paclitaxel, *GEM* gemcitabine, *CG* carboplatin plus gemcitabine, *NR* not yet reached, *GI* gastrointestinal, *HT* hypertension, *VTE* venous thrombosis

[a]Number of patients eligible (Number of patients evaluable for response)

[b]Stage IV and residual disease >1.0 cm

[c]Investigator selected single-agent, non-platinum chemotherapy (pegylated liposomal doxorubicin, gemcitabine, weekly paclitaxel, or topotecan)

[d]Combination of arm 2 and 3

*$P < 0.05$, ** $P < 0.001$, *** $P < 0.0001$ vs. control arm

A phase I–II study of aflibercept in combination with docetaxel was conducted in patients with recurrent ovarian cancer [30]. The objective response rate was 54%, and grade 1–2 hypertension (11%) and grade 2 hypotension (2%) were adverse events specifically associated with aflibercept. Therefore, the combination of aflibercept and docetaxel seems safe and active for patients with recurrent ovarian cancer.

3.2.1.3 Cediranib

Cediranib is a highly potent, small-molecule, oral tyrosine kinase inhibitor of all three VEGF receptors (VEGFR1–3) and c-Kit, which competes for the ATP-binding site within the receptor kinase domain [31, 32]. A phase II trial of cediranib in patients with recurrent ovarian cancer reported a partial response (PR) rate of 17% and median PFS of 5.4 months [33]. These promising results led to a phase III study (ICON6) of patients with platinum-sensitive recurrent disease (Table 3.1) in which the median PFS was significantly prolonged in the platinum-based chemotherapy plus concurrent and maintenance cediranib arm (arm 3) vs. the chemotherapy and placebo (arm 1) (11.0 vs. 8.7 months) [34]. Although the OS analysis is ongoing, early median OS durations for arms 1 and 3 were 21.0 months and 26.3 months, respectively ($P = 0.11$).

3.2.1.4 Pazopanib

Pazopanib is a potent, selective oral multi-targeted receptor tyrosine kinase inhibitor of VEGFR1–3, platelet-derived growth factor receptor (PDGFR)-α and PDGFR-β, and fibroblast growth factor receptor (FGFR) 1–3 [35]. A phase II study (VEG104450) of pazopanib in patients with recurrent ovarian cancer reported a PR rate of 18% [36]. In a phase III trial (AGO-OVAR16), patients with International Federation Gynecology Obstetrics (FIGO) stage II–IV ovarian cancer received maintenance pazopanib or placebo for up to 24 months (Table 3.1) [37]. PFS was significantly prolonged for patients in the maintenance pazopanib arm vs. those in the placebo arm (median PFS: 17.9 vs. 12.3 months), although OS did not differ significantly between the arms at the interim analysis. In a randomized phase II trial (MITO 11), patients with platinum-resistant ovarian cancer received weekly paclitaxel with or without pazopanib; PFS was significantly longer in the paclitaxel/pazopanib group vs. the paclitaxel-only group (median PFS: 6.35 vs. 3.49 months) (Table 3.2) [38]. Two randomized phase II trials to evaluate chemotherapy (paclitaxel or gemcitabine) and combined effects with pazopanib are ongoing in patients with platinum-resistant ovarian cancer.

3.2.1.5 Nintedanib

Nintedanib (BIBF 1120) is a potent, oral tyrosine kinase inhibitor of VEGFR1–3, PDGFR-α and -β, and FGFR1–3. In a placebo-controlled randomized phase II trial of post-chemotherapy maintenance therapy in patients with relapsed ovarian cancer, nintedanib was well tolerated and associated with a potential improvement in PFS (Table 3.2) [39]. A phase III trial (AGO-OVAR12) investigated the combination of standard chemotherapy (paclitaxel/carboplatin) with nintedanib or placebo in patients with newly diagnosed FIGO stage IIB–IV ovarian cancer (Table 3.1) [40] and observed a significantly longer median PFS in the nintedanib group vs. the placebo group (17.2 vs. 16.6 months). The efficacy of nintedanib was particularly notable in patients with a low postsurgical disease burden (FIGO stage IIB–III, ≤1 cm residual postoperative tumor). Although the OS results are pending, further studies are needed to assess the clinical value of nintedanib, particularly in cohorts with lower tumor burdens.

3.2.1.6 Sunitinib

Sunitinib is a potent, oral multi-tyrosine kinase inhibitor that targets VEGFR1–3, PDGFR-α and -β, Flt-3, and c-Kit [41]. Three phase II trials were conducted to evaluate the efficacy and safety of this inhibitor in patients with recurrent ovarian cancer (Table 3.2) [42–44]. However, efficacy seemed to be limited, with response rates of 3–17%, and the common adverse events included hypertension, gastrointestinal events, fatigue, and hand–foot syndrome.

3.2.1.7 Sorafenib

Sorafenib is an oral bis-aryl urea that inhibits c-Raf and b-Raf kinases and VEGFR-2 and -3, PDGFR-β, Flt-3, and c-Kit [45]. In a phase II trial (GOG-0170F) of sorafenib for patients with recurrent ovarian cancer, PR rate of 3% and median PFS and OS of 2.1 months and 16.3 months, respectively, were achieved [46]. However, a randomized phase II study of sorafenib maintenance therapy observed no significant difference in PFS between the sorafenib and placebo arms (Table 3.2) [47].

3.2.1.8 Trebananib

In tumor angiogenesis, angiopoietin-1 and angiopoietin-2 interact with the tyrosine kinase with immunoglobulin-like and EGF-like domains (Tie) 2 receptor, which is expressed on endothelial cells, to mediate blood vessel maturation and stabilization in a VEGF axis-independent pathway [48]. Trebananib (AMG 386), a neutralizing peptibody (i.e., peptide-Fc fusion protein), blocks the binding of both angiopoietin-1 and angiopoietin-2 to the Tie2 receptor, thereby inhibiting angiogenesis [49].

In a randomized phase II trial, trebananib combined with weekly paclitaxel prolonged PFS in patients with recurrent ovarian cancer (Table 3.2) [50]. A phase III trial, Trebananib in Ovarian Cancer-1 (TRINOVA-1), investigated trebananib in addition to single-agent weekly paclitaxel for patients with recurrent ovarian cancer (Table 3.1) [51]. Median PFS was significantly longer in the paclitaxel/trebananib group vs. the paclitaxel/placebo group (7.2 vs. 5.4 months), although the median OS did not statistically differ. Two subsequent phase III trials are ongoing: TRINOVA-2, which evaluates trebananib plus PLD for recurrent, partially platinum-sensitive ovarian cancer, and TRINOVA-3, which investigates trebananib plus first-line chemotherapy (carboplatin/paclitaxel) for FIGO stage III–IV ovarian cancer.

3.2.2 Targeting DNA Repair Mechanisms: Poly(ADP-Ribose) Polymerase (PARP)

PARPs have multiple functions, including DNA repair, cell dysfunction and necrosis, and inflammation (Fig. 3.2) [52]. PARP-1, the most abundant nuclear isoform, plays a vital role in DNA single-strand break (SSB) repair through the base excision repair pathway, whereas residual PARP activity (approximately 10%) is attributed to PARP-2. PARP inhibition causes an accumulation of DNA SSBs and consequent DNA double-strand breaks (DSBs) at replication forks. In

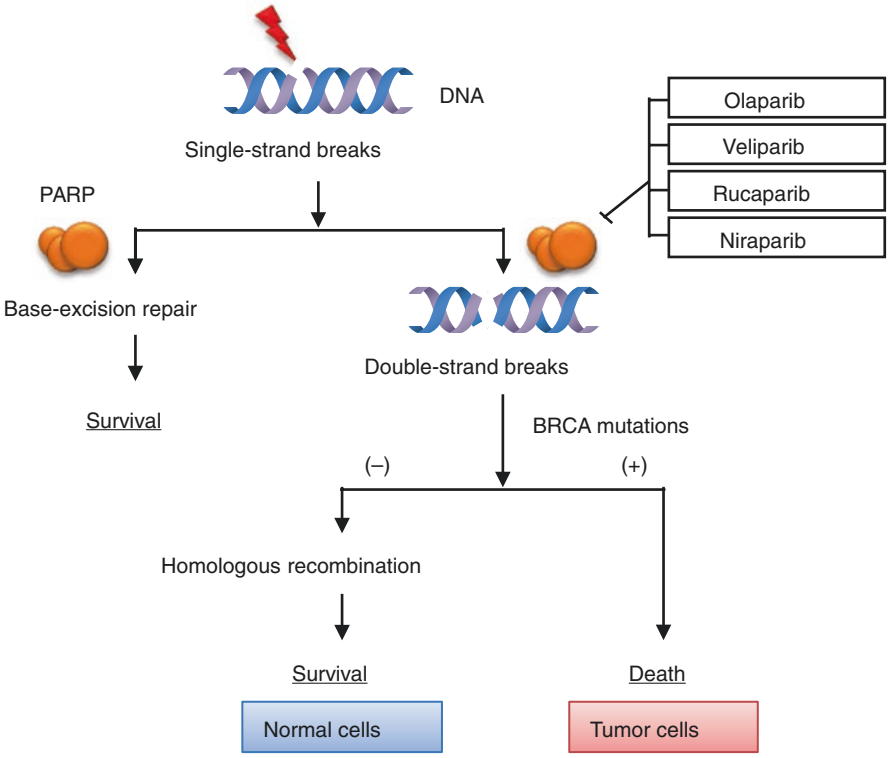

Fig. 3.2 Effect of DNA repair systems on poly(ADP-ribose) polymerase activity. Single-strand breaks lead to the activation of poly(ADP-ribose) polymerases (PARPs). PARP plays a key role in the repair of single-strand breaks. Treatment with a PARP inhibitor induces double-strand breaks and selectively kills homologous recombination-deficient tumor cells. *BRCA* breast cancer susceptibility gene

normal cells, such DSBs are generally repaired by the BRCA1- and BRCA2-dependent homologous recombination (HR) DNA repair pathway. However, these lesions are not repaired in BRCA1- or BRCA2-deficient tumor cells, leading to genomic instability and cell death despite the existence of an alternate non-homologous end-joining pathway for DSB repair.

Female carriers of germline mutations in *BRCA1* on chromosome 17q21 or *BRCA2* on chromosome 13q31 have a higher risk of breast and ovarian cancer development. The lifetime risks of ovarian cancer are 54% for *BRCA1* and 23% for *BRCA2* mutation carriers [53]. Although germline mutations in those genes are seen in 5–10% of all ovarian cancer patients, a loss of HR function (BRCAness), either via genetic or epigenetic events in *BRCA1* or *BRCA2* or alterations in other genes (e.g., *EMSY*, *PTEN*, *RAD51C*, *ATM*, *ATR*, Fanconi anemia genes), are observed in approximately half of high-grade serous ovarian carcinomas [54]. In ovarian cancer, a BRCAness profile may correlate with responses to platinum-based chemotherapy and PARP inhibitors.

3.2.2.1 Olaparib

Olaparib is an oral small-molecule PARP inhibitor that induces synthetic lethality in cells with defective BRCA function [55]. Pooled data from phase I/II trials of olaparib (400 mg twice daily) monotherapy demonstrated an objective response of 36% in germline *BRCA1/2* mutation carriers with recurrent ovarian cancer [56, 57]. An ongoing phase III trial of *BRCA* mutation carriers with platinum-sensitive recurrent ovarian cancer, SOLO3, compares olaparib monotherapy vs. the physician's selected chemotherapy (weekly paclitaxel, topotecan, PLD, or gemcitabine).

The efficacy of olaparib maintenance therapy was evaluated in a randomized phase II study of patients with platinum-sensitive, relapsed, high-grade serous ovarian cancer (Table 3.2) [58, 59]. Among *BRCA* mutation carriers, the median PFS was significantly longer in the olaparib group vs. the placebo group (11.2 vs. 4.3 months), and similar results were observed for wild-type *BRCA* carriers (7.4 vs. 5.5 months). However, OS did not significantly differ between the groups. Phase III confirmatory trials of maintenance olaparib monotherapy are ongoing in *BRCA* mutation carriers with ovarian cancer after first-line platinum-based chemotherapy (SOLO1) and those who have achieved a complete response (CR) or PR with platinum chemotherapy (SOLO2).

A combination therapy of olaparib with chemotherapy (carboplatin/paclitaxel) was tested in patients with platinum-sensitive recurrent, high-grade serous ovarian cancer in a randomized phase II trial (Table 3.2) [60]. PFS was significantly longer in patients treated with olaparib plus chemotherapy followed by maintenance olaparib monotherapy vs. those treated with chemotherapy alone (median PFS, 12.2 vs. 9.6 months), although OS did not differ significantly between the treatment groups. The combined effect of olaparib with targeted agents on patient outcome is also under investigation. In a randomized phase II trial, recurrent platinum-sensitive ovarian cancer patients received olaparib alone or cediranib plus olaparib (Table 3.2) [61]. The median PFS was significantly improved in the combination group vs. the olaparib alone group (17.7 vs. 9.0 months). However, grade 3/4 adverse events were more common with combination therapy. These promising results initiated a randomized phase III trial (NRG-GY004) of platinum-sensitive recurrent ovarian cancer with three treatment arms: (1) carboplatin and paclitaxel (regimen I), gemcitabine (regimen II), or PLD (regimen III), (2) olaparib, and (3) olaparib and cediranib.

3.2.2.2 Other PARP Inhibitors

Besides olaparib, several PARP inhibitors, including veliparib, rucaparib, and niraparib, are being evaluated in clinical trials. Veliparib was tested as a monotherapy for *BRCA*-mutated recurrent ovarian cancer in a phase II trial (GOG-0280), yielding an overall response rate of 26% [62]. A randomized phase II trial of veliparib with low-dose cyclophosphamide did not improve the response rate or median PFS in patients with high-grade serous ovarian cancer (Table 3.2) [63]. A phase III trial of veliparib with first-line chemotherapy (carboplatin/paclitaxel) followed by maintenance veliparib (GOG-3005) is currently recruiting patients with high-grade serous ovarian cancer. Rucaparib (ARIEL3 trial) and niraparib (NOVA trial) are

also currently under evaluation in phase III trials of maintenance treatment after platinum-sensitive recurrent ovarian cancer. These trials are recruiting both sporadic and *BRCA*-mutated ovarian cancer patients.

3.2.3 Targeting the Human Epidermal Growth Factor Receptor Family

The epidermal growth factor receptor (EGFR; HER in humans) family comprises four distinct transmembrane tyrosine kinase receptors: HER-1 (EGFR/erbB1), HER-2/neu (erbB2), HER-3 (erbB3), and HER-4 (erbB4) [64]. These receptors are activated via C-terminal autophosphorylation by ligand binding (although HER2 has no known ligand) and multiple receptor homo- or hetero-dimerization combinations, thus triggering downstream signaling pathways such as the MAPK and PI3K/Akt pathways and thus inducing cancer-cell proliferation, blocking apoptosis, activating invasion and metastasis, and stimulating tumor-induced neovascularization. Accordingly, HERs are attractive targets for anticancer therapies (Fig. 3.3) [64].

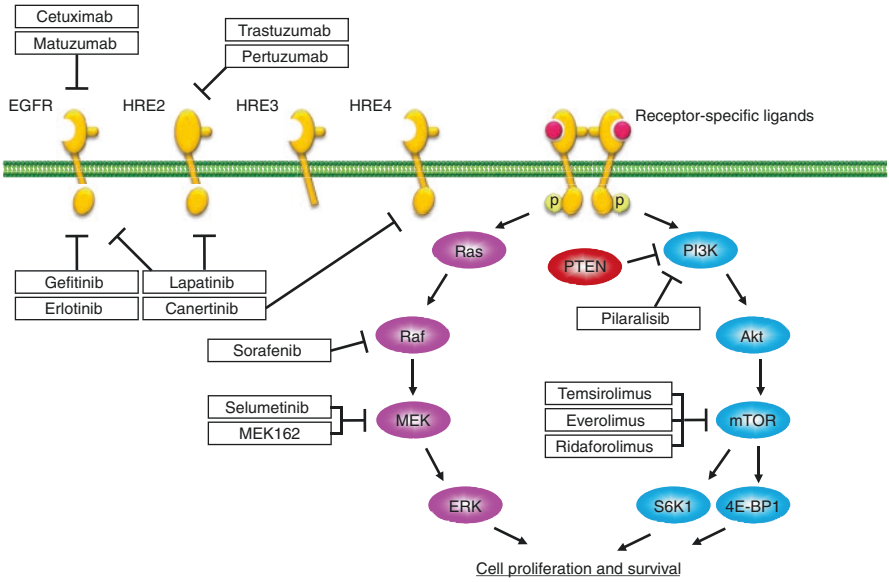

Fig. 3.3 Targeting the human epidermal growth factor receptor family members and their downstream signaling pathways in gynecologic cancer. The human epidermal growth factor receptor (HER) family consists of four distinct transmembrane tyrosine kinase receptors, and receptor-specific ligands selectively bind to each of them. The receptor undergoes homo- or hetero-dimerization that leads to receptor autophosphorylation that activates a series of downstream signaling pathways, such as mitogen-activated protein kinase kinase (MEK)/extracellular signal-regulated kinase (ERK) and phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K)/Akt pathways that control cell growth and apoptotic signaling. *EGFR* epidermal growth factor receptor, *HER* human EGFR, *PTEN* phosphatase and tensin homolog, *mTOR* mammalian target of rapamycin, *S6K1* ribosomal protein S6 kinase 1, *4E-BP1* eukaryotic translation initiation factor 4E binding protein 1, *P* phosphate

HER family members are expressed in many human malignancies, including ovarian cancers, in which a wide range of HER family expression has been reported [EFGR, 4–100% (average, 48%); HER-2, 0–100% (average, 40%); HER-3, 3–90% (average, 48%); and HER-4, 45–92% (average, 71%)] [65]. Overexpression of HER, particularly EGFR and HER-2, may correlate with poor prognosis and decreased therapeutic response, although clinical data are contradictory. Several EGFR and HER-2 inhibitors have been tested in patients with ovarian cancer.

3.2.3.1 EGFR Inhibitors

The EGFR tyrosine kinase inhibitors erlotinib and gefitinib have been tested in phase II trials, which observed limited activities of these agents as monotherapies for recurrent ovarian cancer (response rates, 6% and 0–4%, respectively) [66–68]. The European Organization for Research and Treatment of Cancer-Gynecological Cancer Group (EORTC-GCG) conducted a phase III study of erlotinib (EORTC 55041) in patients with ovarian cancer after first-line, platinum-based chemotherapy (Table 3.1) [69]. Unfortunately, maintenance erlotinib for 2 years after first-line treatment did not improve PFS or OS in these patients.

The monoclonal EGFR-specific antibodies cetuximab and matuzumab block the binding of EGF to its receptor, thus inhibiting ligand-induced receptor autophosphorylation. Both cetuximab and matuzumab were tested in patients with recurrent ovarian cancer in phase II settings, with overall response rates of 4% and 0%, respectively [70, 71]. A phase II trial (GOG-0146P) assessed cetuximab activity in combination with carboplatin for EGFR-positive, recurrent platinum-sensitive ovarian cancer but reported only modest activity, with a PR rate of 35% [72]. Similarly, a phase II trial of cetuximab with carboplatin/paclitaxel as a first-line treatment for FIGO stage III/IV ovarian cancer did not demonstrate PFS prolongation when compared with historical data [73].

3.2.3.2 HER2 Inhibitors

Humanized monoclonal HER2 antibodies, trastuzumab and pertuzumab, were evaluated in phase II trials, which reported limited activity of these agents as monotherapies for recurrent ovarian cancer [74, 75]. A combination of pertuzumab with gemcitabine was tested in a phase II trial of platinum-resistant ovarian cancer patients (Table 3.2) [76] who were randomly allocated to gemcitabine plus placebo or pertuzumab, with objective response rates of 5% and 14%, respectively. Among patients whose tumors exhibited low HER3 mRNA expression, the median PFS was significantly longer with pertuzumab vs. placebo (5.3 vs. 1.4 months), although increased grade $\geq$ 3 neutropenia, diarrhea, and back pain were observed in the former. Pertuzumab was also evaluated together with carboplatin-based chemotherapy in a randomized phase II study of patients with platinum-sensitive, recurrent ovarian cancer (Table 3.2) [77]. No significant differences in PFS or OS were observed between chemotherapy (carboplatin and either paclitaxel or gemcitabine) alone and chemotherapy with pertuzumab. Unfortunately, no differences were observed between the arms in a biomarker analysis of HER3 mRNA expression. These studies suggest that pertuzumab, in combination with chemotherapy, is mainly effective in patients with platinum-resistant ovarian cancer and low HER3 mRNA expression.

3.2.3.3 Other HER Family Inhibitors

Phase II trials of single-agent targeted therapies, including the HER family tyrosine kinase inhibitors lapatinib and canertinib, have shown only modest efficacy [78, 79]. Lapatinib, a dual tyrosine kinase inhibitor of EGFR and HER2, was evaluated in recurrent ovarian cancer patients, although no objective responses were observed [78]. The combination therapy of lapatinib plus topotecan was also tested in a phase II trial in patients with platinum-resistant ovarian cancer, but lacked sufficient activity (no CR, one PR) [80]. Another phase II trial evaluated a pan-HER family tyrosine kinase inhibitor, canertinib, in patients with platinum-resistant recurrent ovarian cancer [79]; although two oral doses of canertinib (50 mg and 200 mg) were evaluated, no responses were observed.

3.2.4 Targeting Mammalian Target of Rapamycin (mTOR)

The PI3K/Akt pathway and the downstream pathway associated with mTOR, an evolutionarily conserved serine/threonine kinase, stimulate cell growth and survival; activation of these pathways suggests drug resistance and poor prognosis in many cancers [81, 82]. In ovarian cancer, PI3K amplification and Akt activation are observed in 12–68% of tumors and are closely associated with upregulated mTOR signaling [54, 82]. Thus, the mTOR pathway might be an attractive therapeutic target.

3.2.4.1 Temsirolimus

Temsirolimus is a specific small-molecule inhibitor of mTOR that acts by binding to FK506-binding protein 12 (FKBP12); the resulting complex inhibits mTOR kinase activity by directly binding the mTOR complex 1 (mTORC1) and interferes with the synthesis of proteins that regulate tumor cell proliferation, growth, and survival [83]. In a phase II clinical trial (GOG-0170I), although temsirolimus exhibited modest activity (PR rate of 9%) in patients with recurrent ovarian cancer, the 6-month PFS was insufficient to warrant inclusion in phase III studies of unselected patients. [84]. A phase II study of temsirolimus in combination with carboplatin/paclitaxel followed by temsirolimus consolidation as a first-line therapy was conducted in patients with stage III or IV ovarian clear cell carcinoma (the subtype with the highest PI3K pathway activation rate), and the results are forthcoming.

3.2.5 Targeting Folate Receptors (FRs)

FRα belongs to a family of single-chain glycosylphosphatidylinositol anchored membrane proteins with high affinities for the binding and unidirectional transport of folate, which is required for DNA replication and cell division, into cells [85]. FRα expression is restricted in normal cells but elevated strongly in various epithelial cancer tissues, including ovarian carcinoma [86]. FRα overexpression is observed in nearly 90% of non-mucinous ovarian cancers and correlates with stage, grade, chemotherapeutic response, and patient outcomes [87]. Therefore, FRα is considered a promising therapeutic target in ovarian cancer.

3.2.5.1 Farletuzumab

Farletuzumab (MORAb-003) is a humanized monoclonal FRα antibody that inhibits folate-dependent cell growth in a dose-dependent manner and induces tumor cell cytotoxicity via antibody-dependent cellular cytotoxicity and complement-dependent cytotoxicity [88]. Farletuzumab also inhibits interactions between cytoplasmic tyrosine kinase Lyn and membrane-signaling complexes, leading to growth reduction in these cells.

In a phase III trial (MORAb-003-004), weekly farletuzumab (1.25 mg/kg or 2.5 mg/kg) was investigated in combination with six cycles of chemotherapy (carboplatin plus paclitaxel or docetaxel) in patients with initial platinum-sensitive relapsed ovarian cancer (Table 3.1) [89]. However, the addition of farletuzumab did not significantly prolong PFS or OS. In a prespecified subgroup of patients with baseline CA125 levels not more than three times the upper limit of normal, farletuzumab 2.5 mg/kg correlated with a longer PFS (median: 13.6 vs. 8.8 months) and OS (median: not estimated vs. 29.1 months) vs. placebo. Therefore, farletuzumab might improve clinical outcomes in some patient subsets.

3.2.5.2 Vintafolide

Vintafolide (EC145) is a water-soluble folate–desacetylvinblastine monohydrazide conjugate that directly targets FR-expressing cells and minimizes exposure of other cells to cytotoxicity [90]. A randomized phase II trial (PRECEDENT) evaluated vintafolide combined with PLD versus PLD alone in patients with platinum-resistant recurrent ovarian cancer (Table 3.2) [91]. The median PFS was significantly prolonged in the vintafolide/PLD arm vs. the PLD-alone arm (5.0 vs. 2.7 months), particularly among patients with an FR-positive lesion rate of 100% (5.5 vs. 1.5 months). These results provide strong rationale for a phase III randomized trial (PROCEED) of vintafolide in combination with PLD vs. PLD plus placebo in patients with platinum-resistant ovarian cancer. However, this trial was suspended based on an interim analysis that demonstrated that vintafolide did not meet the prespecified PFS outcomes.

3.2.6 Other Promising Targets for Ovarian Cancer

The farnesyltransferase inhibitor, lonafarnib; endothelin $_A$ receptor antagonist, zibotentan; protein kinase C-β inhibitor, enzastaurin; methyltransferase inhibitor, decitabine; and polo-like kinase inhibitor, volasertib have been evaluated in randomized phase II trials; however, the effects when combined with chemotherapy were limited in ovarian cancer (Table 3.2) [92–96].

A new classification proposed two main types of epithelial ovarian cancer, type I and type II [97]. Type I tumors include low-grade serous, mucinous, clear cell, and endometrioid carcinomas and malignant Brenner tumors, which typically display mutations in *KRAS*, *BRAF*, *ARID1A*, *PTEN*, *PIK3CA*, *CTNNB1*, and *PPP2R1A*. Type II tumors include high-grade serous carcinoma, malignant mixed mesodermal tumors (carcinosarcoma), and undifferentiated carcinoma, which typically exhibit mutations in *TP53* and *BRCA1/2*. Therefore, type-specific genetic alterations might require consideration with regard to targeted therapies.

Although such mutations are rare in high-grade serous carcinomas, low-grade serous carcinomas frequently (61%) harbor *KRAS* (35%) and *BRAF* (30%) mutations [97], which constitutively activate the mitogen-activated protein kinase kinase (MEK)/extracellular signal-regulated kinase (ERK) signaling cascade. Therefore, GOG conducted a phase II trial (GOG-0239) of the MEK1/2 inhibitor selumetinib in patients with recurrent low-grade serous carcinoma [98], which observed a response rate of 15.4% and median PFS of 11.0 months. This study was followed by a phase III trial of a novel MEK inhibitor, MEK162, versus physician's choice of chemotherapy in patients with recurrent or persistent low-grade serous ovarian cancer.

An activating mutation of the *PIK3CA* gene, which encodes the PI3K catalytic subunit p110α, was detected in 12%–20% of endometrioid carcinomas and 33–40% of clear cell carcinomas [99, 100]. Furthermore, a loss of PTEN activation was observed in 21–31% of endometrioid carcinomas and 40% of clear cell carcinomas [100, 101]. The PI3K/Akt/mTOR cascade has therefore been proposed as a promising therapeutic target in patients with these tumors. A phase II study (GOG-0268) of the mTOR inhibitor temsirolimus in combination with carboplatin and paclitaxel following temsirolimus consolidation as a first-line therapy in patients with clear cell carcinoma of the ovary has been completed; the results are forthcoming.

Mutations in the *ARID1A* gene, also known as *BAF250*, were identified in approximately half of clear cell carcinoma and approximately 30% of endometrial carcinomas [102, 103]. Inhibiting EZH2 methyltransferase activity upregulates *PIK3IP1* expression, which negatively regulates PI3K/Akt signals, thus contributing to synthetic lethality in *ARID1A*-mutated cells [104]. Accordingly, EZH2 inhibition might represent a novel therapeutic strategy for *ARID1A*-mutated ovarian clear cell and endometrioid carcinomas.

3.3 Endometrial Cancer

Endometrial cancer has been classified into two pathogenetic types, type I (estrogen-dependent) and type II (non-estrogen-dependent) [105]. The more common type I tumors comprise low-grade, endometrioid, diploid, hormone-receptor-positive endometrial cancers, which are associated with a good prognosis. In contrast, type II tumors are non-endometrioid, high grade, aneuploid, *TP53*-mutated, hormone-receptor-negative cancers associated with a poor prognosis. A recent genomic characterization of endometrial cancer has revealed that in type I tumors, the PI3K/Akt/mTOR pathway is most frequently dysregulated (e.g., loss of PTEN; >90%), although *KRAS* (~20%) and *FGFR2* mutations (10–16%) are also commonly observed [106–109]. In type II cancers, serous disease exhibits a high frequency of *TP53* mutation (>90%), PI3K/Akt/mTOR pathway activation (~40%), and *HER2* amplification (25–30%), whereas *ARID1A* mutation is frequently observed in clear

cell disease (25%) [108]. These genetic alterations and activated signaling pathways appear to be important to the pathogenesis of endometrial cancer and have therefore been tested as therapeutic targets in several clinical studies.

3.3.1 Targeting the PI3K/Akt/mTOR Pathway

Inhibition of the PI3K/Akt/mTOR pathway, the most commonly upregulated pathway in endometrial cancers, is considered a viable therapeutic target. Accordingly, several specific PI3K/Akt/mTOR pathway inhibitors have been examined in phase II settings (Tables 3.3 and 3.4).

Table 3.3 Randomized Phase II trials of targeted therapy in endometrial cancer

Agents	Patients	Treatment	Response Rate (%)	Median PFS (M)	Median OS (M)	Selected Grade ≥ 3 Adverse Events [a]
mTOR inhibitors						
Recurrent disease						
Temsirolimus [111]	71					
Arm 1	50	Temsirolimus	22	5.6	13.3	6% VTE(any G), 6% fatigue, 4% edema, 8% nausea, 8% vomiting, 6% anorexia, 8% infection, 16% anemia
Arm 2	21	Temsirolimus + Megestrol acetate → Tamoxifen	14	4.2	9.6	24% VTE (any G), 10% pulmonary emboli (any G), 10% fatigue, 10% edema, 5% nausea, 5% vomiting, 5% infection
Ridaforolimus [115]	130					
Arm 1	66	Progestin or Chemotherapy	4	1.9	10.0	[$n = 63$] 2% diarrhea, 5% anemia
Arm 2	64	Ridaforolimus + Megestrol acetate → Tamoxifen	0	3.6 *	9.6	[$n = 63$] 11% diarrhea, 8% asthenia, 19% hyperglycemia, 6% stomatitis, 13% anemia

PFS progression-free survival, *M* months, *OS* overall survival, *mTOR* mammalian target of rapamycin, *VTE* venous thrombosis, *G* grade

[a]Selected adverse events (grade ≥ 3), except for indicated

*$P < 0.05$ vs. control arm

Table 3.4 Phase II trials of targeted therapy in endometrial cancer

Agent	Patients (n) [a]	Response Rate (%)	Stable Disease (%)	Median PFS (M)	Median OS (M)	Selected Grade ≥ 3 Adverse Events
mTOR						
Temsirolimus [110]	60					
chemotherapy-naïve	33 (29)	14	69	7.33	–	12% fatigue, 6% diarrhea, 6% pneumonitis
chemotherapy-treated	27 (25)	4	48	3.25	–	11% fatigue, 11% diarrhea, 11% pneumonitis, 6% dyspnea, 6% hypokalemia
Ridaforolimus [113]	45	11	18	–	–	27% anemia, 11% hyperglycemia
Ridaforolimus [114]	34 (31)	9	53	–	–	15% fatigue, 15% weight loss, 15% hyperglycemia, 12% increased ALT, 9% increased AST, 9% anemia,
Everolims [116]	28	0	43	–	–	29% lymphopenia, 23% fatigue, 11% pain, 11% nausea, 9% anemia
Everolims + Letrozole [118]	38 (35)	31	9	3.0	14	5% thrombocytopenia, 5% increased serum triglyceride
PI3K						
Pilaralisib [119]	67	6	37	–	–	9% rash, 4% diarrhea, 4% increased ALT
EGRF and/or HER2						
Trastuzumab [120]	33	0	36	1.84	7.85	9% gastrointestinal, 9% pulmonary, 6% anemia, 6% cardiovascular
Erlotinib [121]	33 (32)	12	45	–	–	12% diarrhea, 6% pruritus
Gefitinib [122]	26	4	27	1.8	7.1	19% gastrointestinal, 19% fatigue, 15% dermatologic, 15% pain, 12% neurologic, 8% anemia
Lapatinib [123]	30	3	23	1.82	7.33	20% gastrointestinal, 10% metabolic
MEK1/2						
Selumetinib [124]	52	6	25	2.3	8.5	15% fatigue, 10% anemia, 10% pain, 8% extremity edema, 6% dyspnea

Table 3.4 (continued)

Agent	Patients (n) [a]	Response Rate (%)	Stable Disease (%)	Median PFS (M)	Median OS (M)	Selected Grade ≥ 3 Adverse Events
VEGF						
Bevacizumab [125]	52	13	50	4.2	10.5	8% HT, 8% pain, 6% metabolic, 6% musculoskeletal,
Bevacizumab + Temsirolimus [126]	49	24	55	5.6	16.9	37% gastrointestinal, 35% metabolic, 20% constitutional, 18% pain, 14% infection, 12% cardiac, 10% neutropenia, 8% anemia, 6% thrombocytopenia
VEGF, PlGF						
Aflibercept [127]	44	7	32	2.9	14.5	27 HT, 18% pain, 11% gastrointestinal, 11% neurological, 9% metabolic, 9% pulmonary, 7% hemorrhage, 7% constitutional, 7% nausea
TKs (including VEGFR)						
Sorafenib [128]						
carcinoma	40	5	43	3.2	11.4	13% hypertension, 13% hand–foot syndrome, 7% hypophosphatemia, 7% hyponatremia, 5% anemia, 5% rash, 5% diarrhea, 5% fatigue, 5% bleeding, 4% thrombosis
carcinosarcoma	16	0	25	1.8	5.0	
Sunitinib [129]	33	18	18	3	19.4	45% fatigue, 21% HT, 15% hand-foot syndrome, 12% diarrhea, 9% dyspepsia, 9% abdominal pain, 21% neutropenia, 15% leukopenia, 12% lymphopenia, 9% anemia, 9% thrombocytopenia
Cediranib [130]	48	13	38	3.65	12.5	33% HT, 21% fatigue, 15% diarrhea, 6% pulmonary embolus
Brivanib [133]	43	19	28	3.3	10.7	21% HT, 16% GI, 14% metabolic, 12% nausea, 9% coagulation, 9% neurological, 7% pain, 7% vomiting

(continued)

Table 3.4 (continued)

Agent	Patients (n) [a]	Response Rate (%)	Stable Disease (%)	Median PFS (M)	Median OS (M)	Selected Grade ≥ 3 Adverse Events
Nintedanib [134]	32	9	34	3.3	10.1	9% diarrhea
Dovitinib [135]						
FGFR2 mutation (+)	22	5	59	4.1	20.2	17 HT, 9% diarrhea, 8% pulmonary embolism, 8% vomiting, 8% fatigue, 8% skin rash, 8% hypertriglyceridemia, 8% increased lipase, 6% dehydration, 6% diarrhea, 6% thrombocytopenia
FGFR2 mutation (−)	31	16	35	2.7	9.3	
Angiopoietin 1/2						
Trebananib [131]	32	3	25	1.97	6.6	25% abdominal pain, 13% HT, 13% nausea, 9% hyponatremia, 9% ascites
ALK1						
Dalantercept [132]	28	0	57	2.1	14.5	18% GI

PFS progression-free survival, *M* months, *OS* overall survival, *mTOR* mammalian target of rapamycin, *PI3K* phosphatidylinositol-4,5-bisphosphate 3-kinase, *EGFR* epidermal growth factor receptor, *HER* human epidermal growth factor receptor, *MEK* mitogen-activated protein kinase kinase, *VEGF* vascular endothelial growth factor, *PlGF* placental growth factor, *TK* tyrosine kinase, *VEGFR* VEGF receptor, *ALK* activing receptor-like kinase, *ALT* alanine transaminase, *AST* aspartate transaminase, *GI* gastrointestinal, *HT* hypertension

[a]Number of patients eligible (Number of patients evaluable for response)

3.3.1.1 Temsirolimus

In a phase II trial, the mTOR inhibitor temsirolimus was administered to chemotherapy-naïve or chemotherapy-treated patients with recurrent or metastatic endometrial cancer, yielding response rates of 14% and 4%, respectively (Table 3.4) [110]. Based on these trial results, a randomized phase II trial (GOG-0248) of temsirolimus vs. a combination of temsirolimus plus megestrol acetate for 3 weeks, alternating with tamoxifen for 3 weeks, was performed in women with recurrent or metastatic endometrial carcinoma (Table 3.3) [111]. The response rates were 22% and 14% in the temsirolimus and combination arms, respectively, and were similar in patients with and without prior histories of chemotherapy. A next-generation sequence analysis of tumors from enrolled patients revealed that although *AKT1* (4%), *TSC1* (2%), and *TSC2* (2%) mutations were rare, these might predict clinical benefits from temsirolimus. Furthermore, *CTNNB1* mutation (18%) was associated with a longer PFS following temsirolimus therapy [112].

3.3.1.2 Ridaforolimus

Ridaforolimus is a rapamycin analog and selective mTOR inhibitor. Two phase II trials evaluated single-agent i.v. or oral ridaforolimus in patients with advanced or recurrent endometrial cancer and reported PR rates of 11% and 9% and clinical benefit rates of 29% and 62%, respectively (Table 3.4) [113, 114]. Subsequently, a randomized phase II trial compared orally administered ridaforolimus with progestin or chemotherapy (comparator) in patients with advanced endometrial cancer patients (Table 3.3) [115]. The median PFS was significantly longer in the ridaforolimus arm vs. the comparator arm (3.6 vs. 1.9 months), and the most common grade 3/4 adverse events associated with ridaforolimus were diarrhea, hyperglycemia, and anemia.

3.3.1.3 Everolimus

Everolimus, an oral rapamycin analog, was evaluated in a phase II trial in patients with measurable recurrent endometrial carcinoma (Table 3.4) [116]. The confirmed clinical benefit rate was 21%, although no CR or PR cases were observed. Following reports of cross-regulation between the estrogen receptor and PI3K/AKT/mTOR pathways [117], a combination of everolimus with the aromatase inhibitor letrozole was tested in a phase II trial for the treatment of recurrent, pretreated endometrial carcinoma (Table 3.4) [118]. The response and clinical benefit rates were 31% and 40%, respectively. Currently, a randomized phase II trial (GOG-3007) of everolimus with letrozole or hormonal therapy (tamoxifen/medroxyprogesterone acetate) in patients with advanced, recurrent, or persistent endometria carcinoma is ongoing.

3.3.1.4 Pilaralisib

Pilaralisib, a highly selective, reversible, potent ATP-competitive pan-class I PI3K inhibitor, was evaluated in patients with advanced or recurrent endometrial carcinoma in a phase II trial (Table 3.4) [119]. The response rate was 6%, and the most commonly reported treatment-related grade ≥ 3 adverse events were rash, diarrhea, and increased alanine aminotransferase levels.

3.3.2 Targeting the Human Epidermal Growth Factor Receptor Family

HER2 monoclonal antibodies and EGFR tyrosine kinase inhibitors have been tested in endometrial cancer in several phase II trials (Table 3.4). In one phase II trial (GOG-0181B), trastuzumab was administered to patients with HER2-overexpressing tumors [2+ or 3+ by immunohistochemical staining (IHC)] or *HER2* amplification [*HER2*/*CEP 17* ratio > 2.0 by fluorescence in situ hybridization (FISH)] (Table 3.4) [120]. *HER2* amplification was detected in 38% of clear cell carcinomas, 28% of serous carcinomas, and 7% of endometrioid adenocarcinomas; however, no major

tumor responses were observed. An ongoing randomized phase II study of carboplatin and paclitaxel with or without trastuzumab in HER2-positive (3+ by IHC or *HER2*/*CEP 17* ratio > 2.0 by FISH) serous endometrial cancer is based on the high frequency of *HER2* amplification in this cancer subtype.

Erlotinib, gefitinib, lapatinib, and the MEK1/2 inhibitor selumetinib were also evaluated in phase II studies, which reported limited activities of these agents as monotherapies (Table 3.4) [121–124]. Therefore, combinations of these agents with other agents, such as chemotherapies, hormonal therapies, or other targeted agents, might be needed in the context of new treatment strategies targeting HER family members in endometrial cancers.

3.3.3 Targeting Angiogenesis

Bevacizumab was evaluated as a single agent in a phase II trial (GOG-0229E) (Table 3.4) [125] and yielded a response rate of 13% and median PFS and OS of 4.2 and 10.5 months, respectively. The combined activity of bevacizumab with temsirolimus was also assessed in a two-stage phase II study (GOG-0229G) (Table 3.4) [126]. This combination was efficacious, with a response rate of 24% and 6-month PFS rate of 46.9%; however, significant toxicities such as intestinal fistulas and perforation were observed. A randomized phase II trial comparing paclitaxel/carboplatin vs. paclitaxel/carboplatin/bevacizumab and a three-arm randomized phase II study of paclitaxel/carboplatin/bevacizumab, paclitaxel/carboplatin/temsirolimus, and ixabepilone/carboplatin/bevacizumab for endometrial cancer are ongoing.

Other anti-angiogenic drugs, such as aflibercept, sorafenib, sunitinib, cediranib, trebananib, and dalantercept, were tested as single agents in phase II studies of patients with endometrial cancer and yielded response rates of 0%–18% (Table 3.4) [127–132]. Recent studies reported that activating *FGFR2* mutations were more frequent (10–16%) in endometrioid histological subtype tumors vs. serous or clear cell subtype tumors; accordingly, FGFR inhibitors have been tested in clinical trials of endometrial cancer [106–109]. Multi-targeted tyrosine kinase inhibitors such as brivanib, nintedanib, and dovitinib, which target FGFR and VEGFR, yielded response rates of 9–17% (Table 3.4) [133–135]. Clinical trials of FGFR/VEGFR inhibitors for endometrial cancer are ongoing.

3.4 Cervical Cancer

Almost all cervical cancers result from a persistent high-risk human papillomavirus (hrHPV) infection [136]. The estimated lifetime risk of hrHPV infection

is approximately 80%, although most infections are cleared spontaneously by the host immune system. Only a few hrHPV infections become transformative, leading to the persistent expression of hrHPV oncoproteins (E6 and E7). These proteins inactivate the tumor suppressor products of *TP53* and *RB1* and might cause DNA alterations and subsequent cancers. Somatic mutations such as *PIK3CA* [16–37.5% in squamous cell carcinoma (SCC) and 14–16% in adenocarcinoma (AD)], *KRAS* (17.5% in AD), and *EGFR* (7.5% in SCC) have been reported in cervical cancers; these mainly involve signaling pathway components, and their potential status as therapeutic targets has led to several clinical studies.

3.4.1 Targeting Angiogenesis

Recent studies showed that hrHPV oncoproteins contribute to tumor angiogenesis via direct stimulation of *VEGF* and enhanced expressions of VEGF and hypoxia-inducible factor (HIF)-1α, which regulates angiogenesis-promoting genes [137, 138]. However, HIF-1α overexpression has been identified as an independent negative prognostic marker in cervical cancer [139]. Therefore, the VEGF pathway is an attractive therapeutic target in cervical cancer.

Bevacizumab was evaluated as a monotherapy in a phase II trial (GOG-0227C) of patients with previously treated, recurrent cervical cancer (Table 3.5) [140], which reported a response rate of 11% and median PFS of 3.4 months. Subsequently, a randomized phase III trial (GOG-0240) was performed to investigate nonplatinum combination chemotherapy (cisplatin plus paclitaxel or topotecan plus paclitaxel) with or without bevacizumab for the treatment of recurrent, persistent, or metastatic cervical cancer (Table 3.6) [141]. Here, the addition of bevacizumab to combination chemotherapy was associated with an improved median OS (17.0 vs. 13.3 months) but was also associated with an increased incidence of hypertension, grade $\geq$ 3 thromboembolic events, and gastrointestinal fistulas. A phase II trial to evaluate the safety and efficacy of bevacizumab in combination with carboplatin and paclitaxel for patients with recurrent or metastatic cervical cancer is ongoing. In the Radiation Therapy Oncology Group (RTOG) 0417 phase II trial, a combination of bevacizumab with standard chemoradiation was efficacious for bulky FIGO stage IB to IIIB disease (Table 3.5) [142]. However, the role of bevacizumab in a definitive setting with chemoradiation remains to be determined.

The anti-angiogenic multikinase inhibitors (targets include VEGFR) sunitinib and pazopanib were also investigated in patients with cervical cancer, but were found to have limited effects (response rates, 0% and 9%, respectively; Table 3.5) [143, 144].

Table 3.5 Phase II trials of targeted therapy in cervical cancer

Agent	Patients (n) [a]	Response Rate (%)	Stable Disease (%)	Median PFS (M)	Median OS (M)	Selected Grade ≥ 3 Adverse Events
VEGF						
Bevacizumab [140]	46	11	–	3.40	7.29	15% HT, 13% pain, 11% TEE, 9% GI, 7% infection, 7% genitourinary/ renal
Bevacizumab + CCRT [142]	49	–	–	3-year DFS68.7%	3-year OS 81.3%	14% blood/bone marrow, 6% GI
TKs (including VEGFR)						
Sunitinib [143]	19	0	84	3.5	–	24% (4/17) anemia, 47% (8/17) lymphopenia, 16% fistula, 16% fatigue, 16% diarrhea
Pazopanib [144]	74	9	43	18.1 weeks	50.7 weeks	11% diarrhea, 5% abdominal pain, 5% increased alkaline phosphatase
EGRF and/or HER2						
Cetuximab [147]	35	0	31	1.97	6.7	14% dermatologic, 11% GI, 9% constitutional, 6% anemia, 6% infection, 6% vascular, 6% pain
Cetuximab + cisplatin [148]	69	12	–	–	–	23% metabolic, 12% dermatologic, 9% fatigue, 9% gastrointestinal, 9% nausea/vomiting, 7% infection, 7% anemia, 6% leucopenia
Gefitinib [150]	30	0	20	37 days	107 days	13% diarrhea, 7% anorexia
Erlotinib [151]	25	0	16	1.87	4.96	16% anemia, 12% diarrhea, 8% fatigue, 8% nausea, 8% emesis, 8% rash, 8% infection without neutropenia
Erlotinib + CCRT [152]	38 (36)	CR: 94 PR: 6	–	3-year PFS73.8%	3-year OS 80.6%	13% rash, 11% hematological toxicity, 8% diarrhea
Lapatinib [144]	78	5	44	17.1 weeks	39.1 weeks	13% diarrhea, 7% dyspnea, 5% fatigue, 5% anemia

Table 3.5 (continued)

Agent	Patients (n) [a]	Response Rate (%)	Stable Disease (%)	Median PFS (M)	Median OS (M)	Selected Grade ≥ 3 Adverse Events
mTOR						
Temsirolimus [154]	37 (33)	3	58	3.52	–	43% lymphopenia, 16% hyponatremia, 11% hypokalemia, 9% (2/23) hypertriglyceridemia, 8% (2/24) hyperglycemia

PFS progression-free survival, *M* months, *OS* overall survival, *DFS* disease-free survival, *VEGF* vascular endothelial growth factor, *TK* tyrosine kinase, *VEGFR* VEGF receptor, *EGFR* epidermal growth factor receptor, *HER* human epidermal growth factor receptor, *mTOR* mammalian target of rapamycin, *CR* complete response, *PR* partial response, *CCRT* concurrent chemoradiotherapy, *HT* hypertension, *TEE* thromboembolic events, *GI* gastrointestinal

[a]Number of patients eligible (Number of patients evaluable for response)

Table 3.6 Randomized phase III trials of targeted therapy in cervical cancer

Trial	Patients	Treatment	Response Rate (%)	Median PFS (M)	Median OS (M)	Selected Adverse Events [a]
Anti-angiogenic agents						
Recurrent disease						
GOG-0240 [132]	452					
CP vs. TP	229	CP ± bevacizumab	–	7.6 *	15.0	–
	223	TP ± bevacizumab	–	5.7	12.5	–
Chemotherapy ± bevacizumab	225	CP or TP	36	5.9	13.3	[*n* = 219] 2% HT (G ≥ 2), 1% TEE, 26% neutropenia (G ≥ 4), 5% febrile neutropenia
	227	CP or TP + bevacizumab	48 *	8.2 *	17.0 *	[*n* = 220] 3% GI fistula, 25% HT (G ≥ 2), 8% TEE, 35% neutropenia (G ≥ 4), 5% febrile neutropenia
CP ± bevacizumab	114	CP	45	–	14.3	–
	115	CP + bevacizumab	50	–	17.5 *	–
TP ± bevacizumab	111	TP	27	–	12.7	–
	112	TP + bevacizumab	47 *	–	16.2	–

PFS progression-free survival, *M* months, *OS* overall survival, *GOG* Gynecologic Oncology Group, *CP* cisplatin plus paclitaxel, *TP* topotecan plus paclitaxel, *CT* chemotherapy, *I* gastrointestinal, *G* grade, *HT* hypertension, *TEE* thromboembolic events

[a]Selected adverse events (grade ≥ 3), except for indicated

*$P < 0.05$, vs. control arm

3.4.2 Targeting the Human Epidermal Growth Factor Receptor Family

EGFR gene amplification has been reported in 25% of invasive SCCs and was associated with intermediate–high levels of EGFR protein overexpression [145]. Another study reported positive EGFR staining and phosphorylated (p) EGFR in in 35% and 20% of tumors, respectively; these factors were independently associated with a poor response to radiation or concurrent chemoradiation [146]. EGFR staining also was an independent prognostic factor for poor disease-specific survival.

Cetuximab, a chimeric monoclonal EGFR antibody, was investigated as a single agent in a phase II trial (GOG-0227E) of patients with persistent or recurrent cervical cancer, but failed to induce clinical responses (Table 3.5) [147]. A combination of cetuximab with cisplatin was tested in a phase II trial (GOG-0076DD; Table 3.5) [148], but failed to confer additional benefits beyond cisplatin monotherapy. Cetuximab was also investigated in combination with cisplatin-based chemoradiotherapy in a randomized phase II trial of patients with FIGO stage IB2–IIIB cervical cancer [149]; however, cetuximab with chemoradiotherapy yielded no gains in 2-year disease-free survival. Another anti-EGFR antibody, nimotuzumab, is currently being evaluated for cervical cancer.

The EGFR tyrosine kinase inhibitors gefitinib and erlotinib were evaluated in phase II trials of recurrent cervical cancer; however, no objective responses were observed with these agents alone (Table 3.5) [150, 151]. A phase II trial tested a combination of erlotinib with chemoradiotherapy (cisplatin + pelvic radiotherapy) in patients with stage IIB–IIIB cervical SCC (Table 3.5) [152] and yielded promising results, with a CR of 95% and 3-year OS and PFS of 80.6% and 73.8%, respectively. A dual tyrosine kinase inhibitor of EGFR and HER2, lapatinib, was also evaluated in a phase II trial of recurrent disease; however, the activity of this agent as a monotherapy was limited (response rate, 5%) [144].

3.4.3 Targeting Mammalian Target of Rapamycin (mTOR)

PI3K/Akt/mTOR pathway upregulation consequent to *PIK3CA*, *KRAS*, and/or *EGFR* mutation is often observed in cervical cancers [136]. The HPV-encoded oncoprotein E6 also interacts with and degrades tuberous sclerosis complex 2 (TSC2), which can lead to enhanced mTOR activity [153]. Accordingly, the mTOR inhibitor temsirolimus was evaluated in a phase II trial but was not active against cervical cancer (response rate, 3%; Table 3.5) [154]. Molecular markers to indicate which patients would benefit from this treatment are needed.

3.4.4 Targeting HPV E6 and E7 Oncoproteins

Persistent hrHPV infection is a key step in the initiation and development of cervical cancer, and continuous expression of the viral oncoproteins E6 and E7 primarily

maintains malignant phenotypes such as cellular proliferation, prolonged cell-cycle progression, cell death resistance, and immune evasion [136]. Therefore, E6 and E7 are considered potential therapeutic targets of cervical cancer.

Antisense oligonucleotides (ASOs) and short interfering RNAs (siRNAs) have been developed to inhibit E6 and E7 expression [155]. Efficient blockade of E6 and E7 expression was found to induce the accumulation of the tumor suppressor proteins p53 and pRb. Furthermore, siRNA-mediated E6 suppression increased the sensitivity of SiHa cells (HPV16-positive cervical cancer cell line) to cisplatin [156].

Small-molecule reactivation of p53 and induction of tumor cell apoptosis (RITA) can induce p53 accumulation and rescue its tumor suppressor function in cells infected by high-risk HPV16 and HPV18 through an inhibition of E6-mediated proteasomal degradation [157]. Activation of p53 by RITA induces the transcription of proapoptotic p53 targets, resulting in the substantial suppression of cervical carcinoma xenografts in vivo.

Several other approaches to the inhibition of E6 and/or E7 through numerous approaches, such as synthetic peptide ligands, ribozymes, and small molecules, are currently under development [155].

Conclusions

A wide range of novel targeted agents have been developed and subjected to investigation for the treatment of patients with gynecologic cancers. The results of recent studies suggest that angiogenesis inhibitors are the most promising therapies for patients with ovarian and cervical cancers, although the effects of tyrosine kinase inhibitors require further elucidation. PARP inhibitors are also attractive targeted agents for ovarian cancer therapy. A better understanding of tumor molecular biology and identification of predictive biomarkers are essential steps in the selection of treatment strategies that will best improve survival in patients. Therefore, further investigation of the molecular biology and genetics of gynecologic cancers is warranted.

References

1. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer. 2015;136(5):E359–86. doi:10.1002/ijc.29210.
2. Hanahan D, Folkman J. Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. Cell. 1996;86(3):353–64.
3. Lohela M, Bry M, Tammela T, Alitalo K. VEGFs and receptors involved in angiogenesis versus lymphangiogenesis. Curr Opin Cell Biol. 2009;21(2):154–65. doi:10.1016/j.ceb.2008.12.012.
4. Ferrara N, Davis-Smyth T. The biology of vascular endothelial growth factor. Endocr Rev. 1997;18(1):4–25. doi:10.1210/edrv.18.1.0287.
5. Kerbel RS. Tumor angiogenesis. N Engl J Med. 2008;358(19):2039–49. doi:10.1056/NEJMra0706596.

6. Ishigami SI, Arii S, Furutani M, Niwano M, Harada T, Mizumoto M, et al. Predictive value of vascular endothelial growth factor (VEGF) in metastasis and prognosis of human colorectal cancer. Br J Cancer. 1998;78(10):1379–84.
7. Ohta Y, Tomita Y, Oda M, Watanabe S, Murakami S, Watanabe Y. Tumor angiogenesis and recurrence in stage I non-small cell lung cancer. Ann Thorac Surg. 1999;68(3):1034–8.
8. Shimogai R, Kigawa J, Itamochi H, Iba T, Kanamori Y, Oishi T, et al. Expression of hypoxia-inducible factor 1alpha gene affects the outcome in patients with ovarian cancer. Int J Gynecol Cancer. 2008;18(3):499–505. doi:10.1111/j.1525-1438.2007.01055.x.
9. Siddiqui GK, Maclean AB, Elmasry K. Wong te Fong a, Morris RW, Rashid M et al. Immunohistochemical expression of VEGF predicts response to platinum based chemotherapy in patients with epithelial ovarian cancer. Angiogenesis. 2011;14(2):155–61. doi:10.1007/s10456-010-9199-4.
10. Boocock CA, Charnock-Jones DS, Sharkey AM, McLaren J, Barker PJ, Wright KA, et al. Expression of vascular endothelial growth factor and its receptors flt and KDR in ovarian carcinoma. J Natl Cancer Inst. 1995;87(7):506–16.
11. Sher I, Adham SA, Petrik J, Coomber BL. Autocrine VEGF-A/KDR loop protects epithelial ovarian carcinoma cells from anoikis. Int J Cancer. 2009;124(3):553–61. doi:10.1002/ijc.23963.
12. Lu C, Han HD, Mangala LS, Ali-Fehmi R, Newton CS, Ozbun L, et al. Regulation of tumor angiogenesis by EZH2. Cancer Cell. 2010;18(2):185–97. doi:10.1016/j.ccr.2010.06.016.
13. Ellis LM, Hicklin DJ. VEGF-targeted therapy: mechanisms of anti-tumour activity. Nat Rev Cancer. 2008;8(8):579–91. doi:10.1038/nrc2403.
14. Kim KJ, Li B, Houck K, Winer J, Ferrara N. The vascular endothelial growth factor proteins: identification of biologically relevant regions by neutralizing monoclonal antibodies. Growth Factors. 1992;7(1):53–64. doi:10.3109/08977199209023937.
15. Kim KJ, Li B, Winer J, Armanini M, Gillett N, Phillips HS, et al. Inhibition of vascular endothelial growth factor-induced angiogenesis suppresses tumour growth in vivo. Nature. 1993;362(6423):841–4. doi:10.1038/362841a0.
16. Jain RK. Normalization of tumor vasculature: an emerging concept in antiangiogenic therapy. Science. 2005;307(5706):58–62. doi:10.1126/science.1104819.
17. Burger RA, Sill MW, Monk BJ, Greer BE, Sorosky JI. Phase II trial of bevacizumab in persistent or recurrent epithelial ovarian cancer or primary peritoneal cancer: a gynecologic oncology group study. J Clin Oncol. 2007;25(33):5165–71. doi:10.1200/JCO.2007.11.5345.
18. Cannistra SA, Matulonis UA, Penson RT, Hambleton J, Dupont J, Mackey H, et al. Phase II study of bevacizumab in patients with platinum-resistant ovarian cancer or peritoneal serous cancer. J Clin Oncol. 2007;25(33):5180–6. doi:10.1200/JCO.2007.12.0782.
19. Burger RA, Brady MF, Bookman MA, Fleming GF, Monk BJ, Huang H, et al. Incorporation of bevacizumab in the primary treatment of ovarian cancer. N Engl J Med. 2011;365(26):2473–83. doi:10.1056/NEJMoa1104390.
20. Perren TJ, Swart AM, Pfisterer J, Ledermann JA, Pujade-Lauraine E, Kristensen G, et al. A phase 3 trial of bevacizumab in ovarian cancer. N Engl J Med. 2011;365(26):2484–96. doi:10.1056/NEJMoa1103799.
21. Oza AM, Cook AD, Pfisterer J, Embleton A, Ledermann JA, Pujade-Lauraine E, et al. Standard chemotherapy with or without bevacizumab for women with newly diagnosed ovarian cancer (ICON7): overall survival results of a phase 3 randomised trial. Lancet Oncol. 2015;16(8):928–36. doi:10.1016/S1470-2045(15)00086-8.
22. Chan JK, Brady MF, Penson RT, Huang H, Birrer MJ, Walker JL, et al. Weekly vs. every-3-week paclitaxel and carboplatin for ovarian cancer. N Engl J Med. 2016;374(8):738–48. doi:10.1056/NEJMoa1505067.
23. Aghajanian C, Blank SV, Goff BA, Judson PL, Teneriello MG, Husain A, et al. OCEANS: a randomized, double-blind, placebo-controlled phase III trial of chemotherapy with or without bevacizumab in patients with platinum-sensitive recurrent epithelial ovarian, primary peritoneal, or fallopian tube cancer. J Clin Oncol. 2012;30(17):2039–45. doi:10.1200/JCO.2012.42.0505.

24. Pujade-Lauraine E, Hilpert F, Weber B, Reuss A, Poveda A, Kristensen G, et al. Bevacizumab combined with chemotherapy for platinum-resistant recurrent ovarian cancer: the AURELIA open-label randomized phase III trial. J Clin Oncol. 2014;32(13):1302–8. doi:10.1200/JCO.2013.51.4489.
25. Aghajanian C, Goff B, Nycum LR, Wang YV, Husain A, Blank SV. Final overall survival and safety analysis of OCEANS, a phase 3 trial of chemotherapy with or without bevacizumab in patients with platinum-sensitive recurrent ovarian cancer. Gynecol Oncol. 2015;139(1):10–6. doi:10.1016/j.ygyno.2015.08.004.
26. Moroney JW, Sood AK, Coleman RL. Aflibercept in epithelial ovarian carcinoma. Future Oncol. 2009;5(5):591–600. doi:10.2217/fon.09.35.
27. Colombo N, Mangili G, Mammoliti S, Kalling M, Tholander B, Sternas L, et al. A phase II study of aflibercept in patients with advanced epithelial ovarian cancer and symptomatic malignant ascites. Gynecol Oncol. 2012;125(1):42–7. doi:10.1016/j.ygyno.2011.11.021.
28. Gotlieb WH, Amant F, Advani S, Goswami C, Hirte H, Provencher D, et al. Intravenous aflibercept for treatment of recurrent symptomatic malignant ascites in patients with advanced ovarian cancer: a phase 2, randomised, double-blind, placebo-controlled study. Lancet Oncol. 2012;13(2):154–62. doi:10.1016/S1470-2045(11)70338-2.
29. Tew WP, Colombo N, Ray-Coquard I, Del Campo JM, Oza A, Pereira D, et al. Intravenous aflibercept in patients with platinum-resistant, advanced ovarian cancer: results of a randomized, double-blind, phase 2, parallel-arm study. Cancer. 2014;120(3):335–43. doi:10.1002/cncr.28406.
30. Coleman RL, Duska LR, Ramirez PT, Heymach JV, Kamat AA, Modesitt SC, et al. Phase 1–2 study of docetaxel plus aflibercept in patients with recurrent ovarian, primary peritoneal, or fallopian tube cancer. Lancet Oncol. 2011;12(12):1109–17. doi:10.1016/S1470-2045(11)70244-3.
31. Wedge SR, Kendrew J, Hennequin LF, Valentine PJ, Barry ST, Brave SR, et al. AZD2171: a highly potent, orally bioavailable, vascular endothelial growth factor receptor-2 tyrosine kinase inhibitor for the treatment of cancer. Cancer Res. 2005;65(10):4389–400. doi:10.1158/0008-5472.CAN-04-4409.
32. Heckman CA, Holopainen T, Wirzenius M, Keskitalo S, Jeltsch M, Yla-Herttuala S, et al. The tyrosine kinase inhibitor cediranib blocks ligand-induced vascular endothelial growth factor receptor-3 activity and lymphangiogenesis. Cancer Res. 2008;68(12):4754–62. doi:10.1158/0008-5472.CAN-07-5809.
33. Matulonis UA, Berlin S, Ivy P, Tyburski K, Krasner C, Zarwan C, et al. Cediranib, an oral inhibitor of vascular endothelial growth factor receptor kinases, is an active drug in recurrent epithelial ovarian, fallopian tube, and peritoneal cancer. J Clin Oncol. 2009;27(33):5601–6. doi:10.1200/JCO.2009.23.2777.
34. Ledermann JA, Embleton AC, Raja F, Perren TJ, Jayson GC, Rustin GJ, et al. Cediranib in patients with relapsed platinum-sensitive ovarian cancer (ICON6): a randomised, double-blind, placebo-controlled phase 3 trial. Lancet. 2016;387(10023):1066–74. doi:10.1016/S0140-6736(15)01167-8.
35. Davidson BA, Secord AA. Profile of pazopanib and its potential in the treatment of epithelial ovarian cancer. Int J Womens Health. 2014;6:289–300. doi:10.2147/IJWH.S49781.
36. Friedlander M, Hancock KC, Rischin D, Messing MJ, Stringer CA, Matthys GM, et al. A phase II, open-label study evaluating pazopanib in patients with recurrent ovarian cancer. Gynecol Oncol. 2010;119(1):32–7. doi:10.1016/j.ygyno.2010.05.033.
37. du Bois A, Floquet A, Kim JW, Rau J, del Campo JM, Friedlander M, et al. Incorporation of pazopanib in maintenance therapy of ovarian cancer. J Clin Oncol. 2014;32(30):3374–82. doi:10.1200/JCO.2014.55.7348.
38. Pignata S, Lorusso D, Scambia G, Sambataro D, Tamberi S, Cinieri S, et al. Pazopanib plus weekly paclitaxel versus weekly paclitaxel alone for platinum-resistant or platinum-refractory advanced ovarian cancer (MITO 11): a randomised, open-label, phase 2 trial. Lancet Oncol. 2015;16(5):561–8. doi:10.1016/S1470-2045(15)70115-4.
39. Ledermann JA, Hackshaw A, Kaye S, Jayson G, Gabra H, McNeish I, et al. Randomized phase II placebo-controlled trial of maintenance therapy using the oral triple angiokinase

inhibitor BIBF 1120 after chemotherapy for relapsed ovarian cancer. J Clin Oncol. 2011;29(28):3798–804. doi:10.1200/JCO.2010.33.5208.
40. du Bois A, Kristensen G, Ray-Coquard I, Reuss A, Pignata S, Colombo N, et al. Standard first-line chemotherapy with or without nintedanib for advanced ovarian cancer (AGO-OVAR 12): a randomised, double-blind, placebo-controlled phase 3 trial. Lancet Oncol. 2016;17(1):78–89. doi:10.1016/S1470-2045(15)00366-6.
41. Mendel DB, Laird AD, Xin X, Louie SG, Christensen JG, Li G, et al. In vivo antitumor activity of SU11248, a novel tyrosine kinase inhibitor targeting vascular endothelial growth factor and platelet-derived growth factor receptors: determination of a pharmacokinetic/pharmacodynamic relationship. Clin Cancer Res. 2003;9(1):327–37.
42. Biagi JJ, Oza AM, Chalchal HI, Grimshaw R, Ellard SL, Lee U, et al. A phase II study of sunitinib in patients with recurrent epithelial ovarian and primary peritoneal carcinoma: an NCIC clinical trials group study. Ann Oncol. 2011;22(2):335–40. doi:10.1093/annonc/mdq357.
43. Baumann KH, du Bois A, Meier W, Rau J, Wimberger P, Sehouli J, et al. A phase II trial (AGO 2.11) in platinum-resistant ovarian cancer: a randomized multicenter trial with sunitinib (SU11248) to evaluate dosage, schedule, tolerability, toxicity and effectiveness of a multitargeted receptor tyrosine kinase inhibitor monotherapy. Ann Oncol. 2012;23(9):2265–71. doi:10.1093/annonc/mds003.
44. Campos SM, Penson RT, Matulonis U, Horowitz NS, Whalen C, Pereira L, et al. A phase II trial of Sunitinib malate in recurrent and refractory ovarian, fallopian tube and peritoneal carcinoma. Gynecol Oncol. 2013;128(2):215–20. doi:10.1016/j.ygyno.2012.07.126.
45. Wilhelm S, Carter C, Lynch M, Lowinger T, Dumas J, Smith RA, et al. Discovery and development of sorafenib: a multikinase inhibitor for treating cancer. Nat Rev Drug Discov. 2006;5(10):835–44. doi:10.1038/nrd2130.
46. Matei D, Sill MW, Lankes HA, DeGeest K, Bristow RE, Mutch D, et al. Activity of sorafenib in recurrent ovarian cancer and primary peritoneal carcinomatosis: a gynecologic oncology group trial. J Clin Oncol. 2011;29(1):69–75. doi:10.1200/JCO.2009.26.7856.
47. Herzog TJ, Scambia G, Kim BG, Lhomme C, Markowska J, Ray-Coquard I, et al. A randomized phase II trial of maintenance therapy with Sorafenib in front-line ovarian carcinoma. Gynecol Oncol. 2013;130(1):25–30. doi:10.1016/j.ygyno.2013.04.011.
48. Holash J, Maisonpierre PC, Compton D, Boland P, Alexander CR, Zagzag D, et al. Vessel cooption, regression, and growth in tumors mediated by angiopoietins and VEGF. Science. 1999;284(5422):1994–8.
49. Oliner J, Min H, Leal J, Yu D, Rao S, You E, et al. Suppression of angiogenesis and tumor growth by selective inhibition of angiopoietin-2. Cancer Cell. 2004;6(5):507–16. doi:10.1016/j.ccr.2004.09.030.
50. Karlan BY, Oza AM, Richardson GE, Provencher DM, Hansen VL, Buck M, et al. Randomized, double-blind, placebo-controlled phase II study of AMG 386 combined with weekly paclitaxel in patients with recurrent ovarian cancer. J Clin Oncol. 2012;30(4):362–71. doi:10.1200/JCO.2010.34.3178.
51. Monk BJ, Poveda A, Vergote I, Raspagliesi F, Fujiwara K, Bae DS, et al. Anti-angiopoietin therapy with trebananib for recurrent ovarian cancer (TRINOVA-1): a randomised, multicentre, double-blind, placebo-controlled phase 3 trial. Lancet Oncol. 2014;15(8):799–808. doi:10.1016/S1470-2045(14)70244-X.
52. Jagtap P, Szabo C. Poly(ADP-ribose) polymerase and the therapeutic effects of its inhibitors. Nat Rev Drug Discov. 2005;4(5):421–40. doi:10.1038/nrd1718.
53. King MC, Marks JH, Mandell JB. New York breast cancer study G. Breast and ovarian cancer risks due to inherited mutations in BRCA1 and BRCA2. Science. 2003;302(5645):643–6. doi:10.1126/science.1088759.
54. Cancer Genome Atlas Research N. Integrated genomic analyses of ovarian carcinoma. Nature. 2011;474(7353):609–15. doi:10.1038/nature10166.
55. Evers B, Drost R, Schut E, de Bruin M, van der Burg E, Derksen PW, et al. Selective inhibition of BRCA2-deficient mammary tumor cell growth by AZD2281 and cisplatin. Clin Cancer Res. 2008;14(12):3916–25. doi:10.1158/1078-0432.CCR-07-4953.

56. Matulonis UA, Penson RT, Domchek SM, Kaufman B, Shapira-Frommer R, Audeh MW, et al. Olaparib monotherapy in patients with advanced relapsed ovarian cancer and a germline BRCA1/2 mutation: a multi-study analysis of response rates and safety. Ann Oncol. 2016; doi:10.1093/annonc/mdw133.
57. Kaye SB, Lubinski J, Matulonis U, Ang JE, Gourley C, Karlan BY, et al. Phase II, open-label, randomized, multicenter study comparing the efficacy and safety of olaparib, a poly (ADP-ribose) polymerase inhibitor, and pegylated liposomal doxorubicin in patients with BRCA1 or BRCA2 mutations and recurrent ovarian cancer. J Clin Oncol. 2012;30(4):372–9. doi:10.1200/JCO.2011.36.9215.
58. Ledermann J, Harter P, Gourley C, Friedlander M, Vergote I, Rustin G, et al. Olaparib maintenance therapy in platinum-sensitive relapsed ovarian cancer. N Engl J Med. 2012;366(15):1382–92. doi:10.1056/NEJMoa1105535.
59. Ledermann J, Harter P, Gourley C, Friedlander M, Vergote I, Rustin G, et al. Olaparib maintenance therapy in patients with platinum-sensitive relapsed serous ovarian cancer: a pre-planned retrospective analysis of outcomes by BRCA status in a randomised phase 2 trial. Lancet Oncol. 2014;15(8):852–61. doi:10.1016/S1470-2045(14)70228-1.
60. Oza AM, Cibula D, Benzaquen AO, Poole C, Mathijssen RH, Sonke GS, et al. Olaparib combined with chemotherapy for recurrent platinum-sensitive ovarian cancer: a randomised phase 2 trial. Lancet Oncol. 2015;16(1):87–97. doi:10.1016/S1470-2045(14)71135-0.
61. Liu JF, Barry WT, Birrer M, Lee JM, Buckanovich RJ, Fleming GF, et al. Combination cediranib and olaparib versus olaparib alone for women with recurrent platinum-sensitive ovarian cancer: a randomised phase 2 study. Lancet Oncol. 2014;15(11):1207–14. doi:10.1016/S1470-2045(14)70391-2.
62. Coleman RL, Sill MW, Bell-McGuinn K, Aghajanian C, Gray HJ, Tewari KS, et al. A phase II evaluation of the potent, highly selective PARP inhibitor veliparib in the treatment of persistent or recurrent epithelial ovarian, fallopian tube, or primary peritoneal cancer in patients who carry a germline BRCA1 or BRCA2 mutation - an NRG oncology/gynecologic oncology group study. Gynecol Oncol. 2015;137(3):386–91. doi:10.1016/j.ygyno.2015.03.042.
63. Kummar S, Oza AM, Fleming GF, Sullivan DM, Gandara DR, Naughton MJ, et al. Randomized trial of oral cyclophosphamide and Veliparib in high-grade serous ovarian, primary peritoneal, or fallopian tube cancers, or BRCA-mutant ovarian cancer. Clin Cancer Res. 2015;21(7):1574–82. doi:10.1158/1078-0432.CCR-14-2565.
64. Ciardiello F, Tortora G. EGFR antagonists in cancer treatment. N Engl J Med. 2008;358(11):1160–74. doi:10.1056/NEJMra0707704.
65. Lafky JM, Wilken JA, Baron AT, Maihle NJ. Clinical implications of the ErbB/epidermal growth factor (EGF) receptor family and its ligands in ovarian cancer. Biochim Biophys Acta. 2008;1785(2):232–65. doi:10.1016/j.bbcan.2008.01.001.
66. Gordon AN, Finkler N, Edwards RP, Garcia AA, Crozier M, Irwin DH, et al. Efficacy and safety of erlotinib HCl, an epidermal growth factor receptor (HER1/EGFR) tyrosine kinase inhibitor, in patients with advanced ovarian carcinoma: results from a phase II multicenter study. Int J Gynecol Cancer. 2005;15(5):785–92. doi:10.1111/j.1525-1438.2005.00137.x.
67. Schilder RJ, Sill MW, Chen X, Darcy KM, Decesare SL, Lewandowski G, et al. Phase II study of gefitinib in patients with relapsed or persistent ovarian or primary peritoneal carcinoma and evaluation of epidermal growth factor receptor mutations and immunohistochemical expression: a gynecologic oncology group study. Clin Cancer Res. 2005;11(15):5539–48. doi:10.1158/1078-0432.CCR-05-0462.
68. Posadas EM, Liel MS, Kwitkowski V, Minasian L, Godwin AK, Hussain MM, et al. A phase II and pharmacodynamic study of gefitinib in patients with refractory or recurrent epithelial ovarian cancer. Cancer. 2007;109(7):1323–30. doi:10.1002/cncr.22545.
69. Vergote IB, Jimeno A, Joly F, Katsaros D, Coens C, Despierre E, et al. Randomized phase III study of erlotinib versus observation in patients with no evidence of disease progression after first-line platin-based chemotherapy for ovarian carcinoma: a European Organisation for Research and Treatment of Cancer-Gynaecological cancer group, and gynecologic cancer intergroup study. J Clin Oncol. 2014;32(4):320–6. doi:10.1200/JCO.2013.50.5669.

70. Schilder RJ, Pathak HB, Lokshin AE, Holloway RW, Alvarez RD, Aghajanian C, et al. Phase II trial of single agent cetuximab in patients with persistent or recurrent epithelial ovarian or primary peritoneal carcinoma with the potential for dose escalation to rash. Gynecol Oncol. 2009;113(1):21–7. doi:10.1016/j.ygyno.2008.12.003.
71. Seiden MV, Burris HA, Matulonis U, Hall JB, Armstrong DK, Speyer J, et al. A phase II trial of EMD72000 (matuzumab), a humanized anti-EGFR monoclonal antibody, in patients with platinum-resistant ovarian and primary peritoneal malignancies. Gynecol Oncol. 2007;104(3):727–31. doi:10.1016/j.ygyno.2006.10.019.
72. Secord AA, Blessing JA, Armstrong DK, Rodgers WH, Miner Z, Barnes MN, et al. Phase II trial of cetuximab and carboplatin in relapsed platinum-sensitive ovarian cancer and evaluation of epidermal growth factor receptor expression: a gynecologic oncology group study. Gynecol Oncol. 2008;108(3):493–9. doi:10.1016/j.ygyno.2007.11.029.
73. Konner J, Schilder RJ, DeRosa FA, Gerst SR, Tew WP, Sabbatini PJ, et al. A phase II study of cetuximab/paclitaxel/carboplatin for the initial treatment of advanced-stage ovarian, primary peritoneal, or fallopian tube cancer. Gynecol Oncol. 2008;110(2):140–5. doi:10.1016/j.ygyno.2008.04.018.
74. Bookman MA, Darcy KM, Clarke-Pearson D, Boothby RA, Horowitz IR. Evaluation of monoclonal humanized anti-HER2 antibody, trastuzumab, in patients with recurrent or refractory ovarian or primary peritoneal carcinoma with overexpression of HER2: a phase II trial of the gynecologic oncology group. J Clin Oncol. 2003;21(2):283–90.
75. Gordon MS, Matei D, Aghajanian C, Matulonis UA, Brewer M, Fleming GF, et al. Clinical activity of pertuzumab (rhuMAb 2C4), a HER dimerization inhibitor, in advanced ovarian cancer: potential predictive relationship with tumor HER2 activation status. J Clin Oncol. 2006;24(26):4324–32. doi:10.1200/JCO.2005.05.4221.
76. Makhija S, Amler LC, Glenn D, Ueland FR, Gold MA, Dizon DS, et al. Clinical activity of gemcitabine plus pertuzumab in platinum-resistant ovarian cancer, fallopian tube cancer, or primary peritoneal cancer. J Clin Oncol. 2010;28(7):1215–23. doi:10.1200/JCO.2009.22.3354.
77. Kaye SB, Poole CJ, Danska-Bidzinska A, Gianni L, Del Conte G, Gorbunova V, et al. A randomized phase II study evaluating the combination of carboplatin-based chemotherapy with pertuzumab versus carboplatin-based therapy alone in patients with relapsed, platinum-sensitive ovarian cancer. Ann Oncol. 2013;24(1):145–52. doi:10.1093/annonc/mds282.
78. Garcia AA, Sill MW, Lankes HA, Godwin AK, Mannel RS, Armstrong DK, et al. A phase II evaluation of lapatinib in the treatment of persistent or recurrent epithelial ovarian or primary peritoneal carcinoma: a gynecologic oncology group study. Gynecol Oncol. 2012;124(3):569–74. doi:10.1016/j.ygyno.2011.10.022.
79. Campos S, Hamid O, Seiden MV, Oza A, Plante M, Potkul RK, et al. Multicenter, randomized phase II trial of oral CI-1033 for previously treated advanced ovarian cancer. J Clin Oncol. 2005;23(24):5597–604. doi:10.1200/JCO.2005.08.091.
80. Weroha SJ, Oberg AL, Ziegler KL, Dakhilm SR, Rowland KM, Hartmann LC, et al. Phase II trial of lapatinib and topotecan (LapTop) in patients with platinum-refractory/resistant ovarian and primary peritoneal carcinoma. Gynecol Oncol. 2011;122(1):116–20. doi:10.1016/j.ygyno.2011.03.030.
81. Vivanco I, Sawyers CL. The phosphatidylinositol 3-kinase AKT pathway in human cancer. Nat Rev Cancer. 2002;2(7):489–501. doi:10.1038/nrc839.
82. Trinh XB, van Dam PA, Dirix LY, Vermeulen PB, Tjalma WA. The rationale for mTOR inhibition in epithelial ovarian cancer. Expert Opin Investig Drugs. 2009;18(12):1885–91. doi:10.1517/13543780903321508.
83. Rini BI. Temsirolimus, an inhibitor of mammalian target of rapamycin. Clin Cancer Res. 2008;14(5):1286–90. doi:10.1158/1078-0432.CCR-07-4719.
84. Behbakht K, Sill MW, Darcy KM, Rubin SC, Mannel RS, Waggoner S, et al. Phase II trial of the mTOR inhibitor, temsirolimus and evaluation of circulating tumor cells and tumor biomarkers in persistent and recurrent epithelial ovarian and primary peritoneal malignancies: a gynecologic oncology group study. Gynecol Oncol. 2011;123(1):19–26. doi:10.1016/j.ygyno.2011.06.022.

85. Kelemen LE. The role of folate receptor alpha in cancer development, progression and treatment: cause, consequence or innocent bystander? Int J Cancer. 2006;119(2):243–50. doi:10.1002/ijc.21712.
86. Elnakat H, Ratnam M. Role of folate receptor genes in reproduction and related cancers. Front Biosci. 2006;11:506–19.
87. Chen YL, Chang MC, Huang CY, Chiang YC, Lin HW, Chen CA, et al. Serous ovarian carcinoma patients with high alpha-folate receptor had reducing survival and cytotoxic chemoresponse. Mol Oncol. 2012;6(3):360–9. doi:10.1016/j.molonc.2011.11.010.
88. Sato S, Itamochi H. Profile of farletuzumab and its potential in the treatment of solid tumors. Onco Targets Ther. 2016;9:1181–8. doi:10.2147/OTT.S98242.
89. Vergote I, Armstrong D, Scambia G, Teneriello M, Sehouli J, Schweizer C, et al. A randomized, double-blind, placebo-controlled, phase III study to assess efficacy and safety of weekly Farletuzumab in combination with carboplatin and Taxane in patients with ovarian cancer in first platinum-sensitive relapse. J Clin Oncol. 2016; doi:10.1200/JCO.2015.63.2596.
90. Reddy JA, Dorton R, Westrick E, Dawson A, Smith T, Xu LC, et al. Preclinical evaluation of EC145, a folate-vinca alkaloid conjugate. Cancer Res. 2007;67(9):4434–42. doi:10.1158/0008-5472.CAN-07-0033.
91. Naumann RW, Coleman RL, Burger RA, Sausville EA, Kutarska E, Ghamande SA, et al. PRECEDENT: a randomized phase II trial comparing vintafolide (EC145) and pegylated liposomal doxorubicin (PLD) in combination versus PLD alone in patients with platinum-resistant ovarian cancer. J Clin Oncol. 2013;31(35):4400–6. doi:10.1200/JCO.2013.49.7685.
92. Meier W, du Bois A, Rau J, Gropp-Meier M, Baumann K, Huober J, et al. Randomized phase II trial of carboplatin and paclitaxel with or without lonafarnib in first-line treatment of epithelial ovarian cancer stage IIB-IV. Gynecol Oncol. 2012;126(2):236–40. doi:10.1016/j.ygyno.2012.04.050.
93. Cognetti F, Bagnato A, Colombo N, Savarese A, Scambia G, Sehouli J, et al. A phase II, randomized, double-blind study of zibotentan (ZD4054) in combination with carboplatin/paclitaxel versus placebo in combination with carboplatin/paclitaxel in patients with advanced ovarian cancer sensitive to platinum-based chemotherapy (AGO-OVAR 2.14). Gynecol Oncol. 2013;130(1):31–7. doi:10.1016/j.ygyno.2012.12.004.
94. Vergote IB, Chekerov R, Amant F, Harter P, Casado A, Emerich J, et al. Randomized, phase II, placebo-controlled, double-blind study with and without enzastaurin in combination with paclitaxel and carboplatin as first-line treatment followed by maintenance treatment in advanced ovarian cancer. J Clin Oncol. 2013;31(25):3127–32. doi:10.1200/JCO.2012.44.9116.
95. Glasspool RM, Brown R, Gore ME, Rustin GJ, McNeish IA, Wilson RH, et al. A randomised, phase II trial of the DNA-hypomethylating agent 5-aza-2′-deoxycytidine (decitabine) in combination with carboplatin vs carboplatin alone in patients with recurrent, partially platinum-sensitive ovarian cancer. Br J Cancer. 2014;110(8):1923–9. doi:10.1038/bjc.2014.116.
96. Pujade-Lauraine E, Selle F, Weber B, Ray-Coquard IL, Vergote I, Sufliarsky J, et al. Volasertib versus chemotherapy in platinum-resistant or -refractory ovarian cancer: a randomized phase II Groupe des Investigateurs Nationaux pour l'Etude des cancers de l'Ovaire study. J Clin Oncol. 2016;34(7):706–13. doi:10.1200/JCO.2015.62.1474.
97. Shih Ie M, Kurman RJ. Ovarian tumorigenesis: a proposed model based on morphological and molecular genetic analysis. Am J Pathol. 2004;164(5):1511–8.
98. Farley J, Brady WE, Vathipadiekal V, Lankes HA, Coleman R, Morgan MA, et al. Selumetinib in women with recurrent low-grade serous carcinoma of the ovary or peritoneum: an open-label, single-arm, phase 2 study. Lancet Oncol. 2013;14(2):134–40. doi:10.1016/S1470-2045(12)70572-7.
99. Kuo KT, Mao TL, Jones S, Veras E, Ayhan A, Wang TL, et al. Frequent activating mutations of PIK3CA in ovarian clear cell carcinoma. Am J Pathol. 2009;174(5):1597–601. doi:10.2353/ajpath.2009.081000.
100. Willner J, Wurz K, Allison KH, Galic V, Garcia RL, Goff BA, et al. Alternate molecular genetic pathways in ovarian carcinomas of common histological types. Hum Pathol. 2007;38(4):607–13. doi:10.1016/j.humpath.2006.10.007.

101. Hashiguchi Y, Tsuda H, Inoue T, Berkowitz RS, Mok SC. PTEN expression in clear cell adenocarcinoma of the ovary. Gynecol Oncol. 2006;101(1):71–5. doi:10.1016/j.ygyno.2005.09.047.
102. Wiegand KC, Shah SP, Al-Agha OM, Zhao Y, Tse K, Zeng T, et al. ARID1A mutations in endometriosis-associated ovarian carcinomas. N Engl J Med. 2010;363(16):1532–43. doi:10.1056/NEJMoa1008433.
103. Itamochi H, Oumi N, Oishi T, Shoji T, Fujiwara H, Sugiyama T, et al. Loss of ARID1A expression is associated with poor prognosis in patients with stage I/II clear cell carcinoma of the ovary. Int J Clin Oncol. 2015;20(5):967–73. doi:10.1007/s10147-015-0811-x.
104. Bitler BG, Aird KM, Garipov A, Li H, Amatangelo M, Kossenkov AV, et al. Synthetic lethality by targeting EZH2 methyltransferase activity in ARID1A-mutated cancers. Nat Med. 2015;21(3):231–8. doi:10.1038/nm.3799.
105. Bokhman JV. Two pathogenetic types of endometrial carcinoma. Gynecol Oncol. 1983;15(1):10–7.
106. Kandoth C, Schultz N, Cherniack AD, Akbani R, Liu Y, Shen H, et al. Integrated genomic characterization of endometrial carcinoma. Nature. 2013;497(7447):67–73. doi:10.1038/nature12113.
107. Byron SA, Pollock PM. FGFR2 as a molecular target in endometrial cancer. Future Oncol. 2009;5(1):27–32. doi:10.2217/14796694.5.1.27.
108. Morice P, Leary A, Creutzberg C, Abu-Rustum N, Darai E. Endometrial cancer. Lancet. 2016;387(10023):1094–108. doi:10.1016/S0140-6736(15)00130-0.
109. Pollock PM, Gartside MG, Dejeza LC, Powell MA, Mallon MA, Davies H, et al. Frequent activating FGFR2 mutations in endometrial carcinomas parallel germline mutations associated with craniosynostosis and skeletal dysplasia syndromes. Oncogene. 2007;26(50):7158–62. doi:10.1038/sj.onc.1210529.
110. Oza AM, Elit L, Tsao MS, Kamel-Reid S, Biagi J, Provencher DM, et al. Phase II study of temsirolimus in women with recurrent or metastatic endometrial cancer: a trial of the NCIC clinical trials group. J Clin Oncol. 2011;29(24):3278–85. doi:10.1200/JCO.2010.34.1578.
111. Fleming GF, Filiaci VL, Marzullo B, Zaino RJ, Davidson SA, Pearl M, et al. Temsirolimus with or without megestrol acetate and tamoxifen for endometrial cancer: a gynecologic oncology group study. Gynecol Oncol. 2014;132(3):585–92. doi:10.1016/j.ygyno.2014.01.015.
112. Myers AP, Filiaci VL, Zhang Y, Pearl M, Behbakht K, Makker V, et al. Tumor mutational analysis of GOG248, a phase II study of temsirolimus or temsirolimus and alternating megestrol acetate and tamoxifen for advanced endometrial cancer (EC): an NRG oncology/gynecologic oncology group study. Gynecol Oncol. 2016;141(1):43–8. doi:10.1016/j.ygyno.2016.02.025.
113. Colombo N, McMeekin DS, Schwartz PE, Sessa C, Gehrig PA, Holloway R, et al. Ridaforolimus as a single agent in advanced endometrial cancer: results of a single-arm, phase 2 trial. Br J Cancer. 2013;108(5):1021–6. doi:10.1038/bjc.2013.59.
114. Tsoref D, Welch S, Lau S, Biagi J, Tonkin K, Martin LA, et al. Phase II study of oral ridaforolimus in women with recurrent or metastatic endometrial cancer. Gynecol Oncol. 2014;135(2):184–9. doi:10.1016/j.ygyno.2014.06.033.
115. Oza AM, Pignata S, Poveda A, McCormack M, Clamp A, Schwartz B, et al. Randomized phase II trial of Ridaforolimus in advanced endometrial carcinoma. J Clin Oncol. 2015;33(31):3576–82. doi:10.1200/JCO.2014.58.8871.
116. Slomovitz BM, Lu KH, Johnston T, Coleman RL, Munsell M, Broaddus RR, et al. A phase 2 study of the oral mammalian target of rapamycin inhibitor, everolimus, in patients with recurrent endometrial carcinoma. Cancer. 2010;116(23):5415–9. doi:10.1002/cncr.25515.
117. Schiff R, Massarweh SA, Shou J, Bharwani L, Mohsin SK, Osborne CK. Cross-talk between estrogen receptor and growth factor pathways as a molecular target for overcoming endocrine resistance. Clin Cancer Res. 2004;10(1 Pt 2):331S–6S.
118. Slomovitz BM, Jiang Y, Yates MS, Soliman PT, Johnston T, Nowakowski M, et al. Phase II study of everolimus and letrozole in patients with recurrent endometrial carcinoma. J Clin Oncol. 2015;33(8):930–6. doi:10.1200/JCO.2014.58.3401.

119. Matulonis U, Vergote I, Backes F, Martin LP, McMeekin S, Birrer M, et al. Phase II study of the PI3K inhibitor pilaralisib (SAR245408; XL147) in patients with advanced or recurrent endometrial carcinoma. Gynecol Oncol. 2015;136(2):246–53. doi:10.1016/j.ygyno.2014.12.019.
120. Fleming GF, Sill MW, Darcy KM, McMeekin DS, Thigpen JT, Adler LM, et al. Phase II trial of trastuzumab in women with advanced or recurrent, HER2-positive endometrial carcinoma: a gynecologic oncology group study. Gynecol Oncol. 2010;116(1):15–20. doi:10.1016/j.ygyno.2009.09.025.
121. Oza AM, Eisenhauer EA, Elit L, Cutz JC, Sakurada A, Tsao MS, et al. Phase II study of erlotinib in recurrent or metastatic endometrial cancer: NCIC IND-148. J Clin Oncol. 2008;26(26):4319–25. doi:10.1200/JCO.2007.15.8808.
122. Leslie KK, Sill MW, Fischer E, Darcy KM, Mannel RS, Tewari KS, et al. A phase II evaluation of gefitinib in the treatment of persistent or recurrent endometrial cancer: a gynecologic oncology group study. Gynecol Oncol. 2013;129(3):486–94. doi:10.1016/j.ygyno.2013.02.019.
123. Leslie KK, Sill MW, Lankes HA, Fischer EG, Godwin AK, Gray H, et al. Lapatinib and potential prognostic value of EGFR mutations in a gynecologic oncology group phase II trial of persistent or recurrent endometrial cancer. Gynecol Oncol. 2012;127(2):345–50. doi:10.1016/j.ygyno.2012.07.127.
124. Coleman RL, Sill MW, Thaker PH, Bender DP, Street D, McGuire WP, et al. A phase II evaluation of selumetinib (AZD6244, ARRY-142886), a selective MEK-1/2 inhibitor in the treatment of recurrent or persistent endometrial cancer: an NRG oncology/gynecologic oncology group study. Gynecol Oncol. 2015;138(1):30–5. doi:10.1016/j.ygyno.2015.04.005.
125. Aghajanian C, Sill MW, Darcy KM, Greer B, McMeekin DS, Rose PG, et al. Phase II trial of bevacizumab in recurrent or persistent endometrial cancer: a gynecologic oncology group study. J Clin Oncol. 2011;29(16):2259–65. doi:10.1200/JCO.2010.32.6397.
126. Alvarez EA, Brady WE, Walker JL, Rotmensch J, Zhou XC, Kendrick JE, et al. Phase II trial of combination bevacizumab and temsirolimus in the treatment of recurrent or persistent endometrial carcinoma: a gynecologic oncology group study. Gynecol Oncol. 2013;129(1):22–7. doi:10.1016/j.ygyno.2012.12.022.
127. Coleman RL, Sill MW, Lankes HA, Fader AN, Finkler NJ, Hoffman JS, et al. A phase II evaluation of aflibercept in the treatment of recurrent or persistent endometrial cancer: a gynecologic oncology group study. Gynecol Oncol. 2012;127(3):538–43. doi:10.1016/j.ygyno.2012.08.020.
128. Nimeiri HS, Oza AM, Morgan RJ, Huo D, Elit L, Knost JA, et al. A phase II study of sorafenib in advanced uterine carcinoma/carcinosarcoma: a trial of the Chicago, PMH, and California phase II consortia. Gynecol Oncol. 2010;117(1):37–40. doi:10.1016/j.ygyno.2010.01.013.
129. Castonguay V, Lheureux S, Welch S, Mackay HJ, Hirte H, Fleming G, et al. A phase II trial of sunitinib in women with metastatic or recurrent endometrial carcinoma: a study of the Princess Margaret. Chicago and California Consortia Gynecologic oncology. 2014;134(2):274–80. doi:10.1016/j.ygyno.2014.05.016.
130. Bender D, Sill MW, Lankes HA, Reyes HD, Darus CJ, Delmore JE, et al. A phase II evaluation of cediranib in the treatment of recurrent or persistent endometrial cancer: an NRG oncology/gynecologic oncology group study. Gynecol Oncol. 2015;138(3):507–12. doi:10.1016/j.ygyno.2015.07.018.
131. Moore KN, Sill MW, Tenney ME, Darus CJ, Griffin D, Werner TL, et al. A phase II trial of trebananib (AMG 386; IND#111071), a selective angiopoietin 1/2 neutralizing peptibody, in patients with persistent/recurrent carcinoma of the endometrium: an NRG/gynecologic oncology group trial. Gynecol Oncol. 2015;138(3):513–8. doi:10.1016/j.ygyno.2015.07.006.
132. Makker V, Filiaci VL, Chen LM, Darus CJ, Kendrick JE, Sutton G, et al. Phase II evaluation of dalantercept, a soluble recombinant activin receptor-like kinase 1 (ALK1) receptor fusion protein, for the treatment of recurrent or persistent endometrial cancer: an NRG oncology/gynecologic oncology group study 0229N. Gynecol Oncol. 2015;138(1):24–9. doi:10.1016/j.ygyno.2015.04.006.

133. Powell MA, Sill MW, Goodfellow PJ, Benbrook DM, Lankes HA, Leslie KK, et al. A phase II trial of brivanib in recurrent or persistent endometrial cancer: an NRG oncology/gynecologic oncology group study. Gynecol Oncol. 2014;135(1):38–43. doi:10.1016/j.ygyno.2014.07.083.
134. Dizon DS, Sill MW, Schilder JM, McGonigle KF, Rahman Z, Miller DS, et al. A phase II evaluation of nintedanib (BIBF-1120) in the treatment of recurrent or persistent endometrial cancer: an NRG oncology/gynecologic oncology group study. Gynecol Oncol. 2014;135(3):441–5. doi:10.1016/j.ygyno.2014.10.001.
135. Konecny GE, Finkler N, Garcia AA, Lorusso D, Lee PS, Rocconi RP, et al. Second-line dovitinib (TKI258) in patients with FGFR2-mutated or FGFR2-non-mutated advanced or metastatic endometrial cancer: a non-randomised, open-label, two-group, two-stage, phase 2 study. Lancet Oncol. 2015;16(6):686–94. doi:10.1016/S1470-2045(15)70159-2.
136. Steenbergen RD, Snijders PJ, Heideman DA, Meijer CJ. Clinical implications of (epi)genetic changes in HPV-induced cervical precancerous lesions. Nat Rev Cancer. 2014;14(6):395–405. doi:10.1038/nrc3728.
137. Lopez-Ocejo O, Viloria-Petit A, Bequet-Romero M, Mukhopadhyay D, Rak J, Kerbel RS. Oncogenes and tumor angiogenesis: the HPV-16 E6 oncoprotein activates the vascular endothelial growth factor (VEGF) gene promoter in a p53 independent manner. Oncogene. 2000;19(40):4611–20. doi:10.1038/sj.onc.1203817.
138. Tang X, Zhang Q, Nishitani J, Brown J, Shi S, Le AD. Overexpression of human papillomavirus type 16 oncoproteins enhances hypoxia-inducible factor 1 alpha protein accumulation and vascular endothelial growth factor expression in human cervical carcinoma cells. Clin Cancer Res. 2007;13(9):2568–76. doi:10.1158/1078-0432.CCR-06-2704.
139. Birner P, Schindl M, Obermair A, Plank C, Breitenecker G, Oberhuber G. Overexpression of hypoxia-inducible factor 1alpha is a marker for an unfavorable prognosis in early-stage invasive cervical cancer. Cancer Res. 2000;60(17):4693–6.
140. Monk BJ, Sill MW, Burger RA, Gray HJ, Buekers TE, Roman LD. Phase II trial of bevacizumab in the treatment of persistent or recurrent squamous cell carcinoma of the cervix: a gynecologic oncology group study. J Clin Oncol. 2009;27(7):1069–74. doi:10.1200/JCO.2008.18.9043.
141. Tewari KS, Sill MW, Long 3rd HJ, Penson RT, Huang H, Ramondetta LM, et al. Improved survival with bevacizumab in advanced cervical cancer. N Engl J Med. 2014;370(8):734–43. doi:10.1056/NEJMoa1309748.
142. Schefter T, Winter K, Kwon JS, Stuhr K, Balaraj K, Yaremko BP, et al. RTOG 0417: efficacy of bevacizumab in combination with definitive radiation therapy and cisplatin chemotherapy in untreated patients with locally advanced cervical carcinoma. Int J Radiat Oncol Biol Phys. 2014;88(1):101–5. doi:10.1016/j.ijrobp.2013.10.022.
143. Mackay HJ, Tinker A, Winquist E, Thomas G, Swenerton K, Oza A, et al. A phase II study of sunitinib in patients with locally advanced or metastatic cervical carcinoma: NCIC CTG trial IND.184. Gynecol Oncol. 2010;116(2):163–7. doi:10.1016/j.ygyno.2009.08.012.
144. Monk BJ, Mas Lopez L, Zarba JJ, Oaknin A, Tarpin C, Termrungruanglert W, et al. Phase II, open-label study of pazopanib or lapatinib monotherapy compared with pazopanib plus lapatinib combination therapy in patients with advanced and recurrent cervical cancer. J Clin Oncol. 2010;28(22):3562–9. doi:10.1200/JCO.2009.26.9571.
145. Li Q, Tang Y, Cheng X, Ji J, Zhang J, Zhou X. EGFR protein expression and gene amplification in squamous intraepithelial lesions and squamous cell carcinomas of the cervix. Int J Clin Exp Pathol. 2014;7(2):733–41.
146. Noordhuis MG, Eijsink JJ, Ten Hoor KA, Roossink F, Hollema H, Arts HJ, et al. Expression of epidermal growth factor receptor (EGFR) and activated EGFR predict poor response to (chemo)radiation and survival in cervical cancer. Clin Cancer Res. 2009;15(23):7389–97. doi:10.1158/1078-0432.CCR-09-1149.
147. Santin AD, Sill MW, McMeekin DS, Leitao Jr MM, Brown J, Sutton GP, et al. Phase II trial of cetuximab in the treatment of persistent or recurrent squamous or non-squamous cell carcinoma of the cervix: a gynecologic oncology group study. Gynecol Oncol. 2011;122(3):495–500. doi:10.1016/j.ygyno.2011.05.040.

148. Farley J, Sill MW, Birrer M, Walker J, Schilder RJ, Thigpen JT, et al. Phase II study of cisplatin plus cetuximab in advanced, recurrent, and previously treated cancers of the cervix and evaluation of epidermal growth factor receptor immunohistochemical expression: a gynecologic oncology group study. Gynecol Oncol. 2011;121(2):303–8. doi:10.1016/j.ygyno.2011.01.030.
149. de la Rochefordiere A, Kamal M, Floquet A, Thomas L, Petrow P, Petit T, et al. PIK3CA pathway mutations predictive of poor response following standard Radiochemotherapy +/− Cetuximab in cervical cancer patients. Clin Cancer Res. 2015;21(11):2530–7. doi:10.1158/1078-0432.CCR-14-2368.
150. Goncalves A, Fabbro M, Lhomme C, Gladieff L, Extra JM, Floquet A, et al. A phase II trial to evaluate gefitinib as second- or third-line treatment in patients with recurring locoregionally advanced or metastatic cervical cancer. Gynecol Oncol. 2008;108(1):42–6. doi:10.1016/j.ygyno.2007.07.057.
151. Schilder RJ, Sill MW, Lee YC, Mannel R. A phase II trial of erlotinib in recurrent squamous cell carcinoma of the cervix: a gynecologic oncology group study. Int J Gynecol Cancer. 2009;19(5):929–33. doi:10.1111/IGC.0b013e3181a83467.
152. Nogueira-Rodrigues A, Moralez G, Grazziotin R, Carmo CC, Small IA, Alves FV, et al. Phase 2 trial of erlotinib combined with cisplatin and radiotherapy in patients with locally advanced cervical cancer. Cancer. 2014;120(8):1187–93. doi:10.1002/cncr.28471.
153. Lu Z, Hu X, Li Y, Zheng L, Zhou Y, Jiang H, et al. Human papillomavirus 16 E6 oncoprotein interferences with insulin signaling pathway by binding to tuberin. J Biol Chem. 2004;279(34):35664–70. doi:10.1074/jbc.M403385200.
154. Tinker AV, Ellard S, Welch S, Moens F, Allo G, Tsao MS, et al. Phase II study of temsirolimus (CCI-779) in women with recurrent, unresectable, locally advanced or metastatic carcinoma of the cervix. A trial of the NCIC clinical trials group (NCIC CTG IND 199). Gynecol Oncol. 2013;130(2):269–74. doi:10.1016/j.ygyno.2013.05.008.
155. Tan S, de Vries EG, van der Zee AG, de Jong S. Anticancer drugs aimed at E6 and E7 activity in HPV-positive cervical cancer. Curr Cancer Drug Targets. 2012;12(2):170–84.
156. Tan S, Hougardy BM, Meersma GJ, Schaap B, de Vries EG, van der Zee AG, et al. Human papilloma virus 16 E6 RNA interference enhances cisplatin and death receptor-mediated apoptosis in human cervical carcinoma cells. Mol Pharmacol. 2012;81(5):701–9. doi:10.1124/mol.111.076539.
157. Zhao CY, Szekely L, Bao W, Selivanova G. Rescue of p53 function by small-molecule RITA in cervical carcinoma by blocking E6-mediated degradation. Cancer Res. 2010;70(8):3372–81. doi:10.1158/0008-5472.CAN-09-2787.

Immunotherapy for Gynecologic Cancer

4

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Abstract

Immunotherapy is recently drawing attention among various cancer medical treatments. Especially, immune checkpoint inhibitor has been shown to be effective in various cancers including malignant melanoma, prostate cancer, and lung cancer. In gynecological cancer, it has been shown that immune checkpoint inhibition using anti-PD-1 antibody may be effective in a part of the ovarian cancer patients. Presently, multiple clinical trials of anti-PD-1/anti-PD-L1 antibodies for ovarian as well as cervical cancers are underway. Other types of immunotherapies such as cancer vaccines and adoptive cell transfer therapy are also being developed. In recent use of molecular target reagents, personalized treatment biomarkers are in tread. Recent advancements in comprehensive genetic analysis using next-generation sequencing technology have made it possible to obtain a large amount of information about individual cancers, and these advancements have allowed us to distinguish one cancer from others that have a similar pathological appearance and enabled to treat them differently from patient to patient. However, so far, it is still difficult to precisely predict the efficacy of immunotherapy using biomarkers because cancer immunity consists of complicated multiple factors. In the future, it is expected to customize the immunotherapy according to the patients' immune status by precisely evaluating it. Also, optimization of the best combination of different immunotherapies or combination of immunotherapy and other modalities may be put into practice.

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Ovarain cancer • Immune therapy • Microenvironment • Tumor immune escape
Immune checkpoint inhibitor

4.1 Introduction: The Upward Trend of Precision Medicine in Cancer Treatment

4.1.1 Importance of Precision Medical Treatment

Personalized intervention in medical treatments such as cancer chemotherapy is now a growing trend. In personalized medicine, the therapeutic drugs for each patient are selected according to a prediction of effectiveness based on the markers which the tumor or the patient possesses. Such biomarker-based personalized treatment is particularly effective for molecular target drugs. Unlike conventional chemo-reagents such as cisplatin and paclitaxel, molecular target reagents have a clearer point of action, e.g., the inhibition of specific intracellular signals. Therefore, it is relatively easy to find a biomarker that can predict the effect of the drug, and once the biomarker is established, it is not difficult to select the most effective drug for the patient.

Due to a rapid increase in the availability of genetic and proteomic information associated with the characteristics of each cancer case, the trend toward comprehensive analysis and subsequent personalization is a very promising approach in cancer treatment. Personalized medicine is also thought to be effective in lowering healthcare costs because it can theoretically reduce the inefficient use of drugs; this may be particularly relevant because the prices of newly developed drugs tend to be much higher than those of conventional chemo-reagents. In general, the drugs used in the immunotherapy mentioned below are extremely expensive, although they have been shown to be very effective in certain patients. Therefore, the possible use of prediction and personalization in cancer immunotherapy is a pressing issue.

4.1.2 Precision and Personalization

The terms precision medicine and personalized medicine are often confused and used interchangeably. Indeed, these two concepts are closely associated with each other. Naturally, if we can precisely predict the effectiveness of a specific drug for each patient, we can personalize the patient's treatment strategy accordingly. Therefore, the more precise that medicine becomes, the more it moves toward personalization. However, there is a slight difference in nuance between these terms. Precision medicine places more emphasis on the preciseness of the diagnosis and treatment. Thus, the diagnosis is not necessarily personalized, which means that universal treatments could also be used as long as they are effective. On the other hand, personalized medicine pays more attention to the differences among patients and aims to differentiate treatments from patient to patient. Although these ways of

thinking and methodologies look somewhat different, the ultimate goal of both concepts is the same: to offer the best treatment strategy for each individual patient.

Immunotherapy, as stated below, is predominantly personal because immune reactions are quite different among individuals and among tumors. Therefore, reactions to the same immunotherapy (i.e., drug) may be completely different for each patient, more so than for reactions to chemotherapy. In the case of immunotherapy, once the treatment shows a noticeable effect, it virtually cures the patient, which is very rare in chemotherapy. However, currently it is difficult to precisely predict how immunotherapy will work for each patient because immune reactions are so complicated compared with, for example, cytotoxicity resulting from chemotherapy.

4.1.3 Precision Medicine and Biomarkers

Various biomarkers, both genetic and nongenetic, are expected to be effective biomarkers in medical treatments for cancer. For the development of biomarkers, a thorough understanding of tumor biology and the mechanisms of response or resistance to a certain drug are particularly important. Recent advancements in comprehensive genetic analysis using next-generation sequencing technology have made it possible to obtain a large amount of information about individual cancers; these advancements have allowed us to distinguish one cancer from others that have a similar pathological appearance. Thus, in the near future, using a large amount of genetic/nongenetic data to characterize each cancer could become a reality. However, unlike general molecular targeting reagents, which are relatively simple in structure and also in mechanism, immune reactions involve various types of immune cells and are far more complicated than single-molecule reactions. Therefore, obtaining precise predictions of immune response by measuring biomarkers will not be easy.

4.1.4 Immunotherapy as a Personalized Treatment

During the past decade, significant advancements in the understanding of immune reactions, including cancer immunity, have identified the major players and their roles in host-tumor immunity (Fig. 4.1). In general, an immune reaction to cancer is initiated when professional antigen-presenting cells such as dendritic cells (DCs) recognize cancer fragments [1]. DCs are then activated along with several types of maturation signals, which include various cytokines called the “danger” signals. Following activation, antigen-presenting cells (APCs) present tumor-associated antigens on the major histocompatibility complex (MHC) class I and II molecules. The activation of antigen-specific CD4 and CD8 T cells specific for the presented antigenic peptides occurs only when accompanied by the engagement of co-stimulatory receptors such as CD28. The activated T cells then recognize the tumor cells through the T cell receptor (TCR) and the specific tumor peptide on the MHC, which leads to T cell-mediated tumor destruction. Theoretically, any of these steps can be augmented to enhance tumor immunity according to the immune status of an individual (Fig. 4.2).

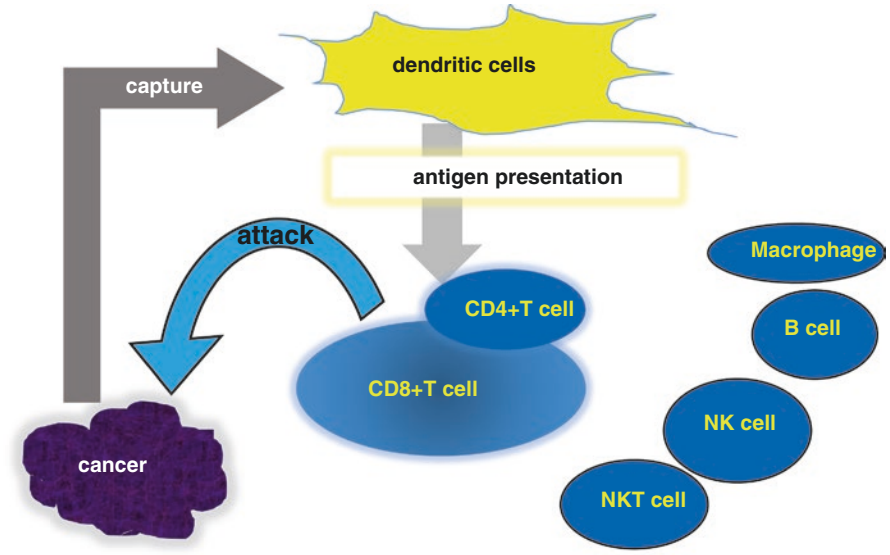

Fig. 4.1 Basic mechanism of tumor immunity

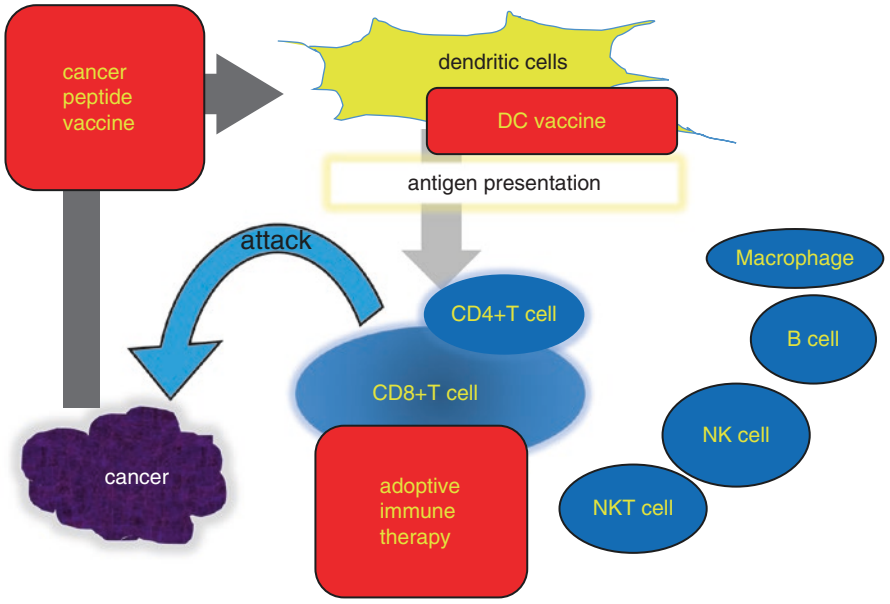

Fig. 4.2 Targeted tumor immune therapies

4.2 Recent Applications of Immunotherapy in Cancer Treatment and Their Role in Precision Medicine

4.2.1 A Sudden Rise of Immunotherapy in Cancer Treatment

Until recently, chemotherapy, including platinum-based reagents and taxanes, has been at the center of medical therapy for solid tumors, including gynecologic cancers; however, the so-called molecular target reagents have begun to drastically change cancer treatment. Among those, immunotherapeutic drugs represent the newest and most promising modality in the field of oncology. Although immune-based cancer treatment has long been viewed as a promising modality, only recently has its clinical efficacy equaled or surpassed conventional chemotherapy.

The development of novel immunotherapy has been achieved by the introduction of the so-called "immune checkpoint inhibitor" drugs, especially those with antibodies that block the PD-L1/PD-1 (programmed cell death ligand-1/programmed cell death-1) immune signal. PD-1 was originally identified as a molecule that is physiologically expressed in specific immune cells, and it is regarded as an inhibitor of immune overreaction such as autoimmune disease [2]. However, later findings indicate that PD-1 plays an important role in host-tumor immunity (Fig. 4.3). Two ligands for PD-1, PD-L1 and PD-L2, were subsequently identified [2], and the expression of PD-L1 in cancer cells was reported in various malignant tumors. In

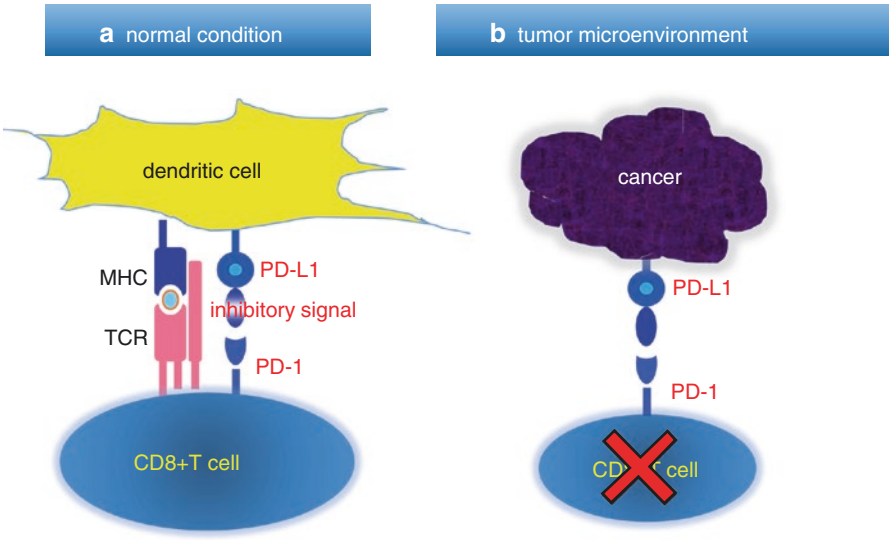

Fig. 4.3 Immune checkpoint molecule PD-L1/PD-1

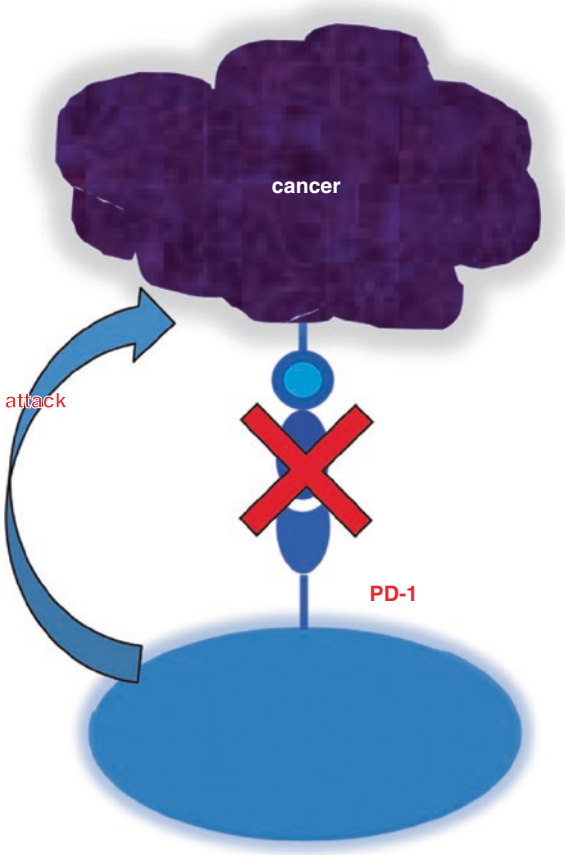

Fig. 4.4 Immune checkpoint inhibition

addition, immune-inhibitory signals via PD-1 on immune cells have been associated with poorer clinical courses of various cancers [2]. Therefore, if we can block the signal by some means, we can expect the restoration of tumor immunity and clinical benefits (Fig. 4.4). In fact, as mentioned below, studies have increasingly reported the clinical effectiveness of immunotherapies targeting the PD-L1/PD-1 signal, namely, the immune checkpoint inhibitor [2].

4.2.2 Representative Clinical Trials Using Anti-PD-1/Anti-PD-L1 Antibodies in Melanoma and Lung Cancer

In 2010, the first Phase I study using an anti-PD-1 antibody, nivolumab, was conducted on various solid tumors including melanoma, non-small cell lung cancer, renal cell carcinoma, and prostate and colorectal cancer [3]. In 2012, a Phase I study

using nivolumab was conducted on 296 patients with non-small cell lung cancer, melanoma, or renal cell carcinoma. The clinical response was surprisingly high given that the mean RR values of this study were 18%, 28%, and 27%, respectively, taking into consideration that the patients had refractory disease [4]. Following this trial, numerous trials for various malignancies have been conducted or are being conducted using both anti-PD-1 and anti-PD-L1 antibodies.

The efficacy of nivolumab and pembrolizumab, another anti-PD-1 antibody for melanoma, which is known to be relatively sensitive to immunotherapy, was investigated in Phase III trials. In a trial with nivolumab, which was used as a first-line treatment, the 1-year overall survival rate was 73% for nivolumab and only 42% for the control dacarbazine [5]. In another Phase III trial for advanced melanoma, nivolumab treatment also showed a threefold higher response rate compared with chemotherapy [6]. Another Phase III trial compared three immunotherapies: pembrolizumab every 2 weeks, pembrolizumab every 3 weeks, and ipilimumab every 3 weeks; the results showed that treatment with pembrolizumab every 2 weeks or every 3 weeks exhibited a better response rate than treatment with ipilimumab every 3 weeks [7].

Lung cancer is another malignancy in which the efficacy of anti-PD-L1/PD-1 therapy has been shown. A randomized Phase III trial comparing nivolumab to docetaxel in patients with advanced NSCLC indicated that the overall survival rate at 1 year was significantly better for the nivolumab group than for the docetaxel group [8, 9]. In early phase trials, anti-PD-L1 antibodies have also shown promising efficacy in patients with NSCLC [10, 11].

4.2.3 Other Immune Checkpoint Inhibitors

The CTLA-4 receptor has a similar function to PD-1, and it is known as another "immune checkpoint molecule." By stimulating CD80/CD86 on antigen-presenting cells, CTLA-4 induces the arrest of effector T cells, which ultimately leads to immunosuppression. Therefore, the abrogation of the CTLA-4 signal by the anti-CTLA-4 antibody can be expected to have antitumor effect by restoring T cell activity [12, 13]. The anti-CTLA-4 antibody ipilimumab was approved by the FDA for metastatic melanoma. Recently, a combination effect of ipilimumab and nivolumab has been reported, suggesting that these two immune checkpoint molecules have independent functions [14, 15].

4.2.4 Precision Medicine in Immune Checkpoint Inhibition

The expression of PD-L1 in tumor tissue is the most investigated candidate for predicting the effectiveness of anti-PD-L1/PD-1 therapy. There have been many studies of the expression of PD-L1 in cancers, including urothelial cancers, gastrointestinal cancer, lung cancer, breast cancer, melanoma, and ovarian cancer [2, 16, 17]. In many of these cancers, PD-L1 expression is correlated with poor patient outcomes, suggesting that PD-L1 expression has a biologically favorable effect on the survival

of cancer cells [18]. However, as a biomarker, PD-L1 expression has not received a stable appraisal. Neither all of the cancers nor all of the cases show an association between the expression of PD-L1 and the effects of anti-PD-L1/PD-1 therapy [2, 19]. This discrepancy is partly due to the unstable evaluation of PD-L1 expression using various anti-PD-L1 antibodies in each study. However, the role of PD-L1 in the cancer immune landscape is not fully understood yet, and further biological clarification is needed to determine if PD-L1 expression could serve as an immunologic biomarker.

Another possible candidate for an immunological biomarker is the mutation burden, that is, the total amount of mutation. Cancers in which immune checkpoint inhibition is effective are known to have more genetic mutations. Moreover, within one cancer type, it is thought that immune checkpoint inhibition may be effective when an individual case has more mutations [2, 20, 21]. In a study of colorectal cancer, the anti-PD-1 antibody pembrolizumab was effective only in patients with a mismatch repair deficiency, who naturally harbor many genetic mutations, while it was not effective in patients without a mismatch repair deficiency and who had fewer mutations [22]. Therefore, the mismatch deficiency or mutation burden of each cancer could be a predictive biomarker in anti-PD-1 therapy. Similarly, it has been reported that patients with more transversion mutations, which are known to be a "smoking signature," were more sensitive to pembrolizumab [23].

Although it is not still clear whether whole mutation burden or specific mutation phenotype can be used as predictive biomarkers, recent advancements in analyzing mutation in a comprehensive way may contribute to the development of a practical biomarker for immunotherapy [24, 25]. Development of biomarkers is also expected to contribute to the personalization of immunotherapy as mentioned below.

4.2.5 Cancer Vaccines

Therapeutic cancer vaccines have been regarded as a potentially promising modality for cancer treatment. These vaccines are usually generated by tumor-specific antigens. By administering these tumor antigens, an immune reaction of specifically targeted tumor cells is elicited, which causes tumor cell distraction by multiple mechanisms including cytotoxic cell-mediated tumor lysis. However, with rare exceptions, most of the past trials of cancer vaccine as a monotherapy failed, suggesting that cancer cells possess the capability to escape from systemic tumor immunity. Nevertheless, it is expected that, in combination with strategies that prevent tumor immune escape such as the anti-PD-1 antibody, cancer vaccines may enhance the effect of immunotherapy. In fact, an animal study has shown that a combination of the stimulator of interferon gene (STING) and anti-PD-1 resulted in enhanced innate immunity and improved response [26].

4.2.6 Precision Medicine in Cancer Vaccine

Cancer vaccines are theoretically an ideal tool for precision and personalized medicine because tumor antigens, which are the main element of cancer vaccines, are thought to vary among cancers. Therefore, by estimating immunogenicity in each case by analyzing the expression of possible tumor antigens, one can predict whether cancer vaccination is suitable for each cancer patient. Additionally, cancer vaccines may be optimized according to the tumor antigens that each cancer expresses.

An apparent target of cancer vaccines that can serve as tumor antigens is nonsynonymous mutations in cancer cells. Mutant proteins resulting from a genetic mutation can be detected by the immune system as non-self epitopes and can elicit an immune reaction to the cancer cells [27]. It is still unclear whether host-tumor immunity depends on the absolute number of mutant proteins or on specific types of mutations. In any case, whole-genome-based analysis of cancer cells may soon clarify what types of mutations contribute to tumor immunity. Based on the results of next-generation sequencing, a personalized vaccine consisting of multiple mutant proteins may be produced in each case [28].

4.2.7 Adoptive Cell Transfer Therapy

Adoptive T cell transfer therapy has long been in use in clinical settings [1]. Initially, autologous lymphocytes are extracted from an excised tumor specimen. They are then cocultured with IL2, which facilitates ex vivo growth. T cells are expanded to as high as one hundred billion and then transferred into patients. Clinical studies have shown that this has a significant clinical effect in at least some types of tumors. However, clinical efficacy has been shown in only a few types of tumors such as melanoma. Obstacles include technical difficulties in expanding effective T cells given costs and time limitations.

4.2.8 Personalization in Adoptive Transfer Therapy

Recently, adoptive transfer therapy has moved into a new stage by adapting to increase patient specificity [29]. This has been enabled by genetically engineering T cells with chimeric antigen receptors (CARs) and by modifying T cell receptors to redirect the specificity of T cells. These strategies have been shown to be effective for personalization by making T cells recognize a specific antigen that is expressed by an individual tumor. There are, however, several issues still to be addressed. First, antigen recognition should lead to functionally effective cytotoxicity. Second, engineered T cells should persist long enough in vivo to exert a clinical effect. Third, they should be effectively trafficked to the target site. In vitro experiments show promising results regarding these issues, but the results should be confirmed in clinical settings.

4.3 Immunotherapy for Gynecologic Cancers

4.3.1 Clinical Trial for Active Immunotherapy in Ovarian Cancer

Several trials have been conducted on CA-125, the most common tumor marker of ovarian cancer, which is expected to serve as a tumor antigen. Although oregovomab, an antibody targeting CA-125, has been demonstrated to elicit anti-CA-125 T cell responses [30], a randomized, placebo-controlled Phase III trial in a maintenance setting of patients with advanced ovarian cancer showed no significant survival benefit [31]. Likewise, farletuzumab, a monoclonal antibody against folate receptor alpha, failed to show apparent efficacy in combination with chemotherapy in large studies [32].

The efficacy of cancer vaccines such as specific peptides, proteins, and DC vaccines has also been investigated in clinical trials. Significant cellular and antibody response to the antigens were observed in most of them, but the clinical benefit of vaccination has not been clearly shown. The primary function of IFN-γ is to augment the antitumor immune response. However, a Phase III trial of IFN-γ plus carboplatin/paclitaxel versus carboplatin/paclitaxel alone for advanced ovarian carcinomas was discontinued early due to the significantly shorter OS time of the patients who were receiving IFN-γ [33, 34].

4.3.2 Immune Checkpoint Inhibition in Ovarian Cancer

We have conducted a first principal investigator-initiated Phase II clinical trial of nivolumab. Two cohorts, 1 or 3 mg/kg, n = 10 each, were tested for 20 platinum-resistant recurrent ovarian cancer patients [35]. The response rate for 3 mg/kg was 20%, including two cases of a durable complete response. The overall response rate for all 20 patients was 15%. The median progression-free survival and overall survival rates were 3.50 months and 20.0 months, respectively. The results of Phase Ib clinical trials with the anti-PD-1 antibody pembrolizumab and the anti-PD-L1 antibody avelumab have also been reported. In a pembrolizumab trial of 26 patients with PD-L1 positive advanced ovarian cancer, the response rate was 11.5% [36]. In another Phase Ib trial of avelumab with 75 patients with recurrent or refractory ovarian cancer, the response rate was 10.7% [37]. Considering that most of the patients recruited for these trials were heavily treated, including for platinum-resistant tumors, the results are thought to be promising and warrant further confirmation. One of 11 patients with ovarian cancer treated with ipilimumab led to an objective response [38]. Table 4.1 lists ongoing trials of immune checkpoint inhibitors in gynecologic cancers.

Table 4.1 Immune checkpoint inhibition trials in gynecological malignancies

Tumor types	Target	Development stage/ study design	Clinical trials identifier
Ovarian cancer	PD-1/CTLA-4	Phase 2/efficacy	NCT02498600
Ovarian carcinoma	PD-1/CD27	Phase 1/2/safety and efficacy	NCT02335918
Ovarian neoplasms	PD-1/IDO	Phase 1/2/safety and efficacy	NCT02327078
Ovarian cancer Cervical cancer	PD-1/CSF1R	Phase 1/2/safety and efficacy	NCT02452424
Ovarian cancer	PD-L1/VEGF PD-L1/chemotherapy	Phase 1/safety	NCT01633970
Ovarian cancer	PD-L1/PARP PD-L1/VEGF	Phase 1/2/safety and efficacy	NCT02484404
Ovarian cancer	PD-L1/TLR 8	Phase 1/2/safety and efficacy	NCT02431559
Cervical cancer	PD-1	Phase 2/efficacy	NCT02257528
Cervical cancer	PD-1/CTLA-4	Phase 1/2/safety and efficacy	NCT02304458
Cervical cancer	PD-1/CD137	Phase 1/2/safety and efficacy	NCT02253992
Cervical cancer	PD-1/LAG3	Phase 1/safety	NCT01968109
Cervical cancer	PD-1/KIR	Phase 1/safety	NCT01714739

4.3.3 Immunotherapy for Endometrial Cancer

Immunotherapy for endometrial cancer is not popular because the prognosis for a patient with this disease is much better than it is for a patient with ovarian cancer, and surgery can cure the patient in a majority of cases. However, it is likely that in specific cases of endometrial cancer, immunotherapy may be very effective. As mentioned above, for colon cancer, pembrolizumab has been shown to be more effective for patients with mismatch repair deficiency compared to those without [22]. Considering that approximately 20–30% of endometrial cancers have a mismatch repair deficiency phenotype, these specific patients could be good candidates for immune checkpoint inhibition, thus enabling precision treatment according to the characteristics of the tumor.

4.3.4 Immunotherapy for Cervical/Vulvar Cancer

Needless to say, preventative immunization for HPV is showing great success as the first ever vaccine-based cancer prevention strategy. However, application of the cancer vaccine to already developed cervical cancers is still underway. Unlike the

preventative HPV vaccine, the main targets of the therapeutic vaccine are the HPV E6 and E7 proteins, which are usually integrated in the cancer genome and expressed in cancer cells [39]. Vaccinia virus-mediated vaccination of the E6/E7 proteins of HPV 16 and 18 was used to treat intraepithelial neoplasia, and the lesions disappeared in 90% of patients [40].

A synthetic long peptide vaccine of HPV16 E6 and E7 has reportedly shown a complete clinical response in 47% of patients with VIN III [41].

4.4 Conclusions and Future Directions

4.4.1 Is Immunotherapy Really Promising?

Although immunotherapy for cancer has long been viewed as a promising modality, and a number of clinical trials using various methodologies have been conducted, its clinical benefit was not apparent for most solid tumors, including gynecologic cancers, until the recent development of immune checkpoint inhibitors. The blockage of immune checkpoints, especially the PD-1/PD-L1 signal, has been shown to be very effective in some cancers, including ovarian cancer, which is obviously promising. Nevertheless, this strategy is not effective for all patients; the response rates differ somewhat from cancer to cancer. Considering that the cost of these drugs is extraordinarily high, we cannot say that immunotherapy is promising from an economic and a social perspective until we are able to distinguish which patients will benefit from it. Precision medicine is expected to reduce the cost of immunotherapy significantly if it is successfully introduced; thus, precision medicine is key to the success of immunotherapy.

Despite the many problems to be addressed, the recent development of new cancer immunotherapies is obviously an epoch-making breakthrough for future cancer medicine. It finally shows that immunotherapy is effective, and actually very effective, at least for a fraction of cancer patients, so that it can now be practically applied to clinical cases.

4.4.2 Problems to Be Solved

Considering that tumor immunology is so complicated, further exploration of basic tumor immunity can lead to new, more effective, and personalized immunotherapies. To this end, there are several issues to be solved. First, a more precise diagnosis of the immune status of each cancer patient should be established. The recent development of comprehensive analyses of genomes, exomes, and proteomes could be applied to the immunology field. In fact, comprehensive analysis of the immunogenic epitope, called immunome analysis, is being developed [20, 42, 43]. The diagnosis of individual immune status should be conducted from both the tumor and the host side because their mutual interaction is the essence of tumor immunity.

Table 4.2 Personalized immune therapy

		Systemic immunity	
		Strong	Weak
Local immunity	Healthy	No need for immune therapy	Active immunization
	Impaired	Anti-PD-1	Combination

Second, identifying the best combination strategy of immunotherapy and conventional therapy, especially chemotherapy, is important. We reported the effect of chemotherapy on the tumor immune microenvironment of ovarian cancer [44]. The treatment of ovarian cancer cell lines with various chemotherapeutic agents induced the expression of the MHC class I antigen, which may facilitate tumor cell recognition by the immune system. However, these chemo-drugs augmented PD-L1 expression at the same time, indicating that chemotherapy induces both immunogenic and immune-inhibitory effects in ovarian cancer. A combination of chemotherapy with an anti-PD-1 antibody led to a significantly prolonged survival rate in a mouse experiment. Likewise, we have shown that INF-γ, which has been viewed as an antitumor cytokine, actually has pro-immune and anti-immune effects, the latter of which is caused by PD-L1 expression [17, 34, 45, 46].

4.4.3 Precision Immunotherapy in the Future

In the near future, we should personalize cancer immunotherapy according to the immune status of the individuals (Table 4.2). There is no need for immunotherapy for those who have both strong systemic and local immunity. However, if a patient does not acquire sufficient immunogenicity from the tumor, then active immunization may be necessary. If a patient has sufficient systemic immunity but suffers from an impaired local immune environment, an immune checkpoint inhibitor may be a good treatment option. Finally, if a patient has both weak systemic and local immunity, simultaneous immune activation and immune checkpoint inhibition may be necessary. Thus, future immunotherapy should be personalized according to the precise immune diagnosis.

References

1. Zamarin D, Jazaeri AA. Leveraging immunotherapy for the treatment of gynecologic cancers in the era of precision medicine. Gynecol Oncol. 2016;141(1):86–94.
2. Hamanishi J, Mandai M, Matsumura N, Abiko K, Baba T, Konishi I. PD-1/PD-L1 blockade in cancer treatment: perspectives and issues. Int J Clin Oncol. 2016;21(3):462–73.
3. Brahmer JR, Drake CG, Wollner I, Powderly JD, Picus J, Sharfman WH, Stankevich E, Pons A, Salay TM, McMiller TL, Gilson MM, Wang C, Selby M, Taube JM, Anders R, Chen L, Korman AJ, Pardoll DM, Lowy I, Topalian SL. Phase I study of single-agent anti-programmed death-1 (MDX-1106) in refractory solid tumors: safety, clinical activity, pharmacodynamics, and immunologic correlates. J Clin Oncol. 2010;28(19):3167–75.

4. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, Powderly JD, Carvajal RD, Sosman JA, Atkins MB, Leming PD, Spigel DR, Antonia SJ, Horn L, Drake CG, Pardoll DM, Chen L, Sharfman WH, Anders RA, Taube JM, McMiller TL, Xu H, Korman AJ, Jure-Kunkel M, Agrawal S, McDonald D, Kollia GD, Gupta A, Wigginton JM, Sznol M. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med. 2012;366(26):2443–54.
5. Robert C, Long GV, Brady B, Dutriaux C, Maio M, Mortier L, Hassel JC, Rutkowski P, McNeil C, Kalinka-Warzocha E, Savage KJ, Hernberg MM, Lebbé C, Charles J, Mihalcioiu C, Chiarion-Sileni V, Mauch C, Cognetti F, Arance A, Schmidt H, Schadendorf D, Gogas H, Lundgren-Eriksson L, Horak C, Sharkey B, Waxman IM, Atkinson V, Ascierto PA. Nivolumab in previously untreated melanoma without BRAF mutation. N Engl J Med. 2015;372(4):320–30.
6. Weber JS, D'Angelo SP, Minor D, Hodi FS, Gutzmer R, Neyns B, Hoeller C, Khushalani NI, Miller Jr WH, Lao CD, Linette GP, Thomas L, Lorigan P, Grossmann KF, Hassel JC, Maio M, Sznol M, Ascierto PA, Mohr P, Chmielowski B, Bryce A, Svane IM, Grob JJ, Krackhardt AM, Horak C, Lambert A, Yang AS, Larkin J. Nivolumab versus chemotherapy in patients with advanced melanoma who progressed after anti-CTLA-4 treatment (CheckMate 037): a randomised, controlled, open-label, phase 3 trial. Lancet Oncol. 2015;16(4):375–84.
7. Robert C, Schachter J, Long GV, Arance A, Grob JJ, Mortier L, Daud A, Carlino MS, McNeil C, Lotem M, Larkin J, Lorigan P, Neyns B, Blank CU, Hamid O, Mateus C, Shapira-Frommer R, Kosh M, Zhou H, Ibrahim N, Ebbinghaus S, Ribas A, KEYNOTE-006 Investigators. Pembrolizumab versus ipilimumab in advanced melanoma. N Engl J Med. 2015;372(26):2521–32.
8. Brahmer J, Reckamp KL, Baas P, Crinò L, Eberhardt WE, Poddubskaya E, Antonia S, Pluzanski A, Vokes EE, Holgado E, Waterhouse D, Ready N, Gainor J, Arén Frontera O, Havel L, Steins M, Garassino MC, Aerts JG, Domine M, Paz-Ares L, Reck M, Baudelet C, Harbison CT, Lestini B, Spigel DR. Nivolumab versus docetaxel in advanced squamous-cell non-small-cell lung cancer. N Engl J Med. 2015;373(2):123–35.
9. Borghaei H, Paz-Ares L, Horn L, Spigel DR, Steins M, Ready NE, Chow LQ, Vokes EE, Felip E, Holgado E, Barlesi F, Kohlhäufl M, Arrieta O, Burgio MA, Fayette J, Lena H, Poddubskaya E, Gerber DE, Gettinger SN, Rudin CM, Rizvi N, Crinò L, Blumenschein Jr GR, Antonia SJ, Dorange C, Harbison CT, Graf Finckenstein F, Brahmer JR. Nivolumab versus docetaxel in advanced nonsquamous non-small-cell lung cancer. N Engl J Med. 2015;373(17):1627–39.
10. Brahmer JR, Tykodi SS, Chow LQ, Hwu WJ, Topalian SL, Hwu P, Drake CG, Camacho LH, Kauh J, Odunsi K, Pitot HC, Hamid O, Bhatia S, Martins R, Eaton K, Chen S, Salay TM, Alaparthy S, Grosso JF, Korman AJ, Parker SM, Agrawal S, Goldberg SM, Pardoll DM, Gupta A, Wigginton JM. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. N Engl J Med. 2012;366(26):2455–65.
11. Herbst RS, Baas P, Kim DW, Felip E, Pérez-Gracia JL, Han JY, Molina J, Kim JH, Arvis CD, Ahn MJ, Majem M, Fidler MJ, de Castro Jr G, Garrido M, Lubiniecki GM, Shentu Y, Im E, Dolled-Filhart M, Garon EB. Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): a randomised controlled trial. Lancet. 2016;387(10027):1540–50.
12. Hamanishi J, Mandai M, Konishi I. Immune checkpoint inhibition in ovarian cancer. Int Immunol. 2016;28(7):339–48.
13. Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, Gonzalez R, Robert C, Schadendorf D, Hassel JC, Akerley W, van den Eertwegh AJ, Lutzky J, Lorigan P, Vaubel JM, Linette GP, Hogg D, Ottensmeier CH, Lebbé C, Peschel C, Quirt I, Clark JI, Wolchok JD, Weber JS, Tian J, Yellin MJ, Nichol GM, Hoos A, Urba WJ. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med. 2010;363(8):711–23.
14. Wolchok JD, Kluger H, Callahan MK, Postow MA, Rizvi NA, Lesokhin AM, Segal NH, Ariyan CE, Gordon RA, Reed K, Burke MM, Caldwell A, Kronenberg SA, Agunwamba BU, Zhang X, Lowy I, Inzunza HD, Feely W, Horak CE, Hong Q, Korman AJ, Wigginton JM, Gupta A, Sznol M. Nivolumab plus ipilimumab in advanced melanoma. N Engl J Med. 2013;369(2):122–33.

15. Postow MA, Chesney J, Pavlick AC, Robert C, Grossmann K, McDermott D, Linette GP, Meyer N, Giguere JK, Agarwala SS, Shaheen M, Ernstoff MS, Minor D, Salama AK, Taylor M, Ott PA, Rollin LM, Horak C, Gagnier P, Wolchok JD, Hodi FS. Nivolumab and ipilimumab versus ipilimumab in untreated melanoma. N Engl J Med. 2015;372(21):2006–17.
16. Hamanishi J, Mandai M, Iwasaki M, Okazaki T, Tanaka Y, Yamaguchi K, Higuchi T, Yagi H, Takakura K, Minato N, Honjo T, Fujii S. Programmed cell death 1 ligand 1 and tumor-infiltrating CD8+ T lymphocytes are prognostic factors of human ovarian cancer. Proc Natl Acad Sci U S A. 2007;104(9):3360–5.
17. Mandai M, Hamanishi J, Abiko K, Matsumura N, Baba T, Konishi I. Anti-PD-L1/PD-1 immune therapies in ovarian cancer: basic mechanism and future clinical application. Int J Clin Oncol. 2016;21(3):456–61.
18. Wu P, Wu D, Li L, Chai Y, Huang J. PD-L1 and survival in solid tumors: a meta-analysis. PLoS One. 2015;10(6):e0131403.
19. Mandal R, Chan TA. Personalized oncology meets immunology: the path toward precision immunotherapy. Cancer Discov. 2016;6(7):703–13.
20. Gubin MM, Artyomov MN, Mardis ER, Schreiber RD. Tumor neoantigens: building a framework for personalized cancer immunotherapy. J Clin Invest. 2015;125(9):3413–21.
21. Martin SD, Coukos G, Holt RA, Nelson BH. Targeting the undruggable: immunotherapy meets personalized oncology in the genomic era. Ann Oncol. 2015;26(12):2367–74.
22. Le DT, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, Skora AD, Luber BS, Azad NS, Laheru D, Biedrzycki B, Donehower RC, Zaheer A, Fisher GA, Crocenzi TS, Lee JJ, Duffy SM, Goldberg RM, de la Chapelle A, Koshiji M, Bhaijee F, Huebner T, Hruban RH, Wood LD, Cuka N, Pardoll DM, Papadopoulos N, Kinzler KW, Zhou S, Cornish TC, Taube JM, Anders RA, Eshleman JR, Vogelstein B, Diaz Jr LA. PD-1 blockade in tumors with mismatch-repair deficiency. N Engl J Med. 2015;372(26):2509–20.
23. Rizvi NA, Hellmann MD, Snyder A, Kvistborg P, Makarov V, Havel JJ, Lee W, Yuan J, Wong P, Ho TS, Miller ML, Rekhtman N, Moreira AL, Ibrahim F, Bruggeman C, Gasmi B, Zappasodi R, Maeda Y, Sander C, Garon EB, Merghoub T, Wolchok JD, Schumacher TN, Chan TA. Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. Science. 2015;348(6230):124–8.
24. McGranahan N, Furness AJ, Rosenthal R, Ramskov S, Lyngaa R, Saini SK, Jamal-Hanjani M, Wilson GA, Birkbak NJ, Hiley CT, Watkins TB, Shafi S, Murugaesu N, Mitter R, Akarca AU, Linares J, Marafioti T, Henry JY, Van Allen EM, Miao D, Schilling B, Schadendorf D, Garraway LA, Makarov V, Rizvi NA, Snyder A, Hellmann MD, Merghoub T, Wolchok JD, Shukla SA, Wu CJ, Peggs KS, Chan TA, Hadrup SR, Quezada SA, Swanton C. Clonal neoantigens elicit T cell immunoreactivity and sensitivity to immune checkpoint blockade. Science. 2016;351(6280):1463–9.
25. Snyder A, Makarov V, Merghoub T, Yuan J, Zaretsky JM, Desrichard A, Walsh LA, Postow MA, Wong P, Ho TS, Hollmann TJ, Bruggeman C, Kannan K, Li Y, Elipenahli C, Liu C, Harbison CT, Wang L, Ribas A, Wolchok JD, Chan TA. Genetic basis for clinical response to CTLA-4 blockade in melanoma. N Engl J Med. 2014;371(23):2189–99.
26. Fu J, Malm IJ, Kadayakkara DK, Levitsky H, Pardoll D, Kim YJ. Preclinical evidence that PD1 blockade cooperates with cancer vaccine TEGVAX to elicit regression of established tumors. Cancer Res. 2014;74(15):4042–52.
27. Battaglia S, Muhitch JB. Unmasking targets of antitumor immunity via high-throughput antigen profiling. Curr Opin Biotechnol. 2016;42:92–7.
28. Hirayama M, Nishimura Y. The present status and future prospects of peptide-based cancer vaccines. Int Immunol. 2016;28:319–28.
29. Spear TT, Nagato K, Nishimura MI. Strategies to genetically engineer T cells for cancer immunotherapy. Cancer Immunol Immunother. 2016;65(6):631–49.
30. Berek JS, Taylor PT, Gordon A, Cunningham MJ, Finkler N, Orr Jr J, Rivkin S, Schultes BC, Whiteside TL, Nicodemus CF. Randomized, placebo-controlled study of oregovomab for consolidation of clinical remission in patients with advanced ovarian cancer. J Clin Oncol. 2004;22(17):3507–16.

31. Berek J, Taylor P, McGuire W, Smith LM, Schultes B, Nicodemus CF. Oregovomab maintenance monoimmunotherapy does not improve outcomes in advanced ovarian cancer. J Clin Oncol. 2009;27(3):418–25.
32. Armstrong DK, White AJ, Weil SC, Phillips M, Coleman RL. Farletuzumab (a monoclonal antibody against folate receptor alpha) in relapsed platinum-sensitive ovarian cancer. Gynecol Oncol. 2013;129(3):452–8.
33. Alberts DS, Marth C, Alvarez RD, Johnson G, Bidzinski M, Kardatzke DR, Bradford WZ, Loutit J, Kirn DH, Clouser MC, Markman M, GRACES Clinical Trial Consortium. Randomized phase 3 trial of interferon gamma-1b plus standard carboplatin/paclitaxel versus carboplatin/paclitaxel alone for first-line treatment of advanced ovarian and primary peritoneal carcinomas: results from a prospectively designed analysis of progression-free survival. Gynecol Oncol. 2008;109(2):174–81.
34. Mandai M, Hamanishi J, Abiko K, Matsumura N, Baba T, Konishi I. Dual faces of IFNγ in cancer progression: a role of PD-L1 induction in the determination of pro- and antitumor immunity. Clin Cancer Res. 2016;22(10):2329–34.
35. Hamanishi J, Mandai M, Ikeda T, Minami M, Kawaguchi A, Murayama T, Kanai M, Mori Y, Matsumoto S, Chikuma S, Matsumura N, Abiko K, Baba T, Yamaguchi K, Ueda A, Hosoe Y, Morita S, Yokode M, Shimizu A, Honjo T, Konishi I. Safety and antitumor activity of anti-PD-1 antibody, nivolumab, in patients with platinum-resistant ovarian cancer. J Clin Oncol. 2015;33(34):4015–22.
36. Varga A, Piha-Paul SA, Ott PA. Antitumor activity and safety of pembrolizumab in patients with PD-L1 positive advanced ovarian cancer: interim results from a phase Ib study. J Clin Oncol. 2015;33(Suppl.) Abstract no. 5510
37. Disis ML, Patel MR, Pant S. Avelumab (MSB0010718C), an anti-PD-L1 antibody, in patients with previously treated, recurrent or refractory ovarian cancer: a phase Ib, open-label expansion trial. J Clin Oncol. 2015;33(Suppl.) Abstract no. 5509
38. Hodi FS, Butler M, Oble DA, Seiden MV, Haluska FG, Kruse A, Macrae S, Nelson M, Canning C, Lowy I, Korman A, Lautz D, Russell S, Jaklitsch MT, Ramaiya N, Chen TC, Neuberg D, Allison JP, Mihm MC, Dranoff G. Immunologic and clinical effects of antibody blockade of cytotoxic T lymphocyte-associated antigen 4 in previously vaccinated cancer patients. Proc Natl Acad Sci U S A. 2008;105(8):3005–10.
39. zur Hausen H. Immortalization of human cells and their malignant conversion by high risk human papillomavirus genotypes. Semin Cancer Biol. 1999;9(6):405–11.
40. Rosales R, López-Contreras M, Rosales C, Magallanes-Molina JR, Gonzalez-Vergara R, Arroyo-Cazarez JM, Ricardez-Arenas A, Del Follo-Valencia A, Padilla-Arriaga S, Guerrero MV, Pirez MA, Arellano-Fiore C, Villarreal F. Regression of human papillomavirus intraepithelial lesions is induced by MVA E2 therapeutic vaccine. Hum Gene Ther. 2014;25(12):1035–49.
41. Kenter GG, Welters MJ, Valentijn AR, Lowik MJ, Berends-van der Meer DM, Vloon AP, Essahsah F, Fathers LM, Offringa R, Drijfhout JW, Wafelman AR, Oostendorp J, Fleuren GJ, van der Burg SH, Melief CJ. Vaccination against HPV-16 oncoproteins for vulvar intraepithelial neoplasia. N Engl J Med. 2009;361(19):1838–47.
42. Gubin MM, Zhang X, Schuster H, Caron E, Ward JP, Noguchi T, Ivanova Y, Hundal J, Arthur CD, Krebber WJ, Mulder GE, Toebes M, Vesely MD, Lam SS, Korman AJ, Allison JP, Freeman GJ, Sharpe AH, Pearce EL, Schumacher TN, Aebersold R, Rammensee HG, Melief CJ, Mardis ER, Gillanders WE, Artyomov MN, Schreiber RD. Checkpoint blockade cancer immunotherapy targets tumour-specific mutant antigens. Nature. 2014;515(7528):577–81.
43. Herbst RS, Soria JC, Kowanetz M, Fine GD, Hamid O, Gordon MS, Sosman JA, McDermott DF, Powderly JD, Gettinger SN, Kohrt HE, Horn L, Lawrence DP, Rost S, Leabman M, Xiao Y, Mokatrin A, Koeppen H, Hegde PS, Mellman I, Chen DS, Hodi FS. Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. Nature. 2014;515(7528):563–7.

44. Peng J, Hamanishi J, Matsumura N, Abiko K, Murat K, Baba T, Yamaguchi K, Horikawa N, Hosoe Y, Murphy SK, Konishi I, Mandai M. Chemotherapy induces programmed cell death-ligand 1 overexpression via the nuclear factor-κB to foster an immunosuppressive tumor microenvironment in ovarian cancer. Cancer Res. 2015;75(23):5034–45.
45. Abiko K, Mandai M, Hamanishi J, Yoshioka Y, Matsumura N, Baba T, Yamaguchi K, Murakami R, Yamamoto A, Kharma B, Kosaka K, Konishi I. PD-L1 on tumor cells is induced in ascites and promotes peritoneal dissemination of ovarian cancer through CTL dysfunction. Clin Cancer Res. 2013;19(6):1363–74.
46. Abiko K, Matsumura N, Hamanishi J, Horikawa N, Murakami R, Yamaguchi K, Yoshioka Y, Baba T, Konishi I, Mandai M. IFN-γ from lymphocytes induces PD-L1 expression and promotes progression of ovarian cancer. Br J Cancer. 2015;112(9):1501–9.

Prevention of Cervical Cancer: Era of HPV Testing and Vaccination

5

Kazuhiko Ino

Abstract

The incidence and mortality of cervical cancer in young women of reproductive ages have recently increased, which is a serious issue worldwide. This chapter will focus on the prevention of cervical cancer with HPV testing and vaccination. It is recognized that strategies for preventing cervical cancer consist of two major steps: preventing infection of oncogenic human papillomavirus (HPV)-16 and HPV-18 by HPV vaccination and secondary prevention by screening using HPV testing and/or cytology. Current cervical cancer screening strategies using cytology combined with HPV testing have been successfully introduced, with shifting from cytology alone to cytology plus HPV cotesting and now to a new paradigm in which HPV testing alone may become a primary screening tool. HPV vaccination is a "primary prevention" tool, and both the bivalent and quadrivalent HPV vaccines have excellent safety and efficacy profiles. Recently, a 9-valent vaccine, targeted against HPV-6, HPV-11, HPV-16, HPV-18, HPV-31, HPV-33, HPV-45, HPV-52, and HPV-58, has been developed, which may possibly protect against over 80% of invasive cervical cancers. Further evidence on the 9-valent HPV vaccine should be accumulated worldwide, and its application is expected as a new strategy. Finally, the WHO recognizes the prevention of cervical cancer and other HPV-related diseases as global public health problems and strongly recommends the HPV vaccination programs. Both HPV vaccination and cancer screening tests are indispensable for cervical cancer prevention.

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The complete eradication of this malignant disease in the world will be realized in the near future by the further development and widespread application of these two strategies.

Keywords
Cervical cancer • Cervical intraepithelial neoplasia (CIN) • Cytology • Human papillomavirus (HPV) • HPV testing • HPV vaccine

5.1 Introduction

Cervical cancer is the fourth most common cancer in women worldwide, with approximately 500,000 estimated new cases annually and nearly 300,000 estimated related deaths in the world [1]. In Japan, over 11,000 cases of cervical cancer are newly diagnosed every year, and more than 3000 women die of the disease, which causes the second greatest number of deaths among gynecologic malignancies. Furthermore, over the last two decades, there has been an increasing trend in cervical cancer mortality among young Japanese women below the age of 50 years [2]. In fact, the incidence rate of cervical cancer in those of younger ages such as in their 20s and 30s has recently increased in Japan, and the mortality of these patients has also increased in parallel. Such situations associated with cervical cancer in young women of reproductive (childbearing or child-rearing) ages are serious issues to share and solve, drawing social attention not only in Japan but also in other developed and developing countries.

Invasive cervical cancer is generally treated by surgery or radiotherapy with/without chemotherapy. While concurrent chemoradiotherapy (CCRT) has been frequently selected for FIGO stage IIB–IVB advanced disease, most patients with stage IA2 through IIB disease are treated with radical hysterectomy in Japan [3]. Despite the generally good prognosis of patients with FIGO stage I–II cervical cancer, significant numbers of patients develop recurrence, and the prognosis of patients with recurrence, metastatic disease, or advanced disease is still poor. Furthermore, most patients who undergo radical surgery or CCRT are likely to suffer from undesirable treatment-related adverse symptoms and/or lose their fertility due to hysterectomy, ovariectomy, or irradiation to the reproductive organs, which results in a lowered quality of life (QOL) even if their disease is cured. In addition, over 9000 patients with precancerous lesions such as cervical intraepithelial neoplasia (CIN)2/3 or microinvasive carcinoma (FIGO stage IA1) are treated with cervical conization every year in Japan, resulting in the possibility of complications on subsequent pregnancy, such as an increased risk of preterm birth, as well as a marked psychological burden for affected women even if their fertility is preserved.

Considering these current situations, to increase the survival rate of cervical cancer patients and improve the posttreatment QOL as well as to protect the health of young women, fundamental and strategic prevention of cervical cancer is an important and continuing global challenge, which could lead to the eradication of this

disease worldwide in the future. This chapter will focus on the prevention of cervical cancer and discuss recent advances, current issues, and future perspectives on HPV testing/cytology and HPV vaccination.

5.2 Prevention of Cervical Cancer: Primary and Secondary Prevention

It is generally recognized based on large-scale global evidence that strategies for preventing cervical cancer consist of two major steps (Fig. 5.1). Primary prevention is the prevention of infection by oncogenic human papillomavirus (HPV), which is directly involved in cervical carcinogenesis and causes nearly all cervical cancers, by HPV vaccination of adolescent girls aged 9–14 years. In contrast, secondary prevention is the early detection of persistent HPV infection into cervical epithelial cells and subsequent precancerous lesions by screening using HPV testing and/or the Papanicolaou (PAP) test (cytology) in women older than 20 years old. Both primary and secondary prevention strategies are indispensable to prevent invasive cervical cancer effectively, reaching a global consensus.

Two highly effective and safe HPV vaccines are available. HPV vaccination is now performed in over 65 countries in the world as the national governmental programs, and its active introduction is strongly recommended by the World Health Organization (WHO) [4] not only in developed countries but also in developing or resource-limited countries where the availability of cytology/HPV testing is limited. More than 200 million HPV vaccinations have been performed worldwide with no significant safety issues, and its effectiveness has been confirmed in countries with high vaccination rates. In contrast, cervical cancer screening systems using HPV testing combined with the PAP test (cytology) have started in some developed countries, but their criteria and methodologies are still diverse among the countries, and have yet to be established worldwide, although their effectiveness has been confirmed.

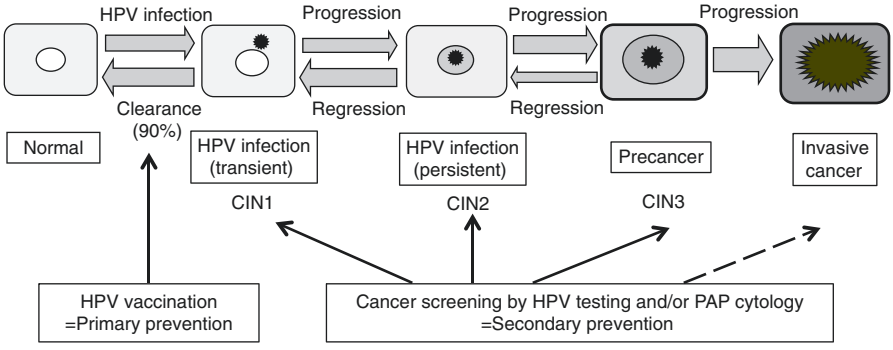

Fig. 5.1 HPV infection and cervical carcinogenesis: role of primary and secondary prevention against progression to invasive cancer

5.3 HPV Infection and Cervical Carcinogenesis

HPV has many types, and its infection is related to various diseases in humans. About 15 types of HPV (HPV-16, HPV-18, HPV-31, HPV-33, HPV-35, HPV-39, HPV-45, HPV-51, HPV-52, HPV-56, HPV-58, HPV-59, HPV-68, HPV-73, and HPV-82) are oncogenic and defined as high-risk HPV, which can cause cervical cancer as well as other HPV-related cancers such as of the vulva, vagina, penis, anus, and oropharynx. HPV is transmitted by sexual contact. HPV infections are common and generally asymptomatic, and it is estimated that 50–80% of healthy sexually active individuals are at risk of HPV infection within their lifetime. However, approximately 90% of women infected initially (incidentally) by HPV may eliminate the infection from their cervical epithelial cells within 2 years, and most women with this transient infection never develop cancer. In contrast, in the remaining 10% of women, persistent HPV infection may occur, and some of those could develop high-grade precancerous lesions, and some may subsequently develop invasive cancer (Fig. 5.1).

Nearly all patients with invasive cervical cancer show evidence of HPV infection. HPV-16 and HPV-18 are the most oncogenic, and these two types are responsible for about 70% of cervical squamous cell carcinomas worldwide. In Japan, HPV-16/HPV-18 were detected in 24% of CIN1, 36% of CIN2/3, and 67% of invasive cervical cancer [5]. More importantly, the detection rate of HPV-16/HPV-18 in invasive cervical cancers varies according to the age and is the highest in patients aged 20–29 years (90.0%) [5]. The next most frequently detected HPV types in cervical cancer are HPV31, HPV-33, HPV-35, HPV-45, HPV-52, and HPV-58. HPV infection with these high-risk types is necessary for the development of cervical cancer, but other factors, such as smoking, immune suppression, and long-term oral contraceptive use, may increase the risk.

Invasive cervical cancer results from the progression of precancerous lesions named CIN or squamous intraepithelial lesion (SIL). CIN is histologically graded into CIN1, CIN2, and CIN3, although most CIN1 and some CIN2 regress. The results of a PAP test are presented according to the Bethesda system, based on cytologic findings: atypical squamous cells of undetermined significance (ASC-US) and low-grade squamous intraepithelial lesions (LSIL) show transient HPV infection (CIN1), while high-grade squamous intraepithelial lesions (HSIL) show persistent HPV infection with cellular atypia (CIN2–3) (Fig. 5.1). In fact, over 70% of ASC-US or CIN1 lesions regress, while 10–30% CIN3 lesions progress to invasive cancer. After screening using cytology, women with abnormal results (ASC-US, LSIL, HSIL, AGC, or more) need colposcopy and biopsy to determine the histological diagnosis and subsequent management/treatment.

Usually, invasive cancer develops from CIN slowly over some years or longer. This long natural history from HPV infection to the development of cervical cancer provides the opportunity for screening to detect this process in precancerous stages and allows the treatment of preinvasive lesions before they become cancerous, which could prevent invasive cancer effectively.

5.4 Limited Effectiveness of Cytology Screening

Historically, cervical cancer screening was conducted using the PAP test (cytology) alone until HPV testing became available. Programs since the 1960s using annual screening with Papanicolaou-stained cervical cytology smears have been successful, and actually, it has contributed to a significant decrease in the mortality rate due to cervical cancer. However, it is now difficult to more effectively reduce the number of deaths from cervical cancer only through this screening measure, mainly due to its relatively lower sensitivity (the percentage of "true-positive" cases that are detected by the screening test). Previous studies showed that the sensitivity for detecting high-grade lesions on a single conventional PAP test is approximately 55–80% [6], and failures to prevent invasive cervical cancer can be attributed to false-negative PAP smears as well as to poor follow-up of abnormal results [7]. False-negative results occasionally occur, especially in pregnant women or in patients with glandular abnormality or precancerous/cancerous lesions of adenocarcinoma. Additionally, in Japan, the proportion of those undergoing such examinations is only 30–40% of targeted women >20 years old, which is lower than those in Western countries, at approximately 70–80%. Recently, the liquid-based cytology technique was developed to improve the sensitivity of screening. Up to now, there has been no evidence that liquid-based cytology significantly reduces the number of deaths compared with the conventional PAP smear test, although there is actually one advantage that the HPV test can be simultaneously conducted on the same preparation for the examination of liquid-based cytology.

5.5 HPV Testing

In consideration of the limitations of cytology, efforts have focused on enhancing the sensitivity of screening to reduce false-negative results and developing new molecular/virological tests to detect high-risk HPV as well as to reduce unnecessary colposcopic examinations. Since 2000, various HPV-DNA tests have been developed, and now some are commercially available for the detection of HPV in cervical specimens [8]. Most of these tests generate a pooled result ("high-risk HPV-positive" or "high-risk HPV-negative") to detect nucleic acids of the 12 HPV types altogether (HPV-16, HPV-18, HPV-31, HPV-33, HPV-35, HPV-39, HPV-45, HPV-51, HPV-52, HPV-56, HPV-58, and HPV-59; some tests also detect HPV-66 and HPV-68). In contrast, HPV genotyping tests that distinguish individual HPV types are also available. HPV infections are particularly common in young women, and the majority clear their infection within 2 years; therefore, the challenge of incorporating HPV testing into cervical screening programs is to balance increasing sensitivity to detect CIN2/3 and minimizing overdiagnosis/treatment of women with transient HPV infections and cervical abnormality that may regress.

Actually, previous studies demonstrated that, compared with cytology, HPV-DNA testing was more sensitive for identifying women who have CIN2/3, with

sensitivities of 84–97%, and that the combination of HPV testing and cytology led to an almost 100% sensitivity. In contrast, it has been noted that HPV-DNA testing generally has a lower specificity compared with cytology. Among women ≥30 years old, cytology had a specificity of 97% compared with 94% for HPV testing. The specificity of HPV-DNA testing is likely be lower among women younger than 30 years old, who have more transient HPV infection.

Now, HPV testing has been approved for use in the following: (1) as a second test (triage) following a cytology result of ASC-US; (2) for primary screening combined with the PAP test for women aged 30 years or older or primary screening by the HPV test alone may be considered; and (3) HPV genotyping tests that distinguish highly oncogenic HPV types, especially HPV-16 and HPV-18, for the further triage of women with a positive pooled result or for risk stratification in patients with CIN1/2.

A recent major clinical trial, "ATHENA HPV Study," demonstrated that incorporating screening with HPV and triage of HPV-positive women by a combination of genotyping for HPV-16/HPV-18 and cytology provided a good balance between maximizing sensitivity (benefit) and specificity by limiting the number of colposcopies (potential harm) [9]. Furthermore, the study showed that primary HPV screening in women ≥25 years is as effective as a hybrid screening strategy that uses cytology if 25–29 years and cotesting if ≥30 years [10]. Further analysis of HPV genotyping from the ATHENA trial supported the identification of HPV-16 in primary screening for all women and demonstrated that the identification of HPV-18 is also warranted with a significant contribution to adenocarcinoma in situ (AIS) and cancer [11].

5.6 Current Cervical Cancer Screening Guidelines Using HPV Test and PAP Cytology

Table 5.1 demonstrates the current cervical cancer screening guidelines in the USA [8, 12]. All normal-risk women should begin cervical cancer screening at age 21. Between the ages of 21 and 29 years, women should be screened using cytology every 3 years. HPV testing is used following an abnormal cytology result. Primary HPV testing can be considered starting at age 25 every 3 years. For women aged 30–65 years, screening can be done using cytology alone every 3 years or HPV cotesting (cytology plus simultaneous HPV test) every 5 years. The guidelines support the discontinuation of screening in women older than 65 years who have three consecutive normal cytology results or two consecutive negative cotest results within the previous 10 years, with the most recent test performed within the past 5 years.

In Japan, the screening system using cytology in combination with the HPV test has not yet been established and is still under investigation by clinical trials. At this time, the guideline proposed by the Japan Association of Obstetricians and Gynecologists in 2012 (Fig. 5.2) is applied for cancer screening targeting women aged 30 years or older in some local areas or cities. According to this guideline,

Table 5.1 Current cervical cancer screening guidelines (2012) in the U.S. [8]

Age (years)	Screening recommendations
21	Initiation of screening
21–29	Cytology every 3 years, or primary HPV testing can be considered starting at age 25 every 3 years; if primary HPV testing is positive, test for HPV16 and HPV18 and refer to colposcopy if positive, or cotesting if negative
30–65	Cytology every 3 years and HPV testing for triage of ASC-US, or HPV cotesting every 5 years and test for HPV16 and HPV18 if normal cytology but HPV-positive, or primary HPV screening every 3 years as indicated above
Discontinuation of screening	Women aged >65 who have 3 or more consecutive negative cytology tests or two consecutive negative cotests within 10 years with the most recent test performed within 5 years; women of any age who have a total hysterectomy and have no history of cervical cancer or precancer should not be screened

From the American Cancer Society (ACS), American Society for Colposcopy and Cervical Pathology (ASCCP), American Society for Clinical Pathology (ASCP), U.S. Preventive Services Task Force (USPSTF), and American College of Obstetricians and Gynecologists (ACOG) with interim guidance from the Society of Gynecologic Oncology and ACOG

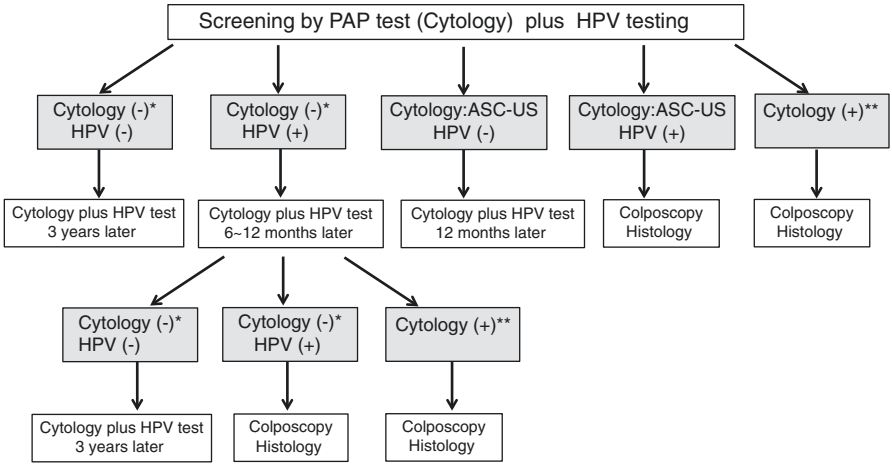

Fig. 5.2 Cervical cancer screening system by PAP test (cytology) in combination with HPV-DNA test in Japan: recommended in 2012 by the Japan Association of Obstetricians and Gynecologists. *Cytology (−): NILM. **Cytology (+): LSIL, HSIL, ASC-H, AGC, or more

women who are both cytology-negative and HPV-negative can be screened 3 years later. Women who are cytology-negative, but HPV-positive, are recommended to undergo cotesting again 6–12 months later. Women with cytology of ASC-US and HPV-positive or cytology of LSIL or more should undergo colposcopy and biopsy. Such studies are expected to establish the appropriate screening system in Japan.

5.7 HPV Vaccines

Two prophylactic vaccines are currently available in many countries worldwide for the primary prevention of cervical cancer and other HPV-related diseases [4, 13]. Both bivalent and quadrivalent vaccines are developed against two main oncogenic HPV genotypes, HPV-16 and HPV-18, responsible for 65–70% of invasive cervical cancer cases. The quadrivalent vaccine is also directed against low-oncogenic types, HPV-6 and HPV-11, that cause anogenital warts (condyloma). The quadrivalent vaccine was first licensed in 2006, followed by licensing of the bivalent vaccine in 2007. It is recommended that HPV vaccine should be administered before the onset of sexual activity (before the first exposure to HPV infection). Both vaccines are prepared from virus like particles that resemble HPV type-specific L1 protein, which contains no viral DNA and, therefore, is noninfectious. Immunologically, HPV vaccine can protect against HPV infecting cervical epithelial cells through humoral immunity mediated by neutralizing antibodies against HPV-16/HPV-18.

Up to now, over 65 countries have introduced HPV vaccine in their national immunization programs for girls aged 9–14 years and in some countries also for boys. Both vaccines are used according to the three-dose immunization schedule at 0, 1(2), and 6 months. After a three-dose schedule, both vaccines are highly immunogenic, and antibody titers remain high for at least 8 years or more. Recent reports have shown that two doses of HPV vaccine in girls aged 9–14 years are non-inferior to three doses in terms of immunogenicity, suggesting the possibility of introducing a two-dose immunization program to such younger girls [4].

5.8 Efficacy of HPV Vaccination and Latest Evidence of HPV Vaccine Benefits

Both vaccines have been evaluated in large Phase III pre-licensed studies, where they can protect against HPV-16/HPV-18 infections at almost 100% in vaccine recipients not already infected with HPV (HPV-naive) and demonstrate high efficacy against HPV-16- or HPV-18-associated precancerous (CIN2/3) lesions in such HPV-naive individuals [14, 15]. It was also observed that the quadrivalent vaccine significantly decreased genital warts.

Recently, many beneficial effects have been reported in several industrialized countries where national HPV vaccination programs had been introduced early since 2007–2008, such as Australia, the UK (England and Scotland), or Denmark, with a three-dose coverage rate of over 70% of the targeted population. In these countries, actually, HPV vaccination has led to marked reductions in the prevalence of vaccine-preventable HPV types, HPV-16 and HPV-18 (and HPV-6/HPV-11 if quadrivalent). Interestingly, this was observed not only in vaccinated women but also in unvaccinated women, suggesting a “herd-immunity effect” [16].

Furthermore, in these countries, there have been some reductions in the prevalence of other HPV types (HPV-31, HPV-33, and HPV-45) that are not specifically targeted by the vaccine, suggesting a "cross-protection effect" [17].

Consistent with such a marked decrease in the HPV infection rates in younger women or girls, HPV vaccination has shown a major impact on the incidence of high-grade cervical abnormalities. In fact, the incidence of CIN3 or AIS in vaccinated generations has decreased to less than 50% during 7–8 years following the introduction of a national HPV vaccination program [18–20]. These findings strongly suggest that the incidence of invasive cervical cancer in younger women must markedly decrease over the next several to 10 years, leading to a subsequent decrease in the mortality rate due to this disease in the near future.

5.9 Global Consensus on Safety of HPV Vaccine

The WHO Global Advisory Committee for Vaccine Safety (GACVS) has repeatedly reviewed the evidence on the safety of HPV vaccines and concluded that both HPV vaccines continue to have an excellent safety profile [4].

As a local adverse event, both vaccines are associated with relatively high rates of injection site reactions, particularly pain, but these are usually of short duration and resolve spontaneously. Systemic adverse events following immunization (AEFI), although it has not yet been confirmed whether they are related to vaccination, include pyrexia (fever), headache, dizziness, myalgia, arthralgia, and gastrointestinal symptoms (nausea, vomiting, abdominal pain). In a comparison of the bivalent and quadrivalent vaccines, systemic reactions were reported at comparable rates. Postvaccination syncope, possibly the vasovagal reflex, has been reported at relatively higher rates but can be minimized and its complications avoided with appropriate care.

There have been no clinically relevant differences reported between vaccinated and unvaccinated groups with regard to new-onset chronic disease, including autoimmune disease, neurological disorders, or immune-mediated diseases. A few case reports showed a link between vaccination and the onset of these chronic conditions; however, a well-conducted population-based study demonstrated no association between HPV vaccine and such conditions [4]. It was also confirmed that Guillain-Barré syndrome and acute disseminated encephalomyelitis (ADEM) after vaccination were within the expected range in a general population. In a large cohort study in Denmark and Sweden, there was no causal relationship between exposure to HPV vaccine and the incidence of autoimmune, neurological, or venous thromboembolic adverse events [21].

Recently, the European Medicines Agency (EMA) confirmed that evidence does not support a causal link between HPV vaccine and the development of two syndromes, complex regional pain syndrome (CRPS) and postural orthostatic tachycardia syndrome (POTS), in girls and young women aged 10–19 years [22].

5.10 Current Issues in Japan on Suspension of Recommendation of HPV Vaccination

Over 3 years have passed since June 2013 when the Ministry of Health, Labour, and Welfare (MHLW) of Japan suspended recommendations for HPV vaccination because of reported cases of suspected adverse events such as chronic pain and motor impairment postvaccination. The Investigative Committee of the MHLW thoroughly and repeatedly analyzed the data and concluded that various postvaccination symptoms including persistent pain or motor impairment are functional physical symptoms (functional somatic syndrome). They also showed that the incidence rate of such adverse events was very low: 176 cases, equivalent to 0.005% of all vaccine recipients (3,380,000) in Japan. Subsequent studies did not provide any scientific or epidemiologic evidence to confirm the causal relationship between these symptoms and HPV vaccine; nevertheless, the suspension of recommendations for vaccination has continued, consequently decreasing the vaccination rate to nearly 0% in Japan [23]. It is of marked concern that if the suspension of vaccine recommendations continues, young Japanese generations will be deprived of the benefits of vaccines for cancer prevention.

The Japanese MHLW in cooperation with the Japan Society of Obstetrics and Gynecology (JSOG) organized 85 cooperative medical institutions covering all areas in Japan to provide treatment for those suffering from any symptoms after HPV vaccination. Furthermore, "Guidelines for the management and treatment of symptoms that occur after HPV vaccine injection" was published in August 2015. Based on this situation, the JSOG published their declaration to demand the immediate resumption of recommendation for HPV vaccination in August 2015 [24]. Furthermore, the Expert Council on Promotion of Vaccination consisting of 15 Japanese academic associations including JSOG also published a statement for the promotion of HPV vaccination in April 2016.

GACVS (WHO) made the following additional comments in December 2015 on such Japanese situations [25]: "Review of clinical data by the national expert committee led to a conclusion that symptoms were not related to the vaccine, but it has not been possible to reach a consensus to resume HPV vaccination. As a result, young women in Japan are being left vulnerable to HPV-related cancers that otherwise could be prevented. Policy decisions based on weak evidence, leading to a lack of use of safe and effective vaccines, can result in real harm."

As is the case in Japan, public concern and incorrect rumors about adverse events as well as broadcasting them by "nonscientific" media may lead to strong resistance to increasing vaccine coverage. A thorough surveillance system of adverse events following vaccination is the most important, but it should be complemented by assessment of the real causal relationship of all suspected adverse events by scientific and epidemiologic analyses.

5.11 Conclusions and Future Perspectives

Current cervical cancer screening strategies as a "secondary prevention" using cytology combined with HPV testing have been successfully introduced, but further efforts are needed for improving the efficiency and effectiveness and preventing increased costs. The importance of HPV testing has been recognized, and its role in cervical screening is shifting from cytology alone to cytology plus HPV cotesting and now to a new paradigm in which HPV testing alone may become a primary screening tool.

HPV vaccination is a "primary prevention" tool, and both the bivalent and quadrivalent HPV vaccines have excellent safety and efficacy profiles. However, vaccination cannot eliminate the need for screening with cytology and/or HPV testing later in life, since both vaccines can protect against HPV-16/HPV-18 infection, but not protect against all high-risk HPV types. Recently, in February 2015, the US Advisory Committee on Immunization Practices (ACIP) recommended 9-valent HPV vaccine [26], a newly developed vaccine targeted against HPV-6, HPV-11, HPV-16, HPV-18, HPV-31, HPV-33, HPV-45, HPV-52, and HPV-58, as one of three HPV vaccines that can be used for routine vaccination. As HPV-16/HPV-18 are responsible for 65–70% and the five additional types (HPV-31, HPV-33, HPV-45, HPV-52, HPV-58) for about 15%, the 9-valent HPV vaccine may possibly protect against over 80% of invasive cervical cancers. Additionally, it has been reported that approximately 40–50% of CIN2/3 are caused by HPV-16/HPV-18 and 25% by HPV-31, HPV-33, HPV-45, HPV-52, or HPV-58. Further evidence on the 9-valent HPV vaccine should be accumulated worldwide, and its application is expected as a new strategy.

Finally, the WHO recognizes the prevention of cervical cancer and other HPV-related diseases as global public health problems and strongly recommends that HPV vaccines should be included in national immunization programs. Both HPV vaccination and cancer screening tests are indispensable for cervical cancer prevention, with a global consensus. The complete eradication of this malignant disease in the world will be realized in the near future by the further development, improvement, and widespread application of these two strategies.

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References

1. GLOBOCAN 2012: estimated cancer incidence, mortality and prevalence worldwide in 2012. International Agency for Research on Cancer, World Health Organization. http://globocan.iarc.fr/Pages/fact_sheets_cancer.aspx
2. Motoki Y, Mizushima S, Taguri M, Takahashi K, Asano R, Kato H, Asai-Sato M, Katayama K, Okamoto N, Hirahara F, Miyagi E. Increasing trends in cervical cancer mortality among young

Japanese women below the age of 50 years: an analysis using the Kanagawa population-based Cancer Registry, 1975-2012. Cancer Epidemiol. 2015;39(5):700–6. doi:10.1016/j.canep.2015.08.001.
3. Yamagami W, Aoki D. Annual report of the committee on gynecologic oncology, the Japan Society of Obstetrics and Gynecology. J Obstet Gynaecol Res. 2015;41:1861–9.
4. Human papillomavirus vaccines: WHO position paper, October 2014. Wkly Epidemiol Rec. 2014;89(43):465–91. http://www.who.int/wer
5. Onuki M, Matsumoto K, Satoh T, Oki A, Okada S, Minaguchi T, Ochi H, Nakao S, Someya K, Yamada N, Hamada H, Yoshikawa H. Human papillomavirus infections among Japanese women: age-related prevalence and type-specific risk for cervical cancer. Cancer Sci. 2009;100(7):1312–6. doi:10.1111/j.1349-7006.2009.01161.x.
6. Nanda K, McCrory DC, Myers ER, Bastian LA, Hasselblad V, Hickey JD, Matchar DB. Accuracy of the Papanicolaou test in screening for and follow-up of cervical cytologic abnormalities: a systematic review. Ann Intern Med. 2000;132(10):810–9.
7. Spence AR, Coggin P, Franco EL. Process of care failures in invasive cervical cancer: a systematic review and meta-analysis. Prev Med. 2007;45:93–106. doi:10.1016/j.ypmed.2007.06.007.
8. Burd EM. Human papillomavirus laboratory testing: the changing paradigm. Clin Microbiol Rev. 2016;29(2):291–319. doi:10.1128/CMR.00013-15.
9. Cox JT, Castle PE, Behrens CM, Sharma A, Wright Jr TC, Cuzick J, Athena HPV Study Group. Comparison of cervical cancer screening strategies incorporating different combinations of cytology, HPV testing, and genotyping for HPV 16/18: results from the ATHENA HPV study. Am J Obstet Gynecol. 2013;208(3):184.e1–11. doi:10.1016/j.ajog.2012.11.020.
10. Wright TC, Stoler MH, Behrens CM, Sharma A, Zhang G, Wright TL. Primary cervical cancer screening with human papillomavirus: end of study results from the ATHENA study using HPV as the first-line screening test. Gynecol Oncol. 2015;136(2):189–97. doi:10.1016/j.ygyno.2014.11.076.
11. Monsonego J, Cox JT, Behrens C, Sandri M, Franco EL, Yap PS, Huh W. Prevalence of high-risk human papilloma virus genotypes and associated risk of cervical precancerous lesions in a large U.S. screening population: data from the ATHENA trial. Gynecol Oncol. 2015;137(1):47–54. doi:10.1016/j.ygyno.2015.01.551.
12. Saslow D, Solomon D, Lawson HW, Killackey M, Kuasingam SL, Cain J, Garcia FA, Moriarty AT, Waxman AG, Wilbur DC, Wentzensen N, Downs Jr LS, Spitzer M, Moscicki AB, Franco EL, Stoler MH, Schiff-man M, Castle PE, Myers ER, ACS-ASCCP-ASCP Cervical Cancer Guideline Committee. American Cancer Society, American Society for Colposcopy and Cervical Pathology, and American Society for Clinical Pathology screening guidelines for the prevention and early detection of cervical cancer. CA Cancer J Clin. 2012;62:147–72. doi:10.3322/caac.21139.
13. Bailey HH, Chuang LT, duPont NC, Eng C, Foxhall LE, Merrill JK, Wollins DS, Blanke CD. American Society of Clinical Oncology statement: human papillomavirus vaccination for cancer prevention. J Clin Oncol. 2016;34(15):1803–12. doi:10.1200/JCO.2016.67.2014.
14. FUTURE II Study Group. Quadrivalent vaccine against human papillomavirus to prevent high-grade cervical lesions. N Engl J Med. 2007;356(19):1915–27.
15. Paavonen J, Naud P, Salmerón J, Wheeler CM, Chow SN, Apter D, Kitchener H, Castellsague X, Teixeira JC, Skinner SR, Hedrick J, Jaisamrarn U, Limson G, Garland S, Szarewski A, Romanowski B, Aoki FY, Schwarz TF, Poppe WA, Bosch FX, Jenkins D, Hardt K, Zahaf T, Descamps D, Struyf F, Lehtinen M, Dubin G, HPV PATRICIA Study Group. Efficacy of human papillomavirus (HPV)-16/18 AS04-adjuvanted vaccine against cervical infection and precancer caused by oncogenic HPV types (PATRICIA): final analysis of a double-blind, randomized study in young women. Lancet. 2009;374(9686):301–14. doi:10.1016/S0140-6736(09)61248-4.
16. Tabrizi SN, Brotherton JM, Kaldor JM, Skinner SR, Liu B, Bateson D, McNamee K, Garefalakis M, Phillips S, Cummins E, Malloy M, Garland SM. Assessment of herd immunity

and cross-protection after a human papillomavirus vaccination programme in Australia: a repeat cross-sectional study. Lancet Infect Dis. 2014;14(10):958–66. doi:10.1016/S1473-3099(14)70841-2.
17. Mesher D, Panwar K, Thomas SL, Beddows S, Soldan K. Continuing reductions in HPV 16/18 in a population with high coverage of bivalent HPV vaccination in England: an ongoing cross-sectional study. BMJ Open. 2016;6(2):e009915. doi:10.1136/bmjopen-2015-009915.
18. Crowe E, Pandeya N, Brotherton JM, Dobson AJ, Kisely S, Lambert SB, Whiteman DC. Effectiveness of quadrivalent human papillomavirus vaccine for the prevention of cervical abnormalities: case-control study nested within a population based screening programme in Australia. BMJ. 2014;348:g1458. doi:10.1136/bmj.g1458.
19. Pollock KG, Kavanagh K, Potts A, Love J, Cuschieri K, Cubie H, Robertson C, Cruickshank M, Palmer TJ, Nicoll S, Donaghy M. Reduction of low- and high-grade cervical abnormalities associated with high uptake of the HPV bivalent vaccine in Scotland. Br J Cancer. 2014;111(9):1824–30. doi:10.1038/bjc.2014.479.
20. Baldur-Felskov B, Munk C, Nielsen TS, Dehlendorff C, Kirschner B, Junge J, Kjaer SK. Trends in the incidence of cervical cancer and severe precancerous lesions in Denmark, 1997-2012. Cancer Causes Control. 2015;26(8):1105–16. doi:10.1007/s10552-015-0603-7.
21. Arnheim-Dahlström L, Pasternak B, Svanström H, Sparén P, Hviid A. Autoimmune, neurological, and venous thromboembolic adverse events after immunisation of adolescent girls with quadrivalent human papillomavirus vaccine in Denmark and Sweden: cohort study. BMJ. 2013;347:f5906. doi:10.1136/bmj.f5906.
22. European Medicine Agency (EMA). HPV vaccines: EMA confirms evidence does not support that they cause CRPS or POTS. 2015. http://www.ema.europa.eu/ema/index.jsp?curl=pages/news_and_events/news/2015/11/news_detail_002436.jsp&mid=WC0b01ac058004d5c1
23. Hanley SJ, Yoshioka E, Ito Y, Kishi R. HPV vaccination crisis in Japan. Lancet. 2015;385(9987):2571. doi:10.1016/S0140-6736(15)61152-7.
24. Fujii T. Declaration to demand the resumption of recommendations for human papillomavirus (HPV) vaccination for cervical cancer prevention. J Obstet Gynaecol Res. 2015; 41(12):1859–60.
25. The World Health Organization, Global Advisory Committee on Vaccine Safety. Statement on safety of HPV vaccines. 2015. http://www.who.int/vaccine_safety/committee/GACVS_HPV_statement_17Dec2015.pdf?ua=1
26. Joura EA, Giuliano AR, Iversen OE, Bouchard C, Mao C, Mehlsen J, Moreira Jr ED, Ngan Y, Petersen LK, Lazcano-Ponce E, Pitisuttithum P, Restrepo JA, Stuart G, Woelber L, Yang YC, Cuzick J, Garland SM, Huh W, Kjaer SK, Bautista OM, Chan IS, Chen J, Gesser R, Moeller E, Ritter M, Vuocolo S, Luxembourg A, Broad Spectrum HPV Vaccine Study. A 9-valent HPV vaccine against infection and intraepithelial neoplasia in women. N Engl J Med. 2015;372(8):711–23. doi:10.1056/NEJMoa1405044.

Pathology, Genomics, and Treatment of Endometrial Cancer

6

Tsukasa Baba

Abstract

Endometrial cancer (EC) is the leading causes of gynecologic malignancy in westernized countries, in which people take fat-rich foods and the estimated incidence rate of EC has kept increasing. EC has been traditionally classified into two categories, Type I and Type II, based on morphologic features. Type I ECs, composed of low-grade endometrioid carcinoma, possess alterations in *PTEN*, *PIK3CA*, *ARID1A*, *K-ras*, *β-catenin*, and/or DNA mismatch repair genes and usually have good prognosis. Type II ECs, mainly composed of serous and clear cell carcinoma, have different gene alterations such as *TP53* and *PPP2R1A* to exhibit aggressive features with poor prognostic outcome. This classification is still convenient to comprehend EC natures roughly or to provide ordinary treatments in the clinical setting, but there are several limitations to investigate EC genomics and to develop a novel therapy for treatment-refractory disease as Type I ECs are not uniform in genetics and Type II ECs are also composed of various histological subtypes. Recent genome-wide analysis provides new concepts as molecular subtyping and genetic predisposition of heterogeneous Type I ECs, which is expected to proceed future personalized medicine. As for Type II ECs, there is no reproducible breakthrough translational achievement, and so that subtype-specific genome-wide analysis with large enough sample size is now warranted for revealing genetics and developing effective therapies for treatment-refractory cases.

Keywords

Endometrial cancer • Serous carcinoma • MELF pattern • Genome-wide analysis

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6.1 Synopsis

The incidence of endometrial cancer is drastically increasing in developed countries and now the most frequent malignancy in women. Although most patients with Type I endometrial cancer show the favorable prognosis, those with Type II such as serous carcinoma exhibit poor clinical outcome. Even in Type I, some cancers show unique invasive process as "MELF" and will metastasize widely. Accordingly, precise pathological analyses and refined genomic analyses are necessary to clarify the heterogeneity of this disease, with relevance to the personalization of treatment.

6.2 Incidence of Endometrial Cancer

Endometrial cancer is one of the leading causes of gynecologic malignancy. It is the fourth most common malignancy among women in the United States, with an estimated 60,050 new cases and 10,470 deaths in 2016 [1], which are 20,000 more new cases and 3000 more deaths compared with those in 2008 (Fig. 6.1a). Estimated incidence rate of whole US female population is 0.033%, while that of Asian-Americans, Native Hawaiians, and Pacific Islanders (AANHPIs) is 0.022% and one-third less than the whole United States ($p < 0.0001$) [2]. AANHPIs are not only designated to convey the distinct genetic background from non-Hispanic white Americans (NHWs) but exhibit a complete different profile of death causes, such as less incidence of obesity−/diabetes-associated heart disease and cerebral infarction from NHWs [2]. Furthermore, the lifetime probability of developing endometrial cancer in Black Americans who share cultural habits with NHWs is 2.5% which is 0.4% less than NHWs but still higher than AANHPIs [3]. Nevertheless, in Japan where it has been westernized rapidly and people are taking more fat-rich foods, the incidence has also increased sixfold in the past three decades. These results indicate that endometrial cancer is developed in the patients bearing genetic factors as well as environmental factors. Although the incidence has kept increasing both in the United States and Japan, the death per incidence rate remains around 16% which are much lower than that of cervical cancer and ovarian cancer (Fig. 6.1b). Low mortality rate of endometrial cancer is owing to the reason that most patients present with low-grade and early-stage disease, but cases of high-grade tumors or preoperatively underdiagnosed low-grade tumors are occasionally accompanied with tumor spread beyond the uterus [4].

6.3 Traditional Classification of Endometrial Cancer Based on Histopathology

Based on pathological features on hematoxylin-eosin-stained specimen, endometrial cancers have been classified into two groups: Type I and Type II [5]. Representative Type I endometrial cancers are low-grade endometrioid carcinomas (G1-2) composed of well-differentiated malignant glandular epithelial cells

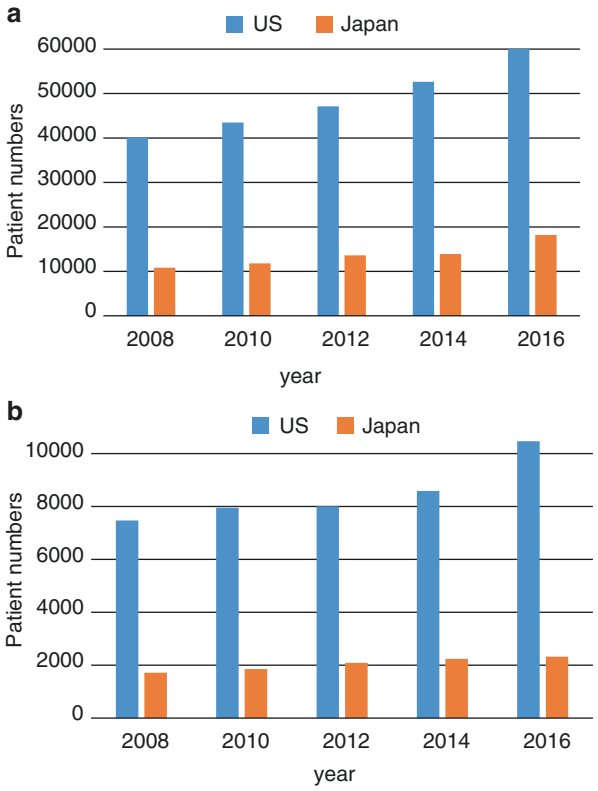

Fig. 6.1 Estimated incidence (**a**) and death (**b**) of endometrial cancers in the United States and Japan. Endometrial cancer is one of the leading causes of gynecologic malignancy with an estimated 60,050 new cases and 10,470 deaths in the United States. Estimated incidence has increased more than 50% for these 8 years both in the United States and Japan (**a**). Although estimated death has kept increasing as well, the death per incidence rate remains around 16% (**b**). Data was obtained from Cancer Statistics of American Cancer Society (http://www.cancer.org) and Cancer Registry and Statistics. Cancer Information Service and National Cancer Center, Japan (http://ganjoho.jp/reg_stat/)

frequently accompanied with squamous metaplasia, so-called morula change, which is different from solid growth of high-grade endometrioid carcinoma (G3). Type I cancers usually feature high expression of estrogen receptor (ER) and a past history of unopposed estrogen caused by anovulation or obesity. Type I cancers possess several alterations in oncogenes, *PTEN*, *PIK3CA*, *ARID1A*, *K-ras*, *β-catenin*, and/or DNA mismatch repair genes and usually have a good prognosis [6, 7].

Type II cancers including high-grade endometrioid carcinoma with solid growth and prominent nuclear atypia, serous papillary carcinoma (SPC), and clear cell carcinoma (CCC) typically arise in old, nonobese women as an estrogen-independent manner. SPC and CCC, respectively, resemble ovarian high-grade serous carcinoma and renal clear cell carcinoma, and both are characterized by very aggressive

progression with poor prognostic outcomes [5, 8]. More than half of Type II cancers exhibit extrauterine spread at the time of diagnosis while less than 5% of Type I cancers [9]. Disease-specific 5-year survival rates of patients bearing SPC and CCC are 55 and 68%, and patients bearing SPC and CCC account for 39 and 8% of those who died of endometrial cancer even though consisting of only 10 and 3% of all endometrial cancer, respectively. Comprehensive surgical staging, including hysterectomy, salpingo-oophorectomy, and retroperitoneal node dissection (Fig. 6.2a),

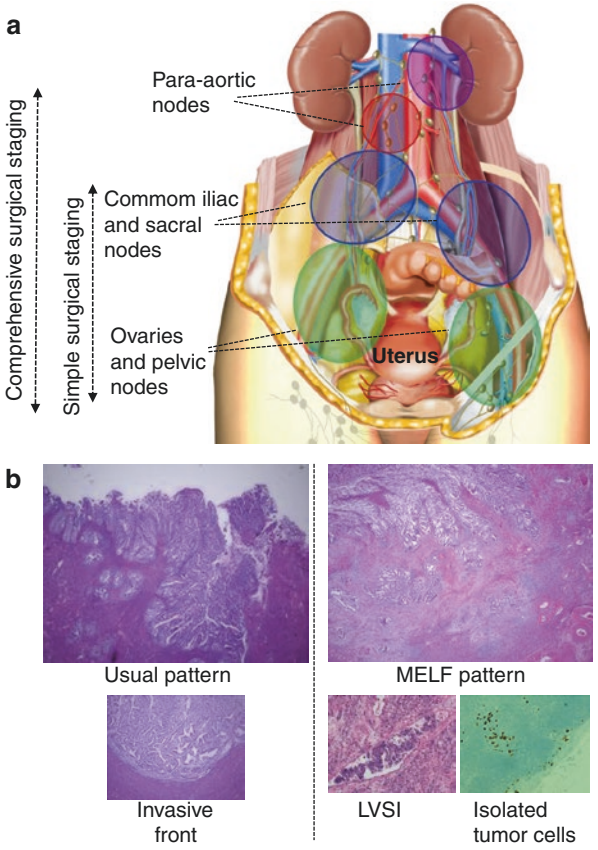

Fig. 6.2 Lesions to be dissected at surgical staging of endometrial cancer (**a**) and two distinct invasive features of Type I low-grade endometrioid carcinoma (**b**). (**a**) Surgical staging for endometrial cancer is carried out to figure out tumor spreads. Simple surgical staging, hysterectomy, salpingo-oophorectomy, and pelvic node dissection are applied for low-risk cases, while comprehensive surgical staging including para-aortic node dissection is for intermediate- or high-risk cases. (**b**) Type I low-grade endometrioid carcinoma (EC) generally exhibits myometrial invasion in a border-pushing expansile manner without lymphovascular invasion (LVSI) (usual pattern, *left*). MELF-pattern Type I ECs exhibit infiltrative myometrial invasion with microcystic, elongated, and fragmented glands surrounded by myxoid and inflamed stroma (*right*). MELF pattern is frequently associated with LVSI and node metastasis with isolated tumor cells which can be detected by cytokeratin staining

followed by adjuvant chemotherapy using carboplatin and paclitaxel is generally recommended for Type II cancers, but the prognostic benefit decreasing the risk of recurrence and improving survival is not satisfactory [9]. Thus, translational analysis of Type II endometrial cancers has been vigorously conducted for clarifying driver genes and developing molecular-targeting therapies.

In Type II endometrial cancers, *TP53*, *PPP2R1A*, *CHD4*, *FBXW7*, *SPOP* mutations, *STK15* and *HER2/neu* amplification, p16 overexpression, downregulation or loss of E-cadherin, and also loss of heterozygosity (LOH) have been reported [5, 7, 10]. These hallmarks of Type II cancers, however, do not entirely explain the aggressive nature of heterogeneous Type II endometrial cancers. Furthermore, there remain two issues concerning Type II histological discrimination, which have made molecular characterization of endometrial cancer uncertain. One is observer variability and the other is inter-tumor heterogeneity. It was frequently demonstrated that inter- or intra-observer reproducibility of histological typing based on morphology was quite poor, and immunohistochemical staining was not enough to compensate the variability [11, 12]. Despite the difficulty of histological discrimination, oncologic molecular mechanism of Type II endometrial cancers has been investigated for long.

6.3.1 Serous Papillary Carcinoma (SPC)

SPC exhibits a complex papillary/glandular architecture with diffuse, prominent nuclear pleomorphism. TP53 is a transcriptional regulator to trigger apoptosis or cell cycle arrest under DNA damage, and *TP53* mutation is observed in more than 90% of SPC and 75% of endometrial intraepithelial carcinoma (EIC, noninvasive SPC) while only in 30% of G3 and less than 10% of G1-2. As high-grade endometrial cancer arose through conditional uterine deletion of Tp53 using the Cre/loxP approach [13], *TP53* mutation is reasonably considered as a primary event of SPC oncogenesis [14]. *p16INK4a* mutation is another hallmark of SPC and EIC. *p16INK4a* mutation is almost 100% observed in serous tubal intraepithelial carcinoma (STIC) and high-grade serous ovarian carcinoma (HGSOC) while not in the precursor of STIC, p53 signature, which is atypical tubular epithelium with *TP53* mutation. These results indicate *p16INK4a* mutation is also not a malignant phonotype driver but an early event of oncogenesis following *TP53* mutation as p53 signature is occasionally identified in benign-appearing endometrium as a latent precancerous lesion of EIC [15, 16] even though oncogenic association of *p16INK4a* mutation has not been clarified in endometrial p53 signature.

Mutations in *PIK3CA* (24%), *FBXW7* (20%), and *PPP2R1A* (18%) in both SPC and EIC are also reported as early events [17]. PIK3CA regulates several malignant phenotypes, such as proliferation, survival, and mobility via PI3K/AKT/mTOR pathway. An F-box protein, FBXW7, is critical in the ubiquitination and targeting of tumor-promoting proteins cyclin E (CCNE1) and PPP2R1A. CCNE1 controls the G1 to S transition of the cell cycle, and *CCNE1* amplification is common in primary resistant and refractory HGSOC [18], and more than half of USC harbor either a

molecular genetic alteration in *FBXW7* or *CCNE1* amplification [17]. PPP2R1A is a regulatory unit of serine/threonine protein phosphatase 2A (PP2A), and its mutant promotes anchorage-independent growth and tumor formation in a dominant-negative manner [19]. EIC is not invasive but has a metastatic ability accompanied with anchorage-independent growth, and half of patients bearing EIC are found to have disease beyond the uterine corpus including omental involvement [20]. The alterations of *PIK3CA*, *FBXW7*, *CCNE1*, and *PPP2R1A* can regulate malignant phenotypes of SPC/EIC, but these alterations are observed only in a certain part of SPC/EIC, which indicates these alterations are not indispensable in the oncogenesis, but the accumulation of these alterations may define the malignant characters of each case and become therapeutic targets. For targeting PI3K/AKT/mTOR pathway, several mTOR and/or PIK3CA inhibitors are currently under evaluation in clinical trials. So far, the efficacy of mTOR inhibitor was demonstrated only for endometrioid carcinoma [21], but in this study, none of 11 SPC cases exhibited any clinical response. As dual blockade of PIK3CA and CCNE1 decreased tumor growth significantly in mouse model [22], multi-targeting might be also necessary in the clinical setting. Concerning the results that two out of two SPC patients showed clinical response to bevacizumab, the efficacy of combination of bevacizumab and temsirolimus, an mTOR inhibitor, was also investigated, but its toxicity was nonnegligibly high [23, 24].

HER2/neu is located upstream to the PIK3CA/AKT/mTOR pathway. *HER2* (erbB-2, the epidermal growth factor Type II receptor) amplification or overexpression is observed most frequently in SPC [25] although the expression rate differs among studies depending on assessing techniques. HER2 expression status is at first determined by immunohistochemistry (IHC), but in cases with equivocal IHC results, fluorescence in situ hybridization (FISH) is employed. *HER2* amplification is frequently observed in African-American patients with worse survival compared with Caucasian patients as those bearing HER2+ breast cancers exhibit poor survival due to metastasis and chemoresistance. HER2 receptor targeting therapy using humanized monoclonal antibody, trastuzumab, is effective in the treatment of HER2+ node-positive breast cancers [26], but it is not so effective for SPC; there were none of 11 HER2+ SPC cases to demonstrate any tumor response [27]. In SPC, frequent *PIK3CA* mutation/amplification would compensate trastuzumab effect, and truncated p95HER2 variant which lacks the trastuzumab-binding domain is frequently observed [28].

6.3.2 Clear Cell Carcinoma (CCC)

Endometrial CCC exhibits characteristic morphology similar to ovarian CCC, a combination of architectural patterns (papillary, glandular, solid, and cystic) and cytoplasmic features (clear and oxyphilic). Nevertheless, as endometrioid carcinoma and SPC display significant morphological overlap with CCC, interobserver discrepancy to differentiate CCC from its mimics occurs frequently [12, 29]. Then, immunostaining is usually employed for differential diagnosis although pathological diagnosis based on morphology is fundamental. In general, endometrial CCC as

well as ovarian counterpart is positive for hepatocyte nuclear factor 1b (HNF1b:100%) and napsin A (93%) and negative for estrogen receptor (ER: 93%), and the combination of these markers is very useful for distinguishing solid/papillary pattern CCC from SPC or G3 [29, 30].

In contrast with the immune profiling, the molecular-genetic background of endometrial CCC is still obscure due to the rarity of pure CCC. Endometrial CCC is basically similar to ovarian CCC, but there exist differences between them: less alterations in *ARID1A* (13–24% vs. 50%) and *PIK3CA* (9–24% vs. 35%). Furthermore, *TP53* mutation is relatively frequent compared with ovarian CCC (33–40% vs. 12%), and the aberrant p53 staining is designated as a poor prognostic factor [25, 29, 30]. Targeted genetic profiling of endometrial CCC identified mutations in genes involved in chromatin remodeling/transcriptional regulation (ARID1A, ZFHX3, and TSPYL2) [29], and loss of BAF250a expression occurs without *ARID1A* mutation in 26% of CCC [31]. Loss of BAF250a itself is not related to poor prognosis in endometrial CCC [32]. However, as ovarian CCC with loss of one or multiple SWI/SNF complex subunits including BAF250a exhibits aggressive behaviors and poor prognosis [33], in endometrial CCC, multiple loss of SWI/SNF complex subunits may also affect the prognostic outcome. In CCC, ubiquitin-mediated proteolysis (*SPOP* and *FBXW7*) and SPC-characteristic genes, *TP53* and *PP2AR1A*, are also highly mutated [29], and *HER2/neu* amplification is identified [25]. These common genetic alterations would provide partial overlapping in tumor phenotypes between SPC and CCC.

6.3.3 Carcinosarcoma (CS)

CS is a biphasic tumor composed of Type II cancer with sarcomatous elements. Although this tumor is a wide-range admixture of nonspecific sarcomatous mesenchyme and high-grade epithelium, SPC, CCC, and G3, this tumor is considered to be of epithelial derivation as a masterpiece of epithelial-mesenchymal transition and to share genetic profiles with Type II cancers. CS harbors relatively high *TP53* mutation (67%), but low *PIK3CA* mutation (22%), and the carcinoma component is considered responsible for the aggressive behavior of CS resulting in a poor survival, while the clinical impact of sarcoma component has been obscure [29]. Most recent integrated analysis revealed that CS shared proteomic features with SPC and EC, and sarcomas with epitherlial-mesenchymal transition features [34]. A multicenter retrospective study for 1192 CS cases demonstrated that carcinoma components tended to spread lymphatically, while sarcoma components tended to spread locoregionally, and high-grade carcinoma component was independently associated with decreased progression-free survival (PFS) [35]. In this study, postoperative chemotherapy was an independent predictor for improved PFS, and characterization of histologic pattern would make drug selection suitable in the treatment of each carcinoma/sarcoma combo: ifosfamide for low grade/homologous (HR 0.21, $p = 0.005$), platinum for high grade/homologous (HR 0.36, $p < 0.001$), and anthracycline for high grade/heterologous (HR 0.30, $p = 0.001$) [35].

6.3.4 Microcystic, Elongated, and Fragmented (MELF) Pattern Invasion

Type I low-grade endometrioid carcinoma (EC) usually presents as an early-stage disease with or without shallow invasion resulting in an excellent outcome. Type I ECs generally exhibit myometrial invasion in a border-pushing expansile manner (usual pattern, Fig. 6.2b), but such invasion stays within inner half of the myometrium without lymphovascular space infiltration (LVSI) [36]. In such FIGO stage IA cases bearing usual pattern of Type I EC, extrauterine spread is so rare that hysterectomy, salpingo-oophorectomy, and pelvic node dissection (Fig. 6.2a) without adjuvant chemotherapy are generally accepted as the standard treatment. In contrast, some exhibit a different way of myometrial invasion in an infiltrative manner with microcystic, elongated, and fragmented glands surrounded by myxoid and inflamed stroma (MELF pattern, Fig. 6.2b), and this type of invasion is usually observed in the invasive front in the outer half of the myometrium. MELF frequency is around 13% (7–36%), and this morphologic pattern is highly associated with LVSI and node metastasis [37–39]. MELF has been considered as a kind of EMT feature due to the morphology of subtle sinus histiocyte-like invasion. Immunophenotyping studies revealed gain of S100A4 and L1CAM and loss or reduction of E-cadherin, CD147, MMP2, and Galectin-3, resulting in loss of cell-cell adhesion and polarity to endow migratory and invasive properties [38, 40–42]. These results indicate MELF is one phenotype of endometrioid carcinoma cells in the phase of EMT, but it is still not clarified whether low-grade ECs with MELF-type invasion are genetically distinct from usual Type I ECs as there is no comprehensive genomic analysis so far.

The clinical impact of MELF is controversial as well. MELF-pattern invasion is not associated with macrometastasis but micrometastasis or isolated tumor cells (ITC, Fig. 6.2b) [39]. Node metastasis is an infamous prognostic factor of endometrial cancer, but the clinical relevance of ITC is obscure since ITC does not increase the recurrence rate. The recurrence rate of cases with or without MELF is considered not different, but MELF cases trend toward decreased time to non-vaginal recurrence due to higher rate of node involvement [38]. MELF pattern invasion exists too sparsely in the tumor frontier to be detected at the preoperative diagnosis with endometrial biopsy or magnetic resonance imaging (MRI). Dynamic contrast-enhanced MRI is very useful for detecting the presence of myometrial invasion, but so far, deep myometrial invasion is detected in more than 10% cases preoperatively diagnosed as FIGO stage IA Type I EC [43]. Although the clinical significance of node dissection in the para-aortic lesion (PAN) is still controversial, underestimation would save PAN at primary surgery resulting in PAN recurrence/residue due to undertreatment. To avoid undertreatment following underestimation, establishment of preoperative diagnosis of MELF in the deep myometrium is warranted.

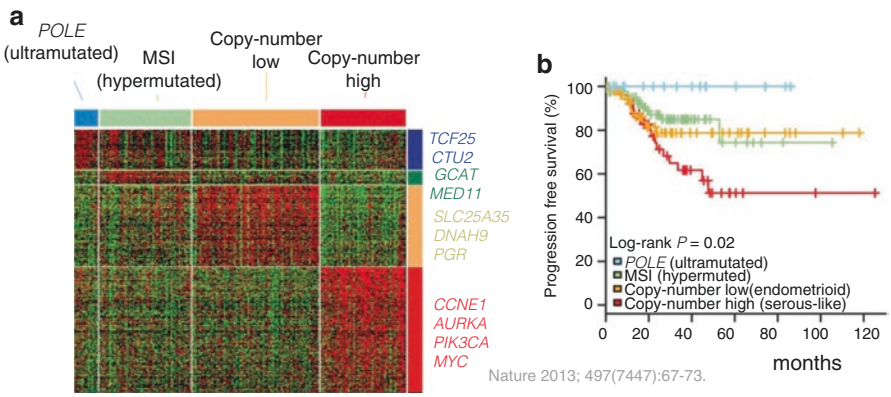

Fig. 6.3 Gene expression across integrated subtypes in endometrial carcinomas (**a**) and prognostic outcome varying among molecular subtypes (**b**). Integrated analysis of somatic mutation rates, frequency of copy number alterations, and microsatellite instability (MSI) status along with the clinical information provides a new insight that endometrial cancers are classified into four distinct molecular subgroups. The subgroups are termed as *POLE* ultramutated, hypermutated (microsatellite unstable: MSI), copy number low (microsatellite stable), and copy number high (serous like). (**a**) These four subgroups, respectively, exhibit characteristic gene expression patterns. (**b**) POLE-mutant tumors have significantly better progression-free survival, whereas copy number high tumors have the poorest outcome

6.4 Genome-Based Classification and Its Clinical Relevance

6.4.1 The Cancer Genome Atlas (TCGA) Project

The International Cancer Genome Consortium (ICGC) was established to analyze the genome-wide abnormalities of 50 kinds of malignant tumors. The Cancer Genome Atlas (TCGA) is a study conducted as the ICGC project in the United States, which initially analyzed brain glioblastoma, non-small cell lung cancer, and high-grade serous ovarian carcinoma [44, 45]. As for endometrial cancer, whole-exome sequencing, SNP array assessing copy number alterations, mRNA expression microarray, DNA methylation microarray, and microRNA microarray were conducted for more than 200 cases [4]. This integrated analysis of somatic mutation rates, frequency of copy number alterations, and microsatellite instability (MSI) status along with the clinical information provided a new insight that endometrial cancers were classified into four distinct molecular subgroups. The subgroups are termed as POLE ultramutated, hypermutated (microsatellite unstable), copy number low (microsatellite stable), and copy number high (serous like) (Fig. 6.3a).

6.4.1.1 POLE Ultramutated Subgroup

6.4% of low-grade ECs and 17.4% of high-grade ECs but none of SPC were designated as POLE ultramutated in TCGA study. POLE ultramutated tumors have somatic mutations in the exonuclease domain of *POLE*, which induce an increased

incidence of C>A transversions resulting in extraordinarily high mutation rate (867–9714 mutations/tumor) [4]. In this subgroup, 190 genes, which encode the pathways of gluconeogenesis, glycolysis, clathrin-mediated endocytosis signaling, tRNA charging, tricarboxylic acid cycle II (eukaryotic), and actin cytoskeleton signaling, are significantly mutated. Although POLE ultramutated tumors are not so many and more than half of them are G3 ECs, the progression-free survival of patients in this subgroup is more favorable than for other molecular subgroups (Fig. 6.3b).

6.4.1.2 Hypermutated (Microsatellite-Unstable) Subgroup

28.6% of low-grade ECs and 54.3% of high-grade ECs were included in this group. Hypermutated microsatellite-unstable tumors carry frequent *MLH1*-promoter methylation and reduced MLH1 gene expression to bring MSI phenotype without somatic copy number alterations [4]. As pathogenic driver genes, 21 genes including *PTEN*, *PIK3CA*, *PIK3R1*, *ARID1A*, *KRAS*, *FGFR2*, and *CTNNB1* are significantly mutated in this subgroup. Subsequently, the RTK (receptor tyrosine kinase)/RAS/β-catenin pathway and the PIK3CA-PIK3R1-PTEN axis are altered in 69.5 and 95.3%, respectively [46]. Large cohort studies indicate there is no significant correlation between MSI status and clinical outcome for endometrial cancer [47, 48].

6.4.1.3 Copy Number Low (Microsatellite-Stable) Subgroup

60.0% of low-grade ECs, 8.7% of high-grade ECs, 2.3% of SPC, and 25% of mixed histology carcinomas were included [4]. In this subgroup, 16 genes including *PTEN*, *PIK3CA*, *CTNNB1*, *ARID1A*, *PIK3R1*, *KRAS*, *FGFR2*, *CHD4*, and *SPOP* are identified as significantly mutated genes. The somatic alterations are frequently observed in the PI3K pathway (92%) and the RTK/RAS/β-catenin pathway (83%), and it is characteristic that somatic mutations in *CTNNB1* are particularly prevalent (52%), but *KRAS* mutations are relatively few (16%) compared with microsatellite-unstable ECs [49, 46].

6.4.1.4 Copy Number High (Serous-Like) Subgroup

5.0% of low-grade ECs, 19.6% of high-grade ECs, 97.7% of SPC, and 75% of mixed histology carcinomas were included in this subgroup. It is noteworthy that one-fifth of tumors histologically classified as G3 ECs are designated as serous like, and Type II concept is validated at the molecular level to a certain extent. The TCGA study described *TP53*, *PIK3CA*, *PTEN*, *PIK3R1*, *PPP2R1A*, *FBXW7*, and *CHD4* as significantly mutated genes [4]. Furthermore, genes involved in chromatin remodeling and ubiquitin-mediated protein degradation are frequently mutated in SPC [50]. Focal amplification in the region of *MYC*, *HER2*, and *CCNE1* is characteristically observed in 23–25% cases [4]. Co-occurrence of mutation and amplification such as *PIK3CA* mutation and *HER2* amplification is frequently observed, which is clinically relevant to make the prognostic outcome of this subgroup worst due to chemoresistance [4, 51]. As the clinical prognosis of this subgroup is worst (Fig. 6.3b), this integrated genome-wide analysis has attracted many researchers to be involved in a lot of studies mining novel serous-like driver

genes. However, due to the lack of another integrated data with similarly large samples, the reproducibility of each study is equivocal, so that independent validation of candidate genes in multiple cohorts as well as functional assessment is necessary to determine the driver genes [52].

6.4.2 Genome-Wide Association Studies (GWAS)

In ECs, there exists a genetic predisposition as risks of those with a family history and first-degree female relatives increase around two- and threefolds, respectively, and there are heritable ECs as Lynch syndrome with autosomal dominant germ line pathogenic variants in DNA mismatch repair genes [53]. To comprehend and estimate genetic risk of EC has been a high priority issue for those with familial episodes.

GWAS is a kind of examination of genetic variants among different individuals to figure out predisposition to a specific disease by focusing on associations with SNPs. GWAS investigates the whole genome for the case-control groups, in contrast to usual methods to test pre-specified genetic regions, and it is a non-biased method to identify SNPs and other variants in DNA associated with a disease. As of 2014, GWAS have discovered more than 1500 common variants associated with various cancers [54], and the GWAS catalog has been updated year by year [55]. GWAS is expected to accelerate novel diagnosis and drug development by integrating genetic studies to identify high-risk SNPs and functional small nucleotide drugs interfering such loci for preventing the disease. However, GWAS frequently holds limitations such as lack of well-defined cases and controls, insufficient sample size, and statistical prematurity.

As for endometrial cancer, more than 30 articles have been published, but due to the lack of statistical power, reproducible results for detecting EC risk loci have not been obtained from individual studies. As meta-analysis methods generate a reasonable summary data from multiple independent GWAS by increasing power and reducing false negatives [56], a couple of meta-analytic GWAS has been conducted for ECs with famous EC GWAS such as ANECS, SEARCH, NSECG, and E2C2 [53, 57]. These two meta-GWAS commonly identified three loci, 17q12 (*HNF1b*), 13q22 (*KLF*), and 6q22 (*NCOA7* and *HEY2*), but there are many discrepancies even between these studies analyzing almost identical datasets [53, 57]. Nevertheless, as functional studies reveal that 13q22 locus regulates *KLF5* linked to uterine development and tumorigenesis [57], meta-GWAS could be considered to shed a new light to develop clinically applicable diagnosis and drugs for ECs in the future.

6.5 Future Perspective

Recent genome-wide analyses have provided a new concept of molecular subtyping and many insights especially for the oncogenesis and the genetic risks of Type I ECs, and the landscape of endometrial cancer research was drastically changed.

However, as for Type II endometrial cancers, almost none other than key molecules in the oncogenic process are clarified. Individual studies have proposed various candidate genes or pathways responsible for the inherently aggressive phenotypes, but TCGA-oriented studies are hardly validated due to the lack of another mega-dataset [52]. Furthermore, TCGA and GWAS revealed the genetic/molecular heterogeneity even in the identical histologic subtype, and there is no such large-scaled analysis for CCC or CS. Recent analysis revealed that HGSOC could be further classified into four subtypes, and the susceptibility to paclitaxel and bevacizumab was different among subtypes [18, 58–60]. For the sake of figuring out "driver" and establishing personal medicine, it is keen to avoid the analysis to put all endometrial cancer together and to proceed subtype-specific genome-wide analysis with large enough sample size.

References

1. Siegel R, Miller KD, Jemal A. Cancer statistics, 2016. CA Cancer J Clin. 2016;66(1):7–30.
2. Torre LA, Sauer AM, Chen Jr MS, Kagawa-Singer M, Jemal A, Siegel RL. Cancer statistics for Asian Americans, Native Hawaiians, and Pacific Islanders, 2016: converging incidence in males and females. CA Cancer J Clin. 2016;66(3):182–202.
3. DeSantis CE, Siegel RL, Sauer AG, Miller KD, Fedewa SA, et al. Cancer statistics for African Americans, 2016: progress and opportunities in reducing racial disparities. CA Cancer J Clin. 2016;66(4):290–308.
4. Kandoth C, Schultz N, Cherniack AD, Akbani R, Liu Y, Shen H, et al. Integrated genomic characterization of endometrial carcinoma. Nature. 2013;497(7447):67–73.
5. Bokhman JV. Two pathogenetic types of endometrial carcinoma. Gynecol Oncol. 1983;15(1):10–7.
6. Lax SF, Kurman RJ. A dualistic model for endometrial carcinogenesis based on immunohistochemical and molecular genetic analyses. Verh Dtsch Ges Pathol. 1997;81:228–32.
7. McConechy MK, Ding J, Cheang MC, Wiegand KC, Senz J, Tone AA, et al. Use of mutation profiles to refine the classification of endometrial carcinomas. J Pathol. 2012;228(1):20–30.
8. Matias-Guiu X, Prat J. Molecular pathology of endometrial carcinoma. Histopathology. 2013;62(1):111–23.
9. Black JD, English DP, Roque DM, Santin AD. Targeted therapy in uterine serous carcinoma: an aggressive variant of endometrial cancer. Womens Health (Lond). 2014;10(1):45–57.
10. McConechy MK, Anglesio MS, Kalloger SE, Yang W, Senz J, Chow C, et al. Subtype-specific mutation of PPP2R1A in endometrial and ovarian carcinomas. J Pathol. 2011;223(5):567–73.
11. Hoang LN, McConechy MK, Köbel M, Han G, Rouzbahman M, et al. Histotype-genotype correlation in 36 high-grade endometrial carcinomas. Am J Surg Pathol. 2013;37(9):1421–32.
12. Gilks CB, Oliva E, Soslow RA. Poor interobserver reproducibility in the diagnosis of high-grade endometrial carcinoma. Am J Surg Pathol. 2013;37(6):874–81.
13. Daikoku T, Hirota Y, Tranguch S, Joshi AR, DeMayo FJ, et al. Conditional loss of uterine Pten unfailingly and rapidly induces endometrial cancer in mice. Cancer Res. 2008;68(14):5619–27.
14. Jia L, Liu Y, Yi X, Miron A, Crum CP, et al. Endometrial glandular dysplasia with frequent p53 gene mutation: a genetic evidence supporting its precancer nature for endometrial serous carcinoma. Clin Cancer Res. 2008;14(8):2263–9.
15. Jarboe EA, Pizer ES, Miron A, Monte N, Mutter GL, Crum CP. Evidence for a latent precursor (p53 signature) that may precede serous endometrial intraepithelial carcinoma. Mod Pathol. 2009;22(3):345–50.

16. Zhang X, Liang SX, Jia L, Chen N, Fadare O, et al. Molecular identification of "latent precancers" for endometrial serous carcinoma in benign-appearing endometrium. Am J Pathol. 2009;174(6):2000–6.
17. Kuhn E, Wu RC, Guan B, Wu G, Zhang J, et al. Identification of molecular pathway aberrations in uterine serous carcinoma by genome-wide analyses. J Natl Cancer Inst. 2012;104(19):1503–13.
18. Patch AM, Christie EL, Etemadmoghadam D, et al. Whole-genome characterization of chemoresistant ovarian cancer. Nature. 2015;521:489–94.
19. Haesen D, Abbasi Asbagh L, Derua R, Hubert A, Schrauwen S, et al. Recurrent PPP2R1A mutations in uterine cancer act through a dominant-negative mechanism to promote malignant cell growth. Cancer Res. 2016;76(19):5719–31.
20. Chan JK, Loizzi V, Youssef M, Osann K, Rutgers J, et al. Significance of comprehensive surgical staging in noninvasive papillary serous carcinoma of the endometrium. Gynecol Oncol. 2003;90(1):181–5.
21. Slomovitz BM, Jiang Y, Yates MS, Soliman PT, Johnston T, et al. Phase II study of everolimus and letrozole in patients with recurrent endometrial carcinoma. J Clin Oncol. 2015; 33(8):930–6.
22. Cocco E, Lopez S, Black J, Bellone S, Bonazzoli E, et al. Dual CCNE1/PIK3CA targeting is synergistic in CCNE1-amplified/PIK3CA-mutated uterine serous carcinomas in vitro and in vivo. Br J Cancer. 2016;115(3):303–11.
23. Aghajanian C, Sill MW, Darcy KM, Greer B, McMeekin DS, et al. Phase II trial of bevacizumab in recurrent or persistent endometrial cancer: a Gynecologic Oncology Group Study. J Clin Oncol. 2011;29(16):2259–65.
24. Alvarez EA, Brady WE, Walker JL, Rotmensch J, Zhou XC, et al. Phase II trial of combination bevacizumab and temsirolimus in the treatment of recurrent or persistent endometrial carcinoma: a Gynecologic Oncology Group Study. Gynecol Oncol. 2013;129(1):22–7.
25. Jones NL, Xiu J, Chatterjee-Paer S, Buckley de Meritens A, Burke WM, et al. Distinct molecular landscapes between endometrioid and nonendometrioid uterine carcinomas. Int J Cancer. 2017;140(6):1396–404.
26. Untch M, Gelber RD, Jackisch C, Procter M, Baselga J, et al. Estimating the magnitude of trastuzumab effects within patient subgroups in the HERA trial. Ann Oncol. 2008;19(6):1090–6.
27. Fleming GF, Sill MW, Darcy KM, McMeekin DS, Thigpen JT, et al. Phase II trial of trastuzumab in women with advanced or recurrent, HER2-positive endometrial carcinoma: a Gynecologic Oncology Group Study. Gynecol Oncol. 2010;116(1):15–20.
28. Growdon WB, Groeneweg J, Byron V, DiGloria C, Borger DR, et al. HER2 over-expressing high grade endometrial cancer expresses high levels of p95HER2 variant. Gynecol Oncol. 2015;137(1):160–6.
29. Hoang LN, McConechy MK, Meng B, McIntyre JB, Ewanowich C, et al. Targeted mutation analysis of endometrial clear cell carcinoma. Histopathology. 2015;66(5):664–74.
30. Fadare O, Desouki MM, Gwin K, Hanley KZ, Jarboe EA, et al. Frequent expression of napsin A in clear cell carcinoma of the endometrium: potential diagnostic utility. Am J Surg Pathol. 2014;38(2):189–96.
31. Wiegand KC, Lee AF, Al-Agha OM, Chow C, Kalloger SE, et al. Loss of BAF250a (ARID1A) is frequent in high-grade endometrial carcinomas. J Pathol. 2011;224(3):328–33.
32. Fadare O, Gwin K, Desouki MM, Crispens MA, Jones III HW, et al. The clinicopathologic significance of p53 and BAF-250a (ARID1A) expression in clear cell carcinoma of the endometrium. Mod Pathol. 2013;26(8):1101–10.
33. Abou-Taleb H, Yamaguchi K, Matsumura N, Murakami R, Nakai H, et al. Comprehensive assessment of the expression of the SWI/SNF complex defines two distinct prognostic subtypes of ovarian clear cell carcinoma. Oncotarget. 2016;7(34):54758–70.
34. Cherniack AD, Shen H, Walter V, Stewart C, Murray BA, et al. Integrated Molecular Characterization of Uterine Carcinosarcoma. Cancer Cell. 2017;31(3):411–23.

35. Matsuo K, Takazawa Y, Ross MS, Elishaev E, Podzielinski I, et al. Significance of histologic pattern of carcinoma and sarcoma components on survival outcomes of uterine carcinosarcoma. Ann Oncol. 2016;27(7):1257–66.
36. Creasman WT, Odicino F, Maisonneuve P, Quinn MA, Beller U, Benedet JL, et al. Carcinoma of the corpus uteri. FIGO 26th Annual Report on the Results of Treatment in Gynecological Cancer. Int J Gynaecol Obstet. 2006;95(Suppl 1):S105–43.
37. Hertel JD, Huettner PC, Pfeifer JD. Lymphovascular space invasion in microcystic elongated and fragmented (MELF)-pattern well-differentiated endometrioid adenocarcinoma is associated with a higher rate of lymph node metastasis. Int J Gynecol Pathol. 2014;33(2):127–34.
38. Joehlin-Price AS, McHugh KE, Stephens JA, Li Z, Backes FJ, et al. The microcystic, elongated, and fragmented (MELF) pattern of invasion: a single institution report of 464 consecutive FIGO grade 1 endometrial Endometrioid adenocarcinomas. Am J Surg Pathol. 2017;41(1):49–55.
39. Pelletier MP, Trinh VQ, Stephenson P, Mes-Masson AM, Samouelian V, et al. Microcystic, elongated and fragmented (MELF) pattern invasion is mainly associated with isolated tumor cell pattern metastases in FIGO grade I endometrioid endometrial cancer (EEC). Hum Pathol. 2016. pii: S0046-8177(16)30306-9. doi:10.1016/j.humpath.2016.10.023. [Epub ahead of print].
40. Han G, Lim D, Leitao Jr MM, Abu-Rustum NR, Soslow RA, et al. Histological features associated with occult lymph node metastasis in FIGO clinical stage I, grade I endometrioid carcinoma. Histopathology. 2014;64(3):389–98.
41. Tahara S, Nojima S, Ohshima K, Hori Y, Kurashige M, et al. S100A4 accelerates the proliferation and invasion of endometrioid carcinoma and is associated with the "MELF" pattern. Cancer Sci. 2016;107(9):1345–52.
42. Kommoss F, Kommoss F, Grevenkamp F, Bunz AK, Taran FA, et al. L1CAM: amending the "low-risk" category in endometrial carcinoma. J Cancer Res Clin Oncol. 2017;143(2):255–62.
43. Fujii S, Kido A, Baba T, Fujimoto K, Daido S, et al. Subendometrial enhancement and peritumoral enhancement for assessing endometrial cancer on dynamic contrast enhanced MR imaging. Eur J Radiol. 2015;84(4):581–9.
44. Cancer Genome Atlas Research Network. Integrated genomic analyses of ovarian carcinoma. Nature. 2011;474:609–15.
45. Cancer Genome Atlas Research Network. Comprehensive genomic characterization defines human glioblastoma genes and core pathways. Nature. 2008;455(7216):1061–8.
46. Le Gallo M, Bell DW. The emerging genomic landscape of endometrial cancer. Clin Chem. 2014;60(1):98–110.
47. Zighelboim I, Goodfellow PJ, Gao F, Gibb RK, Powell MA, et al. Microsatellite instability and epigenetic inactivation of MLH1 and outcome of patients with endometrial carcinomas of the endometrioid type. J Clin Oncol. 2007;25(15):2042–8.
48. Diaz-Padilla I, Romero N, Amir E, Matias-Guiu X, Vilar E, et al. Mismatch repair status and clinical outcome in endometrial cancer: a systematic review and meta-analysis. Crit Rev Oncol Hematol. 2013;88(1):154–67.
49. Byron SA, Gartside M, Powell MA, Wellens CL, Gao F, et al. FGFR2 point mutations in 466 endometrioid endometrial tumors: relationship with MSI, KRAS, PIK3CA, CTNNB1 mutations and clinicopathological features. PLoS One. 2012;7(2):e30801.
50. Le Gallo M, O'Hara AJ, Rudd ML, Urick ME, Hansen NF, et al. Exome sequencing of serous endometrial tumors identifies recurrent somatic mutations in chromatin-remodeling and ubiquitin ligase complex genes. Nat Genet. 2012;44(12):1310–5.
51. Kharma B, Baba T, Mandai M, Matsumura N, Murphy SK, Kang HS, et al. Utilization of genomic signatures to identify high-efficacy candidate drugs for chemorefractory endometrial cancers. Int J Cancer. 2013;133(9):2234–44.
52. Kharma B, Baba T, Matsumura N, Kang HS, Hamanishi J, et al. STAT1 drives tumor progression in serous papillary endometrial cancer. Cancer Res. 2014;74(22):6519–30.
53. Chen MM, O'Mara TA, Thompson DJ, Painter JN, Australian National Endometrial Cancer Study Group (ANECS), et al. GWAS meta-analysis of 16 852 women identifies new susceptibility locus for endometrial cancer. Hum Mol Genet. 2016;25(12):2612–20.

54. Welter D, MacArthur J, Morales J, Burdett T, Hall P, et al. The NHGRI GWAS Catalog, a curated resource of SNP-trait associations. Nucleic Acids Res. 2014;42(Database issue):D1001–6.
55. Wadolowska L, Kowalkowska J, Czarnocinska J, Jezewska-Zychowicz M, Babicz-Zielinska E. Comparing dietary patterns derived by two methods and their associations with obesity in Polish girls aged 13–21 years: the cross-sectional GEBaHealth study. Perspect Public Health. 2016 Nov 29. pii: 1757913916679859. [Epub ahead of print].
56. Evangelou E, Ioannidis JP. Meta-analysis methods for genome-wide association studies and beyond. Nat Rev Genet. 2013;14(6):379–89.
57. Cheng TH, Thompson DJ, O'Mara TA, Painter JN, Glubb DM, et al. Five endometrial cancer risk loci identified through genome-wide association analysis. Nat Genet. 2016;48(6):667–74.
58. Murakami R, Matsumura N, Brown JB, et al. Prediction of taxane and platinum sensitivity in ovarian cancer based on gene expression profiles. Gynecol Oncol. 2016;141:49–56.
59. Gourley C, McCavigan A, Perren T, et al. Molecular subgroup of high-grade serous ovarian cancer (HGSOC) as a predictor of outcome following bevacizumab. J Clin Oncol. 2014;32:5s. abstr 5502
60. Winterhoff BJN, Kommoss S, Oberg AL, et al. Bevacizumab and improvement of progression-free survival (PFS) for patients with the mesenchymal molecular subtype of ovarian cancer. J Clin Oncol. 2014;32:5s. abstr 5509

Diversity in Pathology and Genomics in Ovarian Cancer

7

Noriomi Matsumura

Abstract

Epithelial ovarian cancer comprises of various histologic subtypes including high-grade serous, clear cell, endometrioid, mucinous, and low-grade serous carcinoma. Differences in histologic subtypes reflect distinct biological and clinical features. Recent progress on cancer genome analyses using the rapidly developing sequencing technologies has unveiled molecular background of ovarian cancer.

Keywords

Ovarian cancer • Histology • Genome

7.1 High-Grade Serous Ovarian Carcinoma (HGSOC)

7.1.1 The Cancer Genome Atlas (TCGA) Project

The International Cancer Genome Consortium (ICGC) was established to analyze the genome-wide abnormalities of 50 kinds of malignant tumors. The Cancer Genome Atlas (TCGA) is a study conducted as the ICGC project in the USA, which initially analyzed ovarian, lung, and brain cancers. As for the ovarian cancer, whole-exome sequencing, SNP array (to analyze copy number alterations), mRNA expression microarray, DNA methylation microarray, and microRNA microarray, along with the clinical information, were published for more than 300 HGSOC cases [1]. These analyses identified germline mutations in *BRCA1* (9%) and *BRCA2* (8%). Strikingly, nearly all the HGSOC cases harbored somatic mutations in *TP53* (96%).

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Other somatic mutations included *NF1* (4%), *BRCA1* (3%), *BRCA2* (3%), *CDK12* (3%), and *RB1* (2%). Additionally, extensive copy number aberrations were detected. The integrated analysis revealed pathway deregulation in RB (67%), PI3K/RAS (45%), NOTCH (22%), homologous recombination (51%), and FOXM1 transcriptional network (84%) pathways.

7.1.2 Gene Expression Profile of HGSOC and Drug Sensitivity

The TCGA analysis revealed four gene expression subtypes of HGSOC: mesenchymal, immunoreactive, differentiated, and proliferative [1]. Among these, the immunoreactive subtype characterized by immune-related gene expression showed good prognosis, whereas the mesenchymal subtype characterized by stromal gene expression showed poor prognosis [2]. Recently we showed the mesenchymal subtype might be sensitive to taxane [3]. Additionally, we developed a novel histopathological classification dividing HGSOC into four subtypes correlating with the four gene expression subtypes by focusing on the tumor microenvironment: mesenchymal transition (MT), defined by a remarkable desmoplastic reaction; immune reactive (IR) by lymphocytes infiltrating the tumor; solid and proliferative (SP) by a solid growth pattern; and papilloglandular (PG) by a papillary architecture (Fig. 7.1) [4].

In 2014, two interesting data were presented at the American Society of Clinical Oncology meeting analyzing relationship between the HGSOC gene expression subtypes and the bevacizumab (Bev) sensitivity. Gourley et al. performed mRNA expression microarray analysis of 283 HGSOC cases registered for the ICON7 trial

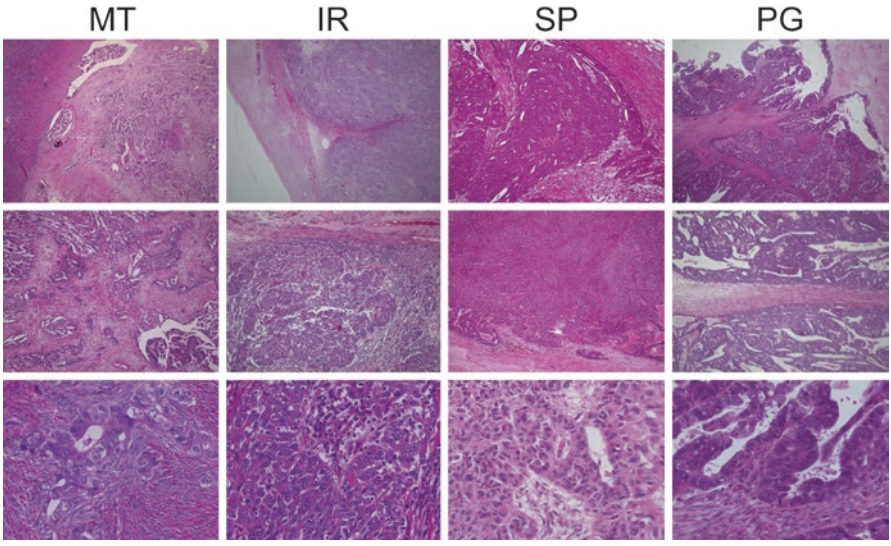

Fig. 7.1 Four histopathological subtypes of HGSOC. *MT* mesenchymal transition, *IR* immune reactive, *SP* solid and proliferative, *PG* papilloglandular. Reused from [4] with permission

[5], which demonstrated improved progression-free survival by addition of Bev to paclitaxel-carboplatin regimen in ovarian cancer [6]. Surprisingly, they found that addition of Bev was significantly associated with poor prognosis in tumors with elevated expression of immune-related genes. Winterhoff et al. also performed mRNA expression microarray analysis of 359 ovarian cancer cases registered for the ICON7 trial [7]. They divided the cases into four gene expression subtypes like the TCGA cases and found that addition of Bev improved survival in proliferative and mesenchymal subtypes. These studies suggest gene expression profile could serve a biomarker to select HGSOC cases Bev should be used for.

7.1.3 Molecular Mechanisms of Chemoresistance in HGSOC

Patch et al. analyzed whole-genome sequencing, transcriptome, DNA methylation, copy number alterations, and microRNA expression of 92 cases of HGSOC by focusing on the mechanism of chemoresistance [8]. They found gene breakage frequently occurs in tumor suppressor genes including *RB1, NF1, RAD51B,* and *PTEN. CCNE1* amplification was common in primary resistant and refractory cases. On the other hand, homologous recombination pathway-deficient cases, having extensive copy number alterations and increased single nucleotide variants, were sensitive to chemotherapy. Mechanism of acquired resistance included gene breakage of tumor suppressor genes, reversion mutation of *BRCA1/2* mutated cases, and upregulation of *BRCA1* gene expression by demethylation of the methylated *BRCA1* promoter region in a primary tumor. Additionally, gene fusion of *ABCB1* with *SLC25A40* promoter caused upregulation of *ABCB1* expression, which can cause increased excretion of chemotherapeutic agents.

7.1.4 PARP Inhibitors

Among the different subtypes of ovarian cancers, the highest rates of germline *BRCA1* and *BRCA2* mutations (8–18%) occur in HGSOC [9, 10]. Germline BRCA mutation-associated ovarian cancers have a relatively distinct clinical behavior characterized by an earlier age at diagnosis, improved survival, higher response rates to platinum, and sensitivity to poly(ADP-ribose) polymerase (PARP) inhibitors [11]. Single-strand DNA breaks, repaired by the mechanism PARP is involved in, are not repaired until DNA replication and generation of double-strand DNA breaks in the presence of a PARP inhibitor. Therefore, following the concept of "synthetic lethality," PARP inhibitors cause apoptosis of homologous recombination (HR)-deficient tumor cells. The clinical proof of this concept in ovarian cancer was first shown in a phase I study of the PARP inhibitor, olaparib [12], and subsequently supported in a phase II trial of olaparib in recurrent ovarian cancer patients with germline BRCA1 or BRCA2 mutation [13].

Ovarian cancers with somatic mutations of *BRCA1/2* are observed in about one-fifth of germline mutation cases [11]. Ovarian cancer patients with somatic BRCA1 or

BRCA2 mutations probably benefit from treatment with PARP inhibitors like those with germline BRCA mutations. The European Medicines Agency approves olaparib as maintenance therapy in platinum-sensitive ovarian cancer including all patients with a germline or somatic BRCA mutation. The number of patients with somatic BRCA mutations analyzed so far is relatively low. Further clinical trials will clarify the clinical significance of somatic BRCA mutations as biomarkers of PARP inhibitors.

It became increasingly apparent that a proportion of ovarian cancers without BRCA mutation also share clinical features of BRCA mutation-associated cases, including platinum sensitivity. This concept, termed as "BRCAness," now indicates a situation where HR DNA repair deficiency is present, but no BRCA1 or BRCA2 mutation is detected. Importantly, PARP inhibitors show activity in clinical trials for ovarian cancer without BRCA mutations [14, 15]. Several groups have studied to find markers of the "BRCAness" by using gene expression profiling, proteomics, or genomic instability scores [11]. Additionally, as the accumulation of RAD51 at the DNA lesion is a marker of HR proficiency, its absence following DNA damage can be a functional biomarker of HR deficiency [16]. Actually, detection of RAD51 foci predicted response to chemotherapy and PARP inhibition [17–19]. The quantification of phosphorylated gamma-H2AX has been used to measure the amount of DNA damage [20]. More recently, SNP array-based signatures of chromosome instability have been reported as biomarkers of HR deficiency and sensitivity to PARP inhibitors [21].

7.2 Ovarian Clear Cell Carcinoma (OCCC)

7.2.1 Gene Expression of OCCC Closely Related to Oxidative Stress

Ovarian clear cell carcinoma (OCCC) is a chemoresistant subtype. Although it is rare in Western countries, it is increasing in Japan. It is often associated with an endometriotic cyst. We analyzed the content of endometriotic cysts and revealed it contained huge amount of iron, oxidative stress marker LPO, and 8-0HdG, a marker of DNA damage caused by oxidative stress. When the content of endometriotic cyst or iron was added to immortalized ovarian surface epithelial cells, intracellular reactive oxygen species (ROS) was elevated. Then the content of endometriotic cyst or iron increased DNA mutations. Therefore, we hypothesized iron-mediated reactive oxygen species may cause DNA mutations and carcinogenesis [22].

Next we conducted a gene expression microarray analysis and identified clear cell-specific genes, termed "OCCC signature." These genes contained *HNF1B*, *SOD2*, *HIF1A*, *IL6*, and *STAT3*. They enriched gene ontology terms related to oxidative stress and glucose metabolism. Many of these genes had HNF1-binding motif in their promoter regions, suggesting that many are downstream genes of HNF1B. Interestingly, the OCCC signature genes were upregulated in immortalized ovarian surface epithelial cells by adding the content of endometriotic cyst or iron [23]. A methylation DNA microarray analysis revealed OCCC is distinct from other

subtypes in terms of the methylation profile. ER pathway genes were hypermethylated and downregulated, while HNF-1 pathway genes were hypomethylated and upregulated [24]. Therefore, the OCCC-specific gene expression seems to be stabilized via the epigenetic mechanism.

7.2.2 Roles of HNF1B in OCCC

We investigated the roles of HNF1B in metabolism of OCCC cells. We found HNF1B increases glucose uptake by increasing GLUT1 expression, a major glucose transporter [25]. We conducted a metabolome analysis and found that the upregulated HNF1B expression enhances anaerobic glucose metabolism, that is, Warburg effect, which is known to cause resistance to oxidative stress. We further analyzed the relationship between HNF1B and oxidative stress in clear cell carcinoma. Knockdown of HNF1B decreased the amount of glutathione, a redox substance. This was due to the decreased intracellular cystine, a substrate for the biosynthesis of glutathione, via the decreased expression of rBAT, a cystine transporter. Then, we found HNF1B knockdown increased intracellular ROS and cytotoxicity by iron-induced oxidative stress. Furthermore, in hypoxia, suppression of HNF1B increased sensitivity to cisplatin. Collectively, HNFB in clear cell carcinoma causes resistance to oxidative stress and platinum [26].

It is known that a germline mutation of *HNF1B* causes hereditary diabetes mellitus and renal hypoplasia. We found clear cell carcinoma is very similar to kidney cancer through the expression of *HNF1B* and its target genes. As sorafenib is effective for kidney cancer, we treated OCCC on nude mice by sorafenib and observed a prominent effect [27]. Then we treated two chemoresistant ovarian clear cell carcinoma patients by sorafenib and observed antitumor effect [28]. Therefore, the similarity of ovarian clear cell carcinoma with kidney cancer implies the efficacy of sorafenib for ovarian clear cell carcinoma.

7.2.3 Genetic Analyses of OCCC

By an analysis of exome sequences of eight OCCC tumors, Jones et al. identified four genes that were mutated in at least two tumors; *PIK3CA*, *KRAS*, *PPP2R1A*, and *ARID1A*. Out of 42 OCCCs, 57% had mutations in *ARID1A* [29]. In another study, *ARID1A* mutations were seen in 55 of 119 OCCCs (46%), 10 of 33 endometrioid carcinomas (30%), and none of the 76 HGSOCs [30]. Out of 97 OCCCs, mutations of *PIK3CA*, *TP53*, *KRAS*, *PTEN*, *CTNNB1*, and *BRAF* occurred in 33%, 15%, 7%, 5%, 3%, and 1% of samples, respectively [31]. Consistently, by an exome sequencing analysis of 39 OCCCs, we recently found *ARID1A* was the top mutated gene and *PIK3CA* was the second one (paper in preparation). The integrated analysis of gene mutations and copy number variations revealed KRAS-PI3K pathway, SWI/SNF complex, and MYC-RB pathway were the most frequently altered pathways.

7.2.4 Analyses of Precursor Lesions of OCCC

ARID1A protein expression was analyzed in endometriosis-associated OCCCs (n = 28) and clear cell adenofibroma-associated OCCCs (n = 14). Among the precursor lesions adjacent to the 23 ARID1A-deficient carcinomas, 86% of the non-atypical endometriosis (12 of 14) and 100% of the atypical endometriosis (14 of 14), benign (3 of 3), and borderline (6 of 6) clear cell adenofibroma components were ARID1A deficient. In contrast, in the 19 patients with ARID1A-intact carcinomas, all of the adjacent precursor lesions were ARID1A positive [32]. In an analysis of 23 endometriosis-associated OCCCs, *PIK3CA* gene mutations were detected in 10/23 (43%) carcinomas. The identical mutations were detected in the adjacent endometriotic epithelium in nine of ten (90%) cases [33]. Using whole-genome sequencing of seven endometriosis-associated OCCCs, *ARID1A* and *PIK3CA* mutations were found in concurrent endometriosis regardless of any cytological atypia when present in the OCCC [34]. Collectively, these data indicate ARID1A and PIK3CA mutations are early event in the carcinogenic process of OCCC, which mutations are usually found in endometriotic lesions adjacent to OCCCs.

Recently, it was reported that *Pik3ca* and *Arid1a* mutations in the ovaries generate clear cell carcinoma in mice [35]. This tumor highly expressed *Hnf1b*. We hypothesize that iron-induced oxidative stress in endometriotic lesions may cause DNA damage, causing mutations of *PIK3CA* and *ARID1A*, which may lead to carcinogenesis of ovarian clear cell carcinoma. HNF1B plays important roles in Warburg effect and resistance to oxidative stress. This may be important for the progression of OCCC in the stressful condition of endometriotic cysts and for the development of platinum resistance. Further epigenetic changes, gene mutations, and copy number alterations may cause stabilization of OCCC-specific gene expression and biological features including chemoresistance.

7.3 Ovarian Endometrioid Carcinoma (OEC)

7.3.1 Genetic Analysis of OEC

Wu et al. analyzed gene mutations in OEC samples with different grades (grade 1; n = 20, grade 2; n = 26, grade 3; n = 26) and found mutations in *CTNNB1* (13, 5, 0%), *APC* (5, 0, 0%), *KRAS* (10, 12, 0%), *PTEN* (20, 8, 0%), *PIK3CA* (20, 8, 0%), and *TP53* (15, 46, 65%), respectively. Therefore, high-grade OECs are likely to harbor *TP53* mutations, while low-grade OECs frequently harbor mutations of Wnt/β-catenin pathway and/or KRAS/PI3K pathway genes. Additionally, inactivation of the *Pten* and *Apc* in murine ovaries resulted in the formation of adenocarcinomas morphologically and biologically similar to human OECs [36]. More recently, *ARID1A* mutations were reported in 10 of 33 EOCs (30%) [30]. Consistently, another group reported mutations of *CTNNB1* (53%), *PIK3CA* (40%), *ARID1A* (30%), *PTEN* (17%), *KRAS* (33%), *PPP2R1A* (17%), and *TP53* (7%) in low-grade (grade 1 and 2) OECs (n = 30) [37].

7.3.2 Genetic Analysis of Synchronous Endometrial and Ovarian Carcinoma

Five to ten percentage of women with OECs present with concurrent endometrial carcinoma. Based on both targeted and exome sequencing of 18 synchronous endometrial and ovarian tumors, most (17/18) cases showed evidence of clonality. Importantly, 10 of 11 cases that fulfilled clinicopathological criteria that would lead to classification as independent endometrial and ovarian primary carcinomas showed evidence of clonality [38]. Therefore, the genome-wide analysis demonstrated that most synchronous endometrial and ovarian carcinoma tumors develop from a clonal origin.

7.4 Mucinous Ovarian Tumors

7.4.1 Genetic Analysis of Mucinous Ovarian Tumors

Ryland et al. performed genetic analysis of a total of 82 mucinous ovarian tumors, which included exome sequencing of 24 tumors and a validation cohort of benign 58 tumors for specific gene regions. Benign, borderline, and carcinoma samples harbored mutations in *BRAF* (0, 10, 23%), *TP53* (9, 14, 52%), and *RNF43* (0, 7, 20%), respectively, in which mutations were associated with progression of the disease. Other recurrent, but not associated with progression, mutations were found in *KRAS* (54%), *CDKN2A* (16%), *ARID1A* (8%), *ELF3* (6%), *GNAS* (6%), *ERBB3* (5%), and *KLF5* (5%) [39]. In another study, *RNF43* mutations were observed with a frequency of 2/22 (9%) in mucinous ovarian borderline tumors and 6/29 (21%) in mucinous ovarian carcinomas [40].

Overexpression and amplification of HER2 is observed in 11/176 (6%) mucinous borderline tumors and 29/154 (19%) mucinous cancers. KRAS mutations were seen in 26/33 (79%) mucinous borderline tumors and 31/71 (44%) mucinous cancers. Importantly, KRAS mutations and HER2 amplification coexisted only in 5% of the cases and were near mutually exclusive [41].

7.4.2 Analysis of Coexisting Brenner Tumors

Brenner tumors are often associated with mucinous cystic neoplasm. DNA from six Brenner tumors with paired mucinous tumors, two Brenner tumors not associated with a mucinous tumor, and two atypical proliferative Brenner tumors was extracted and sequenced using a 358-gene next-generation sequencing assay. There was high concordance of the variants between paired samples (40–75%; $P < 0.0001$), supporting a shared origin or progression. Four of the six mucinous tumors and the two atypical proliferative Brenner tumors showed RAS mutations [42]. These results suggest RAS mutations are required for Brenner tumors to develop mucinous tumors or atypical proliferative Brenner tumors.

7.5 Serous Borderline Tumor (SBT) and low-Grade Serous Ovarian Carcinoma (LGSOC)

It has been well established that LGSOC and HGSOC are fundamentally different types of tumors. LGSOC can develop from SBT. No effective chemotherapy exists for patients with metastatic LGSOCs.

Jones et al. performed exome sequencing of eight LGSOCs and identified a total of 70 somatic mutations in 64 genes in seven of these tumors. The eighth case displayed a mutator phenotype with 783 somatic mutations, including a nonsense mutation in the mismatch repair gene, *MSH2*. Representative mutations were analyzed in an additional nine LGSOCs and 10 SBTs. The genes showing the most frequent mutations were *BRAF* and *KRAS*, occurring in 38% and 19% of the tumors, respectively [43]. Hunter et al. performed copy number analysis and mutation hotspot analysis of *KRAS*, *BRAF*, *NRAS*, *HRAS*, *ERBB2*, and *TP53* for 57 SBTs and 19 LGSOCs. Copy number aberrations were detected in 61% of SBTs and 100% of LGSOCs. Oncogenic RAS/RAF/ERBB2 mutations were detected in 83% (47/57) of SBTs and 63% (12/19) of LGSOCs. Additionally, exome sequencing for 13 SBTs and 10 LGSOCs identified *BRAF*, *KRAS*, *NRAS*, *USP9X*, and *EIF1AX* as the most frequently mutated genes [44]. These results indicate that oncogenic RAS/RAF mutations are crucial in the development of SBT/LGSOC.

7.6 Small Cell Carcinoma of the Ovary, Hypercalcemic Type (SCCOHT)

SCCOHT is a rare disease, but it is the most common undifferentiated ovarian malignancy in women under 40 years of age. Witkowski et al. discovered germline mutations in *SMARCA4* in four families with SCCOHT. Immunohistochemical analysis showed loss of SMARCA4 protein in 38 of 40 SCCOHT cases. Sequencing identified at least one germline or somatic *SMARCA4* mutation in 30 of 32 cases. Therefore, alterations in *SMARCA4* is the major cause of SCCOHT [45].

Fahiminiya et al. performed exome sequencing on 14 SCCOHT tumors and confirmed that *SMARCA4* is the only recurrently mutated gene in SCCOHT. Because alterations in *SMARCA4* have been reported in atypical teratoid/rhabdoid tumors (ATRTs) and malignant rhabdoid tumors (MRTs), they analyzed if SCCOHT was biologically similar to ATRT. By the analysis of 45 SCCOHTs, 65 ATRTs, and 92 HGSOCs, they demonstrated the genomic and epigenomic signatures of SCCOHT were more similar to those of ATRT than HGSC [46].

References

1. Cancer Genome Atlas Research Network. Integrated genomic analyses of ovarian carcinoma. Nature. 2011;474:609–15.
2. Verhaak RG, Tamayo P, Yang JY, et al. Prognostically relevant gene signatures of high-grade serous ovarian carcinoma. J Clin Invest. 2013;123:517–25.

3. Murakami R, Matsumura N, Brown JB, et al. Prediction of taxane and platinum sensitivity in ovarian cancer based on gene expression profiles. Gynecol Oncol. 2016;141:49–56.
4. Murakami R, Matsumura N, Mandai M, et al. Establishment of a novel histopathological classification of high-grade serous ovarian carcinoma correlated with prognostically distinct gene expression subtypes. Am J Pathol. 2016;186:1103–13.
5. Perren TJ, Swart AM, Pfisterer J, et al. A phase 3 trial of bevacizumab in ovarian cancer. N Engl J Med. 2011;365:2484–96.
6. Gourley C, McCavigan A, Perren T, et al. Molecular subgroup of high-grade serous ovarian cancer (HGSOC) as a predictor of outcome following bevacizumab. J Clin Oncol. 2014;32:5s. abstr5502.
7. Winterhoff BJN, Kommoss S, Oberg AL, et al. Bevacizumab and improvement of progression-free survival (PFS) for patients with the mesenchymal molecular subtype of ovarian cancer. J Clin Oncol. 2014;32:5s. abstr 5509.
8. Patch AM, Christie EL, Etemadmoghadam D, et al. Whole-genome characterization of chemoresistant ovarian cancer. Nature. 2015;521:489–94.
9. Risch HA, McLaughlin JR, Cole DE, et al. Prevalence and penetrance of germline BRCA1 and BRCA2 mutations in a population series of 649 women with ovarian cancer. Am J Hum Genet. 2001;68:700–10.
10. Pal T, Permuth-Wey J, Betts JA, et al. BRCA1 and BRCA2 mutations account for a large proportion of ovarian carcinoma cases. Cancer. 2005;104:2807–16.
11. Moschetta M, George A, Kaye SB, Banerjee S. BRCA somatic mutations and epigenetic BRCA modifications in serous ovarian cancer. Ann Oncol. 2016;27(8):1449–55.
12. Fong PC, Boss DS, Yap TA, et al. Inhibition of poly(ADP-ribose) polymerase in tumors from BRCA mutation carriers. N Engl J Med. 2009;361:123–34.
13. Audeh MW, Carmichael J, Penson RT, et al. Oral poly(ADP-ribose) polymerase inhibitor olaparib in patients with BRCA1 or BRCA2 mutations and recurrent ovarian cancer: a proof-of-concept trial. Lancet. 2010;376:245–51.
14. Ledermann J, Harter P, Gourley C, et al. Olaparib maintenance therapy in patients with platinum-sensitive relapsed serous ovarian cancer: a preplanned retrospective analysis of outcomes by BRCA status in a randomised phase 2 trial. Lancet Oncol. 2014;15:852–61.
15. Gelmon KA, Tischkowitz M, Mackay H, et al. Olaparib in patients with recurrent high-grade serous or poorly differentiated ovarian carcinoma or triple-negative breast cancer: a phase 2, multicentre, open-label, non-randomised study. Lancet Oncol. 2011;12:852–61.
16. Michels J, Vitale I, Saparbaev M, et al. Predictive biomarkers for cancer therapy with PARP inhibitors. Oncogene. 2014;33:3894–907.
17. Willers H, Taghian AG, Luo CM, et al. Utility of DNA repair protein foci for the detection of putative BRCA1 pathway defects in breast cancer biopsies. Mol Cancer Res. 2009;7:1304–9.
18. Graeser M, McCarthy A, Lord CJ, et al. A marker of homologous recombination predicts pathologic complete response to neoadjuvant chemotherapy in primary breast cancer. Clin Cancer Res. 2010;16:6159–68.
19. Mukhopadhyay A, Elattar A, Cerbinskaite A, et al. Development of a functional assay for homologous recombination status in primary cultures of epithelial ovarian tumor and correlation with sensitivity to poly (ADP-ribose) polymerase inhibitors. Clin Cancer Res. 2010;16:2344–51.
20. Redon CE, Nakamura AJ, Zhang YW, et al. Histone gammaH2AX and poly (ADPribose) as clinical pharmacodynamic biomarkers. Clin Cancer Res. 2010;16:4532–42.
21. Watkins JA, Irshad S, Grigoriadis A, Tutt AN. Genomic scars as biomarkers of homologous recombination deficiency and drug response in breast and ovarian cancers. Breast Cancer Res. 2014;16:211.
22. Yamaguchi K, Mandai M, Toyokuni S, et al. Contents of endometriotic cysts, especially the high concentration of free iron, are a possible cause of carcinogenesis in the cysts through the iron-induced persistent oxidative stress. Clin Cancer Res. 2008;14:32–40.
23. Yamaguchi K, Mandai M, Oura T, et al. Identification of an ovarian clear cell carcinoma gene signature that reflects inherent disease biology and the carcinogenic processes. Oncogene. 2010;29:1741–52.

24. Yamaguchi K, Huang Z, Matsumura N, et al. Epigenetic determinants of ovarian clear cell carcinoma biology. Int J Cancer. 2014;135:585–97.
25. Okamoto T, Mandai M, Matsumura N, et al. Hepatocyte nuclear factor-1B (HNF-1B) promotes glucose uptake and glycolytic activity in ovarian clear cell carcinoma. Mol Carcinog. 2015;54:35–49.
26. Amano Y, Mandai M, Yamaguchi K, et al. Metabolic alterations caused by HNF1B expression in ovarian clear cell carcinoma contribute to cell survival. Oncotarget. 2015;6:26002–17.
27. Matsumura N, Mandai M, Okamoto T, et al. Sorafenib efficacy in ovarian clear cell carcinoma revealed by transcriptome profiling. Cancer Sci. 2010;101:2658–63.
28. Koshiyama M, Matsumura N, Baba T, et al. Two cases of recurrent ovarian clear cell carcinoma treated with sorafenib. Cancer Biol Ther. 2014;15:22–5.
29. Jones S, Wang TL, Shih IM, et al. Frequent mutations of chromatin remodeling gene ARID1A in ovarian clear cell carcinoma. Science. 2010;330:228–31.
30. Wiegand KC, Shah SP, Al-Agha OM, et al. ARID1A mutations in endometriosis-associated ovarian carcinomas. N Engl J Med. 2010;363:1532–43.
31. Kuo KT, Mao TL, Jones S, et al. Frequent activating mutations of PIK3CA in ovarian clear cell carcinoma. Am J Pathol. 2009;174:1597–601.
32. Yamamoto S, Tsuda H, Takano M, et al. Loss of ARID1A protein expression occurs as an early event in ovarian clear-cell carcinoma development and frequently coexists with PIK3CA mutations. Mod Pathol. 2012;25:615–24.
33. Yamamoto S, Tsuda H, Takano M, et al. PIK3CA mutation is an early event in the development of endometriosis-associated ovarian clear cell adenocarcinoma. J Pathol. 2011;225:189–94.
34. Anglesio MS, Bashashati A, Wang YK, et al. Multifocal endometriotic lesions associated with cancer are clonal and carry a high mutation burden. J Pathol. 2015;236:201–9.
35. Chandler RL, Damrauer JS, Raab JR, et al. Coexistent ARID1A-PIK3CA mutations promote ovarian clear-cell tumorigenesis through pro-tumorigenic inflammatory cytokine signalling. Nat Commun. 2015;6:6118.
36. Wu R, Hendrix-Lucas N, Kuick R, et al. Mouse model of human ovarian endometrioid adenocarcinoma based on somatic defects in the Wnt/beta-catenin and PI3K/Pten signaling pathways. Cancer Cell. 2007;11:321–33.
37. McConechy MK, Ding J, Senz J, et al. Ovarian and endometrial endometrioid carcinomas have distinct CTNNB1 and PTEN mutation profiles. Mod Pathol. 2014;27:128–34.
38. Anglesio MS, Wang YK, Maassen M, et al. Synchronous endometrial and ovarian carcinomas: evidence of clonality. J Natl Cancer Inst. 2016;108:djv428.
39. Ryland GL, Hunter SM, Doyle MA, et al. Mutational landscape of mucinous ovarian carcinoma and its neoplastic precursors. Genome Med. 2015;7:87.
40. Ryland GL, Hunter SM, Doyle MA, et al. RNF43 is a tumour suppressor gene mutated in mucinous tumours of the ovary. J Pathol. 2013;229:469–76.
41. Anglesio MS, Kommoss S, Tolcher MC, et al. Molecular characterization of mucinous ovarian tumours supports a stratified treatment approach with HER2 targeting in 19% of carcinomas. J Pathol. 2013;229:111–20.
42. Tafe LJ, Muller KE, Ananda G, et al. Molecular genetic analysis of ovarian Brenner tumors and associated mucinous epithelial neoplasms. High variant concordance and identification of mutually exclusive RAS driver mutations and MYC amplification. Am J Pathol. 2016;186:671–7.
43. Jones S, Wang TL, Kurman RJ, et al. Low-grade serous carcinomas of the ovary contain very few point mutations. J Pathol. 2012;226:413–20.
44. Hunter SM, Anglesio MS, Ryland GL, et al. Molecular profiling of low grade serous ovarian tumours identifies novel candidate driver genes. Oncotarget. 2015;6:37663–6677.
45. Witkowski L, Carrot-Zhang J, Albrecht S, et al. Germline and somatic SMARCA4 mutations characterize small cell carcinoma of the ovary, hypercalcemic type. Nat Genet. 2014;46:438–43.
46. Fahiminiya S, Witkowski L, Nadaf J, et al. Molecular analyses reveal close similarities between small cell carcinoma of the ovary, hypercalcemic type and atypical teratoid/rhabdoid tumor. Oncotarget. 2016;7:1732–40.

Hereditary Ovarian and Endometrial Cancers: Current Management

8

Akira Hirasawa and Daisuke Aoki

Abstract

Hereditary breast and ovarian cancer (HBOC), Lynch syndrome, and Peutz-Jeghers syndrome (PJS) are including hereditary gynecological tumors. While such tumors share common phenotypes with non-hereditary (sporadic) tumors, they are autosomal dominant diseases; therefore, knowledge of a family's disease history is the first step towards identifying hereditary tumors.

Keywords

Hereditary tumor • Hereditary breast and ovarian cancer • Lynch syndrome • Genetic testing • Risk-reducing salpingo-oophorectomy

8.1 Introduction

The endpoint of clinical research on hereditary tumors is ***to reduce mortality for cancer***. However, effective screening systems have not been established for detecting ovarian cancer; therefore, risk-reducing salpingo-oophorectomy (RRSO) remains the most effective ovarian cancer prevention strategy for mutation carriers. Furthermore, genetic testing for hereditary tumors is used alongside companion diagnostics to select the appropriate chemotherapy regimens, such as poly (ADP-ribose) polymerase (PARP) inhibitors for carriers of the *BRCA1* and/or *BRCA2* (*BRCA1/2*) mutations.

In this chapter, hereditary tumors, genetic testing, cancer prevention for unaffected mutation carriers, and companion diagnostics for ovarian cancer patients with *BRCA1/2* mutations are described.

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8.2 Carcinogenesis of Hereditary Tumors

Both genetic and environmental factors can cause cancers (Fig. 8.1). Germ-line mutations are the underlying cause of hereditary tumors, many of which are autosomal dominant diseases. If a parent harbors a mutant allele, 50% of the mutation carrier's children are likely to be mutation carriers (Fig. 8.2). The two-hit

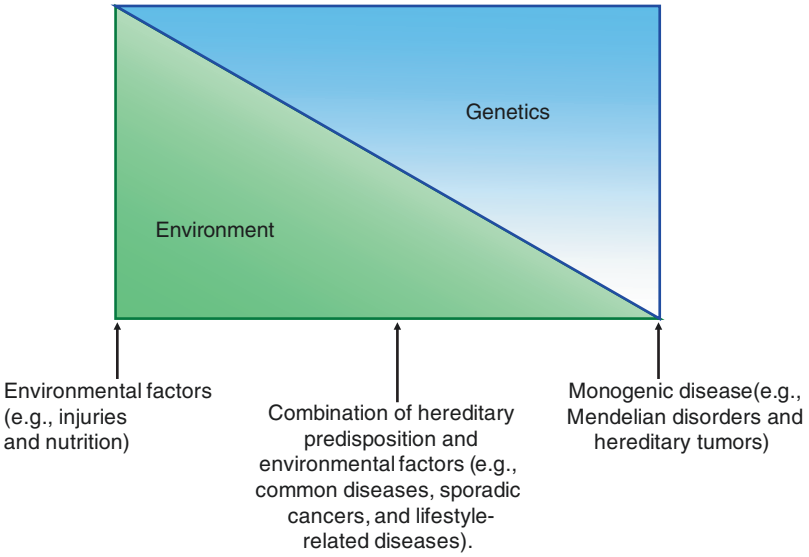

Fig. 8.1 Environmental and genetic factors in diseases. Many diseases, including cancer, are influenced by both environmental and genetic factors. Hereditary tumors are mainly caused by genetic factors. Examples of environmental factors for carcinogenesis are chemicals, smoking, ultraviolet light exposure, diet, viruses, and hormones

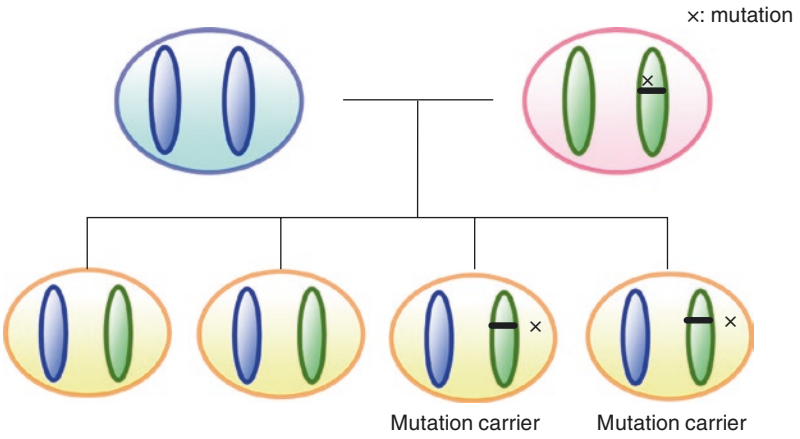

Fig. 8.2 Autosomal dominant inheritance pattern. Many hereditary tumors show an autosomal dominant pattern. Germ-line mutations are represented by (×). Fifty percent of the offspring of a mutantion carriers are also likely to carry mutations

hypothesis formulated by Alfred Knudson [1] stated that multiple hits are necessary to cause cancer (Fig. 8.3); this may explain why hereditary tumors frequently involve onset at a younger age and tend to exhibit multiple lesions and bilateral diseases more frequently (Fig. 8.4) [1].

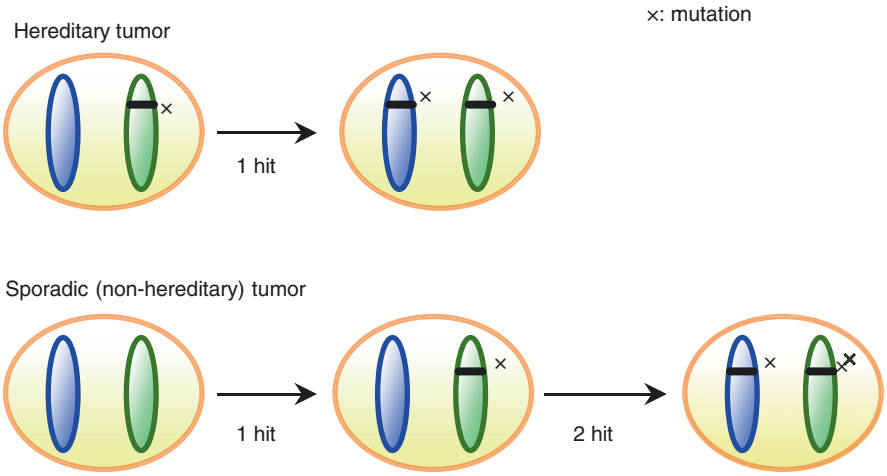

Fig. 8.3 Two-hit theory (Knudson's hypothesis). This hypothesis was based on statistical models from retinoblastoma patients. In this representation, two hits are required for carcinogenesis. Carriers of hereditary germ-line mutations already harbor the first 'hit' and the second hit follows after birth

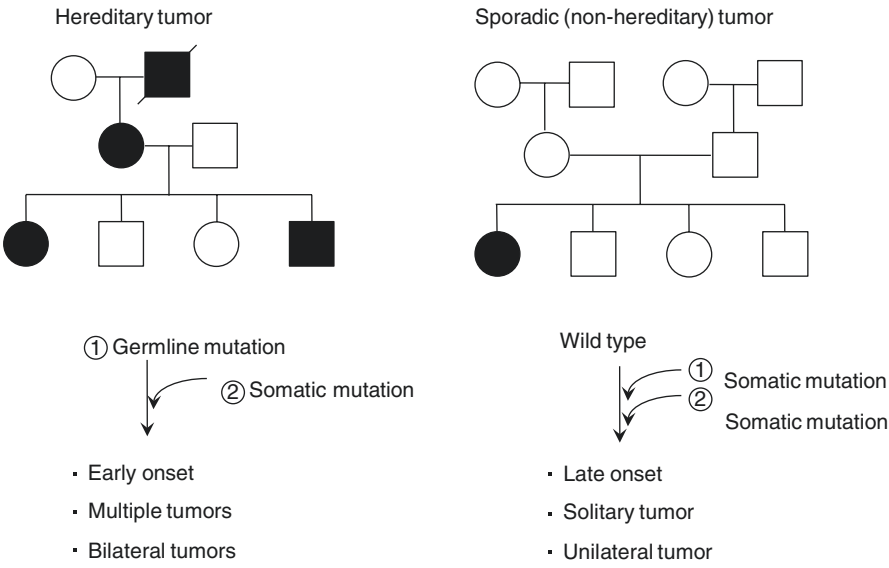

Fig. 8.4 Hereditary and non-hereditary tumors. A comparison of hereditary vs. sporadic (non-hereditary) tumors. Hereditary tumors have different characteristics than sporadic tumors; multiple affected persons can be found in the pedigree. Moreover, onset at a younger age, multiple tumors, and bilateral tumors can occur. This phenomenon can be explained by the two-hit theory

Table 8.1 Hereditary gynecologic cancers

Syndrome	Related tumors and typical phenotype	Associated gene
Hereditary breast and ovarian cancer syndrome	Breast cancer (including male breast cancer)	*BRCA1*
	Ovarian cancer, fallopian tube cancer, peritoneal cancer	*BRCA2*
	Prostate cancer	
	Pancreatic cancer	
Lynch syndrome	Colorectal cancer	*MSH2*
	Endometrial cancer	*MLH1*
	Ovarian cancer	*PMS2*
	Small intestinal cancer	*MSH6*
	Renal pelvic, or ureteral cancer	
	Gastric cancer	
	Hepatobiliary cancer	
	Sebaceous neoplasms of the skin in Muir-Torre syndrome	
Peutz-Jeghers syndrome	Gastrointestinal polyposis	*STK11*
	Mucocutaneous pigmentation	
	Colorectal, stomach and small bowel cancers	
	Adenoma malignum of the cervix	
	Sertoli cell tumors of the testes	
	Sex cord tumors with annular tubules	
	Ovarian tumor	
	Breast cancer	
	Pancreatic cancer	
Cowden syndrome	Breast cancer	*PTEN*
	Thyroid cancer	
	Macrocephaly	
	Endometrial carcinoma	

8.3 Hereditary Gynecologic Cancers

Hereditary gynecologic cancers involve HBOC, Lynch syndrome, PJS, Cowden syndrome and Li–Fraumeni syndrome. Table 8.1 presents a list of hereditary gynecologic cancers with related tumors and associated genes.

8.4 Hereditary Breast and Ovarian Cancer

Pathogenic germ-line variants in *BRCA1/2* produce an increased risk of cancer in the breasts, ovaries, fallopian tubes, peritoneum, prostate, and pancreas. Individuals with male breast cancer are more commonly associated with families in which mutations in *BRCA2* are more prevalent compared with *BRCA1*. Mutations in *BRCA1/2* should be suspected in individuals with a personal or family history (i.e.,

Table 8.2 Factors in the clinical diagnosis of hereditary breast and ovarian cancer [2]

Breast cancer diagnosed at the age of 50 years or younger
Ovarian cancer
Multiple primary breast cancers in either the same or contralateral breast
Comorbid breast and ovarian cancers
Male breast cancer
Triple-negative (estrogen receptor negative, progesterone receptor negative, and HER2 negative) breast cancer
Pancreatic cancer with breast or ovarian cancer in the same individual or on the same side of the family
Ashkenazi Jewish ancestry
Two or more relatives with breast cancer, one under the age of 50
Three or more relatives with breast cancer at any age
A previously identified *BRCA1* or *BRCA2* pathogenic variant in the family

"Breast cancer" includes both invasive cancer and ductal carcinoma in situ (DCIS). "Ovarian cancer" includes epithelial ovarian cancer, fallopian tube cancer, and primary peritoneal cancer

Table 8.3 The lifetime risk for hereditary breast and ovarian-related cancers in individuals carrying pathogenic variants of *BRCA1/2* [2]

Cancer type	Risk (%)
Breast cancer	40–80
Ovarian cancer	11–40
Male breast cancer	1–10
Prostate cancer	Up to 39
Pancreatic cancer	1–7

in a first-, second-, or third-degree relative in either lineage) on the basis of any of the criteria listed in Table 8.2 [2].

Approximately 10–15% of patients with ovarian cancers harbor *BRCA1/2* germline mutations [3, 4]. Table 8.3 shows the lifetime risk for HBOC-related cancers in patients who carry *BRCA1/2* mutations [2]. Hence, gynecologists are likely to frequently encounter patients who are *BRCA1/2* germ-line mutation carriers. Therefore, gynecologists who work in primary care are required to evaluate the genetic risks of HBOC in their patients and families.

Ovarian serous carcinoma is frequently observed in *BRCA1/2* mutation carriers, and ovarian cancers with *BRCA1/2* mutations have been reported to exhibit unique chemosensitivity and prognosis [5–7]. For example, recently developed PARP inhibitors are more effective against *BRCA1/2*-mutated ovarian cancer [8]. Therefore, *BRCA1/2* genetic testing is increasingly being performed in conjunction with companion diagnostics.

8.5 Lynch Syndrome

Lynch syndrome is caused by germ-line mutations in the mismatch repair (MMR) genes; *MLH1*, *MSH2*, *MSH6*, or *PMS2*. These mutations increase the risk of colon cancer as well as cancers of the endometrium, ovary, stomach, small intestine,

Table 8.4 Cancer risks in individuals ≤70 years with Lynch syndrome compared to the general population [2]

Cancer type	General population risk (%)	Lynch syndrome (*MLH1* and *MSH2* heterozygotes)	
		Risk (%)	Mean age of onset (years)
Colon	4.80	52–82	44–61
Endometrium	2.70	25–60	48–62 years
Stomach	<1	6–13	56 years
Ovary	1.40	4–12	42.5 years
Hepatobiliary tract	<1	1.4–4	Not reported
Urinary tract	<1	1–4	~55 years
Small bowel	<1	3–6	49 years
Brain/central nervous system	<1	1–3	~50 years
Sebaceous neoplasms	<1	1–9	Not reported

hepatobiliary tract, urinary tract, brain, and skin. Table 8.4 lists the characteristics of individuals with Lynch syndrome [2]. Colorectal and endometrial cancers are frequently found among carriers of MMR genes mutations, followed by gastric and ovarian cancers. While the risks of other Lynch syndrome-related cancers are lower, they remain elevated compared to the general population. Microsatellite instability (MSI) within tumor tissues and lower or absent expression of proteins encoded by MMR genes increase the probability of developing Lynch syndrome. Therefore, MSI or protein expression with immunohistochemistry (IHC) of MMR genes are frequently employed to screen Lynch syndrome before genetic testing of MMR genes.

8.6 Detecting Hereditary Tumors in Clinical Practice and Introducing Genetic Counseling

It is important for a primary care physician to determine the family histories of individuals with hereditary tumors; therefore, thorough interviews are necessary. If the primary physician suspects that a patient's tumor is hereditary in nature, screening of the patient's family should be considered, at least up to second-degree relatives (i.e., grandparents, uncles, aunts, nephews, nieces, and grandchildren). Moreover, genetic counseling is recommended in such cases [9, 10].

The American Congress of Obstetricians and Gynecologists has released criteria for identifying patients who are predisposed to HBOC, and for whom genetic risk assessment is recommended [11]; Table 8.5 lists these criteria. Furthermore, the Amsterdam II Criteria are applied for the clinical screening of Lynch syndrome (Table 8.6) [12]. MSI and/or IHC tests can be performed in patients suspected of

Table 8.5 Criteria for genetic risk assessment by the American Congress of Obstetricians and Gynecologists [11]

Patients with an approximate chance greater than 20–25% of having an inherited predisposition to breast and ovarian cancer, and for whom genetic risk assessment is recommended:
• Women with a personal history of both breast and ovarian cancers[a]
• Women with ovarian cancer[a] who has a close relative[b] with ovarian cancer, premenopausal breast cancer, or both
• Women with ovarian cancer[a] who are of Ashkenazi Jewish ancestry
• Women with breast cancer at age 50 years or younger who have a close relative[b] with ovarian cancer[a] or male breast cancer at any age
• Women of Ashkenazi Jewish ancestry in whom breast cancer was diagnosed at age 40 years or younger
• Women with a close relative[b] known to have a *BRCA1* or *BRCA2* mutation

[a]Cancer of the peritoneum and fallopian tubes should be considered part of the spectrum of hereditary breast and ovarian cancer syndromes
[b]Close relative is defined as a first-degree relative (mother, sister, daughter) or second-degree relative (grandmother, granddaughter, aunt, niece)

Table 8.6 The Amsterdam II criteria for the clinical screening of Lynch syndrome [12]

• Three or more family members (one of whom is a first-degree relative of the other two) with HNPCC-related cancers
• Two successive affected generations
• One or more of the HNPCC-related cancers diagnosed before the age of 50 years
• Exclusion of familial adenomatous polyposis

HNPCC hereditary nonpolyposis colorectal cancer

having Lynch syndrome. Finally, genetic testing for *BRCA1/2* or MMR genes can differentiate the diagnosis of HBOC or Lynch syndrome.

8.7 Cancer Prevention and Risk Reduction Strategies

The endpoint of hereditary tumor research is ***to reduce mortality for cancers in mutation carriers*** who are at risk. RRSO is ***recommend*** for *BRCA1/2* mutation carriers. RRSO reduces the risk of ovarian cancer for unaffected *BRCA1/2* mutation carriers by 71–96% [13–18], and is usually performed after the completion of childbearing and during premenopausal years. However, premenopausal bilateral oophorectomy produces adverse effects; early-stage menopausal symptoms such as hot flashes, fatigue, shoulder stiffness, and palpitations can give rise to coital pain, atrophic vaginitis, urethritis, urinary incontinence, skin atrophy, and obesity. Long-term problems, such as osteoporosis or osteopenia, dyslipidemia, and arteriosclerosis, can also occur. Such adverse effects require monitoring by physicians who work on women health care [19].

Furthermore, according to recent NCCN guidelines, RRSO is also ***recommend*** in mutation carriers of MMR genes (*MSH2*, *MLH1, MSH6, PMS2*, *EPCAM*), and is ***considered*** in mutation carriers of *RAD51C*, *RAD51D* and *BRIP1* [20].

8.8 Current State of HBOC Research

Recently, multi-gene assaying has been introduced that can analyze that status of multiple suspect genes simultaneously. Furthermore, genetic testing for hereditary tumors is applied not only for assisting in cancer diagnosis but also for companion diagnostics. *BRCA1/2* testing is used as a companion diagnostic for PARP inhibitors. Furthermore, MSI screening may be used to predict the sensitivity of PD-1 (anti-programmed death-1) antibody because significant responses of cancers with MSI to anti–PD-1 inhibitors in patients who failed conventional therapy [21].

Conclusion

If clinicians suspect that an unusual number or pattern of cancers within a family may be caused by an inherited cancer predisposition genes, genetic counseling can be provided and genetic testing can be offered to find out inherited cancer genes. Gynecologists can play key roles in identifying women with hereditary cancer syndrome; this may help reduce mortality for mutation carriers.

References

1. Knudson Jr AG. Mutation and cancer: statistical study of retinoblastoma. Proc Natl Acad Sci U S A. 1971;68:820–3.
2. Petrucelli N, Daly MB, Feldman GL. *BRCA1* and *BRCA2* hereditary breast and ovarian cancer. In: Pagon RA, Adam MP, Ardinger HH, et al., editors. GeneReviews. 2013. http://www.ncbi.nlm.nih.gov/books/NBK1247/. Accessed 21 Sept 2016.
3. Pal T, Permuth-Wey J, Betts JA, Krischer JP, Fiorica J, Arango H, et al. BRCA1 and BRCA2 mutations account for a large proportion of ovarian carcinoma cases. Cancer. 2005;104:2807–16.
4. Risch HA, McLaughlin JR, Cole DE, Rosen B, Bradley L, Kwan E, et al. Prevalence and penetrance of germline BRCA1 and BRCA2 mutations in a population series of 649 women with ovarian cancer. Am J Hum Genet. 2001;68:700–10.
5. Bolton KL, Chenevix-Trench G, Goh C, Sadetzki S, Ramus SJ, Karlan BY, et al. Association between BRCA1 and BRCA2 mutations and survival in women with invasive epithelial ovarian cancer. JAMA. 2012;307:382–90.
6. Alsop K, Fereday S, Meldrum C, de Fazio A, Emmanuel C, George J, et al. BRCA mutation frequency and patterns of treatment response in BRCA mutation-positive women with ovarian cancer: a report from the Australian ovarian cancer study group. J Clin Oncol. 2012;30:2654–63.
7. Yang D, Khan S, Sun Y, Hess K, Shmulevich I, Sood AK, et al. Association of BRCA1 and BRCA2 mutations with survival, chemotherapy sensitivity, and gene mutator phenotype in patients with ovarian cancer. JAMA. 2011;306:1557–65.
8. Ledermann J, Harter P, Gourley C, Friedlander M, Vergote I, Rustin G, et al. Olaparib maintenance therapy in platinum-sensitive relapsed ovarian cancer. N Engl J Med. 2012;366:1382–92.

9. Moyer VA, U.S. Preventive Services Task Force. Risk assessment, genetic counseling, and genetic testing for BRCA-related cancer in women: U.S. preventive services task force recommendation statement. Ann Intern Med. 2014;160:271–81.
10. Lu KH, Wood ME, Daniels M, Burke C, Ford J, Kauff ND, et al. American Society of Clinical Oncology expert statement: collection and use of a cancer family history for oncology providers. J Clin Oncol. 2014;32:833–40.
11. American College of Obstetricians and Gynecologists, ACOG Committee on Practice Bulletins–Gynecology, ACOG Committee on Genetics, Society of Gynecologic Oncologists. ACOG practice bulletin no. 103: hereditary breast and ovarian cancer syndrome. Obstet Gynecol. 2009;113:957–66.
12. Vasen HF, Watson P, Mecklin JP, Lynch HT. New clinical criteria for hereditary nonpolyposis colorectal cancer (HNPCC, Lynch syndrome) proposed by the international collaborative group on HNPCC. Gastroenterology. 1999;116:1453–6.
13. Finch A, Beiner M, Lubinski J, Lynch HT, Moller P, Rosen B, et al. Salpingo- oophorectomy and the risk of ovarian, fallopian tube, and peritoneal cancers in women with a *BRCA1* or *BRCA2* mutation. JAMA. 2006;296:185–92.
14. Rebbeck TR, Lynch HT, Neuhausen SL, Narod SA, Van't Veer L, Garber JE, et al. Prophylactic oophorectomy in carriers of *BRCA1* or *BRCA2* mutations. N Engl J Med. 2002;346:1616–22.
15. Kauff ND, Satagopan JM, Robson ME, Scheuer L, Hensley M, Hudis CA, et al. Risk-reducing salpingo-oophorectomy in women with a *BRCA1* or *BRCA2* mutation. N Engl J Med. 2002;346:1609–15.
16. Domchek SM, Friebel TM, Neuhausen SL, Wagner T, Evans G, Isaacs C, et al. Mortality after bilateral salpingo-oophorectomy in BRCA1 and BRCA2 mutation carriers: a prospective cohort study. Lancet Oncol. 2006;7:223–9.
17. Kauff ND, Domchek SM, Friebel TM, Robson ME, Lee J, Garber JE, et al. Risk-reducing salpingo-oophorectomy for the prevention of BRCA1- and BRCA2-associated breast and gynecologic cancer: a multicenter, prospective study. J Clin Oncol. 2008;26:1331–7.
18. Rutter JL, Wacholder S, Chetrit A, Lubin F, Menczer J, Ebbers S, et al. Gynecologic surgeries and risk of ovarian cancer in women with BRCA1 and BRCA2 Ashkenazi founder mutations: an Israeli population-based case-control study. J Natl Cancer Inst. 2003;95:1072–8.
19. Hirasawa A, Masuda K, Akahane T, Tsuruta T, Banno K, Makita K, et al. Experience of risk-reducing salpingo-oophorectomy for a BRCA1 mutation carrier and establishment of a system performing a preventive surgery for hereditary breast and ovarian cancer syndrome in Japan: our challenges for the future. Jpn J Clin Oncol. 2013;43:515–9.
20. NCCN. Clinical practice guidelines in oncology, genetic/familial high-risk assessment: breast and ovarian version 1.2017. https://www.nccn.org.
21. Le DT, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, et al. PD-1 blockade in tumors with mismatch-repair deficiency. N Engl J Med. 2015;372(26):2509–20.

Molecular Pathology and Novel Therapy for Uterine Sarcomas

9

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Abstract

Patients with uterine leiomyosarcoma (Ut-LMS) typically present with vaginal bleeding, pain, and a pelvic mass. Typical presentations with hypercalcemia or eosinophilia have been reported. Radiographic evaluation with combined positron emission tomography (PET)/computed tomography (CT) may assist in the diagnosis and surveillance of women with Ut-LMS. A recently developed risk assessment index is highly predictive of disease-specific survival. Ovarian preservation does not appear to negatively impact outcome, and the addition of adjuvant therapy after surgical treatment does not seem to improve survival. It is noteworthy that homozygous-deficient mice for a proteasome β1i subunit, PSMB9/β1i, spontaneously develop Ut-LMS with a disease prevalence of ~37% by 12 months of age. The *PSMB9/β1i* gene is transcribed from a promoter containing an interferon (IFN)-γ-response factor element (IRF-E); thus, the IFN-γ-signal markedly induces *PSMB9/β1i* expression. Furthermore, recent reports demonstrated the loss of ability to induce PSMB9/β1i expression, which is an IFN-γ-inducible factor, in human Ut-LMS tissues and Ut-LMS cell lines. Analysis of human Ut-LMS shows somatic mutations in the IFN-γ-signal pathway; thus the loss of PSMB9/β1i induction is attributable to a defect in the earliest steps of the IFN-γ signal pathway. The discovery of an impaired key cell signaling pathway may provide new targets for diagnostic approaches and therapeutic intervention.

Keywords

Uterus • Leiomyosarcoma • psmb9/β1i • IFN-γ signaling pathway

9.1 Introduction

Smooth muscle tumors (SMTs) have been traditionally divided into benign leiomyomas (LMA) and malignant leiomyosarcomas (LMS) based on cytological atypia, mitotic activity, and other criteria. Uterine LMS (Ut-LMS), which are some of the most common neoplasms of the female genital tract, are relatively rare SMTs, having an estimated annual incidence of 0.64 per 100,000 women [1]. They account for approximately one-third of uterine sarcomas and are considered to be aggressive malignancies with a 5-year survival rate of only 53% for tumors confined to the uterus [2, 3]. Gynecological malignant tumors, for instance, breast cancer and endometrial carcinomas, are strongly promoted by female hormones, but the rate of hormone receptor expression is reported to be significantly less in human Ut-LMS compared with normal uterine smooth muscle (Ut-SM) cells. These low receptor expressions were found to not correlate with the promotion of initial disease development or with the overall survival of patients with Ut-LMS; however, molecular targeting therapies against tumors have recently shown remarkable achievements [4, 5]. It is noteworthy that, when adjusting for stage and mitotic count, Ut-LMS has a significantly worse prognosis than carcinosarcoma [6]. As Ut-LMS is resistant to

chemotherapy and radiotherapy, and thus surgical intervention is virtually the only means of treatment for this disease, developing an efficient adjuvant therapy is expected to improve the prognosis of the disease [7–9]. A trend toward prolonged disease-free survival is seen in patients with matrix metalloproteinase (MMP)-2-negative tumors [10]. Although typical presentations with hypercalcemia or eosinophilia have been reported, this clinical abnormality is not an initial risk factor for Ut-LMS. Homozygous-deficient mice for a proteasome β1i subunit, PSMB9/β1i, exhibit tissue- and substrate-dependent defect in physiological function of immunoproteasome, and *Psmb9/β1i*$^{-/-}$ female mice are shown to develop Ut-LMS, with a disease prevalence of 36% by 12 months of age [11, 12]. Furthermore, a recent report showed that the loss of PSMB9/β1i expression in human Ut-LMS tissues is probably attributable to a defect in the earliest steps of the IFN-γ signal pathway. Defective PSMB9/β1i expression may initiate the development of spontaneous human Ut-LMS [12, 13]. Because there is no effective therapy for unrespectable human Ut-LMS, these findings may enable the development of diagnostics and specific molecular therapies to treat this disease.

9.2 PSMB9/β1i-Deficient Mice Exhibit Spontaneous Development of Uterine LMS

Although gynecological malignant tumors, for instance, breast cancer and endometrial carcinomas, are strongly promoted by female hormones, the rate of hormone receptor expression is reported to be significantly less in Ut-LMS than in normal uterine smooth muscle (Ut-SM). As apoptotic mechanisms have also been implicated in many human malignant tumors, investigating the dysregulation of the expression of apoptotic and/or cell cycle regulators in Ut-LMS is required to identify molecular pathways that could possibly be important in the development of human Ut-LMS. Although the significant differential expression of apoptotic and cell cycle regulatory factors, including initiation factor, in human Ut-LMS, has all been reported and compared to normal Ut-SM, there exists no scientific evidence to show that abnormal expression of these factors directly correlates to the initiation and promotion of human Ut-LMS [14–19].

The targeted disruption of *Psmb9/β1i* results in the impairment of tissue- and substrate-dependent physiological function of immunoproteasome [11]. *Psmb9/β1i*$^{-/-}$ mice were reported to be prone to the development of uterine neoplasms [12]. The percentage of mice with overt tumors increased with age after 6 months, with a cumulative prevalence of disease in female mice of 37% by 12 months of age and no apparent plateau at this late observation time. Ut-LMS was observed in *Psmb9/β1i* $^{-/-}$ female mice but not in their parental mice, C57BL/6 mice [12] (Fig. 9.1). Histological examinations of *Psmb9/β1i*$^{-/-}$ uterine neoplasms revealed common characteristic abnormalities of human Ut-LMS (Fig. 9.1). The tumors lacked lymphoid infiltrates, which is a sign of immune recognition, and consisted of uniform elongated Ut-SM cells arranged into bundles. The nuclei of the tumor cells varied in size and shape; furthermore, mitosis was frequent. In contrast, Ut-SM cells of

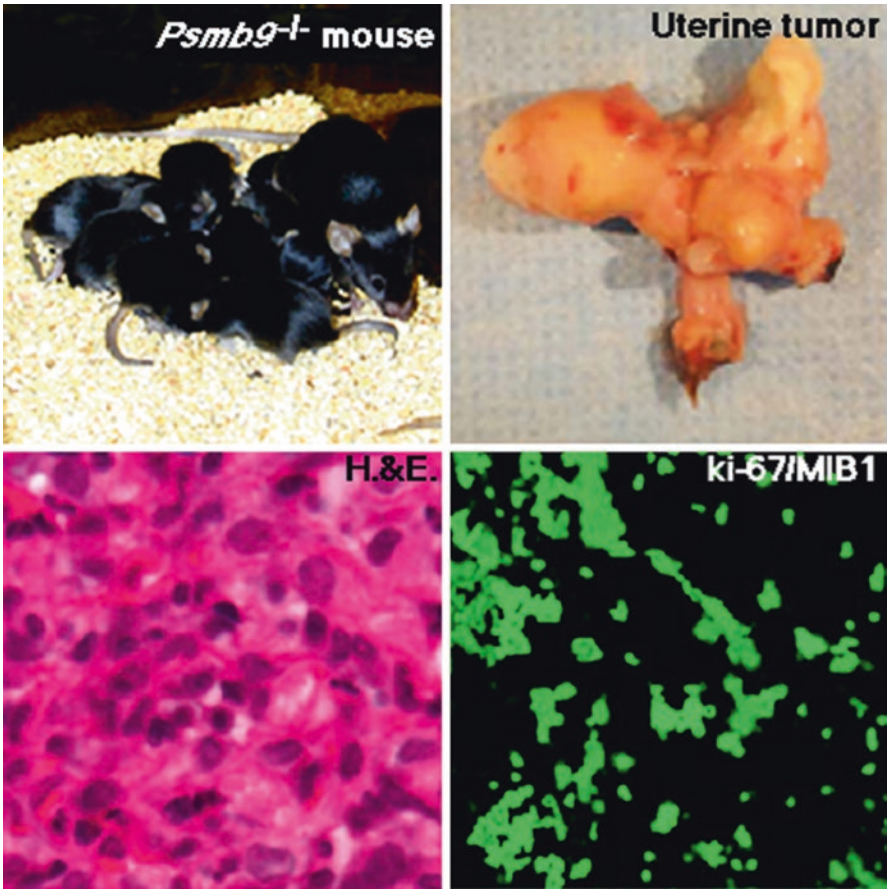

Fig. 9.1 Development of uterine neoplasms in *Psmb9/β1i* $^{-/-}$ mice. Uterine neoplasms in *Psmb9/β1i* $^{-/-}$ mice. Photographs of H&E staining and immunohistochemistry (IHC) with anti-ki-67/MIB1 antibody show the characters of uterine neoplasm. Histological examinations of the *Psmb9*$^{-/-}$ uterine tumors revealed the common characteristic abnormalities of uterine leiomyosarcomas (LMS). Presentation of these histological data in this manuscript was approved by Prof. Susumu Tonegawa (Picore Inst. and Dept. of Biology, M.I.T., MA)

C57BL/6 mice were normal in appearance, and relatively few Ki-67-positive cells, the proliferating cells of solid tumors, were observed in the basal cell layer of normal Ut-SM, whereas most basal cells vividly expressed Ki-67 in *Psmb9/β1i*$^{-/-}$ mice [12]. These histopathological examinations indicate the abnormal proliferation of *Psmb9/β1i*$^{-/-}$ Ut-SM cells in the basal cell layer of normal Ut-SM. In *Psmb9/β1i*$^{-/-}$ mice, immunoproteasomal activity against hydrophobic and basic substrates but not acidic substrates was lower in the muscle [11]. Furthermore, flow cytometric analysis showed no difference in the expression of MHC class I molecules. Importantly, spontaneous murine Ut-LMS was particularly detected, but no other tumor

progression was observed at high/low incidences in both male and female *Psmb9/β1i*$^{-/-}$ mice; therefore, PSMB9/β1i expression, rather than providing an escape from immune surveillance, seems to play an important role in the spontaneous development of murine Ut-LMS.

9.3 Correlation Between Defective PSMB9/β1i Expression and Human Uterine LMS

Several reports suggest that IFN-γ-induced restoration of antigen-processing machinery improves antitumor-specific antigen cytotoxic T-lymphocyte (CTL) recognition in some patients; thus, approaches to activate this pathway may be of benefit to patients with PSMB9/β1i deficiency. Furthermore, it should be demonstrated whether human Ut-LMS shows a weak expression of PSMB9/β1i. The effects of IFN-γ on PSMB9/β1i expression were examined using five human Ut-LMS cell lines [13]. PSMB9/β1i expression were not markedly induced by IFN-γ treatment in human Ut-LMS cell lines, although cervical epithelial adenocarcinoma cell lines and normal human Ut-SM cells underwent strong induction of PSMB9/β1i following IFN-γ treatment [13]. Furthermore, the experiments, performed separately at several medical facilities, revealed a serious loss in the ability to induce PSMB9/β1i expression in human Ut-LMS tissues in comparison with normal myometrium tissues located in the same tissue sections: normal myometrium, total 72 cases; LMA, total 51 cases; bizarre LMA, total three cases; and LMS, total 58 cases [13]. In addition, immunohistochemistry (IHC) showed marked PSMB9/β1i expression in cervical epithelial adenocarcinoma tissues as well as cell lines treated with IFN-γ.

The defect was localized to Janus-activated kinase 1 (JAK1) activation, which acts upstream in the IFN-γ signal pathway since IFN-γ treatment could not strongly induce JAK1 kinase activity in human Ut-LMS cell lines. Sequence analysis demonstrated that the loss of IFN-γ responsiveness in the human Ut-LMS cell line was attributable to the inadequate kinase activity of JAK1 due to a G781E somatic mutation in the ATP-binding region on human JAK1 molecule [13].

9.4 Mutations in IFN-γ Signaling Pathway in Human Uterine LMS Tissues

IFN-γ treatment markedly increased the expression of PSMB9/β1i, a subunit of the proteasome, which alters the proteolytic specificity of proteasomes. After binding of IFN-γ to the type II IFN receptor, JAK1 and JAK2 are activated and phosphorylate the signal transducer and activator of transcription 1 (STAT1) on the tyrosine residue at position 701 (Tyr701) and the serine residue at position 727 (Ser727) [20, 21] (Fig. 9.2). The phosphorylated STAT1 forms homodimers that translocate to the nucleus and bind IFN-γ-activated site (GAS) elements in the promoters of IFN-γ-regulated genes [20, 21] (Fig. 9.2).

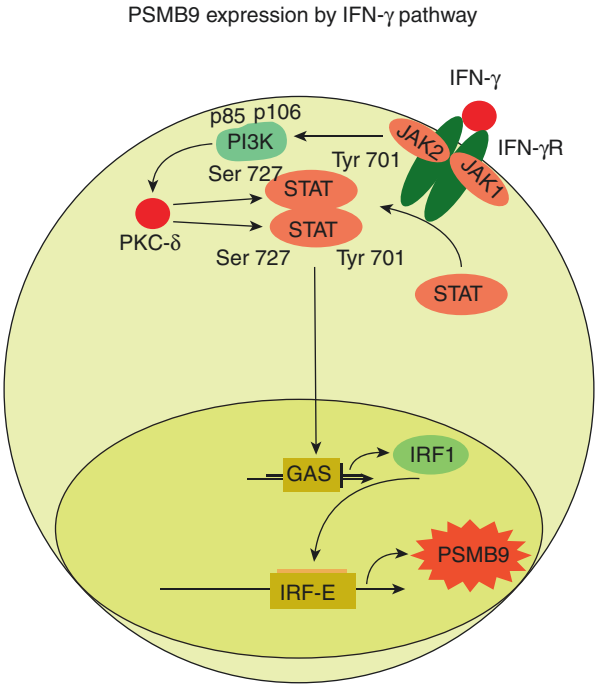

Fig. 9.2 Key role of the IFN-γ signaling pathway on PSMB9/β1i expression in human myometrium. After the binding of interferon-γ (IFN-γ) to the type II IFN receptor, Janus-activated kinase 1 (JAK1) and JAK2 are activated and phosphorylate signal transducer and activator of transcription 1 (STAT1) on the tyrosine residue at position 701 (Tyr701). The tyrosine-phosphorylated form of STAT1 forms homodimers that translocate to the nucleus and bind to IFN-γ-activated site (GAS) elements that are present in the promoters of IFN-γ-regulated genes. The IFN-γ-activated JAKs also regulate, through yet unknown intermediates, activation of the catalytic subunit (p110) of phosphatidylinositol 3-kinase (PI3K). PI3K activation ultimately results in downstream activation of protein kinase C-δ (PKC-δ), which in turn regulates phosphorylation of STAT1 on the serine residue at position 727 (Ser727). The phosphorylation of Ser727 is not essential for the translocation of STAT1 to the nucleus or for the binding of STAT1 to targeting DNA, but is required for full transcriptional activation. IFNGR1, IFN-γ receptor subunit 1; IFNGR2, IFN-γ receptor subunit 2

Genetic alterations in tyrosine kinases have previously been firmly implicated in tumorgenesis, but only a few serine/threonine kinases are known to be mutated in human cancers [22–25]. For instance, mice carrying homozygous deletion of *Pten* alleles developed widespread smooth muscle cell hyperplasia and abdominal leiomyosarcomas [26] and JUN oncogene amplification and overexpression block adipocytic differentiation in highly aggressive sarcomas [27]. Most frequently, leiomyosarcomas (LMS) have appeared in the uterus, retroperitoneum, or extremities, and although histologically indistinguishable, they have different clinical courses and chemotherapeutic responses. The molecular basis for these differences remains unclear. Therefore, the examination of human Ut-LMS tissues (32 human Ut-LMS tissue sections and normal myometrium tissue sections located in the same tissue) was performed to detect somatic

(tumor-specific) mutations in the IFN-γ signal cascade. The genetic approach has already addressed that somatic mutations in JAK1 molecule correlate to the initiation of several cancer progressions and other disorders [28–30]. Overall, nearly 37.5% (6/16) of human Ut-LMS tissues had mutations in the catalytic regions, the ATP-binding region or kinase-active site of JAK1. Furthermore, the genetic approach has already revealed that somatic mutations in the PSMB9/β1i molecule or its enhancer region correlate to the initiation of several cancer progressions and other disorders; 31.3% (5/16) of human Ut-LMS tissues had somatic mutations in the *Psmb9* promoter region, which is required for transcriptional activation [31–35]. In addition, genetic examination has already demonstrated that somatic mutations in the STAT1 molecule correlate to the initiation of several disorders [36–39]. Nearly 37.5% (6/16) of human Ut-LMS tissues had somatic mutations in the STAT1 intermolecular region. Although the genetic approach has already addressed that marked JAK2 activation causes myelo- and lymphoproliferative disease, no somatic mutation in the ATP-binding region and kinase-active site of JAK2 was detected in human Ut-LMS [40–44]. In a recent report, high-resolution genome-wide array comparative genomic hybridization (CGH) analysis of human Ut-LMS cases gave gene-level information about the amplified and deleted regions that may play a role in the development and progression of human Ut-LMS. Among the most intriguing genes, whose copy number sequence was revealed by CGH, were loss of *JAK1* (genome locus 1p31 ~ p32) and *PSMB9* (genome locus 6p21.3) [45, 46]. The discovery of these mutational defects in a key cell signaling pathway may be an important development in the pathogenesis of human Ut-LMS.

It is probable that the list of new elements involved in IFN-mediated signal cascade will continue to grow during the next few years, whereas the contributions of known pathways might need to be reevaluated. At present, it seems that the activation of more than one signaling pathway is required for the generation of different biological properties of IFNs, and no signaling cascade alone is sufficient for the generation of any given biological endpoint. For example, the physiological functions of the STAT, NF-κB, and p38 signaling pathways are required for antiviral effects or antitumor effects of IFNs, but activation of these pathways alone is not sufficient to elicit an antiviral or antitumor response [47, 48]. Such a requirement for multiple signaling pathways also seems to be the case for IFN-dependent antiproliferative responses and might reflect the synergistic effects of various signals at the levels of gene transcription and translation; therefore, additional genetic analysis is required to completely elucidate the mutational activation of a key cell signaling pathway in human Ut-LMS.

9.5 Potential Role of Anti-Oncogenic Function by PSMB9/ β1i

The growth of cell lines with JAK1 kinase activity is strongly inhibited by IFN-γ treatment, whereas the growth of JAK1-deficient cell lines is unaffected [49]. Similarly, the cell cycle distribution pattern of freshly explanted tumor cells derived from JAK1-deficient tumors shows no response to IFN-γ treatment [49].

The growth of the original SKN human Ut-LMS cells, which had defective JAK1 activity, was unaffected by IFN-γ treatment (population doubling time (PDT) = 15.2 h) [13]. In contrast, the growth of JAK1-transfected SKN cells, which had strong exogenous JAK1 activity, was prevented by IFN-γ treatment (PDT = 18.1 h). Interestingly, analysis of PSMB9/β1i-transfected SKN cells showed that exogenous PSMB9/β1i expression resulted in cell growth arrest (PDT = 17.9 h) [13]. Conversely, the growth of PSMB9/β1i-transfected SKN cells was unaffected by IFN-γ treatment (PDT = 18.0 h). In SKN-PSMB9/β1i transfectants, there is a correlation between the levels of exogenous PSMB9 expression and the degree of suppression of the transformed phenotype [13, 50]. The physiological function of PSMB9/β1i with revertant-inducing activity on SKN cells has been demonstrated.

Microarray analysis provides insight into the gene expression changes associated with malignant transformation. To investigate whether stable PSMB9/β1i expression contributes to cell growth phenotype in SKN cells, the experiment (using Affymetrix Human GeneChip HG U133 Plus2.0) demonstrated the gene expression profile of SKN cells transfected with plasmid without insert (pCEP9) compared with *PSMB9/β1i* coding DNA (pCEP9-PSMB9/β1i). Microarray analysis has elucidated that PSMB9 expression dramatically influences the expression pattern of cell cycle regulators, especially anti-oncogenic factor interferon regulatory factor 1 (IRF-1), which directly correlates to progressively worsen with the increasing stage and grade of the tumor [50–52] (Fig. 9.3). In the farther study, we show that PSMB9/β1i may negatively regulate progression of human Ut-LMS independently of its role in the proteasome. Moreover, several lines of evidence indicate that although CALPONIN h1 does not directly influence tumorigenesis, it clearly affects PSMB9/β1i-induced cellular morphological changes [51].

The downregulation of MHC expression, including the *Psmb9/β1i* gene, is one of the biological mechanisms that tumor cells use to evade host immune surveillance. Recently, the incidence of IFN-γ unresponsiveness in human tumors was examined in several cancers and revealed that around 33% of each group exhibited a reduction in IFN-γ sensitivity [53]. Nevertheless, PSMB9/β1i expression, rather

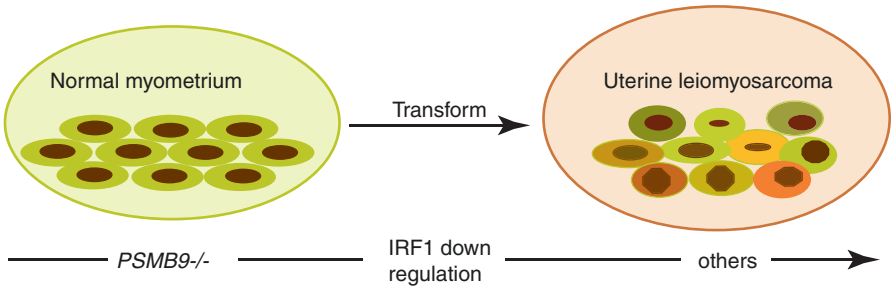

Fig. 9.3 Model for the initiation of sarcomagenesis of uterine leiomyosarcoma. The initiation of sarcomagenesis of uterine leiomyosarcoma development is attributed to defect in PSMB9/β1i expression, which results in marked cell proliferation

than providing an escape from immune surveillance, seems to play an important role in the negative regulation of Ut-LMS cell growth. Defective PSMB9/β1i expression is likely to be a risk factor for the development of human Ut-LMS, as it is in PSMB9/β1i-deficient mice.

9.6 Tumor Suppressor and Oncogenic Pathways Involved in Sarcomagenesis

Tumor protein 53 (TP53) anti-oncogenic pathway is one of the most well-characterized signal cascades in tumorigenesis [54]. *TP53* gene encodes a transcription factor required for the activation of numerous DNA damage cell cycle checkpoint response and apoptotic factors, and thus its activities are often ablated in many malignant tumors. In addition to the loss of TP53 physiological functions via inherited germ line mutations, the TP53 signaling pathway is commonly disrupted by point mutations in the *TP53* gene during sporadic sarcomagenesis [55, 56]. However, even though *TP53* gene alterations are widely regarded to have a significant impact on sarcomagenesis, many soft tissue sarcomas retain wild-type TP53 but phenotypically display a loss of TP53 physiological function. These research observations suggest that changes in other components of TP53 signal cascade, such as amplification of MDM2, a negative regulator of TP53 signal pathway, may result in TP53 inactivation [57, 58]. Furthermore, mice and humans with elevated levels of MDM2 due to a high frequency single nucleotide polymorphism in the *MDM2* promoter (Mdm2SNP309) are both more susceptible to sarcoma formation [59]. Additionally, deletion or silencing of $P19^{Arf}$ ($P14^{ARF}$ in human), an inhibitor of the MDM2/TP53 axis, often results in the development of soft tissue sarcomas. Together, these findings indicate that while inactivation of the TP53 signal pathway is observed in the vast majority of human soft tissue sarcomas, the mechanisms leading to disruption of the pathway vary greatly.

The retinoblastoma (RB) signal pathway represents a second major anti-oncogenic pathway that is deregulated in many soft tissue sarcomas. Individuals inheriting a germ line *RB* mutation typically develop cancers of the eye early in life. However, in addition to retinal malignant tumors, these children have a significantly higher propensity to develop soft tissue sarcomas than the general population [60]. While the inheritance of germ line *RB* alterations increases the risk of soft tissue sarcoma, there are also numerous examples of sporadic sarcomas harboring spontaneous mutations and deletions in RB, particularly osteosarcomas and rhabdomyosarcomas [61]. Furthermore, $P16^{INK4A}$, a negative regulator of the CDK/CYCLIN complexes that phosphorylate and activate RB, is often deleted in soft tissue sarcomas [62]. These findings may illustrate the importance of RB signaling pathway in sarcomagenesis. Although we previously demonstrated that the abnormal expression of TP53 and Ki-67 and mutations in TP53 molecule were frequently associated with human Ut-LMS, the defective expression of PSMB9/β1i appeared to be more characteristic of human Ut-LMS than these factors [63].

Conclusion

To improve the prognosis of human Ut-LMS, research experiments were performed to identify the key role of pro- or anti-oncogenic factors that have an important function in their pathogenesis and that could serve as molecular targets for tumor treatment. For this purpose, several research facilities conducted a microarray procedure between human Ut-LMS and normal myometrium and showed that several known pro-oncogenic factors, such as brain-specific polypeptide PEP-19 and c-kit, may be associated with the pathogenesis of human Ut-LMS [64–66]. However, in terms of the tumorgenesis of human Ut-LMS, merely comparing the expression of potential pro-oncogenic factors between normal and malignant tissues is not sufficient because the results obtained may be the consequence of malignant transformation and, therefore, not necessarily the cause.

For almost all types of cancer studied to date, it seems as if the transition from a normal, healthy cell to a malignant tumor cell is a stepwise progression that requires genetic changes in several different oncogenes and tumor suppressors. In order to generate a malignant cell, a series of somatic mutations must occur in the same cell. Since the likelihood of any gene becoming mutated is very low, it stands to reason that the chance of several different mutations occurring in the same cell is highly unlikely. For this reason, cells in an elderly body have had more time to accumulate the changes needed to form cancer cells, whereas those in a child are much less likely to have acquired the requisite genetic changes. Importantly, clinical experiments have revealed loss of the ability to induce PSMB9/β1i expression in human Ut-LMS tissues in comparison with normal myometrium tissues. The discovery of somatic mutational defects in the IFN-γ-signaling pathway may be important for the initial development of human Ut-LMS. It is noteworthy that stable PSMB9/β1i expression contributes to cell proliferation, which directly correlates to the progressive deterioration with increasing stage and grade of the tumor. Recent advances in our understanding of the biology of human Ut-LMS have concentrated on the impaired IFN-γ signaling pathway. It is clear that mutations in key regulatory genes (tumor suppressors and proto-oncogenes) alter the behavior of cells and can potentially lead to the unregulated growth seen in malignant tumor. Therefore, continued improvement of our knowledge of the molecular biology of murine and human Ut-LMS may ultimately lead to novel therapies and improved outcome.

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References

1. Zaloudek C, Hendrickson MR. In: Kurman RJ, editor. Mesenchymal tumors of the uterus: Blaustein's pathology of the female genital tract. 5th ed. New York: Springer; 2002. p. 561–78.
2. Gadducci A, Landoni F, Sartori E, Zola P, Maggino T, Lissoni A, Bazzurini L, Arisio R, Romagnolo C, Cristofani R. Uterine leiomyosarcoma: analysis of treatment failures and survival. Gynecol Oncol. 1996;62:25–32.
3. Nordal R, Thoresen S. Uterine sarcomas in Norway 1956–1992: incidence, survival and mortality. Eur J Cancer. 1997;33:907–11.
4. Leitao MM, Soslow RA, Nonaka D, Olshen AB, Aghajanian C, Sabbatini P, Dupont J, Hensley M, Sonoda Y, Barakat RR, Anderson S. Tissue microarray immunohistochemical expression of estrogen, progesterone, and androgen receptors in uterine leiomyomata and leiomyosarcoma. Cancer. 2004;101:1455–62.
5. Perez EA, Pusztai L, Van de Vijver M. Improving patient care through molecular diagnostics. Semin Oncol. 2004;31:14–20.
6. Miettinen M, Fetsch JF. Evaluation of biological potential of smooth muscle tumours. Histopathology. 2006;48:97–105.
7. Brooks SE, Zhan M, Cote T, Baquet CR. Surveillance, epidemiology, and end results analysis of 2677 cases of uterine sarcoma 1989–1999. Gynecol Oncol. 2004;93:204–8.
8. Dusenbery KE, Potish RA, Judson P. Limitations of adjuvant radiotherapy for uterine sarcomas spread beyond the uterus. Gynecol Oncol. 2004;94:191–6.
9. TI W, Chang TC, Hsueh S, Hsu KH, Chou HH, Huang HJ, Lai CH. Prognostic factors and impact of adjuvant chemotherapy for uterine leiomyosarcoma. Gynecol Oncol. 2006;100:166–72.
10. Bodner-Adler B, Bodner K, Kimberger O, Czerwenka K, Leodolter S, Mayerhofer K. MMP-1 and MMP-2 expression in uterine leiomyosarcoma and correlation with different. J Soc Gynecol Investig. 2003;10:443–6.
11. Van Kaer L, Ashton-Rickardt PG, Eichelberger M, Gaczynska M, Nagashima K, Rock KL, Goldberg AL, Doherty PC, Tonegawa S. Altered peptidase and viral-specific T cell response in LMP2 mutant mice. Immunity. 1994;1:533–41.
12. Hayashi T, Faustman DL. Development of spontaneous uterine tumors in low molecular mass polypeptide-2 knockout mice. Cancer Res. 2002;62:24–7.
13. Hayashi T, Kobayashi Y, Kohsaka S, Sano K. The mutation in the ATP-binding region of JAK1, identified in human uterine leiomyosarcomas, results in defective interferon-gamma inducibility of TAP1 and LMP2. Oncogene. 2006;25:4016–26.
14. Zhai YL, Kobayashi Y, Mori A, Orii A, Nikaido T, Konishi I, Fujii S. Expression of steroid receptors, Ki-67, and p53 in uterine leiomyosarcomas. Int J Gynecol Pathol. 1999;18:20–8.
15. Miyajima K, Tamiya S, Oda Y, Adachi T, Konomoto T, Toyoshiba H, Masuda K, Tsuneyoshi M. Relative quantitation of p53 and MDM2 gene expression in leiomyosarcoma; real-time semi-quantitative reverse transcription-polymerase chain reaction. Cancer Lett. 2001;164:177–88.
16. Raspollini MR, Pinzani P, Simi L, Amunni G, Villanucci A, Paglierani M, Taddei GL. Uterine leiomyosarcomas express KIT protein but lack mutation(s) in exon 9 of c-KIT. Gynecol Oncol. 2005;98:334–5.
17. Alhopuro P, Ylisaukko-Oja SK, Koskinen WJ, Bono P, Arola J, Jarvinen HJ, Mecklin JP, Atula T, Kontio R, Makitie AA, Suominen S, Leivo I, Vahteristo P, Aaltonen LM, Aaltonen LA. The MDM2 promoter polymorphism SNP309T-->G and the risk of uterine leiomyosarcoma, colorectal cancer, and squamous cell carcinoma of the head and neck. J Med Genet. 2005;42:694–8.
18. Leiser AL, Anderson SE, Nonaka D, Chuai S, Olshen AB, Chi DS, Soslow RA. Apoptotic and cell cycle regulatory markers in uterine leiomyosarcoma. Gynecol Oncol. 2005;101:86–91.

19. Anderson SE, Nonaka D, Chuai S, Olshen AB, Chi D, Sabbatini P, Soslow RA. p53, epidermal growth factor, and platelet-derived growth factor in uterine leiomyosarcoma and leiomyomas. Int J Gynecol Cancer. 2006;16:849–53.
20. Parmar S, Platanias LC. Interferons. Cancer Treat Res. 2005;126:45–68.
21. Platanias LC. Mechanisms of type-I- and type-II-interferon-mediated signalling. Nat Rev Immunol. 2005;5:375–86.
22. Futreal PA, Coin L, Marshall M, Down T, Hubbard T, Wooster R, Rahman N, Stratton MR. A census of human cancer genes. Nat Rev Cancer. 2004;4:177–83.
23. Jones PA, Baylin SB. The epigenomics of cancer. Cell. 2007;128:683–92.
24. Lengyel E, Sawada K, Salgia R. Tyrosine kinase mutations in human cancer. Curr Mol Med. 2007;7:77–84.
25. Pajares MJ, Ezponda T, Catena R, Calvo A, Pio R, Montuenga LM. Alternative splicing: an emerging topic in molecular and clinical oncology. Lancet Oncol. 2007;8:349–57.
26. Hernando E, Charytonowicz E, Dudas ME, Menendez S, Matushansky I, Mills J, Socci ND, Behrendt N, Ma L, Maki RG, Pandolfi PP, Cordon-Cardo C. The AKT-mTOR. Pathway plays a critical role in the development of leiomyosarcomas. Nat Med. 2007;13:748–53.
27. Mariani O, Brennetot C, Coindre JM, Gruel N, Ganem C, Delattre O, Stern MH, Aurias A. Oncogene amplification and overexpression block Adipocytic differentiation in highly aggressive sarcomas. Cancer Cell. 2007;11:361–74.
28. Briscoe J, Rogers NC, Witthuhn BA, Watling D, Harpur AG, Wilks AF, Stark GR, Ihle JN, Kerr IM. Kinase-negative mutants of JAK1 can sustain interferon-gamma-inducible gene expression but not an antiviral state. EMBO J. 1996;15:799–809.
29. Rossi MR, Hawthorn L, Platt J, Burkhardt T, Cowell JK, Ionov Y. Identification of inactivating mutations in the JAK1, SYNJ2, and CLPTM1 genes in prostate cancer cells using inhibition of nonsense-mediated decay and microarray analysis. Cancer Genet Cytogenet. 2005;161:97–103.
30. Haan S, Margue C, Engrand A, Rolvering C, Schmitz-Van de Leur H, Heinrich PC, Behrmann I, Haan C. Dual role of the Jak1 FERM and kinase domains in cytokine receptor binding and in stimulation-dependent Jak activation. J Immunol. 2008;180:998–1007.
31. Heward JM, Allahabadia A, Sheppard MC, Barnett AH, Franklyn JA, Gough SC. Association of the large multifunctional proteasome (LMP2) gene with graves' disease is a result of linkage disequilibrium with the HLA haplotype DRB.1*0304-DQB.1*02-DQA1*0501. Clin Endocrinol. 1999;51:115–8.
32. Satoh E, Mabuchi T, Satoh H, Asahara T, Nukui H, Naganuma H. Reduced expression of the transporter associated with antigen processing 1 molecule in malignant glioma cells, and its restoration by interferon-gamma and -beta. J Neurosurg. 2006;104:264–71.
33. Krämer U, Illig T, Grune T, Krutmann J, Esser C. Strong associations of psoriasis with antigen processing LMP and transport genes TAP differ by gender and phenotype. Genes Immun. 2007;8:513–7.
34. Liu Y, Li HJ, Qiu XT, Guo HW, Li YH, Zhang Q. Molecular characterization, expression and mapping of porcine LMP2 and MECL-1 genes. DNA Seq. 2007;18:257–64.
35. Mehta AM, Jordanova ES, van Wezel T, HW U, Corver WE, Kwappenberg KM, Verduijn W, Kenter GG, van der Burg SH, Fleuren GJ. Genetic variation of antigen processing machinery components and association with cervical carcinoma. Genes Chromosomes Cancer. 2007;46:577–86.
36. Dupuis S, Dargemont C, Fieschi C, Thomassin N, Rosenzweig S, Harris J, Holland SM, Schreiber RD, Casanova JL. Impairment of mycobacterial but not viral immunity by a germline human STAT1 mutation. Science. 2001;293:300–3.
37. Dupuis S, Jouanguy E, Al-Hajjar S, Fieschi C, Al-Mohsen IZ, Al-Jumaah S, Yang K, Chapgier A, Eidenschenk C, Eid P, Al Ghonaium A, Tufenkeji H, Frayha H, Al-Gazlan S, Al-Rayes H, Schreiber RD, Gresser I, Casanova JL. Impaired response to interferon-alpha/beta and lethal viral disease in human STAT1 deficiency. Nat Genet. 2003;33:388–91.

38. Chapgier A, Boisson-Dupuis S, Jouanguy E, Vogt G, Feinberg J, Prochnicka-Chalufour A, Casrouge A, Yang K, Soudais C, Fieschi C, Santos OF, Bustamante J, Picard C, de Beaucoudrey L, Emile JF, Arkwright PD, Schreiber RD, Rolinck-Werninghaus C, Rösen-Wolff A, Magdorf K, Roesler J, Casanova JL. Novel STAT1 alleles in otherwise healthy patients with mycobacterial disease. PLoS Genet. 2006;2:131.
39. Scarzello AJ, Romero-Weaver AL, Maher SG, Veenstra TD, Zhou M, Qin A, Donnelly RP, Sheikh F, Gamero AM. A mutation in the SH2 domain of STAT2 prolongs tyrosine phosphorylation of STAT1 and promotes type I IFN-induced apoptosis. Mol Biol Cell. 2007;18:2455–62.
40. Baxter EJ, Scott LM, Campbell PJ, East C, Fourouclas N, Swanton S, Vassiliou GS, Bench AJ, Boyd EM, Curtin N, Scott MA, Erber WN, Green AR. Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative disorders. Lancet. 2005;365:1054–61.
41. Campbell PJ, Scott LM, Buck G, Wheatley K, East CL, Marsden JT, Duffy A, Boyd EM, Bench AJ, Scott MA, Vassiliou GS, Milligan DW, Smith SR, Erber WN, Bareford D, Wilkins BS, Reilly JT, Harrison CN, Green AR. Definition of subtypes of essential thrombocythaemia and relation to polycythaemia vera based on JAK2 V617F mutation status: a prospective study. Lancet. 2005;366:1945–53.
42. James C, Ugo V, Le Couédic JP, Staerk J, Delhommeau F, Lacout C, Garçon L, Raslova H, Berger R, Bennaceur-Griscelli A, Villeval JL, Constantinescu SN, Casadevall N, Vainchenker W. A unique clonal JAK2 mutation leading to constitutive signalling causes polycythaemia vera. Nature. 2005;434:1144–8.
43. Kralovics R, Passamonti F, Buser AS, Teo SS, Tiedt R, Passweg JR, Tichelli A, Cazzola M, Skoda RC. A gain-of-function mutation of JAK2 in myeloproliferative disorders. N Engl J Med. 2005;352:1779–90.
44. Tefferi A, Lasho TL, Gilliland G. JAK2 mutations in myelo-proliferative disorders. N Engl J Med. 2005;353:1416–7.
45. Lee JW, Kim YG, Soung YH, Han KJ, Kim SY, Rhim HS, Min WS, Nam SW, Park WS, Lee JY, Yoo NJ, Lee SH. The JAK2 V617F mutation in de novo acute myelogenous leukemias. Oncogene. 2006;25:1434–6.
46. Svarvar C, Larramendy ML, Blomqvist C, Gentile M, Koivisto-Korander R, Leminen A, Butzow R, Bohling T, Knuutila S. Do DNA copy number changes differentiate uterine from nonuterine leiomyosarcomas and predict metastasis? Mod Pathol. 2006;19:1068–82.
47. Ramana CV, Gil MP, Schreiber RD, Stark GR. Stat1-dependent and -independent pathways in IFN-gamma-dependent signaling. Trends Immunol. 2002;23:96–101.
48. Sizemore N, Agarwal A, Das K, Lerner N, Sulak M, Rani S, Ransohoff R, Shultz D, Stark GR. Inhibitor of kappaB. Kinase is required to activate a subset of interferon gamma-stimulated genes. Proc Natl Acad Sci U S A. 2004;101:7994–8.
49. Sexl V, Kovacic B, Piekorz R, Moriggl R, Stoiber D, Hoffmeyer A, Liebminger R, Kudlacek O, Weisz E, Rothammer K, Ihle JN. Jak1 deficiency leads to enhanced Abelson-induced B-cell tumor formation. Blood. 2003;101:4937–43.
50. Hayashi T, Horiuchi A, Sano K, Hiraoka N, Kasai M, Ichimura T, Sudo T, Tagawa Y, Nishimura R, Ishiko O, Kanai Y, Yaegashi N, Aburatani H, Shiozawa T, Konishi I. Potential role of LMP2 as tumor-suppressor defines new targets for uterine leiomyosarcoma therapy. Sci Rep. 2011;1:180. doi:10.1038/srep00180.
51. Hayashi T, Horiuchi A, Sano K, Hiraoka N, Kasai M, Ichimura T, Sudo T, Nishimura R, Ishiko O, Shiozawa T, Kanai Y, Yaegashi N, Aburatani H, Konishi I. Potential role of LMP2 as an anti-oncogenic factor in human uterine leiomyosarcoma: morphological significance of calponin h1. FEBS Lett. 2012;586(13):1824–31.
52. Hayashi T, Horiuchi A, Sano K, Yaegashi N, Konishi I. Uterine leiomyosarcoma tumorigenesis in Lmp2-deficient mice: involvement of impaired anti-oncogenic factor IRF1. Anticancer Res. 2015;35(9):4665–79.

53. Kaplan DH, Shankaran V, Dighe AS, Stockert E, Aguet M, Old LJ, Schreiber RD. Demonstration of an interferon gamma-dependent tumor surveillance system in immunocompetent mice. Proc Natl Acad Sci U S A. 1998;95:7556–61.
54. Vogelstein B, Lane D, Levine AJ. Surfing the p53 network. Nature. 2000;408(6810):307–10.
55. Raycroft L, Wu HY, Lozano G. Transcriptional activation by wild-type but not transforming mutants of the p53 anti-oncogene. Science. 1990;249(4972):1049–51.
56. Wang LL. Biology of osteogenic sarcoma. Cancer J. 2005;11(4):294–305.
57. Oliner JD, Kinzler KW, Meltzer PS, George DL, Vogelstein B. Amplification of a gene encoding a p53-associated protein in human sarcomas. Nature. 1992;358(6381):80–3.
58. Oliner JD, Pietenpol JA, Thiagalingam S, Gyuris J, Kinzler KW, Vogelstein B. Oncoprotein MDM2 conceals the activation domain of tumour suppressor p53. Nature. 1993; 362(6423):857–60.
59. Ito M, Barys L, O'Reilly T, Young S, Gorbatcheva B, Monahan J, Zumstein-Mecker S, Choong PF, Dickinson I, Crowe P, Hemmings C, Desai J, Thomas DM, Lisztwan J. Comprehensive mapping of p53 pathway alterations reveals an apparent role for both SNP309 and MDM2 amplification in sarcomagenesis. Clin Cancer Res. 2011;17(3):416–26.
60. Deshpande A, Hinds PW. The retinoblastoma protein in osteoblast differentiation and osteosarcoma. Curr Mol Med. 2006;6(7):809–17.
61. Toguchida J, Ishizaki K, Sasaki MS, Nakamura Y, Ikenaga M, Kato M, Sugimot M, Kotoura Y, Yamamuro T. Preferential mutation of paternally derived RB gene as the initial event in sporadic osteosarcoma. Nature. 1989;338(6211):156–8.
62. Oda Y, Yamamoto H, Takahira T, Kobayashi C, Kawaguchi K, Tateishi N, Nozuka Y, Tamiya S, Tanaka K, Matsuda S, Yokoyama R, Iwamoto Y, Tsuneyoshi M. Frequent alteration of p16(INK4a)/p14(ARF) and p53 pathways in the round cell component of myxoid/round cell liposarcoma: p53 gene alterations and reduced p14(ARF) expression both correlate with poor prognosis. J Pathol. 2005;207(4):410–21.
63. Kawano M, Hayashi T. Biological analyses for understanding of the uterine sarcomagenesis. Global J Res Anal. 2016;5(5):310–1.
64. Kanamori T, Takakura K, Mandai M, Kariya M, Fukuhara K, Kusakari T, Momma C, Shime H, Yagi H, Konishi M, Suzuki A, Matsumura N, Nanbu K, Fujita J, Fujii S. PEP-19 overexpression in human uterine leiomyoma. Mol Hum Reprod. 2003;9:709–17.
65. Wang Z, Shen D, Parsons DW, Bardelli A, Sager J, Szabo S, Ptak J, Silliman N, Peters BA, Van der Heijden MS, Parmigiani G, Yan H, Wang TL, Riggins G, Powell SM, Willson JK, Markowitz S, Kinzler KW, Vogelstein B, Velculescu VE. Mutational analysis of the tyrosine phosphatome in colorectal cancers. Science. 2004;304:1164–6.
66. Ylisaukko-oja SK, Kiuru M, Lehtonen HJ, Lehtonen R, Pukkala E, Arola J, Launonen V, Aaltonen LA. Analysis of fumarate hydratase mutations in a population-based series of early onset uterine leiomyosarcoma patients. Int J Cancer. 2006;119:283–7.

Recurrent Pregnancy Loss: Current Evidence and Clinical Guideline

10

Mayumi Sugiura-Ogasawara

Abstract

Recurrent pregnancy loss (RPL) is defined as two or more pregnancy demise at any gestational age. The estimated frequencies of recurrent miscarriage, three or more miscarriages, and RPL are 0.9 and 4.2% in the Japanese general population. Identifiable causes are antiphospholipid syndrome (APS), uterine anomaly, and parental and embryonic chromosome abnormality.

APS is the most important treatable etiology. Lupus anticoagulant is priority to anticardiolipin antibody in obstetric APS. However, it is not established what kinds of assays to be measured for detecting antiphospholipid antibodies. Randomized control trial is needed to examine the merit of surgery for patients with septate uterine and the advantage of preimplantation genetic diagnosis for patients with translocation. There are no established treatment methods though many treatments such as heparin, aspirin, progesterone, and prednisone are offered for unexplained patients. The live birth rates are about 80% in patients with previous two miscarriages, 70% in patients with three miscarriages, 60% in patients with four miscarriages, and 50% in patients with five miscarriages without medication. This information is important before subsequent pregnancy.

Keywords

Recurrent pregnancy loss • Recurrent miscarriage • Antiphospholipid antibody Translocation • Uterine anomaly • Embryonic karyotype • Preimplantation genetic diagnosis

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10.1 Etiology

Miscarriage is the most common pregnancy complication with the prevalence of 15% (definition by European Society of Human Reproduction, American Society of Reproductive Medicine, and World Health Organization) [1].

Recurrent miscarriage (*RM*): Three or more consecutive clinical miscarriages occurring before 20 weeks postmenstruation (before 22 weeks in Japan).

Recurrent pregnancy loss (*RPL*): Two or more pregnancy losses (demise) at any gestational age.

The estimated frequencies of RM and RPL are 0.9 and 4.2% in the Japanese general population [2]. Ninety-five percent of patients repeated early miscarriage in our previous study. Nonvisualized pregnancy (biochemical pregnancy and failed pregnancy of unknown location combined) is not included in the RM and RPL. Recently, it was found to contribute negatively to the chance for live birth by the relative risk of 0.90 (95% CI 0.83–0.97) [3].

Several guidelines recommend test for antiphospholipid syndrome (APS), uterine anomalies, and parental and embryonic abnormal karyotype in clinical practice (Table 10.1) [4]. APS, uterine anomalies, and abnormal chromosomes in either partner are established causes of RPL [4–8]. Only about 30% of cases have an identifiable cause and the remaining 69% was unexplained in our study (Fig. 10.1a) [6], and it is well known that the cause remains unexplained in over a half of the cases [4, 8]. The abnormal embryonic karyotype was found in 41.1% of patients in whom both conventional causes and karyotype of aborted conceptus could be examined in 482 patients (Fig. 10.1b) [9]. Therefore, the prevalence of truly unexplained, of cases with normal embryonic karyotype, was only 24.5%. An abnormal embryonic karyotype is usually included in unexplained because the embryonic karyotype is seldom analyzed clinically.

Table 10.1 Recommended test for patients with recurrent pregnancy loss

Cause	Recommended tests	Secondary RPL	Clinical features
Antiphospholipid antibody	Lupus anticoagulant (at least two kinds of reagent such as dRVVT and aPTT) Anticardiolipin antibodies Anti-β2-glycopritein I antibodies	Rare	Intrauterine fetal death Recurrent early miscarriage Pre-eclampsia
Uterine anomaly	Ultrasound, sonohysterograpy and hysterosalpingography	Rare	Recurrent early miscarriage Intrauterine fetal death Preterm birth Breech presentation
Abnormal chromosome	Chromosome analysis of father and mother	Yes	Early miscarriage
Abnormal embryonic karyotype	Chromosome analysis of products of conception	Yes	Early miscarriage

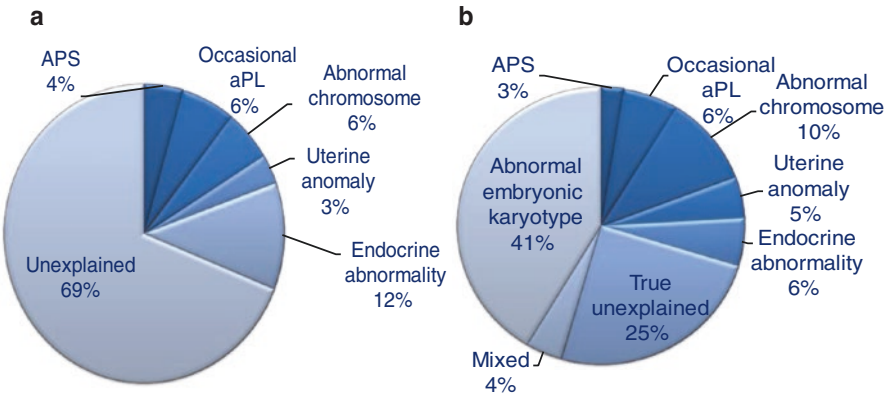

Fig. 10.1 Comparison of the distribution of causes (**a**) 1676 patients in our previous study. Sugiura-Ogasawara et al. Fertil Steril. 2010. (**b**) 482 patients with RM, including those with an abnormal embryonic karyotype. Sugiura-Ogasawara et al. Hum Reprod. 2012

The distribution of each cause depends on the characteristics of patients such as women's age or the number of previous miscarriages. Women's age, obesity, assisted conception, smoking, and alcohol are associated with RPL.

The "causes of RPL" should be strong predictors for subsequent miscarriage in the prospective study. It has not been established whether endocrine disorders such as hypothyroidism, diabetes mellitus and polycystic ovarian syndrome, thrombophilia, immune dysfunction, infection, and psychological stress may contribute to RPL because there were a limited number of randomized control trials concerning these issues.

10.2 Antiphospholipid Syndrome

APS is the most important treatable etiology. Low-dose aspirin plus heparin combined therapy is accepted as the standard treatment for patients with APS [10–12]. However, the live birth rate is limited to be 70–80%. The international classification criteria for the diagnosis of APS include obstetric clinical features as follows: [13].

1. Three or more consecutive unexplained miscarriages before the 10 week of gestation.
2. One or more unexplained death of a morphologically normal fetus at 10 weeks of gestation or later.
3. One or more premature births of a morphologically normal fetus at 34 weeks of gestation or earlier, associated with severe preeclampsia or placental insufficiency.

10.2.1 Fetal Death is Priority to Early Miscarriage

Recommended tests are lupus anticoagulant (LA) by at least two kinds of reagent such as dilute activated partial thromboplastin time (aPTT) and dilute Russell's viper venom time (RVVT) and β2glycoprotein I (β2GPI)-dependent anticardiolipin antibodies (aCL) IgG/IgM or anti-β2GPI antibodies IgG/IgM (Table 10.1) [13, 14]. Patients can be diagnosed as having APS when positive for at least one antiphospholipid antibody (aPL) persistent for 12 weeks to avoid pseudo positivity. The 99th percentile in healthy controls is recommended as the cutoff for the assays. The incidence of APS was 4.5% in our RPL previous study according to the international criteria [6].

The prospective studies concerning treatment methods are listed in Table 10.2. [8]. Combination of unfractionated heparin and low-dose aspirin is the standard treatment method [10–12, 15–20]. However, there were differences of assays and cutoff values to diagnose for APS among all facilities. The kinds of assays and titer might influence on the pregnancy outcome. The methods for detecting obstetric APS have not been established.

Table 10.2 Assays for antiphospholipid antibodies and cutoff values and live birth rate according to treatment in patients with antiphospholipid antibodies

	Anticardiolipin antibody	Lupus anticoagulant	Case (n)	Control (n)	Live birth rate %	
Cowchock et al. [10]	IgG > 30 IgM > 11	dRVVT or aPTT	A + scUFH (26)	A + PSL (19)	73.1	68.4
Silver et al. [15]	IgG > 8 IgM > 5	dRVVT	A + PSL (12)	A (22)	100	100
Kutteh et al. [11]	IgG > =27 IgM > =27	No	A + scUFH (25)	A (25)	80.0[a]	44.0
Rai et al. [12]	IgG > 5 IgM > 3	RVVT aPTT (exclude SLE)	A + scUFH (45)	A (45)	71.1[a]	42.2
Pattison et al. [16]	IgG > =5 IgM > =5	aPTT, dRVVT, KCT	A (20)		80	
Farquharson et al. [17]	IgG > 9 IgM > 5	dRVVT	A + scLMWH (51)	A (47)	78.4	72.3
Franklin and Kutteh [18]	IgG > 20 IgM > 20	dRVVT	A + LMWH (25)		76.0	
Noble and Kutteh [19]	IgG > 20 IgM > 20	dRVVT, aPTT	A + scLMWH (25)	A + scUFH (25)	84	80
Laskin et al. [20]	IgG > 15 IgM > 25	dRVVT, aPTT, KCT, dPT (include ANA, thrombophilia)	A + scLMWH (45)	A (43)	77.8	79.1

A low dose aspirin, *scUFH* subcutaneous unfructionate heparin, *PSL* prednisolone, *LMWH* low molecular weight heparin

[a]Significant difference

LA is well known to be better correlated with pregnancy morbidity than aCL [21, 22]. The PROMISE study concluded that LA, but not classical aCL, was a predictor of adverse pregnancy outcomes [21]. Harris et al. also confirmed that classical CL IgG and IgM were rarely associated with adverse pregnancy outcomes [22]. Both aPTT and RVVT are suitable for assay of LA, and two tests with different assay principles are recommended [13, 14]. Therefore, a combination of aPTT-based LA and dRVVT-based LA could be used in daily clinical practice.

We conducted a prospective study to examine whether a positive test result for β2GPI-dependent aCL might predict adverse pregnancy by 10 weeks of gestation in 1125 pregnant women without complications; results obtained using a cutoff value of 1.9 (99th percentile in healthy volunteers) were found to have a predictive value for intrauterine fetal death, intrauterine growth restriction, and preeclampsia [23]. However, in the study, it could not be ascertained whether β2GPI-dependent aCL might have been of predictive value for early miscarriage, because the sampling was conducted only at about 10 weeks of gestation. On the other hand, we established a test for LA by 5×–diluted aPTT with the mixing test (LA-aPTT) and proved that treatment could improve the subsequent live birth in patients with a positive test result [24]. The ascertainment of each assay to improve live birth rate has not been performed in obstetric APS though the clinical significance of the assay is to improve live birth rate. The true antigens of antiphospholipid antibodies are not phospholipids, but phospholipid-binding plasma proteins such as β2GPI, prothrombin, kininogen, protein C, and protein S [25, 26]. In fact, there are over ten commercially available methods in Japan. We determined clinical significance of LA-aPTT (StaClot) and phosphatidylserine-dependent antiprothrombin antibody but not aCL IgG, IgM, IgA, β2GPI IgG, IgM, and IgA (Phadia). Standardization is needed for detecting obstetric APS to improve the live birth rate [27].

Regarding antinuclear antibody (ANA), the frequency was significantly higher in 225 patients with two miscarriages than that in 740 normal pregnant controls; however, the ANA positive and ANA negative did not predict the subsequent miscarriage rate [28].

We usually carry out LA-aPTT, LA-RVVT, and β2GPI-dependent aCL in clinical practice. The prevalence of at least one positive test is 10.7%, and in 4.5%, the positive finding is sustained for 12 weeks until APS is diagnosed. Precise calculation of the gestational weeks can be made from the basal body temperature chart. Combined treatment with low-dose aspirin and heparin calcium at 10,000 IU/day (twice a day) should be started from 4 weeks of gestation. We discontinue aspirin by 35 weeks of gestation and continue heparin until the onset of labor.

Regarding occasional aPL, but not APS, it is not yet established how to treat them. The live birth rate with low-dose aspirin was 84.6% (44/52) and that was 95.7% (44/46) when miscarriage cases caused by an abnormal embryonic karyotype were excluded [29].

10.3 Congenital Uterine Anomaly

A 3.2–10.4% likelihood of having a major uterine anomaly except arcuate uterus is reported in patients with RPL [30–33]. The variation largely depends on the methods and the criteria selected for the diagnoses. The associations between arcuate uterus and RPL remain controversial.

Affected patients have been offered surgery in an attempt to restore the uterine anatomy. The live birth rates after surgery in studies including a relative large number of patients are summarized in Table 10.3 [30, 31]. 35–66% of patients with bicornuate or septate uteri give live births after correctional surgery [34–40]. All studies had no controls without surgery.

In contrast, we conducted a case-control study to examine the live birth rate without surgery in 1676 patients with a history of 2–12 consecutive miscarriages whose subsequent pregnancies were ascertained at least one time in our medical records [6]. Of the 42 patients with a septate or bicornuate uterus not treated by any kind of surgery, 59.5% (25) had a successful outcome, while this was the case in 71.7% (1096/1528) women with normal uteri at the subsequent first pregnancy (p = 0.084). The normal chromosomal karyotype rates in the aborted concepti in cases with anomalies were significantly higher than that in those without anomalies (84.6% vs. 42.5%, p = 0.006). 78.0% of patients (32/41) with anomalies and 85.5% of patients (1307/1528) patients with normal uteri could cumulatively have live babies within the follow-up period (not significant). Major uterine anomalies clearly have a negative impact on the reproductive outcome in women with RPL, being associated with a higher risk of further miscarriage with a normal embryonic karyotype. The large defect/cavity ratio was predictor of the subsequent miscarriage. Ghi et al. showed poorer outcome of 33.3% than our data [41].

We conducted the first multi-center prospective study to compare the live birth rate between with and without surgery in 170 patients with RPL associated with anomalies [42]. In 124 patients with a septate uterus, the live birth rate at the first pregnancy after ascertainment of anomalies with surgery tended to be higher than that in those without surgery (81.3% vs. 61.5%). The infertility rates were similar in both groups. Surgery showed no benefit in improving live birth rate in 46 patients with a bicornuate uterus, though it tended to decrease the preterm birth rate. A randomized control trial (RCT) is necessary to compare the live birth rates, also taking into consideration the infertility rate.

Table 10.3 Live birth rate with and without surgery in patients with congenital uterine anomalies

	Surgery							No surgery	
	Makino et al. [34]	Candiani et al. [35]	Ayhan et al. [36]	DeCherney et al. [37]	Daly et al. [38]	Hickok et al. [39]	Kormanyos et al. [40]	Sugiura-Ogasawara et al. [6]	Ghi et al. [41]
Type of anomaly	Arcuate, septate	Septate Bicornuate	Septate Bicornuate	Septate	Septate	Septate	Septate	Septate Bicornuate	Septate Subseptate
Indication	Recurrent SAB	Recurrent SAB Infertility	Recurrent SAB Preterm delivery	Recurrent SAB	Recurrent SAB Preterm delivery	Pregnancy loss Complication of pregnancy Infertility	2 or more SAB	2 or more SAB	First pregnancy
Method of surgery	Abdominal	Tompkins Jones Te Linde Strassman	Tompkins Jones Strassman	Resectoscope	Scissors	Resectoscope	Resectoscope	–	–
Live birth rate per pregnancy	84.8% (39/46)	68% (45/66) septate 76% (50/66) bicornuate	65% (30/46) septate 83% (45/54) bicornuate	80% (63/72) successful resection	80% (60/75)	77.3% (17/22)	68.8% (33/48) Cumulative 71.8% (51/71)		
Live birth rate per patient	54.9% (39/71)	66.0% (95/144)		61.2% (63/103)		84.6% (22/26)	35.1% (33/94) Cumulative 54.3% (51/94)	59.5% (25/42) Cumulative 78.0% (32/41)	33.3% (8/24)

10.4 Abnormal Chromosomes in Either Partner

A review of the data including 22,199 couples with a history of two or more miscarriages indicated that the rate of chromosomal structural rearrangements was 4.7% [43].

We conducted the first prospective study of 1284 couples to examine whether translocations constituted a risk factor for RPL [7]. Our findings indicated a live birth rate of 31.9% (15/47) in the first pregnancy after ascertainment of the carrier status, and a cumulative live birth rate was 68.1% (32/47), which is much less than that in cases with normal chromosomes (71.7%, 849/1184). We concluded that the prognosis of RPL patients with reciprocal translocations is poor, given that the study was conducted over a 17-year period and included severe cases with a history of 10–13 miscarriages.

Franssen et al. reported cumulative live birth rates with reciprocal translocations, Robertsonian translocations, and a normal karyotype of 83.0%, 82.0%, and 84.1%, respectively, based on a prospective case-control study [44]. They concluded that the chance of having a healthy child was as high as that in noncarrier couples, despite the higher risk of miscarriage.

The live birth rate with preimplantation genetic diagnosis (PGD) was reported to be 14–58% [45–50]. The live birth rate with natural conception was reported to be 32–65% on the first trial and 68–83% cumulatively [7, 44, 51]. The live birth rates with PGD in reciprocal translocation carriers are comparable to or sometimes lower than those with a subsequent first natural conception. The live birth rate with the use of new technology, microarray comparative genomic hybridization (array CGH) or single nucleotide polymorphism microarray, is also comparable to those with a subsequent first natural conception [49, 50].

We conducted the first cohort study to compare the live birth rate [52]. PGD was found to reduce the miscarriage rate significantly. However, infertility rate of PGD was significantly higher (18.9% vs. 3.8%). Cumulative live birth rates were 67.6% (25/37) and 65.4% (34/52), respectively, in the groups undergoing and not undergoing PGD. The cost of PGD was US$7956 per patient.

While PGD significantly prevented further miscarriages, there was no merit in improving the live birth rate. Couples should be fully informed of the advantages and disadvantages of PGD, such as the reduction in the miscarriage rate, higher cost, and IVF failure.

10.5 Abnormal Embryonic (Fetal) Karyotypes

Embryonic aneuploidy is the most common cause of RPL (Fig. 10.1). G-banding technique is used in clinical practice. An array CGH approach indicated about 80% of abnormality in the aborted embryo.

The rate of normal embryonic karyotype was significantly higher in patients with RPL than in patients with sporadic miscarriage [53]. The 16, 22, and 21 trisomies were the most common and there was no monosomy except 45,X (Fig. 10.2a). The miscarriage rate increased and the normal embryonic karyotype rate decreased

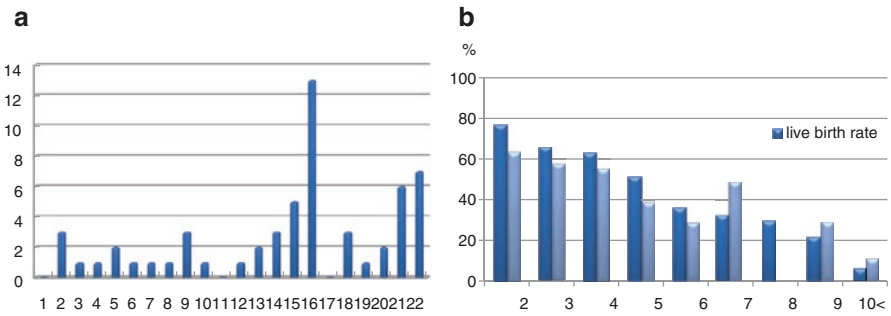

Fig. 10.2 Abnormal embryonic karyotype. (**a**) The number of chromosome. (**b**) The miscarriage rate increased and the normal embryonic karyotype rate decreased according to the number of previous miscarriages

according to the number of previous miscarriages in our previous study (Fig. 10.2b). The live birth rate of patients with a previous abnormal embryonic karyotype was significantly higher than that in patients with a previous normal embryonic karyotype. The embryonic karyotype can be a good predictor of subsequent success. Further examination could be saved if the second miscarriage was caused by the abnormal embryonic karyotype.

The live birth rate with preimplantation genetic screening (PGS) for aneuploidy was reported to be 4–47% [54–56]. The previous studies with the use of PGS lack appropriate controls. The subsequent live birth rates in unexplained patients, including patients caused by an abnormal embryonic karyotype, with previous two, three, four, and five miscarriages are 80%, 70%, 60%, and 50% with no medication, respectively [57]. The cumulative live birth rate was 85% [7]. This information is important for genetic counseling. The RCT is necessary in patients with RPL associated with aneuploidy.

10.6 Genetics and Thrombophilia in Truly Unexplained Patients

Single nucleotide polymorphisms (SNPs) of 187 candidate genes were reported to be associated with RPL. Factor V Leiden and prothrombin mutation have been most frequently examined. The frequencies of FV Leiden mutation, prothrombin mutation, and protein S deficiency are higher in patients with fetal loss than in controls [58, 59]. However, no association between protein S deficiency and early RPL was found [59]. Thrombophilia is speculated to cause fetal loss through placental dysfunction. It is important to distinguish between early miscarriage and fetal loss because many parts of RPL are early miscarriage, less than 10 weeks' gestation.

Gris et al. showed that treatment with low molecular weight heparin improved live birth rate in patients with FV Leiden or protein S deficiency and a history of one fetal loss [60]. However, sample size was relatively small. Recent RCT indicated that dalteparin had no effect in reducing pregnancy complications such as RPL,

preeclampsia, small gestational age and placental abruption, and venous thromboembolism in patients with a history of thrombophilia and pregnancy complications [61].

Annexin A5 is present on villi surface and a placental anticoagulant protein. Four cross-sectional studies have shown positive associations between *ANXA5* SNPs and RPL. Our previous cross-sectional study confirmed *ANXA5* SNP5 as a risk factor for RPL [62]. The presence/absence of the risk allele did not predict further miscarriage.

Six cross-sectional studies showed that the frequency of patients with low levels of coagulation factor XII (FXII) activity is significantly higher in patients with RPL than in controls. In the assay for FXII activity, aPTT is measured after mixing tested plasma and FXII-deficient plasma. LA prolongs aPTT and results in FXII activity decrease. It is well known that aPLs or anti-XII antibody reduces FXII activity. Our recent study proved that LA-aPTT but not β2GPI-dependent aCL reduced FXII activity by about 23% and that there was no difference of mean value of FXII activity between patients and controls after excluding patients with aPLs [63]. The study also suggested CT genotype of XII gene as a risk factor for RPL. However, both CT genotype and low FXII activity did not predict the subsequent miscarriage.

These studies mean that risk factors with small ORs identified in the cross-sectional study may be of little clinical relevance. It is speculated that patients with a number of risk alleles with small relative risks might be more likely to suffer from unexplained RPL. The effect of measurement and treatment of thrombophilia has not been established.

10.7 Treatment Methods for Unexplained Patients

However, patients with unexplained reason desire to receive medication. Paternal immunization, low-dose aspirin and heparin combined therapy, and progesterone had no effect of improving the live birth rate [64–66]. The subsequent live birth rates in unexplained patients, including patients caused by an abnormal embryonic karyotype, with previous two, three, four, and five miscarriages are 80%, 70%, 60%, and 50% with no medication, respectively [57]. The cumulative live birth rate was 85% [7]. It is important to make the patients aware that no medications have been established to improve the live birth rate shown above.

Several couples in our experience divorced or gave up trying to conceive after RPL, because they had the misconception that it would be impossible for them to have a living baby [2]. Psychological support with tender loving care might be the most important to encourage such couples to continue to conceive until a live birth results.

Disclosure None of the authors have any conflicts of interests to report.

References

1. Kolte AM, Bernardi LA, Christiansen OB, Quenby S, Farquharson RG, Goddijn M, Stephenson MD, ESHRE Special Interest Group for Early Pregnancy. Terminology for pregnancy loss prior to viability: a consensus statement from the ESHRE early pregnancy special interest group. Hum Reprod. 2015;30(3):495–8.

2. Sugiura-Ogasawara M, Suzuki S, Ozaki Y, Katano K, Suzumori N, Kitaori T. Frequency of recurrent spontaneous abortion and its influence on further marital relationship and illness: the Okazaki Cohort Study in Japan. J Obstet Gynaecol Res. 2013;39(1):126–31.
3. Kolte AM, van Oppenraaij RH, Quenby S, Farquharson RG, Stephenson M, Goddijn M, Christiansen OB, ESHRE Special Interest Group for Early Pregnancy. Non-visualized pregnancy losses are prognostically important for unexplained recurrent miscarriage. Hum Reprod. 2014;29(5):931–7.
4. Branch DW, Gibson M, Silver RM. Clinical practice: recurrent miscarriage. N Engl J Med. 2010;363:1740–7.
5. Farquharson RG, Pearson JF, John L. Lupus anticoagulant and pregnancy management. Lancet. 1984;28:228–9.
6. Sugiura-Ogasawara M, Ozaki Y, Kitaori T, Kumagai K, Suzuki S. Midline uterine defect size correlated with miscarriage of euploid embryos in recurrent cases. Fertil Steril. 2010;93:1983–8.
7. Sugiura-Ogasawara M, Ozaki Y, Sato T, Suzumori N, Suzumori K. Poor prognosis of recurrent aborters with either maternal or paternal reciprocal translocation. Fertil Steril. 2004;81:367–73.
8. Sugiura-Ogasawara M, Ozaki Y, Katano K, Kitaori T. Contemporary prevention and treatment of recurrent pregnancy loss. In: Bashiri A, Harlev A, Agarwal A, editors. Recurrent pregnancy loss. Cham: Springer International; 2016. p. 155–63.
9. Sugiura-Ogasawara M, Ozaki Y, Katano K, Suzumori N, Kitaori T, Mizutani E. Abnormal embryonic karyotype is the most frequent cause of recurrent miscarriage. Hum Reprod. 2012;27:2297–303.
10. Cowchock FS, Reece EA, Balaban D, Branch DW, Plouffe L. Repeated fetal losses associated with antiphospholipid antibodies: a collaborative randomized trial comparing prednisone with low-dose heparin treatment. Am J Obstet Gynecol. 1992;166(5):1318–23.
11. Kutteh WH. Antiphospholipid antibody-associated recurrent pregnancy loss: treatment with heparin and low-dose aspirin is superior to low-dose aspirin alone. Am J Obstet Gynecol. 1996;174:1584–9.
12. Rai R, Cohen H, Dave M, Regan L. Randomised controlled trial of aspirin and aspirin plus heparin in pregnant women with recurrent miscarriage associated with phospholipid antibodies (or antiphospholipid antibodies). BMJ. 1997;314:253–7.
13. Miyakis S, Lockshin MD, Atsumi T, et al. International consensus statement of an update of the classification criteria for definite antiphospholipid syndrome (APS). J Thromb Haemost. 2006;4:295–306.
14. Pengo V, Tripod A, Reber G, Rand JH, Ortel TL, Galli M, De Groot PG. Update of the guidelines for lupus anticoagulant detection. J Thromb Haemost. 2009;7:1737–40.
15. Silver RK, MacGregor SN, Sholl JS, Hobart JM, Neerhof MG, Ragin A. Comparative trial of prednisone plus aspirin versus aspirin alone in the treatment of anticardiolipin antibody-positive obstetric patients. Am J Obstet Gynecol. 1993;169(6):1411–7.
16. Pattison NS, Chamley LW, Birdsall M, Zanderigo AM, Liddell HS, McDougall J. Does aspirin have a role in improving pregnancy outcome for women with the antiphospholipid syndrome? A randomized controlled trial. Am J Obstet Gynecol. 2000;183(4):1008–12.
17. Farquharson RG, Quenby S, Greaves M. Antiphospholipid syndrome in pregnancy: a randomized, controlled trial of treatment. Obstet Gynecol. 2002;100(3):408–13.
18. Franklin RD, Kutteh WH. Antiphospholipid antibodies (APA) and recurrent pregnancy loss: treating a unique APA positive population. Hum Reprod. 2002;17(11):2981–5.
19. Noble LS, Kutteh WH, Lashey N, Franklin RD, Herrada J. Antiphospholipid antibodies associated with recurrent pregnancy loss: prospective, multicenter, controlled pilot study comparing treatment with low-molecular-weight heparin versus unfractionated heparin. Fertil Steril. 2005;83(3):684–90.
20. Laskin CA, Spitzer KA, Clark CA, Crowther MR, Ginsberg JS, Hawker GA, Kingdom JC, Barrett J, Gent M. Low molecular weight heparin and aspirin for recurrent pregnancy loss: results from the randomized, controlled HepASA Trial. J Rheumatol. 2009;36(2):279–87.
21. Lockshin MD, Kim M, Laskin CA, Guerra M, Branch DW, Merrill J, Petri M, Porter TF, Sammaritano L, Stephenson MD, Buyon J, Salmon JE. Prediction of adverse pregnancy

outcome by the presence of lupus anticoagulant, but not anticardiolipin antibody, in patients with antiphospholipid antibodies. Arthritis Rheum. 2012;64(7):2311–8.
22. Harris EN, Spinnato JA. Should anticardiolipin tests be performed in otherwise healthy pregnant women? Am J Obstet Gynecol. 1991;165(5 Pt 1):1272–7.
23. Katano K, Aoki K, Sasa H, et al. beta 2-Glycoprotein I-dependent anticardiolipin antibodies as a predictor of adverse pregnancy outcomes in healthy pregnant women. Hum Reprod. 1996;11:509–12.
24. Ogasawara MS, Aoki K, Katano K, et al. Factor XII but not protein C, protein S, antithrombin III or factor XIII as a predictor of recurrent miscarriage. Fertil Steril. 2001;75:916–9.
25. Matsuura E, Igarashi Y, Fujimoto M, Ichikawa I, Koike T. Anticardiolipin cofactor(s) and differential diagnosis of autoimmune disease. Lancet. 1990;21:177–8.
26. Roubey RAS. Autoantibodies to phospholipid-binding plasma proteins: a new view of lupus anticoagulant and other "antiphospholipid" autoantibodies. Blood. 1994;84:2854–67.
27. Kitaori T, Sugiura-Ogasawara M, Oku K, Papisch W, Ebara T, Ozaki Y, Katano K, Atsumi T. Determination of clinically significant tests for antiphospholipid antibodies and cutoff levels for obstetric antiphospholipid syndrome. Lupus. 2015;24:1505–19.
28. Ogasawara M, Aoki K, Kajiura S, Yagami Y. Are antinuclear antibodies predictive of recurrent miscarriage? Lancet. 1996;347:1183–4.
29. Sugiura-Ogasawara M, Ozaki Y, Nakanishi T, Sato T, Suzumori N, Nozawa K. Occasional antiphospholipid antibody positive patients with recurrent pregnancy loss also merit aspirin therapy: a retrospective cohort-control study. Am J Reprod Immunol. 2008;59:235–41.
30. Sugiura-Ogasawara M, Ozaki Y, Suzumori N. Müllerian anomalies and recurrent miscarriage. Curr Opin Obstet Gynecol. 2013;25:293–8.
31. Sugiura-Ogasawara M, Ozaki Y, Katano K, Suzumori N, Mizutani E. Uterine anomaly and recurrent pregnancy loss. Semin Reprod Med. 2011;29:514–21.
32. Chan YY, Jayaprakasan K, Zamora J, Thornton JG, Raine-Fenning N, Coomarasamy A. The prevalence of congenital uterine anomalies in selected and high-risk populations: a systematic review. Hum Reprod Update. 2011;17:761–71.
33. Saravelos SH, Cocksedge KA, Li TC. Prevalence and diagnosis of congenital uterine anomalies in women with reproductive failure: a critical appraisal. Hum Reprod Update. 2008;14:415–29.
34. Makino T, Umeuchi M, Nakada K, Nozawa S, Iizuka R. Incidence of congenital uterine anomalies in repeated reproductive wastage and prognosis for pregnancy after metroplasty. Int J Fertil. 1992;37(3):167–70.
35. Candiani GB, Fedele L, Parazzini F, Zamberletti D. Reproductive prognosis after abdominal metroplasty in bicornuate or septate uterus: a life table analysis. Br J Obstet Gynaecol. 1990;97(7):613–7.
36. Ayhan A, Yücel I, Tuncer ZS, Kişnişçi HA. Reproductive performance after conventional metroplasty: an evaluation of 102 cases. Fertil Steril. 1992;57(6):1194–6.
37. DeCherney AH, Russell JB, Graebe RA, Polan ML. Resectoscopic management of müllerian fusion defects. Fertil Steril. 1986;45(5):726–8.
38. Daly DC, Maier D, Soto-Albors C. Hysteroscopic metroplasty: six years' experience. Obstet Gynecol. 1989;73(2):201–5.
39. Hickok LR. Hysteroscopic treatment of the uterine septum: a clinician's experience. Am J Obstet Gynecol. 2000;182(6):1414–20.
40. Kormányos Z, Molnár BG, Pál A. Removal of a residual portion of a uterine septum in women of advanced reproductive age: obstetric outcome. Hum Reprod. 2006;21(4):1047–51.
41. Ghi T, De Musso F, Maroni E, Youssef A, Savelli L, Farina A, Casadio P, Filicori M, Pilu G, Rizzo N. The pregnancy outcome in women with incidental diagnosis of septate uterus at first trimester scan. Hum Reprod. 2012;27(9):2671–5.
42. Sugiura-Ogasawara M, Lin BL, Aoki K, Maruyama T, Nakatsuka M, Ozawa N, Sugi T, Takeshita T, Nishida M. Does surgery improve live birth rates in patients with recurrent miscarriage caused by uterine anomalies? J Obstet Gynaecol. 2015;35(2):155–8.

43. De Braekeler M, Dao TN. Cytogenetic studies in couples experiencing repeated pregnancy losses. Hum Reprod. 1990;5:519–28.
44. Franssern MTM, Korevaar JC, van der Veen F, et al. Reproductive outcome after chromosome analysis in couples with two or more miscarriages: case-control study. BMJ. 2006;332:759–62.
45. Lim CK, Jun JH, Min DM, Lee HS, Kim JY, Koong MK, Kang IS. Efficacy and clinical outcome of preimplantation genetic diagnosis using FISH for couples of reciprocal and Robertsonian translocations: the Korean experience. Prenat Diagn. 2004;24:556–61.
46. Otani T, Roche M, Mizuike M, Colls P, Escudero T, Munne S. Preimplantation genetic diagnosis significantly improves the pregnancy outcome of translocation carriers with a history of recurrent miscarriage and unsuccessful pregnancies. Reprod Biomed Online. 2006;13:869–74.
47. Feyereisen E, Steffann J, Romana S, Lelorc'h M, Ray P, Kerbrat V, Tachdjian G, Frydman R, Frydman N. Five years-experience of preimplantation genetic diagnosis in the Parisian Center: outcome of the first 441 started cycles. Fertil Steril. 2007;87:60–73.
48. Fischer J, Colls P, Escudero T, Munné S. Preimplantation genetic diagnosis (PGD) improves pregnancy outcome for translocation carriers with a history of recurrent losses. Fertil Steril. 2010;94:283–9.
49. Fiorentino F, Bono S, Biricik A, Nuccitelli A, Cotroneo E, Cottone G, Kokocinski F, Michel CE, Minasi MG, Greco E. Application of next-generation sequencing technology for comprehensive aneuploidy screening of blastocysts in clinical preimplantation genetic screening cycles. Hum Reprod. 2014;29(12):2802–13.
50. Idowu D, Merrion K, Wemmer N, Mash JG, Pettersen B, Kijacic D, Lathi RB. Pregnancy outcomes following 24-chromosome preimplantation genetic diagnosis in couples with balanced reciprocal or Robertsonian translocations. Fertil Steril. 2015;103(4):1037–42.
51. Hirshfeld-Cytron J, Sugiura-Ogasawara M, Stephenson MD. Management of recurrent pregnancy loss associated with a parental carrier of a reciprocal translocation: a systematic review. Semin Reprod Med. 2011;29:470–81.
52. Ikuma S, Sato T, Sugiura-Ogasawara M, Nagayoshi M, Tanaka A, Takeda S. Preimplantation genetic diagnosis and natural conception: a comparison of live birth rates in patients with recurrent pregnancy loss associated with translocation. PLoS One. 2015;10(6):e0129958.
53. Ogasawara M, Aoki K, Okada S, Suzumori K. Embryonic karyotype of abortuses in relation to the number of previous miscarriages. Fertil Steril. 2000;73:300–4.
54. Platteau P, Staessen C, Michiels A, Van Steirteghem A, Liebaers I, Devroey P. Preimplantation genetic diagnosis for aneuploidy screening in patients with unexplained recurrent miscarriages. Fertil Steril. 2005;83:393–7.
55. Wilding M, Forman R, Hogewind G, Di Matteo L, Zullo F, Cappiello F, Dale B. Preimplantation genetic diagnosis for the treatment of failed in vitro fertilization-embryo transfer and habitual abortion. Fertil Steril. 2004;81(5):1302–7.
56. Munné S, Chen S, Fischer J, Colls P, Zheng X, Stevens J, Escudero T, Oter M, Schoolcraft B, Simpson JL, Cohen J. Preimplantation genetic diagnosis reduces pregnancy loss in women aged 35 years and older with a history of recurrent miscarriages. Fertil Steril. 2005;84(2):331–5.
57. Katano K, Suzuki S, Ozaki Y, Suzumori N, Kitaori T, Sugiura-Ogasawara M. Peripheral natural killer cell activity as a predictor of recurrent pregnancy loss: a large cohort study. Fertil Steril. 2013;100(6):1629–34.
58. Preston FE, Rosendaal FR, Walker ID, Briët E, Berntorp E, et al. Increased fetal loss in women with heritable thrombophilia. Lancet. 1996;348:913–6.
59. Rey E, Kahn SR, David M, Shrier I. Thrombophilic disorders and fetal loss: a meta-analysis. Lancet. 2003;361:901–8.
60. Gris JC, Mercier E, Quéré I, Lavigne-Lissalde G, Cochery-Nouvellon E, Hoffet M, Ripart-Neveu S, Tailland ML, Dauzat M, Marès P. Low-molecular-weight heparin versus low-dose aspirin in women with one fetal loss and a constitutional thrombophilic disorder. Blood. 2004;103:3695–9.

61. Rodger MA, Hague WM, Kingdom J, Kahn SR, Karovitch A, Sermer M, Clement AM, Coat S, Chan WS, Said J, Rey E, Robinson S, Khurana R, Demers C, Kovacs MJ, Solymoss S, Hinshaw K, Dwyer J, Smith G, McDonald S, Newstead-Angel J, McLeod A, Khandelwal M, Silver RM, Le Gal G, Greer IA, Keely E, Rosene-Montella K, Walker M, Wells PS. TIPPS Investigators. Antepartum dalteparin versus no antepartum dalteparin for the prevention of pregnancy complications in pregnant women with thrombophilia (TIPPS): a multinational open-label randomised trial. Lancet. 2014;384(9955):1673–83.
62. Hayashi Y, Sasaki H, Suzuki S, Nishiyama T, Kitaori T, Mizutani E, Suzumori N, Sugiura-Ogasawara M. Genotyping analyses for polymorphisms of *ANXA5* gene in patients with recurrent pregnancy loss. Fertil Steril. 2013;100(4):1018–24.
63. Asano E, Ebara T, Yamada-Namikawa C, Kitaori T, Suzumori N, Katano K, Ozaki Y, Nakanishi M, Sugiura-Ogasawara M. Genotyping analysis for the 46 C/T polymorphism of coagulation factor XII and the involvement of factor XII activity in patients with recurrent pregnancy loss. PLoS One. 2014;9(12):e114452.
64. Ober C, Karrison T, Odem RR, Barnes RB, Branch DW, Stephenson MD, Baron B, Walker MA, Scott JR, Schreiber JR. Mononuclear-cell immunisation in prevention of recurrent miscarriages: a randomised trial. Lancet. 1999;354(9176):365–9.
65. Kaandorp SP, Goddijn M, van der Post JAM, Hutten BA, Verhoeve HR, Hamulyak K, Mol BW, Folkeringa N, Nahuis M, Papatsonis DNM, et al. Aspirin plus heparin or aspirin alone in women with recurrent miscarriage. N Engl J Med. 2010;362:1586–96.
66. Coomarasamy A, Williams H, Truchanowicz E, Seed PT, Small R, Quenby S, Gupta P, Dawood F, Koot YE, Bender Atik R, Bloemenkamp KW, Brady R, Briley AL, Cavallaro R, Cheong YC, Chu JJ, Eapen A, Ewies A, Hoek A, Kaaijk EM, Koks CA, Li TC, MacLean M, Mol BW, Moore J, Ross JA, Sharpe L, Stewart J, Vaithilingam N, Farquharson RG, Kilby MD, Khalaf Y, Goddijn M, Regan L, Rai R. A randomized trial of progesterone in women with recurrent miscarriages. N Engl J Med. 2015;373(22):2141–8.

Genomic Approach for Recurrent Pregnancy Loss: Prevention Feasible?

11

Aisaku Fukuda

Abstract

Recurrent pregnancy loss (RPL) affects 2–5% of all couples. RPL brings not only a grief for the patient but also physical damage on female reproductive organs. Furthermore, no matter how early in the pregnancy is, the loss of a baby is almost the same as the loss of a child. Therefore, a significant role for evaluation after just two losses in patients with no prior live births is strongly recommended. There are a small number of etiologies such as genetic, anatomic, immunologic, endocrine, and others, generally accepted for RPL. On the other hand, it has been widely accepted that aneuploidy is the most common cause of miscarriage. Although each etiologic factor has its treatment strategies, any treatment cannot prevail the aneuploidy. Preimplantation genetic screening (PGS) has been proposed as a method for reducing miscarriage by selecting euploid embryos for transfer. Previous PGS study by FISH failed to demonstrate its validity. However, PGS using recent technologies such as CCS has been showing its validity in preventing miscarriages. PGS with recent technology is feasible to reduce subsequent miscarriages in RPL patients. The drawback factor is that patients have to have ART toward the next conception.

Keywords

Recurrent pregnancy loss • Habitual abortion • Aneuploidy • PGS • ART

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11.1 Background

Recurrent pregnancy loss (RPL), also referred to as recurrent abortion (RA), recurrent spontaneous miscarriage, or habitual abortion, is practically defined as three or more consecutive pregnancy losses of clinically recognized pregnancies prior to 20 weeks of gestation excluding ectopic, molar, and biochemical pregnancies. RPL is defined as two or more failed pregnancies in the United States [1]. This definition is applied to the indication for clinical treatment in many advanced countries due to diminishing the number of babies born in each family. The incidence of RPL should be approximately 1 in 300 pregnancies based on the incidence of sporadic pregnancy loss. RPL brings not only a grief for the patient but also physical damage on female reproductive organs and affects 2–5% of all couples [2]. Furthermore, no matter how early in the pregnancy is, the loss of a baby is almost the same as the loss of a child to the patient. Therefore, RPL has serious influences on all the mental and physical feelings associated with it. RPL only compounds these feelings and can lead to increasing stress, anger, frustration, a feeling of loneliness and despair, emptiness and a feeling of a lack of self-worth, and failure. Although no definite

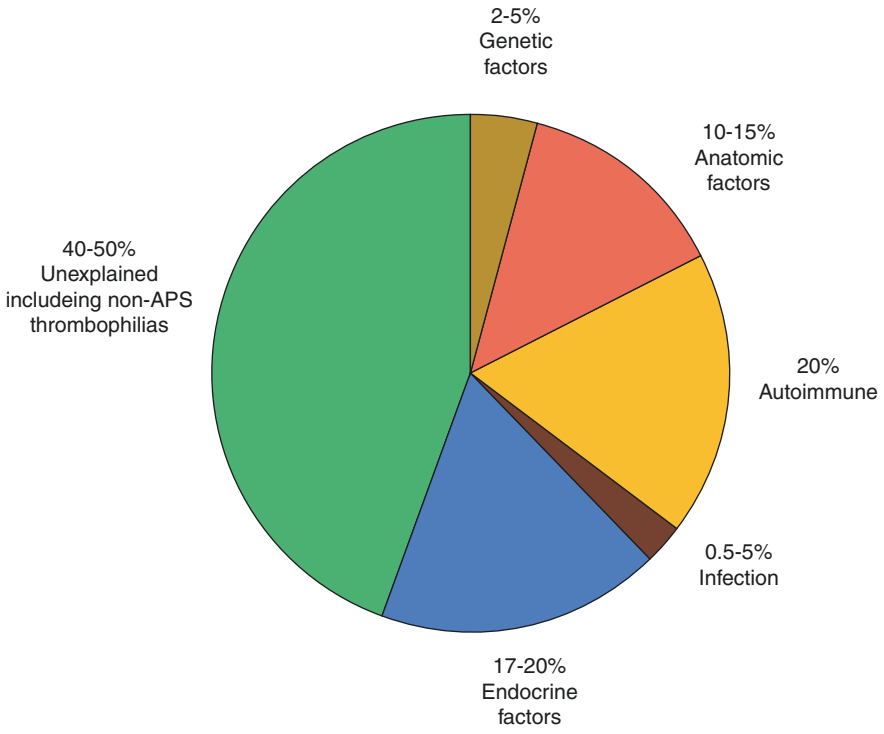

Fig. 11.1 Etiology of recurrent pregnancy loss. APS, antiphospholipid antibody syndrome (figure 1 from Rev Obstet Gynecol. 2009 Spring;2(2):76–83)

data have determined the probability of finding an etiology for RPL in a population with two versus three or more miscarriages, it has been suggested that the risk of subsequent miscarriage in the next pregnancies is 30% after two losses, compared with 33% after three losses among patients without a history of a live birth [3]. Diagnostic testing and therapeutic intervention for RPL as a clinical entity are based on understanding of the elevation of risk for subsequent fetal loss and the treatable etiology for the disorder. Therefore, a significant role for evaluation after just two losses in patients with no prior live births is strongly recommended in general. If fetal cardiac activity was identified prior to a loss, preventive evaluation should be considered in the woman older than 35 years, or the couple has had infertility treatment. There are a small number of etiologies generally accepted for RPL (Fig. 11.1).

11.2 Genetic Factors

Approximately 2–4% of RPL is associated with a parental balanced structural chromosome rearrangement, most commonly balanced reciprocal or Robertsonian translocations. These structural rearrangement disorders are already approved to be treated by preimplantation genetic diagnosis (PGD) through assisted reproductive technology (ART) to avoid subsequent miscarriage. There are some other structural abnormalities associated with RPL such as chromosomal inversions, insertions, and mosaicism. There might be some association with RPL including some single gene defects such as cystic fibrosis or sickle cell anemia. Parental karyotyping should be performed to evaluate RPL with genetic counseling. Appropriate genetic counseling is indicated in all cases of RPL associated with parental chromosomal abnormalities. Definitive treatment may need ART with PGD depending on the particular diagnosis. Preimplantation genetic screening (PGS) is explained in the following explanations in details. Application of donor gametes on treatment may be suggested in cases involving genetic anomalies that always result in embryonic aneuploidy such as Robertsonian translocations involving homologous chromosomes.

11.3 Anatomic Factors

Approximately 10–15% of causes of RPL are derived from anatomical malformation of female reproductive organs and are generally thought to induce miscarriage by insufficient vasculature of the endometrium, prompting abnormal and inadequate placentation. There are some suggested causes which are congenital uterine anomalies, intrauterine adhesions, and uterine fibroids or polyps. These abnormalities are thought to be potential causes of RPL through interruption of vascular supply of the endometrium. The most closely linked to RPL is uterine septum, congenital uterine anomaly, with as much as a 76% risk of spontaneous pregnancy loss among affected patients. Other Müllerian anomalies, including unicornuate, didelphic, and bicornuate uteri, have been associated with smaller increases in the risk for RPL. Arcuate uterus may or may not be causing RPL. The presence of intrauterine adhesions, sometimes associated

with Asherman syndrome, may significantly impact placentation and result in early pregnancy loss. Intramural fibroids larger than 5 cm, as well as submucosal fibroids of any size, may be associated with RPL. Myomectomy should be considered in cases of submucosal fibroids or any type of fibroids larger than 5 cm, especially in the patient under infertility treatment. Significantly, improvement of live birth rates has been shown by myomectomy from 57 to 93% [4]. Myomectomy could be performed via open laparotomy, laparoscopy, or hysteroscopy. Congenital anomalies caused by prenatal exposure to diethylstilbestrol (DES) are well known with reference to RPL. However, influence of DES administration is becoming less clinically significant since most affected patients move beyond their reproductive ages. Uterine anatomic anomalies should be evaluated by either office hysteroscopy or hysterosalpingography (HSG). If these abnormalities are determined, resection of intrauterine adhesions or intrauterine septa should be performed hysteroscopically. Successful hysteroscopic septum resection brings the patients nearly normal pregnancy outcomes, with term delivery rates of around 75% and live birth rates of around 85% [5].

11.4 Endocrine Factors

Endocrine disorders such as luteal insufficiency, polycystic ovary syndrome (PCOS), diabetes mellitus, thyroid gland disease, and hyperprolactinemia might be associated with RPL in approximately 17–20% [6]. Luteal insufficiency has been identified to be an inadequate progesterone production by the corpus luteum and insufficient endometrial maturation for implantation. However, definite influence of luteal insufficiency on RPL is controversial, and endometrial biopsies for diagnosis of luteal insufficiency are getting less performed. Insulin resistance with resultant hyperinsulinemia may play a role on RPL of the patients complicated with PCOS as well as type II diabetes mellitus, because treatment of those patients with insulin-sensitizing drug, metformin, decreased the rate of spontaneous pregnancy loss [7]. There is an evidence of PCOS in at least 40% of women with RPL. Poorly controlled type I diabetes mellitus is also associated with an increased risk of spontaneous abortion. Untreated hypothyroidism is clearly associated with spontaneous miscarriage and RPL, but the relation between antithyroid antibodies and RPL in euthyroid patients is currently under investigation. There are data to suggest that euthyroid women with antithyroid antibodies, especially those undergoing infertility treatment, are likely to become clinically hypothyroid when they achieved pregnancy. Because pregnancy outcomes in these women may improve with early (possibly prenatal) thyroid hormone replacement, similar approaches are presently being studied among women with RPL [8, 9]. Evaluation of endocrine disorders should include measurement of the thyroid-stimulating hormone (TSH) level. Other testings that might be indicated based on the patient's presentation include insulin resistance testing, ovarian reserve testing, serum prolactin in the presence of irregular menses, antithyroid antibody testing, and, very rarely, luteal phase endometrial biopsies. Therapy with insulin-sensitizing agents for the treatment of RPL that occurs in the presence of PCOS has recently gained popularity.

11.5 Infections

The role of infectious diseases in RPL is not clarified yet, but proposed an incidence of 0.5–5% [6, 10]. There are some candidate infectious diseases such as *Listeria monocytogenes*, *Toxoplasma gondii*, rubella, herpes simplex virus (HSV), measles, cytomegalovirus, and coxsackie viruses. Infectious diseases may cause pregnancy loss by the following mechanisms such as direct infection of the uterus, fetus, or placenta, placental insufficiency, chronic endometritis/endocervicitis, amnionitis, or intrauterine miscellaneous infections. Infections of mycoplasma, ureaplasma, *Chlamydia trachomatis*, *L. monocytogenes*, and HIV are speculated to play a role in RPL. Chronic infection is the most pertinent risk for RPL secondary to acute stage in an immunocompromised patient. Evaluation for chronic infections may be warranted for those patients. Overall, prevention of infectious diseases is not necessary, but favorable for the patient of RPL to relieve their anxiety.

11.6 Immune Factors

11.6.1 Alloimmune (Histocompatibility) Disorder

It is reasonable to infer that there are immunologic events that must occur to allow the mother to carry the fetus throughout gestation without rejection, because a fetus is not genetically identical to its mother. Therefore, there may be abnormalities within these immunologic mechanisms that could lead to both sporadic and recurrent pregnancy loss. In spite of the intense interest in this potential etiology for RPL, there is no consensus on appropriate diagnostic workup or therapy. Therapies such as paternal leukocyte immunization, intravenous immune globulin, third-party donor cell immunization, and trophoblast membrane infusions have been shown to provide no significant improvement in live birth rates and are only available for use in some areas [11].

11.6.2 Antiphospholipid Antibody Syndrome (APS)

Antiphospholipid antibody syndrome (APS) is characterized by the presence of at least one clinical and one laboratory criterion [12]. If you need details of APS, please refer to the literature. APS has strong association with RPL, but particularly in the second trimester. The relation of APS on RPL is not clarified yet, but is thrombophilia which is the most frequently acquired risk factor. There is a risk of thrombophilia with a prevalence of 3–5% in the general population. Evaluation for RPL related to APS should include testing for anticardiolipin antibodies and lupus anticoagulant at least. Treatment recommendations include low-dose aspirin (LDA: 81–100 mg/d) and/or low-molecular-weight heparin. LDA should be started before conception or with a positive pregnancy test, but heparin should be started with a positive pregnancy test. Heparin is safe during pregnancy because of large complex of molecules that do not cross the placenta.

11.7 Non-APS Thrombophilia

This problem happens mainly in Caucasian people. Inherited and combined inherited/acquired thrombophilias are common with more than 15% of the white population carrying an inherited thrombophilic mutation. The factor V Leiden mutation is the most common. This is the mutation in the promoter region of the prothrombin gene and mutations in the gene encoding methylenetetrahydrofolate reductase (MTHFR). These common mutations are associated with mild thrombotic risks, and it remains controversial whether homozygous MTHFR mutations are associated with vascular disease at all. The potential association between RPL and heritable thrombophilias is based on the theory that impaired placental development and function. This mechanism causes venous and/or arterial thrombosis and thereafter induces miscarriage. Pregnancy losses by this type of thrombophilia take place at greater than 10 weeks of gestation rather than prior to 10 weeks of gestation, because maternal blood begins to flow within intervillous spaces of the placenta at approximately 10 weeks of gestation. Of course, the transfer of nutrition from the maternal blood to the fetal tissues depends on uterine blood flow regardless of gestational age. Therefore, thrombotic events occurring at any gestational age play a role for thrombophilias in pregnancy losses [13]. The heritable thrombophilias associated with RPL include hyperhomocysteinemia resulting from MTHFR mutations, activated protein C resistance associated with factor V Leiden mutations, protein C and protein S deficiencies, prothrombin promoter mutations, and antithrombin mutations. Acquired thrombophilias associated with RPL include hyperhomocysteinemia and activated protein C resistance. Definite causative links between these heritable and acquired conditions have yet to be solidified. However, testings for factor V Leiden mutation, protein S levels, prothrombin promoter mutations, homocysteine levels, and global activated protein C resistance are appropriate targets for the selection of treatments. Once diagnosis is determined, appropriate therapy for heritable or acquired thrombophilias should be initiated. Specific for individual disorder should be performed as follows: supplementation of folic acid for those patients with hyperhomocysteinemia and prophylactic anticoagulation in cases of isolated defects with no personal or family history of thrombotic complications. Therapeutic anticoagulation should be performed in cases of combined thrombophilia defects.

11.8 Unexplained Factors

11.8.1 Alcohol, Smoking, and Caffeine

Patients are often particularly concerned about the possibility that environmental exposures may have caused their pregnancy losses, because of its propensity to result in feelings of responsibility and guilt. Association of RPL and occupational and/or environmental exposures to organic solvents, medications, ionizing radiation, and toxins have been suggested as always, but it is difficult to draw strong conclusions from because they tend to be retrospective and confounded by alternative or additional

environmental exposures. Exposures such as smoking, alcohol, and caffeine are three major particular topics which are of widespread use and a modifiable issue. Maternal alcoholism (or frequent consumption of intoxicating amounts of alcohol) is known with higher rates of spontaneous pregnancy loss, but a connection with more moderate ingestion remains controversial [14, 15]. Cigarette smoking could apparently increase the risk of spontaneous abortion based on the ingestion of nicotine, a strong vasoconstrictor that is known to reduce uterine and placental blood flow. Nevertheless, the relation between smoking and pregnancy loss remains still controversial [16, 17]. As to caffeine intake, there are some evidence that caffeine, even in amounts as low as three to five cups of coffee per day, may increase the risk of spontaneous pregnancy loss with a dose-dependent manner [18–20]. The association of caffeine, alcohol, and nicotine intake with RPL is even weaker than their associations with sporadic loss.

11.8.2 Miscellaneous Factors

Direct and indirect interventions for patients with RPL are outlined in the previous information. However, when all known and potential causes for RPL are screened, almost half of patients will remain without a definitive diagnosis. The optimal management of these patients is often as unclear as the etiology of their RPL. Progesterone has been shown to be beneficial in decreasing the miscarriage rate among women who have experienced at least three losses. Low-dose aspirin (LDA) therapy has also been investigated as a potential therapy for unexplained RPL. Its use prior to and during pregnancy has only been proven to increase live birth rates among those women with previous miscarriages beyond 13 weeks of gestation. Actually, the most effective therapy for patients with unexplained RPL is often the most simple: antenatal counseling and psychological support. These remedies have been shown to have subsequent pregnancy success rates of 86% when compared with success rates of 33% in women provided without additional antenatal care [21–24].

11.9 Aneuploid Embryo and RPL

Although exact causes of RPL have not been elucidated and still unexplained or idiopathic, some of the cause can be explained by various factors such as described above. Quintessential possibility of these causes for idiopathic RPL is that these couples are producing more aneuploid embryos, leading to higher miscarriage occurrence. The role of chromosomal abnormalities in miscarriage has been widely reported, with 50–70% of first-trimester miscarriages attributed to aneuploidy. Furthermore, it has been demonstrated that analyses of fetal chromosomes miscarried could explain 80% of unexplained RPL in older women [25]. A higher rate of aneuploidy in RPL patients has been confirmed by many authors [10, 26–34]. Preimplantation genetic screening (PGS) has been proposed as a method for reducing miscarriage by selecting euploid embryos for transfer, because of the prevalence of aneuploidy in first-trimester losses and the increased prevalence of aneuploidy in

the RPL population. The current standard of care for patients with unexplained RPL espoused by the American Society for Reproductive Medicine is expectant management [35]. However, the emotional trauma that can accompany clinical miscarriages and a perceived urgency to conceive felt by many RPL patients lead them toward alternative treatment options, including assisted reproductive technology, and specifically to in vitro fertilization (IVF) and PGS. Therefore, PGS for the indication of idiopathic RPL is that euploidy embryos could be selected for embryo transfer, leading to a decreased pregnancy loss rate in idiopathic RPL patients. All studies using PGS for this indication have evaluated that the miscarriage rate after this procedure has shown a decrease [36–39]. Again, it has been widely well accepted that aneuploidy is the most common genetic abnormality in embryos and also the most common cause of miscarriage [40, 41]. No matter how good treatment the patient is offered, if the embryo implanted was aneuploidy, it never works.

11.10 Is PGS Really Helpful for RPL Patients?

A term, aneuploidy, has been used to describe a loss or gain of genetic material of a chromosome(s) since the first human with aneuploidy. Since then, aneuploidy has been demonstrated to be a very common cause, accounting for no less than 15–20% of all clinical pregnancies. The majority of aneuploid embryos will never result in a clinical pregnancies and live birth, making aneuploidy the leading cause of miscarriage, but some are compatible with live birth, making aneuploidy the leading cause of congenital malformations and mental retardation. Aneuploidy has been identified as a significant factor contributing to IVF cycle failures, specifically implantation failure and/or spontaneous miscarriage in the field of assisted reproductive technology (ART) [42]. However, recent advances in reproductive medicine and molecular cytogenetics have completely changed the treatment protocol designed for infertile couples suffering from recurrent aneuploid losses. Genetic testings such as chorionic villus sampling, amniocentesis, and NIPT (noninvasive prenatal test) from maternal blood have been available prenatally. When these techniques are applied, if unfavorable results are revealed, a subsequent termination of living fetuses would still be necessary. Fluorescence in situ hybridization (FISH) was the main methodology of PGD or PGS over the past two decades, and aneuploidy detected by FISH technology with reference to infertility was reported in the beginning [43–45]. In spite of the confirmation of the high rate of aneuploidy in both repeated IVF failures and miscarriages, improvement of IVF outcomes with PGS by FISH was not demonstrated successfully [46–49]. These earlier studies were typically performed with the use of FISH evaluation of cleavage-stage embryos and typically tested only 7–12 chromosomes. In one meta-analysis [50], four observational studies [41, 51–53] were evaluated in which fertile patients with RPL underwent day 3 cleavage-stage biopsy of 1–2 cells and were compared with natural conception RPL patients. All four studies performed FISH screening 3–9 chromosomes. The spontaneous abortion rate (SABR) ranged from 0 to 10% (mean 9%) in RPL patients with PGS compared with 14–52% (mean 28%) with natural conception ($P = 0.0013$).

Thereafter, array CGH technology appeared by analyzing all 24 chromosomes, as opposed to FISH, allowing more accurate results when detecting for aneuploidy. There are several methods of comprehensive chromosome screening (CCS), including single nucleotide polymorphism (SNP) array, CGH, and quantitative polymerase chain reaction (PCR) [50]. Comparison of FISH with SNP array showed up to a 60% false-positive rate with FISH. When FISH was compared to CCS, it was found that mosaicism was three times more common in FISH [54]. Therefore, the European Society of Human Reproduction (ESHRE) recently recommended that this technique should be replaced by comprehensive methods of screening [55]. In conclusion, PGS by CCS should be applied to RPL patients in modern ART era.

11.11 Application of PGS on RPL Patients

It is difficult to find the ideal control group for RPL studies to determine if PGS is beneficial to reduce miscarriage. It is the question if the RPL couple should be compared with other couples undergoing PGS, with or without infertility, or only those with a history of RPL when PGS is applied and found that PGS using FISH significantly reduced miscarriage rates, from 36% expected rate to 13%. Patients that were offered PGS but rejected it had a 44% miscarriage rate, which is also another way to compare RPL patients using PGS with an appropriate control. This beneficial effect of PGS for RPL was observed in both fertile and infertile RPL patients undergoing IVF [40]. However, these studies used FISH, evaluated a limited number of chromosomes, and used day 3 embryo biopsy, which very recent evidence suggests it can negatively affect the implantation potential of the biopsied embryo, whereas blastocyst biopsy does not seem to be detrimental [56]. The clinical effectiveness of IVF and PGS compared with expectant management, which is the current standard of care in the treatment of RPL patients, has not been investigated with longitudinal prospective studies or randomized clinical trials. Furthermore, IVF–PGS is an expensive treatment option, and the cost-effectiveness of IVF–PGS compared with expectant management needs to be investigated. However, recent following report demonstrated the beneficial effect of PGS on RPL patients. It concluded that patients with RPL initiating PGS have a significantly higher LBR compared to expected management with no significant difference in miscarriage rate. Miscarriage rate would likely be lower if all IVF patients intending PGS completed the cycle as intended since aneuploidy is a common cause of first-trimester miscarriage. Of course, further studies are needed to investigate the cost-effectiveness of this treatment strategy for fertile RPL patients.

11.12 Justification of Applying PGS on RPL

It is widely recognized that aneuploidy is the leading cause of implantation failure and miscarriage in both fertile and infertile couples seeking to achieve a pregnancy. Cytogenetic analysis of previous miscarriages is an important component in the assessment of couples with a history of pregnancy loss because it can guide

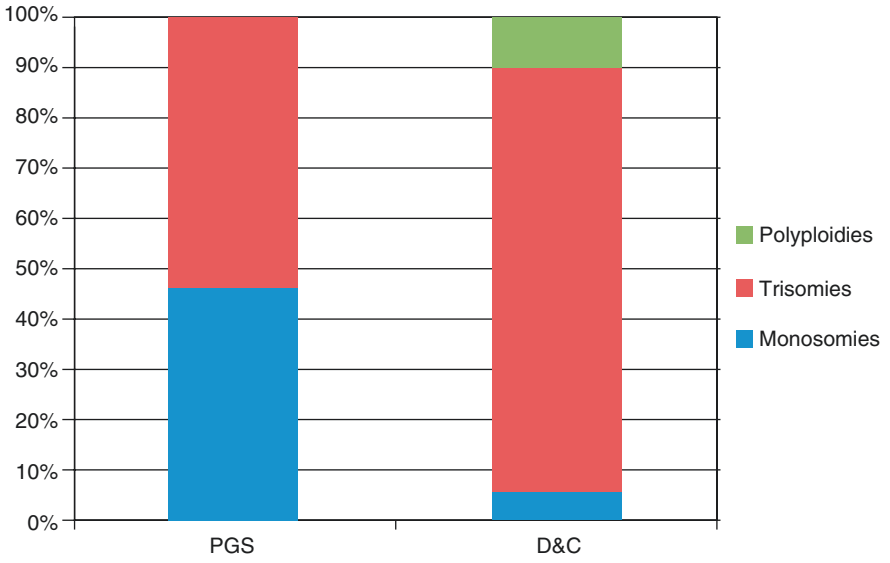

Fig. 11.2 This comparison shows how much percentage of miscarried abnormality overlaps the disorders of PGS samples (figure from Fertil Steril. 2015;104:1460–1466.e12 with permission)

subsequent treatment. Furthermore, the field of PGS for aneuploidy screening has also provided an opportunity to understand cell division errors, which has eliminated a potential implantation failure due to aneuploidy. Additionally, the use of PGS has created a positive impact on IVF success rates in certain cases, a worldwide push toward single-embryo transfer, and a reduction in multiple births after ART. The most common numerical chromosomal abnormality (NCA) occurs both before implantation after IVF/PGS and after implantation (D&C) in an infertile population pursuing pregnancy (Fig. 11.2).

Although the incidence of monosomies and trisomies was observed before implantation, it was rarely observed in these embryos implanted. These results advocate PGS technology as an advantageous facilitator that helps circumvent the inheritance of aneuploidy. Performing PGS will significantly reduce the incidence of NCA, thereby decreasing the likelihood of implantation failure and/or miscarriage after IVF. With this knowledge, implantation and pregnancy rates per transfer can be expected to be increased after an IVF cycle(s) by means of excluding unnecessary embryo transfer, preventing both implantation failure and early miscarriages. Many researchers have analyzed the frequency of NCA among human fetuses by analyzing chorionic villi after a miscarriage and report an incidence of NCA ranging between 40 and 80%. However, in general, these reports are biased by the high spontaneous loss rate of chromosomally abnormal pregnancies before a pregnancy is clinically recognized, as well as the lack of patients universally electing for cytogenetic analysis of their POCs. Remarkably, when these results were compared with tissue collected after D&C, monosomies were rarely observed, and trisomies most

frequently were shown in chromosomes 22, 16, 21, 15, and 19 (order reflects frequency). NCAs are present at a high frequency, rooting from early development. Previous molecular genetic analyses of chromosome abnormalities occurring in miscarriages have revealed that most aneuploid events arise during female meiosis, usually as a consequence of nondisjunction in the first meiotic division. Direct observation of female meiotic divisions (via polar body analysis) and early embryonic stages has shown that, before implantation, a wide range of aneuploidies are present. Historically most investigations have focused on trisomies, especially those compatible with live birth. On the basis of those analyses, three "rules" of human nondisjunction were formulated: first, regardless of the specific chromosome, most trisomies originate during oogenesis; second, for most chromosomes, maternal MI errors are more common than maternal meiosis II (MII) errors; and third, the proportion of cases of maternal origin increases with age [57]. PGS continues to evolve, but the current PGS technique does not detect certain polyploidies. The efficacy of genomic technologies could identify abnormal embryos that otherwise could appear to be morphologically normal. PGS assists the decision process before embryo transfer by detecting for any NCA, which potentially avoids early pregnancy loss. Discreet application of embryonic screening could maximize implantation and live birth rates and minimize the incidence of miscarriages related to chromosomal abnormalities [58, 59]. Moreover, understanding of the etiology of reproductive loss can alleviate the feelings of guilt or irresponsibility in the patient under infertility treatment [60]. Nevertheless, further studies may provide guidance into optimizing or even improving oocyte quality, such as pronuclear transfer, maternal spindle transfer, or nuclear genome transfer, and into noninvasive ways to identify reproductively competent embryos to improve clinical outcome [61, 62].

11.13 Summary

1. Etiology of recurrent pregnancy loss is not well identified, but there are some candidates with effective treatment. However, any treatment cannot overcome aneuploid embryo.
2. Chromosome errors, aneuploidy, in human embryos are a major cause of ART failure, miscarriage, obstetric complications, stillbirth, and infertility and result in the birth of affected children.
3. Accurate technology for detecting chromosomally normal, euploid embryos exists and is now available for clinical use.
4. Recent technologies such as array CGH, qPCR, and NGS can detect all 24 chromosomes with high accuracy and should be applied for PGS in the patients suffering IVF failure and/or recurrent pregnancy losses.
5. PGS can reduce the time to a live birth by selecting only euploid embryos and reduce the incidence of miscarriage.
6. PGS makes it possible to perform a single euploid embryo transfer that maximizes a chance of live birth without the risk of a multiple pregnancy.
7. Any treatment plan for RPL cannot prevail over aneuploid embryos.

8. The patients have no embryo transfer because no euploid embryos prefer to know the reality. They can move on for the future rather than wait for failure or possible miscarriage or the birth of an affected child.
9. Yes, PGS is feasible for prevention of recurrent pregnancy loss; however, ART is necessary for the patient who conceived naturally.

References

1. Practice Committee of the American Society for Reproductive Medicine. Definitions of infertility and recurrent pregnancy loss. Fertil Steril. 2008;90:S60.
2. Stephenson M, Kutteh W. Evaluation and management of recurrent early pregnancy loss. Clin Obstet Gynecol. 2007;50:132–45.
3. The American College of Obstetricians and Gynecologists. Management of recurrent early pregnancy loss. ACOG Practice Bulletin No. 24. Washington, DC: The American College of Obstetricians and Gynecologists; 2001.
4. Bajekal N, Li TC. Fibroids, infertility and pregnancy wastage. Hum Reprod Update. 2000;6:614–20.
5. Grimbizis GF, Camus M, Tarlatzis BC, et al. Clinical implications of uterine malformations and hysteroscopic treatment results. Hum Reprod Update. 2001;7:161–74.
6. Fox-Lee L, Schust DJ. Recurrent pregnancy loss. In: Berek JS, editor. Berek and Novak's gynecology. Philadelphia: Lippincott Williams & Wilkins; 2007. p. 1277–322.
7. Rai R, Backos M, Rushworth F, Regan L. Polycystic ovaries and recurrent miscarriage—a reappraisal. Hum Reprod. 2000;15:612–5.
8. Poppe K, Velkeniers B, Glinoer D. The role of thyroid autoimmunity in fertility and pregnancy. Nat Clin Pract Endocrinol Metab. 2008;4:394–405.
9. Negro R, Formoso G, Coppola L, et al. Euthyroid women with autoimmune disease undergoing assisted reproduction technologies: the role of autoimmune disease and thyroid function. J Endocrinol Investig. 2007;30:3–8.
10. Stephenson MD. Frequency of factors associated with habitual abortion in 197 couples. Fertil Steril. 1996;66:24–9.
11. Porter TF, LaCoursiere Y, Scott JR. Immunotherapy for recurrent miscarriage. Cochrane Database Syst Rev. 2006;2:CD000112.
12. Derksen RHWM. The obstetric antiphospholipid syndrome. J Reprod Immunol. 2008;77:41–50.
13. Burton G, Hempstock J, Jauniaux E. Nutrition of the human fetus during the first trimester—a review. Placenta. 2001;22:S70–6.
14. Windham GC, Von Behren J, Fenster L, et al. Moderate maternal alcohol consumption and risk of spontaneous abortion. Epidemiology. 1997;8:509–14.
15. Rasch V. Cigarette, alcohol, and caffeine consumption: risk factors for spontaneous abortion. Acta Obstet Gynecol Scand. 2003;82:182–8.
16. Kline J, Levin B, Kinney A, et al. Cigarette smoking and spontaneous abortion of known karyotype: precise data but uncertain inferences. Am J Epidemiol. 1995;141:417–27.
17. Ness RB, Grisso JA, Hirschinger N. Cocaine and tobacco use and the risk of spontaneous abortion. N Engl J Med. 1999;340:333–9.
18. Mills JL, Holmes LB, Aarons JH. Moderate caffeine use and the risk of spontaneous abortion and intrauterine growth retardation. JAMA. 1993;269:593–7.
19. Cnattingius S, Signorello LB, Anneren G, et al. Caffeine intake and the risk of first-trimester spontaneous abortion. N Engl J Med. 2000;343:1839–45.
20. Domínguez-Rojas V, de Juanes-Pardo JR, Astasio-Arbiza P, et al. Spontaneous abortion in a hospital population: are tobacco and coffee intake risk factors? Eur J Epidemiol. 1994;10:665–8.

21. Haas DM, Ramsey PS. Progestogen for preventing miscarriage. Cochrane Database Syst Rev. 2008;2:CD003511.
22. Rai R, Backos M, Baxter N, et al. Recurrent miscarriage—an aspirin a day? Hum Reprod. 2000;15:2220–3.
23. Tulppala M, Marttunen M, Söderström-Anttila V, et al. Low-dose aspirin in prevention of miscarriage in women with unexplained or autoimmune related recurrent miscarriage: effect on prostacyclin and thromboxane A_2 production. Hum Reprod. 1997;12:1567–72.
24. Stray-Pedersen B, Stray-Pedersen S. Etiologic factors and subsequent reproductive performance in 195 couples with a prior history of habitual abortion. Am J Obstet Gynecol. 1984;148:140–6.
25. Marquard K, Westphal L, Milki A, Lathi R. Etiology of recurrent pregnancy loss in women over the age of 35. Fertil Steril. 2010;94:1473–7.
26. Daniely M, Aviram-Goldring A, Barkai G, Goldman B. Detection of chromosomal aberration in fetuses arising from recurrent spontaneous abortion by comparative genomic hybridization. Hum Reprod. 1998;13:805–9.
27. Fritz B, Hallermann C, Olert J, Fuchs B, Bruns M, Aslan M, et al. Cytogenetic analyses of culture failures by comparative genomic hybridisation (CGH)—re-evaluation of chromosome aberration rates in early spontaneous abortions. Eur J Hum Genet. 2001;9:539–47.
28. Pellicer A, Rubio C, Vidal F, Minguez Y, Gimenez C, Egozcue J, et al. In vitro fertilization plus preimplantation genetic diagnosis in patients with recurrent miscarriage: an analysis of chromosome abnormalities in human preimplantation embryos. Fertil Steril. 1999;71:1033–9.
29. Simon C, Rubio C, Vidal F, Gimenez C, Moreno C, Parrilla JJ, et al. Increased chromosome abnormalities in human preimplantation embryos after in vitro fertilization in patients with recurrent miscarriage. Reprod Fertil Dev. 1998;10:87–92.
30. Vidal F, Gimenez C, Rubio C, Simon C, Pellicer A, Santalo J, et al. FISH preimplantation diagnosis of chromosome aneuploidy in recurrent pregnancy wastage. J Assist Reprod Genet. 1998;15:310–3.
31. Sullivan AE, Silver RM, LaCoursiere DY, Porter TF, Branch DW. Recurrent fetal aneuploidy and recurrent miscarriage. Obstet Gynecol. 2004;104:784–8.
32. Stephenson MD, Awartani KA, Robinson WP. Cytogenetic analysis of miscarriages from couples with recurrent miscarriage: a case-control study. Hum Reprod. 2002;17:446–51.
33. Ogasawara M, Aoki K, Okada S, Suzumori K. Embryonic karyotype of abortuses in relation to the number of previous miscarriages. Fertil Steril. 2000;73:300–4.
34. Carp H, Toder V, Aviram A, Daniely M, Mashiach S, Barkai G. Karyotype of the abortus in recurrent miscarriage. Fertil Steril. 2001;75:678–82.
35. Practice Committee of the American Society for Reproductive Medicine. Evaluation and treatment of recurrent pregnancy loss: a committee opinion. Fertil Steril. 2012;98:1103–11.
36. Werlin L, Rodi I, DeCherney A, Marello E, Hill D, Munné S. Preimplantation genetic diagnosis as both a therapeutic and diagnostic tool in assisted reproductive technology. Fertil Steril. 2003;80:467–8.
37. Rubio C, Simon C, Vidal F, Rodrigo L, Pehlivan T, Remohi J, et al. Chromosomal abnormalities and embryo development in recurrent miscarriage couples. Hum Reprod. 2003;18:182–8.
38. Garrisi JG, Colls P, Ferry KM, Zheng X, Garrisi MG, Munné S. Effect of infertility, maternal age, and number of previous miscarriages on the outcome of preimplantation genetic diagnosis for idiopathic recurrent pregnancy loss. Fertil Steril. 2009;92:288–95.
39. Munné S, Chen S, Fischer J, Colls P, Zheng X, Stevens J, et al. Preimplantation genetic diagnosis reduces pregnancy loss in women aged 35 years and older with a history of recurrent miscarriages. Fertil Steril. 2005;84:331–5.
40. Palermo GD, Munne S, Colombero LT, Cohen J, Rosenwaks Z. Genetics of abnormal human fertilization. Hum Reprod. 1995;10(Suppl 1):120–7.
41. Drugan A, Koppitch 3rd FC, Williams 3rd JC, Johnson MP, Moghissi KS, Evans MI. Prenatal genetic diagnosis following recurrent early pregnancy loss. Obstet Gynecol. 1990;75:381–4.
42. Pellestor F, Andreo B, Anahory T, Hamamah S. The occurrence of aneuploidy in human: lessons from the cytogenetic studies of human oocytes. Eur J Med Genet. 2006;49:103–16.

43. Kamiguchi Y, Rosenbusch B, Sterzik K, Mikamo K. Chromosomal analysis of unfertilized human oocytes prepared by a gradual fixation-air drying method. Hum Genet. 1993;90(5):533–41.
44. Munne S, Lee A, Rosenwaks Z, Grifo J, Cohen J. Diagnosis of major chromosome aneuploidies in human preimplantation embryos. Hum Reprod. 1993;8(12):2185–91.
45. Delhanty JD, Griffin DK, Handyside AH, Harper J, Atkinson GH, Pieters MH, et al. Detection of aneuploidy and chromosomal mosaicism in human embryos during preimplantation sex determination by fluorescent in situ hybridisation, (FISH). Hum Mol Genet. 1993;2(8):1183–5.
46. Mastenbroek S, Twisk M, van Echten-Arends J, Sikkema-Raddatz B, Korevaar JC, Verhoeve HR, et al. In vitro fertilization with preimplantation genetic screening. N Engl J Med. 2007;357(1):9–17.
47. Staessen C, Verpoest W, Donoso P, Haentjens P, Van der Elst J, Liebaers I, et al. Preimplantation genetic screening does not improve delivery rate in women under the age of 36 following single-embryo transfer. Hum Reprod. 2008;23(12):2818–25.
48. Treff NR, Levy B, Su J, Northrop LE, Tao X, Scott Jr RT. SNP microarray-based 24 chromosome aneuploidy screening is significantly more consistent than FISH. Mol Hum Reprod. 2010;16(8):583–9.
49. Twisk M, Mastenbroek S, Hoek A, Heineman MJ, van der Veen F, Bossuyt PM, et al. No beneficial effect of preimplantation genetic screening in women of advanced maternal age with a high risk for embryonic aneuploidy. Hum Reprod. 2008;23(12):2813–7.
50. Musters AM, Repping S, Korevaar JC, Mastenbroek S, Limpens J, van der Veen F et al. Pregnancy outcome after preimplantation genetic screening or natural conception in couples with unexplained recurrent miscarriage: a systematic review of the best available evidence. Fertil Steril. 2011;95:2153–57 (2157.e1–3).
51. Wilding M, Forman R, Hogewind G, di Matteo L, Zullo F, Cappiello F, et al. Preimplantation genetic diagnosis for the treatment of failed in vitro fertilization–embryo transfer and habitual abortion. Fertil Steril. 2004;81:1302–7.
52. Platteau, P., Staessen, C., Michiels, A., Van Steirteghem, A., Liebaers, I., and Devroey, P. Preimplantation genetic diagnosis for aneuploidy screening in patients with unexplained recurrent miscarriages. Fertil Steril. 2005;83:393–397. (quiz 525–6).
53. Mantzouratou A, Mania A, Fragouli E, Xanthopoulou L, Tashkandi S, Fordham K, et al. Variable aneuploidy mechanisms in embryos from couples with poor reproductive histories undergoing preimplantation genetic screening. Hum Reprod. 2007;22:1844–53.
54. Wilton L, Voullaire L, Sargeant P, Williamson R, McBain J. Preimplantation aneuploidy screening using comparative genomic hybridization or fluorescence in situ hybridization of embryos from patients with recurrent implantation failure. Fertil Steril. 2003;80(4):860–8.
55. Harper J, Coonen E, De Rycke M, Fiorentino F, Geraedts J, Goossens V, et al. What next for preimplantation genetic screening (PGS)? A position statement from the ESHRE PGD Consortium Steering Committee. Hum Reprod. 2010;25(4):821–3.
56. Treff NR, Ferry KM, Zhao T, Su J, Forman EJ, Scott Jr RT. Cleavage stage embryo biopsy significantly impairs embryonic reproductive potential while blastocyst biopsy does not: a novel paired analysis of cotransferred biopsied and nonbiopsied sibling embryos. Fertil Steril. 2011;96:S2.
57. Hassol T, Hall H, Hunt P. The origin of human aneuploidy: where we have been, where we are going. Hum Mol Genet. 2007;16:R203–8.
58. Scott RT, Upham KM, Forman EJ, Hong KH, Scott KL, Taylor D, et al. Blastocyst biopsy with comprehensive chromosome screening and fresh embryo transfer significantly increases in vitro fertilization implantation and delivery rates: a randomized controlled trial. Fertil Steril. 2013;100:697–703.
59. Lee E, Illingworth P, Wilton L, Chambers GM. The clinical effectiveness of preimplantation genetic diagnosis for aneuploidy in all 24 chromosomes (PGD-A): systematic review. Hum Reprod. 2015;30:473–83.

60. Kong GW, Chung TK, Lok IH. The impact of supportive counselling on women's psychological wellbeing after miscarriage—a randomized controlled trial. Br J Obstet Gynaecol. 2014;121:1253–62.
61. Botros L, Sakkas D, Seli E. Metabolomics and its application for non-invasive embryo assessment in IVF. Mol Hum Reprod. 2008;14:679–90.
62. Rodriguez-Purata JD, Lee J, Whitehouse M, Moschini RM, Knopman J, Duke M, Sandler B, Copperman A. Embryo selection versus natural selection: how do outcomes of comprehensive chromosome screening of blastocysts compare with the analysis of products of conception from early pregnancy loss (dilation and curettage) among an assisted reproductive technology population? Fertil Steril. 2015;104:1460–1466.e12.

Prenatal Diagnosis of the Human Embryo and Fetus

12

Shigehito Yamada and Hidehiko Miyake

Abstract

During the prenatal period, several approaches, such as imaging and genetic tests, can be used to identify diagnostic clues. Owing to recent progress in imaging modalities, we can now image smaller embryos and fetuses with a higher resolution. Here, we describe the diagnostic imaging modalities for living or dead embryos and fetuses. In addition, we provide information on the latest genetic testing methods with a focus on both the technical and ethical aspects.

Keywords

Clinical ethics • Prenatal diagnosis • Diagnostic imaging

12.1 Imaging of the Human Embryo and Fetus

12.1.1 Prenatal Imaging of Human Embryos and Fetuses

Fetal ultrasound was developed in the late 1950s as A-mode, in the 1970s as B-mode, in the 1980s as real-time imaging, and in the 1990s as 3D imaging [1, 2]. Currently, ultrasonography is commonly performed throughout pregnancy. The gestational sac at approximately 5 weeks, the yolk sac at 5.5 weeks, flickering cardiac motion at 6 weeks, etc. are observed by transvaginal ultrasonography. Embryos and early

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Table 12.1 Representative formula for estimation of fetal weight

Name (year)	Formula	Ref.
Warsof (1977)	$\log_{10}BW = -1.599 + (0.144 \times BPD) + (0.032 \times AC) - (0.000111 \times BPD^2 \times AC)$	[3]
Shepard (1982)	$\log_{10}BW = -1.7492 + (0.166 \times BPD) + (0.046 \times AC) - [(2.646 \times AC \times BPD)/1000]$	[4]
Hadlock (1985)	$\log_{10}BW = 1.335 - (0.0034 \times AC \times FL) + (0.0316 \times BPD) + (0.0457 \times AC) + (0.1623 \times FL)$	[5]
Aoki (1985)	$EFBW = (1.25647 \times BPD^3) + (3.50665 \times FTA \times FL) + 6.30994$	[6]
Shinozuka (1987)	$EFBW = (1.07 \times BPD^3) + (3.42 \times APTD \times TTD \times FL)$	[7]
Modified Shinozuka (2000)	$EFBW = (1.07 \times BPD^3) + (0.30 \times AC^2 \times FL)$	[8]

AC abdominal circumference, *APTD* anterior-posterior trunk diameter, *BPD* biparietal diameter, *BW* body weight, *FL* femur length, *FTA* fetal trunk cross-sectional area, *TTD* transverse trunk diameter

fetuses within 12 weeks of gestation are usually examined by transvaginal ultrasonography, and fetuses more than 12 weeks are examined by transabdominal ultrasonography. Ultrasonography plays several roles in the examination of embryos and fetuses. One of its roles is the measurement of embryos and fetuses to determine gestational age and estimate fetal weight. A formula to estimate fetal weight was first suggested in the late 1970s [3]. Several formulas have been proposed and are widely used since then [4–8] (Table 12.1), and new formulas for estimating fetal weight are also frequently suggested [9, 10]. Detection (including assessment) of congenital fetal anomalies is also another role of ultrasonography. The first application of ultrasonography to the diagnosis of congenital disease is to evaluate anencephaly [11], and various anomalies can currently be detected using ultrasonography. For effective screening of morphological anomalies, a definition of optimal fetal anatomy survey has been published in the guidelines from the International Society of Ultrasound in Obstetrics and Gynecology (ISUOG) [12]. During the 1980s and 1990s, certain studies found that soft markers in ultrasonography, which are not harmful themselves, indicate an elevated risk of chromosomal abnormalities [13–15]. At present, soft markers combined with maternal serum can achieve a high detection rate for aneuploidy [16].

When an abnormality is detected during prenatal US examination or when there is an increased risk for neurodevelopmental disabilities, MRI is the next imaging choice for further examination. Fetal MRI can reveal abnormalities of the central nervous system that are not apparent on prenatal US in approximately 20% of cases [17]. If an atrial width of ≥10 mm is detected at the glomus of the choroid plexus on an axial ultrasound image at the level of the thalamus, it is considered to represent ventriculomegaly [18]; thus, an MRI scan would be performed for further diagnosis. Fetal MRI reveals additional abnormalities in up to 50% of ventriculomegaly cases identified in US images [19, 20]. Commonly associated anomalies include agenesis of the corpus callosum, migrational anomalies, and ventricular hemorrhage. Holoprosencephaly (HPE) can also be detected by ultrasound and MRI; however, milder cases of lobar HPE can be difficult to detect considering the wide spectrum of HPE symptoms. Cortical malformations are characteristics of

target diseases in fetal MRI, although the normal smoothness of the cortex in the second trimester makes it difficult to be distinguished from that in migrational disorders [21]. Intracranial tumors are quite rare and can be detected by fetal MRI, mostly as a supratentorial lesion with mixed/high signal intensity on T2-weighted sequences.

MRI is also effective for other congenital anomalies. For congenital diaphragmatic hernia (CDH), which occurs in 1 in 4000 live births, fetal MRI is superior to US in distinguishing the subtypes of CDH [22]. The analysis of fetal lung volume is important in CDH for evaluation of prognosis, and MRI is commonly used to calculate the volume during evaluation. This function of MRI can also be applied to other diseases involving lung mass, such as pulmonary sequestrations, congenital pulmonary airway malformations, and, very rarely, neoplasms. Furthermore, MRI is used in the diagnosis of ventral abdominal wall defects such as gastroschisis and omphalocele. Axial T2-weighted sequences are the most helpful for revealing defects in the abdominal wall and the position of the umbilical cord, whereas T1-weighted sequences are useful in tracing the bowel [23, 24]. Musculoskeletal system evaluation is still one of the challenging aspects of using fetal MRI. Diffusion tensor imaging (DTI) has recently been applied in clinical MRI, and muscle fibers can be imaged by DTI in adults [25]. Although fetal muscle imaging using DTI has started only in experimental animals [26], it will be applied for fetal MRI in the near future.

Despite the risk to the fetus by exposure to ionizing radiation, X-ray computed tomography (CT) plays an important diagnostic role during the prenatal period, especially for fetal skeletal diseases (SDs). The sensitivity to SDs in ultrasonography screening is limited, ranging between 40 and 60% [27, 28], and diagnostic three-dimensional ultrasonography offers a better sensitivity of around 80% [29–31]. MRI is no more effective than ultrasonography for further diagnosis of SDs [32]. In recent years, fetal skeletal CT has been used for visualizing the fetal skeleton [33, 34]. A low-dose CT protocol with 3D reconstruction has been suggested for decreasing the adverse effects of X-ray, and fine images can be obtained for accurate diagnosis [32, 35].

12.1.2 Autopsy Imaging of Human Embryos and Fetuses

For dead human embryos and fetuses, additional imaging modalities can be applied. Classically, solid reconstruction and fine drawing have been the primary approaches used, for example, the wax plate technique using serial histological sections of human embryos was the first 3D morphological imaging technique developed by Gustav Born [8]. Recently, the 3D reconstruction of serial sections is performed using computer graphic methods; therefore, the 3D reconstruction becomes easier and quicker than before [36]. The 2D image stacks generated from serial sections have a high resolution; however, they have issues of section registration and distortion. A solution to this problem is by using episcopic fluorescence image capture (EFIC), a novel imaging modality for the generation of high-resolution 3D reconstructed images [37]. In EFIC imaging, tissue autofluorescence is used to image the

block face prior to cutting each section. Although the samples are sliced and lost during the procedure, the optical resolution of EFIC has been reported to reach approximately 5–6 μm [38].

MRI is a useful imaging modality not only for living prenatal embryos and fetuses but also for dead embryos and fetuses as autopsy imaging. Since it takes a longer time to capture images, images with a higher resolution can be obtained. The imaging time for high-resolution images ranges from several hours to several days. MR devices should be selected depending on the sample size; MR microscopy, clinical MRI, and experimental MRI are suitable for small-sized embryos, larger fetuses, and embryos or fetuses with an intermediate size, respectively (Fig. 12.1a–c) [39, 40].

X-ray imaging is also used for dead embryos and fetuses. Since there is no need to consider the influence of radiation exposure, it is possible to devote a longer time to imaging. Conventional (absorption-contrast) X-ray CT (cCT) is used for fetal skeletal imaging (Fig. 12.1d). Phase-contrast X-ray CT (pCT) is another method of X-ray imaging [40]. Owing to the characteristics of X-rays as electromagnetic waves, phase-contrast X-ray imaging is able to visualize the phase shift of X-rays passing through the samples and reconstruct 2D or 3D images of the samples in combination with CT. An embryo or early fetus is mostly composed of soft tissue because of the absence of a bony structure and thus is suitable for pCT (Fig. 12.1e).

Ultrasonography of living embryos and fetuses is now commonly performed, and many malformations can be diagnosed during the early prenatal period. In cases of pregnancy termination, not all of the aborted fetuses are dissected and pathologically diagnosed because it is technically difficult to dissect small fetuses. The imaging modalities presented here can be used for autopsy imaging of embryos and fetuses in the future. If clues for diseases the fetus may have had can be identified by imaging, the appropriate genetic tests can be performed, and a final accurate diagnosis can be obtained. With the final diagnosis, parents would have sufficient information about their lost pregnancy and can receive appropriate genetic counseling for the next pregnancy.

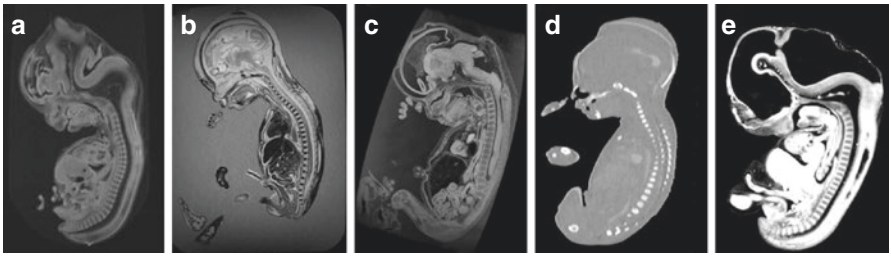

Fig. 12.1 (**a**) MR microscopy at 2.34 T; digital resolution is 40 μm^3. (**b**) Clinical MRI at 3 T; digital resolution is 200 μm^3. (**c**) Experimental MRI at 7 T; digital resolution is 35 μm^3. (**d**) Conventional (absorption-contrast) X-ray CT; digital resolution is 200 μm^3. (**e**) Phase-contrast X-ray CT; digital resolution is 6.5 μm^3

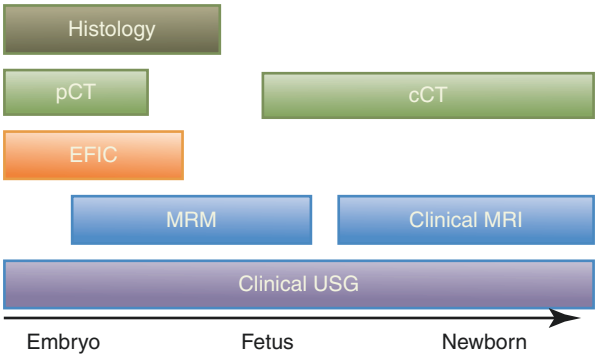

Fig. 12.2 Relationship between sample size and 3D imaging techniques for the human embryo, fetus, and newborn. *pCT* phase-contrast X-ray computed tomography, *cCT* conventional (absorption-contrast) X-ray computed tomography, *EFIC* episcopic fluorescence image capture, *MRM* magnetic resonance microscopy, *clinical MRI* magnetic resonance imaging for routine clinical use, *clinical USG* ultrasonography for routine clinical use

The imaging modalities described in this section are summarized in Fig. 12.2. The appropriate modalities for imaging of dead embryo or fetus should be used depending on the period of the pregnancy.

12.2 Genetic Analysis of the Human Embryo and Fetus

The amniotic fluid, chorionic villi, and umbilical cord blood have been used for genetic analyses of human embryos and fetuses. Recently, DNA fragments derived from villus cells have been identified in the maternal blood [41, 42], and the genetic information of the fetus could be determined from maternal blood analysis; this approach is called noninvasive prenatal genetic testing (NIPT). In comparison with maternal serum analysis, NIPT for aneuploidy has considerably higher sensitivity and specificity [43]. However, NIPT is a screening test with the potential for false-positive and false-negative results because cell-free DNA (cfDNA) could also be derived from multiple sources such as placental mosaicism, maternal conditions including cancer, or fetal and/or maternal copy number variation (CNV) [44].

The cell samples obtained from the amniotic fluid and chorionic villi are used for both screening and diagnostic tests. Several laboratory techniques can be used for prenatal genetic diagnosis. Traditional karyotype analysis is most commonly used to examine cells obtained by chorionic villus sampling (CVS) and amniocentesis (AC). This method is appropriate for the diagnosis of aneuploidies and large rearrangements. The diagnostic accuracy of traditional karyotype analysis is greater than 99% for aneuploidy and chromosomal abnormalities larger than 5–10 Mb [45]. Fluorescence in situ hybridization (FISH) analysis can detect specific chromosomes or chromosomal regions by using fluorescently labeled probes. The turnaround for FISH results (usually within 2 days) is faster than that for conventional karyotyping

results (7–14 days, including the cell culture period). FISH is commonly used as a screening panel for chromosomes 13, 18, 21, X, and Y. It is considered a screening test because false-positive and false-negative results have been reported with FISH [46–48]. Therefore, clinical diagnosis using FISH results should be supported with other clinical and laboratory analyses, such as abnormal ultrasonography, positive screening test using maternal serum and/or soft markers, or confirmatory traditional metaphase chromosome analysis or chromosomal microarray analysis (CMA), as described in the next paragraph.

CMA can detect small chromosomal aneuploidies that cannot be identified by conventional karyotyping [49]. The duplicated or deleted regions of DNA are called CNV. CMA can be performed without cell or tissue culture; thus, the results are obtained in approximately 3–7 days. Since CMA can also be performed with nonviable cells, which are not suitable for conventional karyotyping analysis, cases of fetal death or stillbirth can be examined by this technique [49]. CMA can identify nearly all abnormalities except for balanced translocations and triploidy. When CMA is compared with conventional karyotyping in the detection of structural abnormalities by prenatal ultrasonography, approximately 6% of the fetuses were identified with chromosomal abnormalities by CMA; however, conventional karyotype analysis presented normal results [50, 51]. Therefore, CMA should be the primary test if a structural abnormality is detected by fetal ultrasonography, as recommended by the American Congress of Obstetricians and Gynecologists (ACOG) [49].

In the late 1980s, single-gene disorders were diagnosed using fetal samples. At first, prenatal diagnosis of β-thalassemia was performed using amplified fetal DNA [52], and then the number of diagnosable diseases or genes has increased. Whole-genome sequencing using DNA samples from the amniotic fluid was performed in the next-generation sequencing (NGS) era [53]. Whole-exome sequencing (WES) is also a choice for fetal genetic analysis because coding exons sequenced in WES are only 2% of the genome but contain 85% of disease-coding mutations. Prenatal WES using fetal blood samples has been performed since 2013 [54]. In the late 2000s, massive parallel sequencing (MPS) using NGS opened the way to NIPT [55]. Now, NIPT for aneuploidy is widely used in the world [56], and some fetal single-gene diseases can also be detected using cell-free fetal DNA (cffDNA) obtained from maternal blood [57, 58]. Although the number of diseases that can be detected using cffDNA is gradually expanding, cffDNA analyses are screening tests and do not replace diagnostic testing, as mentioned in the guidelines of professional societies [59–64].

12.3 Ethical Issue of Prenatal Diagnosis

Prenatal diagnosis involves certain ethical issues, and recent progress in fetal genetic testing also presents additional ethical challenges. The four principle-based ethics are (1) respect for autonomy, (2) beneficence, (3) non-maleficence, and (4) justice. All women have reproductive rights to make the final reproductive decision following

appropriate counseling or advice based on these principles. Respect for autonomy requires that parents are given accurate information so they can make a properly informed decision [65]. For example, the specific steps in genetic counseling for pre-NIPT include pretest education, counseling, and informed consent; the screening or testing procedure; a laboratory component that includes test interpretation; and, finally, the disclosure of results to the patient within a context that includes the appropriate education, counseling, and follow-up [64]. Moreover, since termination for fetal abnormality can have long-lasting psychological consequences, identifying women vulnerable to poor psychological adjustment and promoting coping strategies associated with lower levels of grief may be beneficial [66]. In Japan, prenatal genetic screening and testing are offered to women whose child is at risk for severe child-onset diseases and/or chromosomal abnormalities, in accordance with criteria provided by Japanese professional societies. Some people claim that the detection of congenital anomalies should reduce cases of disabilities; the response of the WHO to this claim is that the availability of genetic tests must not be allowed to create an illusion that most disabilities are preventable and therefore unacceptable to society [67].

In the near future, we should be able to detect the whole genomic information, epigenetic condition, and molecular structure of a fetus with ease. The analysis results would contain information about not only life-threatening diseases but also the general phenotype. Furthermore, fetal therapy and genome editing will be practically performed. In the context of prenatal diagnosis, we should consider our ethical responsibilities to two patients: the mother and the fetus. The more we possess, the harder quandaries lead.

References

1. Rao R, Platt LD. Ultrasound screening: status of markers and efficacy of screening for structural abnormalities. Semin Perinatol. 2016;40(1):67–78.
2. Benson CB, Doubilet PM. The history of imaging in obstetrics. Radiology. 2014;273(2 Suppl):S92–110.
3. Warsof SL, Gohari P, Berkowitz RL, Hobbins JC. The estimation of fetal weight by computer-assisted analysis. Am J Obstet Gynecol. 1977;128(8):881–92.
4. Shepard MJ, Richards VA, Berkowitz RL, Warsof SL, Hobbins JC. An evaluation of two equations for predicting fetal weight by ultrasound. Am J Obstet Gynecol. 1982;142(1):47–54.
5. Hadlock FP, Harrist RB, Sharman RS, Deter RL, Park SK. Estimation of fetal weight with the use of head, body, and femur measurements—a prospective study. Am J Obstet Gynecol. 1985;151(3):333–7.
6. Aoki M. Fetal weight calculation; Osaka University method. In: Ciba Y, editor. Ultrasound in obstetrics and gynaecology. Kyoto: Kinpoudo; 1990. p. 95–107.
7. Shinozuka N, Okai T, Kohzuma S, Mukubo M, Shih CT, Maeda T, et al. Formulas for fetal weight estimation by ultrasound measurements based on neonatal specific gravities and volumes. Am J Obstet Gynecol. 1987;157(5):1140–5.
8. Shinozuka N, Akamatsu N, Sato S, Kanzaki T, Takeuchi H, Natori M, et al. Ellipse tracing fetal growth assessment using abdominal circumference: JSUM standardization committee for fetal measurements. J Med Ultrasound. 2000;8(2):87–94.
9. Schild RL, Fell K, Fimmers R, Gembruch U, Hansmann M. A new formula for calculating weight in the fetus of < or = 1600 g. Ultrasound Obstet Gynecol. 2004;24(7):775–80.

10. Kalantari M, Negahdari A, Roknsharifi S, Qorbani M. A new formula for estimating fetal weight: the impression of biparietal diameter, abdominal circumference, mid-thigh soft tissue thickness and femoral length on birth weight. Iran J Reprod Med. 2013;11(11):933–8.
11. Cunningham ME, Walls WJ. Ultrasound in the evaluation of anencephaly. Radiology. 1976;118(1):165–7.
12. Salomon LJ, Alfirevic Z, Berghella V, Bilardo C, Hernandez-Andrade E, Johnsen SL, et al. Practice guidelines for performance of the routine mid-trimester fetal ultrasound scan. Ultrasound Obstet Gynecol. 2011;37(1):116–26.
13. Benacerraf BR, Frigoletto Jr FD, Greene MF. Abnormal facial features and extremities in human trisomy syndromes: prenatal US appearance. Radiology. 1986;159(1):243–6.
14. Benacerraf BR, Frigoletto Jr FD, Cramer DW. Down syndrome: sonographic sign for diagnosis in the second-trimester fetus. Radiology. 1987;163(3):811–3.
15. Benacerraf BR, Nadel A, Bromley B. Identification of second-trimester fetuses with autosomal trisomy by use of a sonographic scoring index. Radiology. 1994;193(1):135–40.
16. Dey M, Sharma S, Aggarwal S. Prenatal screening methods for aneuploidies. N Am J Med Sci. 2013;5(3):182–90.
17. Rossi AC, Prefumo F. Additional value of fetal magnetic resonance imaging in the prenatal diagnosis of central nervous system anomalies: a systematic review of the literature. Ultrasound Obstet Gynecol. 2014;44(4):388–93.
18. Cardoza JD, Goldstein RB, Filly RA. Exclusion of fetal ventriculomegaly with a single measurement: the width of the lateral ventricular atrium. Radiology. 1988;169(3):711–4.
19. Salomon LJ, Ouahba J, Delezoide AL, Vuillard E, Oury JF, Sebag G, et al. Third-trimester fetal MRI in isolated 10- to 12-mm ventriculomegaly: is it worth it? Br J Obstet Gynaecol. 2006;113(8):942–7.
20. Morris JE, Rickard S, Paley MN, Griffiths PD, Rigby A, Whitby EH. The value of in-utero magnetic resonance imaging in ultrasound diagnosed foetal isolated cerebral ventriculomegaly. Clin Radiol. 2007;62(2):140–4.
21. Lyons K, Cassady C, Jones J, Paldino M, Mehollin-Ray A, Guimaraes C, et al. Current role of fetal magnetic resonance imaging in neurologic anomalies. Semin Ultrasound CT MR. 2015;36(4):298–309.
22. Mehollin-Ray AR, Cassady CI, Cass DL, Olutoye OO. Fetal MR imaging of congenital diaphragmatic hernia. Radiographics. 2012;32(4):1067–84.
23. Shinmoto H, Kashima K, Yuasa Y, Tanimoto A, Morikawa Y, Ishimoto H, et al. MR imaging of non-CNS fetal abnormalities: a pictorial essay. Radiographics. 2000;20(5):1227–43.
24. Shinmoto H, Kuribayashi S. MRI of fetal abdominal abnormalities. Abdom Imaging. 2003;28(6):877–86.
25. Longwei X. Clinical application of diffusion tensor magnetic resonance imaging in skeletal muscle. Muscles Ligaments Tendons J. 2012;2(1):19–24.
26. Zhang L, Allen J, Hu L, Caruthers SD, Wickline SA, Chen J. Cardiomyocyte architectural plasticity in fetal, neonatal, and adult pig hearts delineated with diffusion tensor MRI. Am J Physiol Heart Circ Physiol. 2013;304(2):H246–52.
27. Doray B, Favre R, Viville B, Langer B, Dreyfus M, Stoll C. Prenatal sonographic diagnosis of skeletal dysplasias. A report of 47 cases. Ann Genet. 2000;43(3–4):163–9.
28. Parilla BV, Leeth EA, Kambich MP, Chilis P, MacGregor SN. Antenatal detection of skeletal dysplasias. J Ultrasound Med. 2003;22(3):255–8.
29. Garjian KV, Pretorius DH, Budorick NE, Cantrell CJ, Johnson DD, Nelson TR. Fetal skeletal dysplasia: three-dimensional US—initial experience. Radiology. 2000;214(3):717–23.
30. Krakow D, Williams 3rd J, Poehl M, Rimoin DL, Platt LD. Use of three-dimensional ultrasound imaging in the diagnosis of prenatal-onset skeletal dysplasias. Ultrasound Obstet Gynecol. 2003;21(5):467–72.
31. Ruano R, Molho M, Roume J, Ville Y. Prenatal diagnosis of fetal skeletal dysplasias by combining two-dimensional and three-dimensional ultrasound and intrauterine three-dimensional helical computer tomography. Ultrasound Obstet Gynecol. 2004;24(2):134–40.

32. Victoria T, Epelman M, Coleman BG, Horii S, Oliver ER, Mahboubi S, et al. Low-dose fetal CT in the prenatal evaluation of skeletal dysplasias and other severe skeletal abnormalities. Am J Roentgenol. 2013;200(5):989–1000.
33. Sohda S, Hamada H, Oki A, Iwasaki M, Kubo T. Diagnosis of fetal anomalies by three-dimensional imaging using helical computed tomography. Prenat Diagn. 1997;17(7):670–4.
34. Bonnefoy O, Delbosc JM, Maugey-Laulom B, Lacombe D, Gaye D, Diard F. Prenatal diagnosis of hypochondroplasia: three-dimensional multislice computed tomography findings and molecular analysis. Fetal Diagn Ther. 2006;21(1):18–21.
35. Adler-Levy Y, Yagel S, Nadjari M, Bar-ziv Y, Simanovsky N, Hiller N. Use of low dose computed tomography with 3D reconstructions for the prenatal evaluation of suspected skeletal dysplasia. Isr Med Assoc J. 2015;17(1):42–6.
36. Born G. Die Plattenmodelliermethode. Arch Mikrosk Anat. 1883;22:584–99.
37. Yamada S, Itoh H, Uwabe C, Fujihara S, Nishibori C, Wada M, et al. Computerized three-dimensional analysis of the heart and great vessels in normal and holoprosencephalic human embryos. Anat Rec (Hoboken). 2007;290(3):259–67.
38. Weninger WJ, Mohun T. Phenotyping transgenic embryos: a rapid 3-D screening method based on episcopic fluorescence image capturing. Nat Genet. 2002;30(1):59–65.
39. Yamada S, Samtani RR, Lee ES, Lockett E, Uwabe C, Shiota K, et al. Developmental atlas of the early first trimester human embryo. Dev Dyn. 2010;239(6):1585–95.
39. Yamaguchi Y, Miyazaki, R Kamatani M, Uwabe C, Makishima H, Nagai M, et al. Three-dimensional models of the segmented human fetal brain generated by magnetic resonance imaging. Congenital Anomalies, in press.
40. Matsuda Y, Ono S, Otake Y, Handa S, Kose K, Haishi T, et al. Imaging of a large collection of human embryo using a super-parallel MR microscope. Magn Reson Med Sci. 2007;6(3):139–46.
41. Momose A, Takeda T, Itai Y, Hirano K. Phase-contrast X-ray computed tomography for observing biological soft tissues. Nat Med. 1996;2(4):473–5.
42. Yoneyama A, Yamada S, Takeda T. Fine biomedical imaging using x-ray phase-sensitive technique. In: Gargiulo GD, McEwan A, editors. Advanced biomedical engineering, vol. 2. Rijeka, Croatia: InTech; 2011. p. 107–28.
43. Palomaki GE, Deciu C, Kloza EM, Lambert-Messerlian GM, Haddow JE, Neveux LM, et al. DNA sequencing of maternal plasma reliably identifies trisomy 18 and trisomy 13 as well as Down syndrome: an international collaborative study. Genet Med. 2012;14(3):296–305.
44. Snyder MW, Gammill HS, Shendure J. Copy-number variation and false positive results of prenatal screening. N Engl J Med. 2015;373(26):2585.
45. Jackson LG, Zachary JM, Fowler SE, Desnick RJ, Golbus MS, Ledbetter DH, et al. A randomized comparison of transcervical and transabdominal chorionic-villus sampling. The U.S. National Institute of Child Health and Human Development Chorionic-Villus Sampling and Amniocentesis Study Group. N Engl J Med. 1992;327(9):594–8.
46. Toutain J, Epiney M, Begorre M, Dessuant H, Vandenbossche F, Horovitz J, et al. First-trimester prenatal diagnosis performed on pregnant women with fetal ultrasound abnormalities: the reliability of interphase fluorescence in situ hybridization (FISH) on mesenchymal core for the main aneuploidies. Eur J Obstet Gynecol Reprod Biol. 2010;149(2):143–6.
47. Tepperberg J, Pettenati MJ, Rao PN, Lese CM, Rita D, Wyandt H, et al. Prenatal diagnosis using interphase fluorescence in situ hybridization (FISH): 2-year multi-center retrospective study and review of the literature. Prenat Diagn. 2001;21(4):293–301.
48. Bryndorf T, Lundsteen C, Lamb A, Christensen B, Philip J. Rapid prenatal diagnosis of chromosome aneuploidies by interphase fluorescence in situ hybridization: a one-year clinical experience with high-risk and urgent fetal and postnatal samples. Acta Obstet Gynecol Scand. 2000;79(1):8–14.
49. American College of Obstetricians and Gynecologists Committee on Genetics. Committee Opinion No. 581: the use of chromosomal microarray analysis in prenatal diagnosis. Obstet Gynecol. 2013;122(6):1374–7.

50. Baer RJ, Flessel MC, Jelliffe-Pawlowski LL, Goldman S, Hudgins L, Hull AD, et al. Detection rates for aneuploidy by first-trimester and sequential screening. Obstet Gynecol. 2015;126(4):753–9.
51. Aagaard-Tillery KM, Malone FD, Nyberg DA, Porter TF, Cuckle HS, Fuchs K, et al. Role of second-trimester genetic sonography after Down syndrome screening. Obstet Gynecol. 2009;114(6):1189–96.
52. Pirastu M, Ristaldi MS, Cao A. Prenatal diagnosis of beta thalassaemia based on restriction endonuclease analysis of amplified fetal DNA. J Med Genet. 1989;26(6):363–7.
53. Talkowski ME, Ordulu Z, Pillalamarri V, Benson CB, Blumenthal I, Connolly S, et al. Clinical diagnosis by whole-genome sequencing of a prenatal sample. N Engl J Med. 2012;367(23):2226–32.
54. Yang Y, Muzny DM, Reid JG, Bainbridge MN, Willis A, Ward PA, et al. Clinical whole-exome sequencing for the diagnosis of mendelian disorders. N Engl J Med. 2013;369(16):1502–11.
55. Chiu RW, Chan KC, Gao Y, Lau VY, Zheng W, Leung TY, et al. Noninvasive prenatal diagnosis of fetal chromosomal aneuploidy by massively parallel genomic sequencing of DNA in maternal plasma. Proc Natl Acad Sci U S A. 2008;105(51):20458–63.
56. Hui L, Bianchi DW. Recent advances in the prenatal interrogation of the human fetal genome. Trends Genet. 2013;29(2):84–91.
57. Lench N, Barrett A, Fielding S, McKay F, Hill M, Jenkins L, et al. The clinical implementation of non-invasive prenatal diagnosis for single-gene disorders: challenges and progress made. Prenat Diagn. 2013;33(6):555–62.
58. Lewis C, Hill M, Chitty LS. Non-invasive prenatal diagnosis for single gene disorders: experience of patients. Clin Genet. 2014;85(4):336–42.
59. Benn P, Borrell A, Chiu RW, Cuckle H, Dugoff L, Faas B, et al. Position statement from the Chromosome Abnormality Screening Committee on behalf of the Board of the International Society for Prenatal Diagnosis. Prenat Diagn. 2015;35(8):725–34.
60. Practice Bulletin No. 163 Summary: screening for fetal aneuploidy. Obstet Gynecol. 2016;127(5):979–81.
61. Wilson KL, Czerwinski JL, Hoskovec JM, Noblin SJ, Sullivan CM, Harbison A, et al. NSGC practice guideline: prenatal screening and diagnostic testing options for chromosome aneuploidy. J Genet Couns. 2013;22(1):4–15.
62. Gregg AR, Gross SJ, Best RG, Monaghan KG, Bajaj K, Skotko BG, et al. ACMG statement on noninvasive prenatal screening for fetal aneuploidy. Genet Med. 2013;15(5):395–8.
63. Committee Opinion Summary No. 640: cell-free DNA screening for fetal aneuploidy. Obstet Gynecol. 2015;126(3):691–2.
64. Gregg AR, Skotko BG, Benkendorf JL, Monaghan KG, Bajaj K, Best RG, et al. Noninvasive prenatal screening for fetal aneuploidy, 2016 update: a position statement of the American College of Medical Genetics and Genomics. Genet Med. 2016;18(10):1056–65.
65. Howe D. Ethics of prenatal ultrasound. Best Pract Res Clin Obstet Gynaecol. 2014;28(3):443–51.
66. Lafarge C, Mitchell K, Fox P. Perinatal grief following a termination of pregnancy for foetal abnormality: the impact of coping strategies. Prenat Diagn. 2013;33(12):1173–82.
67. Wertz DC, Fletcher GF, Berg K, WHO Human Genetics Programme. Review of ethical issues in medical genetics: report of consultants to WHO/DC. Wertz, J.C. Fletcher, K. Berg. Geneva: World Health Organization; 2003. p. 102.

Pathology and Genomics in Gestational Trophoblastic Neoplasia

13

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Abstract

Gestational trophoblastic diseases (GTDs) originate from placental tissue and are rare tumors with a current cure rate of greater than 90% with the right diagnosis and clinical management. GTDs are generally divided into two categories: (1) hydatidiform moles, presenting abnormal villous proliferation with chromosomal aberrations, and (2) rare gestational trophoblastic neoplasms (GTNs) including choriocarcinoma, placental site trophoblastic tumor, and epithelioid trophoblastic tumor. Persistent gestational trophoblastic disease/tumor most commonly occurs following molar pregnancy; however, it may follow any GTD.

Hydatidiform mole was previously diagnosed in the second trimester; however, it is now diagnosed in first trimester specimens, based on the availability of accurate and sensitive tests for the detection of hCG and on the use of early ultrasonographic examination. Often, a diagnosis is made before classical clinical signs and symptom develop. In daily practice, histological diagnosis of GTD continues to have some degree of diagnostic misclassification with a fairly high degree of inter- and intraobserver variability. Studies evaluating the concomitant use of histology with $p57^{KIP2}$ immunohistochemistry, and/or genotyping, have further refined diagnoses of hydatidiform mole. Beyond hydatidiform mole, the even rarer tumors of the GTN family require broad knowledge of the clinical and histological features, as well as the application of immunohistochemical markers directed toward the various types of trophoblast, to arrive at the correct diagnosis.

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This chapter focuses on recent advances in the pathogenesis; pathological diagnostic features, including immunohistochemistry; and genetic findings, of GTD, along with a review of the clinical management.

Keywords
Hydatidiform mole • Choriocarcinoma • PSTT • ETT

13.1 Molar Pregnancies

WHO classification (the 4th edition, 2014) of gestational trophoblastic disease (GTD) includes neoplasms, molar pregnancies, nonneoplastic lesions, and abnormal (non-molar) villous lesions [1] (Table 13.1). Molar pregnancies include complete hydatidiform mole (CM), partial hydatidiform mole (PM), and invasive mole. Hydatidiform mole is an abnormal placenta with villous hydrops and variable degrees of abnormal trophoblastic hyperplasia which can be distinguished by means of gross morphologic and histopathological examination along with cytogenetic analyses.

The prevalence of hydatidiform mole varies by country, with the highest incidence in Southeast Asia (3.8–13/1000 pregnancies) and the lowest incidence in the USA and Europe (0.5–1.84/1000 pregnancies) [1–3]. In Japan, the incidence is decreasing from 2.5/1000 pregnancies in 1974 to 1.65/1000 pregnancies in 2000 [4].

Significant risk factors are maternal age >40 years, previous spontaneous abortion and previous history of hydatidiform mole, and Asian ethnicity and genetics. Some studies suggest that vitamin A deficiency and nutritional and socioeconomic factors may increase the risk of molar pregnancy [1, 3, 5].

Persistent GTD is determined by hCG values that have plateaued or are increasing after curettage for hydatidiform mole. Further clinical and imaging studies are indicated to exclude invasive mole or high-risk GTD including choriocarcinoma [5–7]. The incidences of persistent GTD are 15–29% following CM and 0–4% after PM [1, 6]. It is crucial to diagnostically separate the non-molar, hydropic abortions from hydatidiform moles for prognostic and clinical management purposes.

Table 13.1 Classification of gestational trophoblastic disease (WHO classification, 2014)

Neoplasms	Choriocarcinoma
	Placental site trophoblastic tumour
	Epithelioid trophoblastic tumour
Molar pregnancies	Hydatidiform mole
	• Complete
	• Partial
	• Invasive
Non-neoplastic lesions	Exaggerated placental site
	Placental site nodule and plaque
Abnormal (nonmolar) villous lesions	

The clinical presentation of a CM has changed considerably over the past three decades. CM was once easily diagnosed in the second trimester, and certain symptoms were common at the time of presentation, including prominent uterine enlargement, anemia, toxemia, hyperemesis, hyperthyroidism, and respiratory failure. However, the diagnosis is now typically made in the first trimester often before classic clinical symptoms develop. This is based on the availability of accurate and sensitive tests for hCG and the widespread use of both transabdominal and transvaginal ultrasonographies [5, 6].

Although abortion specimens with hydropic chorionic villi are routinely encountered in general and gynecological pathology practice, the histological features of early CM (at less than 12-week gestational age), PM, and hydropic abortus often overlap and have a low sensitivity and specificity, especially for PM [8].

The development of ancillary diagnostic testing methods, including immunohistochemical detection of imprinted genes/products, DNA ploidy analysis, and, most recently, DNA short tandem repeat (STR) genotyping, has advanced the study of GTD during the past three decades [9–11]. Diagnostic algorisms have been proposed in pathological diagnosis of PTD with the concomitant use of traditional histopathological assessment and ancillary studies for higher diagnostic accuracy [9, 10].

Clinical and pathological diagnostic features of hydatidiform moles and nonmolar hydropic abortus are summarized in Table 13.2.

13.1.1 Compete Hydatidiform Mole

WHO classification (2014) defines CM as a nonneoplastic, proliferative disorder of the placenta, resulting in villous hydrops and trophoblastic hyperplasia without embryonic development and having androgenic diploid karyotype (diploid paternal-only genome) [1].

Clinical findings are vaginal bleeding in the second trimester, prominent uterine enlargement, and marked elevation of serum hCG (>100 × 10^3 mIU/mL). Ultrasonography shows the absence of fetus and "snow storm" pattern. These are characteristic features of well-developed, classic CM [6].

Genetically, the majority of CM cases have a diploid, paternal-only genome with the karyotypes of 46XX or 46XY. Two paternal haploid chromosome sets consist of either monosomic/homozygous (80–90%) or dispermic/heterozygous (10–20%) origin (Fig. 13.1) [1, 3]. Rarely, tetraploid CM may exist with four paternal haploid sets in the genome.

Macroscopically, classic CM consists of bulky, bloody tissue with uniformly hydropic "grapelike" villi (Fig. 13.2) [1, 5, 6]. The edematous villi range from a few millimeters to over 10 mm in diameter. Fetal and normal placenta are absent apart from rare exceptions [12, 13]. Early complete mole (ECM) of 6.5 to 12 weeks of gestational age is typically normal grossly [6].

Histological features of classic CM differ from those of ECM. Knowledge of the gestational age is useful in determining the appropriate histological criteria to be

Table 13.2 Diagnostic features of hydatidiform moles

Features	CM	ECM (6.5–12 weeks of gestation)	PM	Hydropic abortus
Karyotype	46XX, 46XY (paternal-only)	46XX, 46XY (paternal-only)	69XXX, 69XXY, 69XYY	46XX, 46XY
Pretreatment hCG (mIU/mL)	$>100 \times 10^3$	Normal or $<100 \times 10^3$	Normal or $<100 \times 10^3$	Normal
Ultrasound	Snow storm pattern	–	Focal cystic change	–
Gestational sac and fetus	Absence	Absence	Rarely presence	Presence
Macroscopy	Overall hydropic change	No gross abnormality	Focal hydropic change	No gross abnormality
Villous shape				
Outline	Round to oval	Polypoid Cauliflower shapes Crab-shaped	Scalloped with pseudo-inclusionfjord-like invagination	Round to oval
Enlargement	Marked	Normal size	Some enlarged, but often not prominent	Often marked
Cistern	Prominent	Uncommon	Variable, usually not prominent	Variable, usually not prominent
Trophoblastic hyperplasia				
Extent	Multifocal	Circumferencial	Focal, syncytiotrophoblast knuckles (sprouts)	None
Amount	Abundant	Increase	Minimal	None
Atypia	Marked	Mild to moderate	Limited to mild	None
Normal villi	None or few	Some	Numerous	Sometimes
Apoptosis in villous stroma	Marked	Marked to mild	Rare	None
Vasculature and nucleated RBCs	Absent, generally	Absent, capillary may be present	Common	Absent, generally
p57KIP2 immunostain in cytotrophoblast and villous stromal cells	Negative	Negative	Positive	Positive
Persistent trophoblastic disease	15–29%	15–29%	0–4%	0%

CM complete hydatidiform mole, *ECM* early complete hydatidiform mole, *PM* partial hydatidiform mole, *HA* hydropic abortus, *hCG* human chorionic gonadotropin

applied. Classic CM presents with diffuse villous enlargement and edema, frequent cistern formation, and conspicuous trophoblastic hyperplasia. Cistern formation is cavitation in the center of the villi produced by necrosis of the mesenchyme (Fig. 13.3a). Villous stromal vessels are usually absent. The villi are usually round to

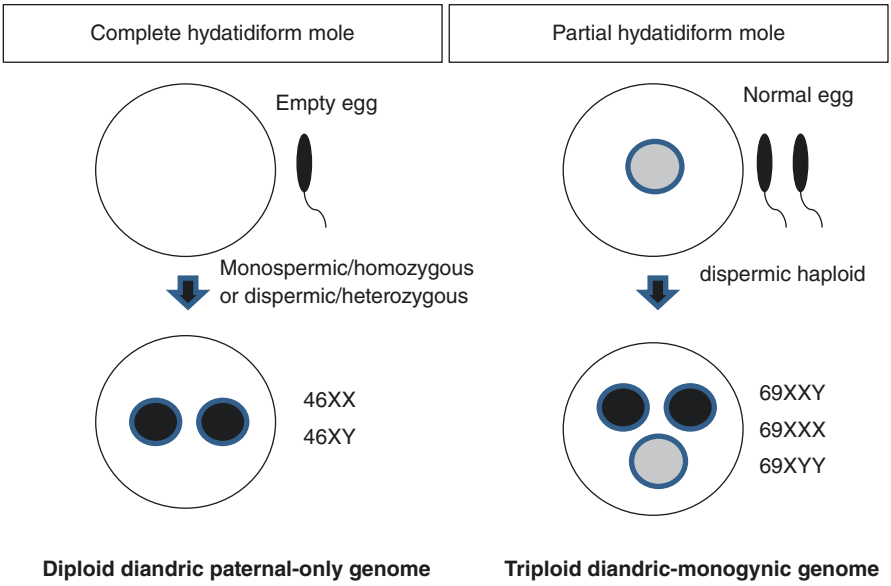

Fig. 13.1 Pathogenesis of complete hydatidiform mole and partial hydatidiform mole

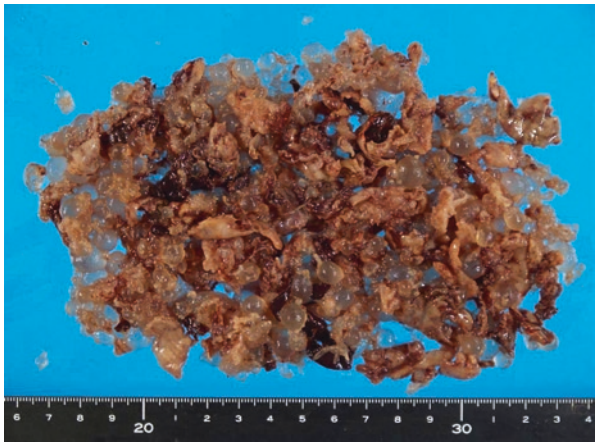

Fig. 13.2 Gross appearance of complete hydatidiform mole presenting diffuse grapelike villous swelling

oval. Trophoblastic hyperplasia is circumferential, and significant cytological atypia of all three trophoblasts is almost always present (Fig. 13.3b). Mitotic figures are usually found. There are no fetal tissue and normal placental structures excluding very rare exceptions. In contrast, ECM shows minimal hydropic change and cistern formation is rare [14, 15]. The villi have irregular shapes called polypoid, cauliflower-like, or crab-shaped (Fig. 13.3c). Trophoblastic hyperplasia is mild to moderate in degree, and trophoblast shows circumferential or random distribution on the villi. An exaggerated placental site (molar implantation site) with atypical trophoblast is often present. The villous stroma is abnormally cellular with prominent apoptosis (karyorrhexis)

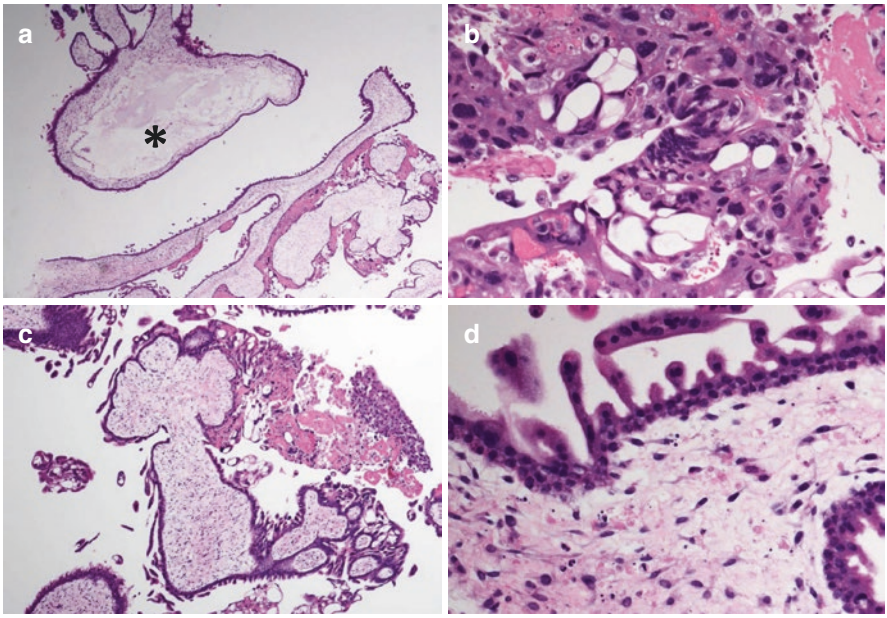

Fig. 13.3 Histological appearance of complete hydatidiform mole. (**a**) Swollen villi with necrosis of the mesenchyme produce cistern (*), and left lower side villi show polypoid, crab-shaped irregular outline. (**b**) The implantation site trophoblast presenting conspicuous trophoblastic atypia. (**c**) Early complete hydatidiform mole. Characteristic irregular villous contour resembling cauliflower-like shape. (**d**) Early complete hydatidiform mole. Hypercellular myxoid villous stroma with karyorrhexis (apoptosis) accompanied by modest circumferential trophoblastic proliferation

and myxoid change (Fig. 13.3d) [14, 15]. Capillary vessels can be seen in the stroma with or without nucleated red blood cells. Stromal fibrosis is absent.

The prevalence of persistent gestational trophoblastic disease is 15–29%, and 2–3% of the patients develop choriocarcinoma after CM [1, 3, 6]. The risk of subsequent CM is 1–1.8% and 10–18% after two consecutive CMs [16, 17]. The rare case of recurrent, familial, and biparental CM develops as a result of abnormal imprinting and overexpression of the paternal genome related to maternal mutation of *NALP7/NLRP7* and more rarely *KHDC3L* [3, 18].

13.1.2 Partial Hydatidiform Mole (PM) (Figs. 13.5, 13.6 and 13.7a–d)

PM is defined as a hydatidiform mole with a spectrum of villous populations ranging from normal size to substantial hydrops with mild, focal trophoblastic hyperplasia. Most cases present diandric-monogynic triploid genome [1, 3, 5, 6, 19].

Clinical presentations are vaginal bleeding, missed or incomplete abortion in the late first or early second trimester, normal to mild elevated serum hCG ($<100 \times 10^3$ mIU/mL), and a focal cystic change in the placenta on ultrasound. Fetal tissues or gestational sac can be seen [19].

Genetically, PM has a triploid diandric-monogynic genome with karyotype of 69XXY (70%), 69XXX (27%), and 69XYY (3%) (Fig. 13.1) [1, 3, 19].

Grossly, hydropic vesicles admixed with normal placental tissue are characteristic features (Fig. 13.4). Gestational sac, abnormal fetus, and normal fetus might be found.

Histological features are hydropically enlarged villi with occasional central cistern and oval or irregular outline, called "fjord-like" or "scalloping" (Fig. 13.5a). Trophoblastic stromal inclusions are commonly found and the result of villous irregular shape (Fig. 13.5b). Circumferential trophoblastic hyperplasia is not conspicuous; however, focal mild syncytiotrophoblastic hyperplasia called "knuckles" or "sprouts"

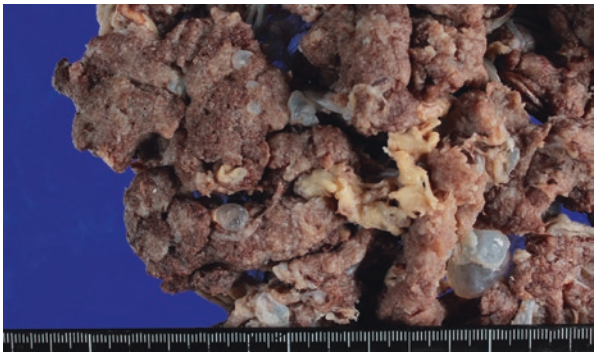

Fig. 13.4 Gross appearance of partial hydatidiform mole, 25 weeks of gestation with triploidy revealed by DNA genotyping. Scattered hydropic vesicles are seen in normal placental villi

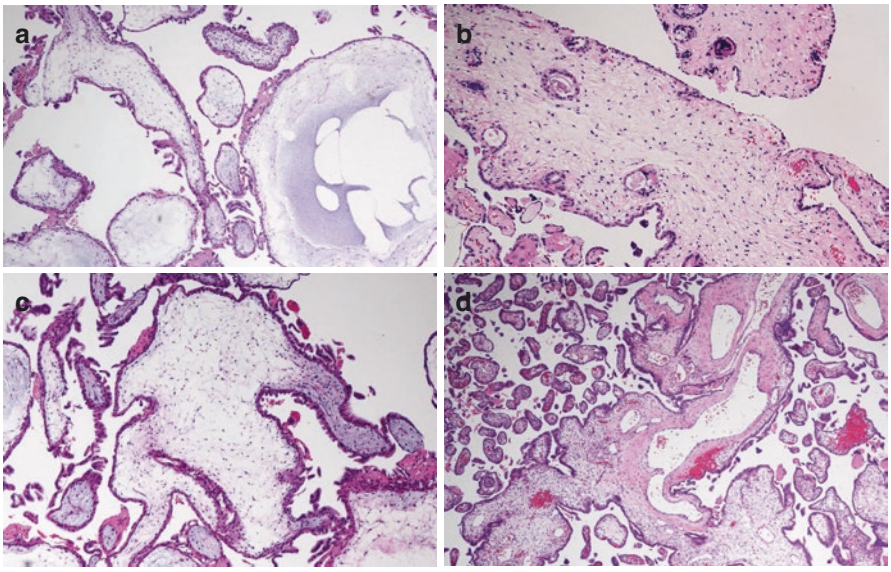

Fig. 13.5 Histologic appearance of partial hydatidiform mole. (**a**) A mixed population of villi with shape differences in villous size. (**b**) Trophoblastic inclusions and villous stromal fibrosis. (**c**) "Fjord-like" irregular-shaped villi with predominantly mild syncytiotrophoblastic proliferation like "sprouts". (**d**) Pseudoangiomatous change showing dilated vessels in the villous stroma

is a characteristic feature (Fig. 13.5c). Cytologic atypia is minimal to mild. Villous stroma sometimes contains pseudoangiomatoid change (Fig. 13.5d). Stromal fibrosis and nucleated red blood cells in villous stromal vessels, which are less common, but present, in CM, are commonly found in PM [1, 3, 15, 19].

The prevalence of persistent GTD is 0.5–5% in PM, especially in the case of invasive PM [16, 17]. The risk of developing choriocarcinoma is 0–0.5% [1, 3, 20]. Differentiating PM from CM is important because CM has higher risk (15–29%) of persistent GTD. There are cases in which the morphological distinction between PM and CM, especially ECM, may be difficult because of inter-observer variation of commonly observed features, for example, edematous villi with irregular outline, mild trophoblastic hyperplasia, cistern formation, and trophoblastic pseudo-inclusions.

13.1.3 Invasive Hydatidiform Mole

Invasive hydatidiform mole is defined as CM or PM that invades the myometrium and/or uterine vessels (Fig. 13.6) [1, 21]. Clinically, these cases present with vaginal bleeding with persistent elevation of serum hCG after primary evacuation of a hydatidiform mole. Uterine perforation caused by invasive hydatidiform mole has been

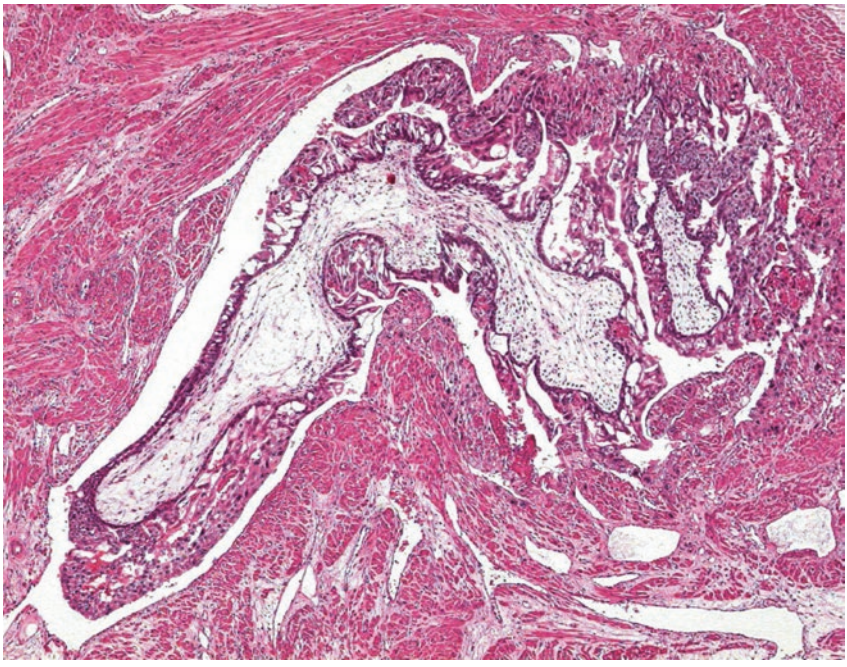

Fig. 13.6 Invasive complete hydatidiform mole. Molar tissue is identified in the myometrium

reported. Histologically, molar villi invading into myometrium are the diagnostic requirement. Histological features of CM or PM are same in the case of invasive hydatidiform mole. The finding of extravillous trophoblast (mainly intermediate trophoblast) without villi invading into superficial myometrium and maternal spiral artery is commonly seen in noninvasive moles and does not form the basis for a diagnosis of invasive hydatidiform mole. The incidence of invasive CM is higher than that of PM.

13.1.4 Ancillary Studies for the Diagnosis of Hydatidiform Mole

13.1.4.1 Immunohistochemistry

$p57^{KIP2}$ is an effective marker for differentiating CM from partial hydatidiform mole (PM) and hydropic abortus [3, 9–11]. $p57^{KIP2}$ is a cyclin-dependent kinase inhibitor encoded by the paternally imprinted and maternally expressed gene *CDKN1C* on chromosome 11p15.5. Due to its preferential expression from the maternal allele, the gene is silent in androgenic CM, PM, hydropic non-molar abortuses, and trisomies. These latter cases all show normal p57 protein expression pattern: positive nuclear staining in cytotrophoblast and villous stromal cells. In contrast, CM shows absent nuclear $p57^{KIP2}$ staining in cytotrophoblast and villous stromal cells because CM has only paternal genome, without any maternal genome (Fig. 13.7a, b). It is important to mention that the syncytiotrophoblast is negative and intermediate

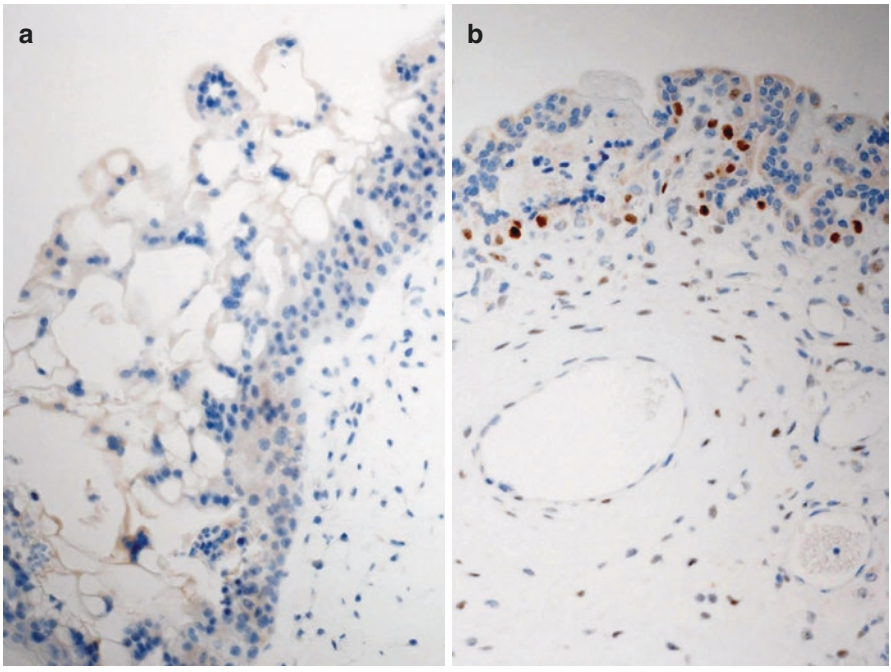

Fig. 13.7 Immunohistochemical staining of p57. (**a**) Complete hydatidiform mole. Absence of nuclear staining in cytotrophoblast and villous stromal cells. (**b**) Partial hydatidiform mole. Presence of nuclear staining in cytotrophoblast and villous stromal cells

trophoblast is positive for $p57^{KIP2}$ in all CM, PM, and non-molar villi. This may be a pitfall for the reviewer due to inexperience in recognizing the different types of trophoblast, especially the cytotrophoblast and intermediate trophoblast, resulting in inaccurate interpretation of immunohistochemical staining pattern. It is important to carefully note which cell types show nuclear-positive staining for $p57^{KIP2}$. Studies have revealed that p57 expression is highly correlated with DNA genotyping and it is a reliable marker for the diagnosis of CM. However, there are some exceptions of CM showing normal p57 pattern. For example, twin gestation with CM and normal fetus, rare CM of mosaic androgenic/biparental mosaic/chimeric gestations, and CM with retained maternal chromosome 11 are included. On the other hand, PM with p57-negative pattern based on loss of maternal chromosome rarely occurs [10]. Cell cycle proteins or proliferation markers (Ki-67, PCNA, ESF-1, CDK2, cyclin E) of molar pregnancies have also been studied and show variable results; however, none of them demonstrate the high sensitivity and specificity of p57 for use in routine diagnosis of hydatidiform mole [9].

13.1.4.2 Ploidy Analysis

Ploidy analysis has been used for decades in determining the number of haploid sets of chromosomes [9]. Karyotype analysis by chromosome G-band can also rule out chromosomal trisomy syndromes presenting with histological findings mimicking a molar gestation. The problems of ploidy analysis are: (1) it is unable to identify the paternal origin and cannot differentiate triploid PM from non-molar digynic triploidy; (2) fresh tissue is needed for karyotyping, whereas ploidy analysis can be performed on both fresh and fixed tissues; and (3) the rate of correct diagnosis of CM by FISH is only 38.5% because of technical difficulties.

13.1.4.3 Short Tandem Repeat (STR) Genotyping

Short tandem repeats (STRs) are prevalent, genetically stable, and repetitive noncoding DNA sequences. The number of repeats at each STR locus differs between individuals, and this feature is used for comparing the allelic profiles of maternal and molar tissue. Unstained tissue section from formalin-fixed paraffin-embedded tissue block(s) of maternal tissue and the chorionic villi are used to analyze the DNA genotype. After DNA extraction, PCR amplification using a commercially available kit is performed. By comparing the alleles of maternal and villous tissue at each STR locus, the presence and copy number of maternal and paternal alleles in the villi can be detected. With this technique, CM contains only paternal alleles of either homozygous or heterozygous pattern in at least two STR loci. One maternal allele and a duplicate quantity of one paternal allele at every STR locus are present in homozygous PM, and two different paternal alleles in addition to one maternal allele in at least two loci are detected in heterozygous PM [9–11].

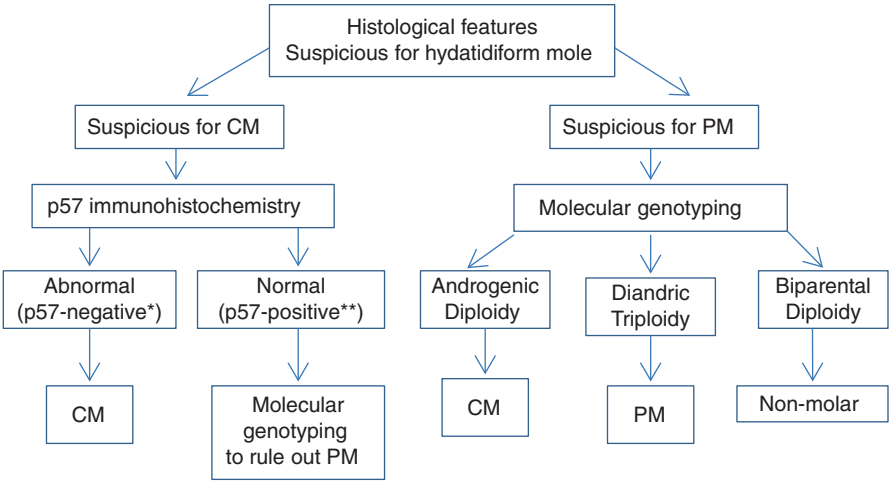

Fig. 13.8 Diagnostic algorithm for hydatidiform moles integrating immunohistochemistry and molecular genotyping [10]

Diagnostic algorithm of hydatidiform moles presented by Banet et al. is shown in Fig. 13.8 [10].

STR genotyping has the advantage of being able to precisely distinguish the paternal origin of DNA material in molar gestation. As such, it can accurately categorize the genotypes of molar pregnancies, for example, it can separate diandric triploid PM from non-molar digynic triploidy. In addition, genotyping does not require fresh tissue and can be performed retrospectively using formalin-fixed paraffin-embedded tissue.

Studies of DNA genotyping for CM by using STR loci detected by PCR have been reported in the USA [9, 11]; however, it has never been reported in Japan, while analysis of CM by using single-nucleotide polymorphism (SNP) genotyping, which is the measurement of genetic variations of SNPs, between maternal and villous tissues has been reported [22].

13.2 Gestational Trophoblastic Neoplasms

Gestational trophoblastic neoplasms (GTNs) include choriocarcinoma, placental site trophoblastic tumor (PSTT), and epithelioid trophoblastic tumor (ETT). GTNs arise from different subtypes of trophoblast and have unique clinical, pathological, and genetic features. Clinical and histological features are summarized in Table 13.3.

Table 13.3 Diagnostic features of gestational trophoblastic tumors

Features	Choriocarcinoma	PSTT	ETT
Age	Reproductive age (ave. 30 y.o.)	20–63 y.o. (ave. 30 y.o.)	15–48 y.o. (ave.36 y.o.)
Antecedent pregnancy	Term pregnancy	Term pregnancy	Term pregnancy
	CM		
Interval time from index gestation	A few months to 14 years (ave.: 2 months after term pregnancy and 13 months after CM)	2 weeks to 17 years (median: 12–18 months)	1–25 years (ave. 6.2 years)
Clinical presentation	Vaginal bleeding	Missed abortion	Vaginal bleeding
	Persistent GTD	Amenorrhea	
Pretreatment hCG (mIU/mL)	$>10 \times 10^3$	$<1 \times 10^3$	$<3 \times 10^3$
Gross appearance	Circumscribed or Invasive hemorrhagic mass	Expansile to infiltrative solid large mass	Expansile solid mass
Tumor location	Corpus	Corpus	Cervix, Lower uterine segment, Corpus
Tumor border	Infiltrative	Infiltrative	Pushing
Tumor cells and cytologic atypia	Villous IT, ST and CT with marked atypia	Implantation site type IT, sometimes enlarged cells with moderate to marked atypia	Chorionic type IT, with mild to moderate atypia
Tumor growth pattern	Prominent hemorrhage and necrosis, trimorphic pattern of all three types of trophoblast	Tumor cells split myometrial smooth muscle fibers at tumor periphery and replacing vascular wall	Sheets, nests and cords, Geographic necrosis, Colonizing mucosal surface epithelium
Hyaline-like material	Absence	Presence, sometimes	Prominent
Stroma	No intrinsic tumor stroma or vasculature	Intimately infiltrates muscle fibers	Presence of nearby decidualized stromal cells
Immunohistochemistry	hCG, hPL, Ki-67 labeling index of >90%	hPL Mel-CAM, hCG (focal), Ki-67 labeling index of <10%	p63, hPL (focal), MEL-CAM (focal), Ki-67 labeling index of >10%

PSTT placental site trophoblastic tumor, *ETT* epithelioid trophoblastic tumor, *ave.* average, *y.o.* years old, *CM* complete hydatidiform mole, *GTD* gestational trophoblastic disease, *CT* cytotrophoblast, *ST* syncytiotrophoblast, *IT* intermediate trophoblast

13.2.1 **Choriocarcinoma** (Fig. 13.9a–d)

Choriocarcinoma is defined as a malignant tumor which consisted of mononuclear intermediate trophoblast or cytotrophoblast and multinucleated syncytial trophoblast without villi [1, 3, 5, 9].

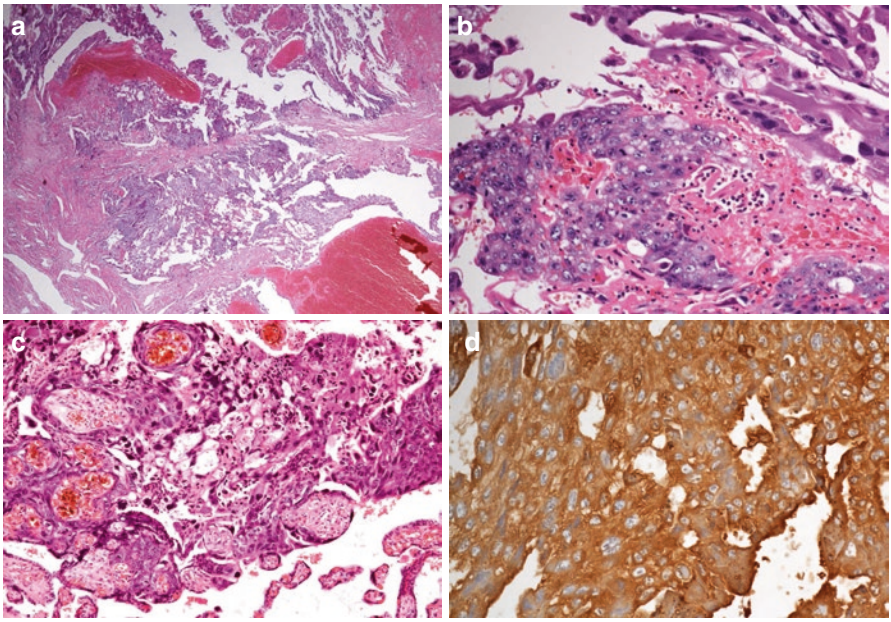

Fig. 13.9 Histologic appearance of choriocarcinoma. (**a**, **b**) The tumor cells invade into the myometrium with hemorrhage and necrosis. High-power view shows biphasic atypical trophoblast consisting of syncytial trophoblast (**b**, right upper side) and intermediate trophoblast or cytotrophoblast (**b**, left lower side). (**c**) Intraplacental choriocarcinoma in term placenta. Biphasic atypical trophoblast and nonneoplastic villi in the background. The fetus presented severe anemia caused by fetomaternal transfusion. (**d**) Immunohistochemical staining of hCG

Incidence of choriocarcinoma is 2–7/100,000 pregnancies in the USA and Europe and higher in Asia with 5–63/100,000 pregnancies. These rates are 1/20–1/40 of hydatidiform mole. In Japan, the incidence of choriocarcinoma as well as hydatidiform moles is decreasing. The risk of complete hydatidiform mole (CM) progress to choriocarcinoma is 2–3% and that of partial hydatidiform mole (PM) is 0–0.5%. Antecedent pregnancies include CM in 50%, missed abortion in 25%, and term pregnancy in 25% [1, 3, 5, 6]. Histological diagnosis of postmolar choriocarcinoma has been less common because treatment is administered based on only serological and imaging studies before hysterectomy. Interval time from index gestation is a few months to 14 years (average after normal pregnancy, 2 months; after CM, 13 months) [1, 3, 5]. Rarely intraplacental or in situ choriocarcinoma develops in full-term placentas with occasional concurrent metastatic disease. Marked fetal anemia caused by fetomaternal transfusion based on intraplacental choriocarcinoma has been reported [23].

Choriocarcinoma occurs in women of reproductive age with average of 30 years of age. Most common symptoms are vaginal bleeding and/or extrauterine hemorrhage caused by metastasis. Marked elevation of serum hCG, more than 10×10^3 mIU/mL, is always present. Persistent gestational trophoblastic disease

progressing to choriocarcinoma after CM is detected by persistent elevation of serum hCG [1, 3, 5].

Grossly, the tumor is typically a bulky, destructive red mass with prominent hemorrhage and necrosis. The tumor invades into the myometrium and occasionally multiple tumor nodules exist. In the case of choriocarcinoma after an ectopic pregnancy, tumor may develop in the extrauterine adnexa. Even in metastatic sites, marked hemorrhage and necrosis are common. Intraplacental choriocarcinoma presents typically with white nodules simulating placental infarction and may be quite difficult to recognize as a tumor [23].

Microscopically, choriocarcinoma presents with marked hemorrhage and necrosis in the center of the tumor, and tumor cells exist at the peripheral part of the mass and invade into the myometrium and vessels. The tumor cells are composed of malignant syncytiotrophoblast and intermediate trophoblast or cytotrophoblast with striking cytologic atypia (Fig. 13.9a, b) [1]. This pattern is called "biphasic pattern," although trimorphic proliferation of all three trophoblasts commonly occurs. Syncytiotrophoblast is a hyperchromatic, multinucleated cell, with polymorphic, relatively broad, and eosinophilic cytoplasm. Intermediate trophoblast or cytotrophoblast is a hyperchromatic mononuclear cell with prominent nucleoli and frequent mitotic figures showing solid and sheet-like proliferation patterns [1, 3, 9]. Normal or abnormal chorionic villi are absent, except in the case of intraplacental choriocarcinoma (Fig. 13.9c) [23].

Immunohistochemically, all tumor cells strongly express cytokeratin, hCG (Fig. 13.9d), and high rate of Ki-67 labeling index with more than 90% positivity. Intermediate trophoblast is positive for Mel-CAM (CD146) and MUC-4 [1, 9].

Differential diagnoses include exaggerated placental site, invasive CM, PSTT, ETT, and poorly differentiated carcinoma with trophoblastic differentiation. Exaggerated placental site is a nonneoplastic implantation site change consisting of intermediate trophoblast. It is occasionally similar to choriocarcinoma, especially in curettage specimens. The cells of exaggerated placental site have mild to moderate cytologic atypia and rare mitosis. Ki-67 labeling index of less than 1% in exaggerated implantation site reaction is helpful to differentiate it from choriocarcinoma. Invasive CM can be excluded when hydropic villi are not found; however, distinguishing these entities can become problematic when only scant tissue samples are available [1, 9].

Untreated choriocarcinoma frequently metastasizes to the vagina, lung, liver, brain, and kidney. Scores categorizing the severity of disease based on FIGO/WHO classification system (Table 13.4) including various factors from the history and clinical examination give a combined score that predicts the potential of resistance to single-agent chemotherapy [1, 24]. Most patients with GTN following a hydatidiform mole have a FIGO/WHO score of 0–6, indicating low risk of developing GTN resistant to single-agent chemotherapy with methotrexate or actinomycin D, and high-risk GTN (score $\geq$7) cases are considered clinically as choriocarcinoma. Patients with high-risk score or resistance with single-agent therapy are treated with combination-agent chemotherapy, generally methotrexate, actinomycin D, and etoposide [3]. Over 90% of patients are cured by combined and sequential chemotherapy [1, 3, 5].

Table 13.4 FIGO/WHO scoring system of prognostic and predictive parameter for trohpblastic tumours

Prognostic factor	0	1	2	4
Age	<40	≥40		
Antecedent pregnancy	Mole	Abortion	Term pregnancy	
Interval, months from index gestation	<4	4–6	7–12	>12
Pretreatment hCG (mIU/mL)	$<10^3$	10^3–10^4	10^4–10^5	$>10^6$
Largest tumor size, including uterus	<3 cm	3–5 cm	>5 cm	
Site of metastasis	Lung	Spleen, kidney	GI tract	Brain, liver
Number of metastasis		1–4	5–8	>8
Previous failed chemotherapy			Single agent	Two or more agents
Total score				

Low-risk, score ≤6; high-risk, score ≥7; *hCG* human chorionic gonadotropin

13.2.2 Placental Site Trophoblastic Tumor (PSTT)

Placental site trophoblastic tumor (PSTT) is a rare trophoblastic tumor consisting of neoplastic implantation site intermediate trophoblast [1, 25, 26]. The incidence of PSTT in gestational trophoblastic disease is 0.23–3%. The patient's age in PSTT ranges from 20 to 63 years, with mean age of 30 years. In most cases, the antecedent pregnancies are term pregnancies, and interval time to diagnosis for the index gestation ranged from 2 months to 17 years (mean, 12–18 months). The fetus of antecedent pregnancy of PSTT tends to be overwhelmingly female (M:F = 2:11). The most common clinical presentation is vaginal bleeding. Pretreatment serum hCG is usually less than 1000 mIU/mL. Eighty percent of reported cases are FIGO stage I, and 10–20% of cases are FIGO stage II with metastasis to the lung, adnexa, pelvic lymph node, and parametrium.

Cytogenetically, the absence of the Y chromosome in PSTT with a haploid pair of X chromosomes has been reported, and the paternal X chromosome is considered to be related to tumorigenesis of PSTT [27].

Macroscopically, PSTT presents as an endomyometrial nodular mass of 1–10 cm in diameter. The tumor is relatively well circumscribed and white-tan to light yellow in color and invades into the myometrium in 50% of the cases. Focal hemorrhage and necrosis are present in nearly half of the cases (Fig. 13.10a).

Histologically, the tumor cells have an infiltrative growth pattern with cords or sheet-like aggregates (Fig. 13.10b). The most charachteristic features of PSTT are infiltrative tumor cells splitting individual myometrial smooth muscle fibers at the peripheral part of the tumor and replacing the vascular wall of myometrial vessels. These cells are neoplastic implantation site intermediate trophoblast with abundant eosinophilic or clear cytoplasm and marked hyperchromatic, convoluted large nuclei. Syncytiotrophoblast with multinucleated cells is commonly scattered; however, mononuclear monotonous intermediate trophoblast forms the vast majority of the tumor. Mitotic activity is relatively low ranging from 2 to 4 per 10

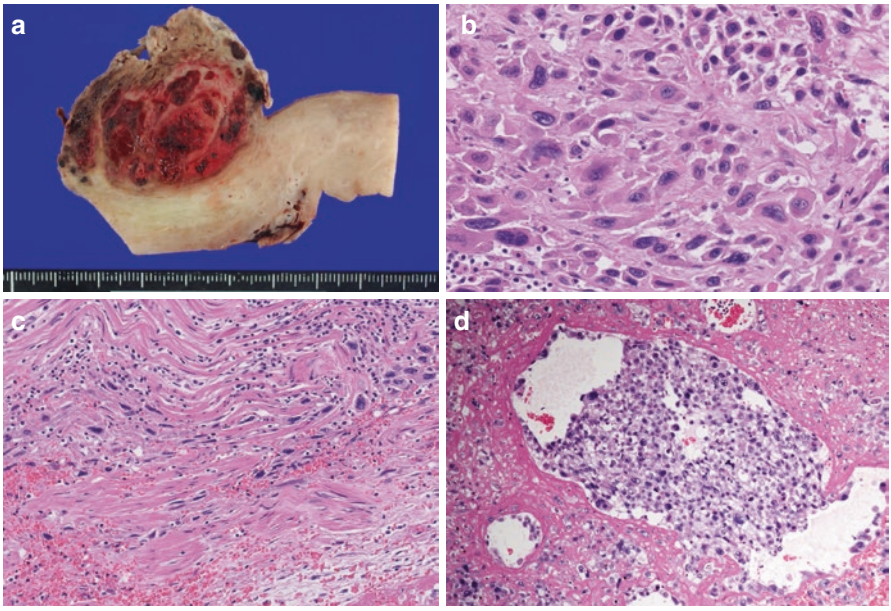

Fig. 13.10 Placental site trophoblastic tumor (PSTT). (**a**) Gross appearance of PSTT. Discrete solid mass in the endomyometrium presenting focal hemorrhage. (**b**) Sheet of atypical intermediate cells with eosinophilic abundant cytoplasm and large convoluted nuclei. (**c**) Tumor cells infiltrate and split existing smooth muscle fibers at the tumor periphery. (**d**) Tumor cells replacing the vascular wall of the myometrial vessel

high-power fields; however, cases with higher mitotic activity are found occasionally. Hemorrhage and necrosis are not uncommon [9, 25, 26].

Immunohistochemically, tumor cells are diffusely positive for Mel-CAM (CD146) (Fig. 13.11a), hPL, MUC-4, and HLA-G and negative for p63 (Fig. 13.11b). Expression of hCG (Fig. 13.11c) and inhibin is focal (Fig. 13.11a, b). Ki-67 labeling index is 10–30% in most of the cases (Fig. 13.11d) [28].

Differential diagnoses include exaggerated placental site, choriocarcinoma, ETT, epithelioid leiomyoma/leiomyosarcoma, and poorly differentiated carcinoma. Differentiation from exaggerated placental site is the most frequent problem on routine pathological diagnosis. Exaggerated placental site is also composed of implantation site-type intermediate trophoblast with cytomorphological similarities with PSTT. However, exaggerated placental site does not present as a nodular lesion with increased mitotic activity. Ki-67 labeling index is an effective marker with 0–2% positivity in exaggerated placental site and 10–30% in PSTT.

Most patients are cured by hysterectomy [3]. The previously described scoring systems are not used for PSTT. The recurrence rate is 25–30% and half of these patients may die of PSTT. Histological parameters of worse prognosis are tumor cells with clear cytoplasm, depth of invasion, tumor size, necrosis, and high mitotic count (>5 per 10 HPF) [26].

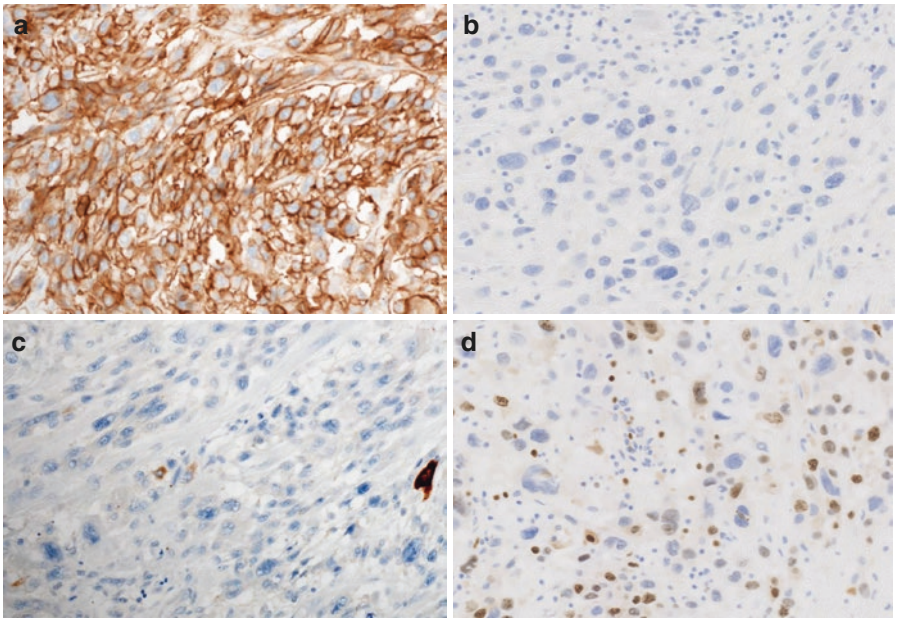

Fig. 13.11 Immunohistochemical staining for PSTT. (**a**) Mel-CAM (CD146). The tumor cells diffusely express. (**b**) p63, negative. (**c**) hCG. Most of the tumor cells are negative with scattered positive cells. (**d**) Ki-67 labeling index is about 30%

13.2.3 Epithelioid Trophoblastic Tumor

Epithelioid trophoblastic tumor (ETT) is defined as a trophoblastic tumor consisting of neoplastic intermediate trophoblast arising from the chorion laeve [1, 9, 29, 30]. The patient ages range from 15 to 48 years (mean of 36 years). Common symptom is vaginal bleeding. In most cases, antecedent pregnancy is term pregnancy, and interval time ranges from 1 to 1.5 years with average of 6.2 years. Serum hCG level shows only a mild to moderate elevation of less than 2500 mIU/mL. Thirty-five percent of reported cases already had metastases at the time of initial diagnosis.

Genetically, the absence of a Y chromosome with a haploid pair of X chromosomes in ETT as well as PSTT has been reported [31].

ETT is distributed as follows: 30% in the uterine corpus, 50% in the fundus or cervix, and 20% in extrauterine sites including the small intestine and lung. The tumor size is 0.5–4 cm in diameter, forming discrete nodule or a cystic hemorrhagic mass. The tumor cells deeply invade into the myometrium. The cut surface of the tumor is solid and has white-tan to brown color with various amounts of hemorrhage and necrosis [1, 9, 29, 30].

Histologically, ETT is a nodular lesion of medium-sized mononuclear tumor cells arranged in nests or cords to a sheet-like appearance. The tumor cells are relatively uniform with small, round to oval nuclei, eosinophilic or clear cytoplasm, and

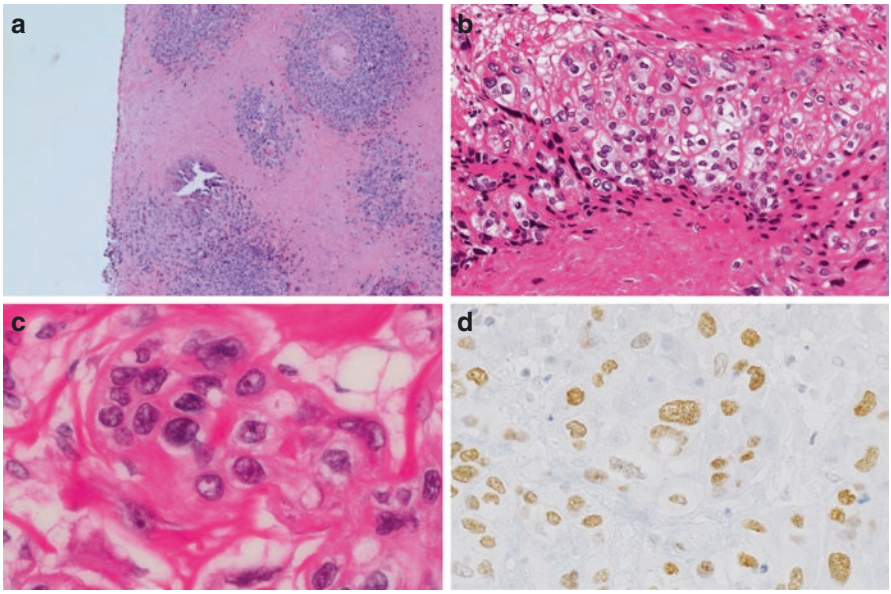

Fig. 13.12 Epithelioid trophoblastic tumor. (**a**) The tumor is characterized by geographic necrosis. (**b**) A nest of tumor cells with a relatively uniform populations of mononuclear intermediate trophoblastic cells with necrosis and fibrinoid deposition. (**c**) The tumor cells with eosinophilic to clear cytoplasm and moderate nuclear atypia. Deposition of hyaline-like material in the background. (**d**) The tumor cells express nuclear positivity for p63. (**b**, **d**, courtesy of Dr. Takako Kiyokawa; **c**, courtesy of Dr. Yuichiro Sato)

distinct cell membranes. Cytologic atypia is mild to moderate and mitotic rate is 0–9/10 HPF. Hyaline-like material in the center of the tumor or between tumor cells is the most characteristic feature. Extensive geographic necrosis is also a common feature (Fig. 13.12a–c). Histological findings are occasionally similar to squamous cell carcinoma. In cases of cervical involvement of ETT, a cervical mucosal lesion simulating high-grade squamous intraepithelial lesion is often seen [1, 9, 29, 30].

Immunohistochemistry is very helpful in differentiating ETT from its mimics, squamous cell carcinoma, PSTT, and placental site nodule [9, 28]. ETT is diffusely positive for p63 (Fig. 13.12d), HLA-G, and inhibin-alpha. Mel-CAM (CD146) and hPL are expressed focally, whereas PSTT is p63 negative and shows diffuse strong positive with Mel-CAM and hPL. Squamous cell carcinoma is positive for p63 and negative for trophoblastic markers. ETT shows Ki-67 labeling index higher than 10%, whereas Ki-67 labeling index of placental site nodule is less than 10%.

The main treatment for ETT is surgical, and the FIGO/WHO scoring system is not used for ETT. The prognosis of ETT is similar to that of PSTT. The rate of metastasis is 25%, and 10% of these patients may die of disease. The survival rates are 100% in patients without metastasis and 50–60% in patients with metastasis [3]. Histological predictor of a worse prognosis is higher mitotic counts (> 6/10 HPF) [1, 29, 30].

References

1. Kurman RJ, Carcangiu ML, Herrington CS, Young RH. WHO classification of tumours of female reproductive organs. 4th ed. Lyon: IARC; 2014. p. 158–67.
2. Eysbouts YK, Bulten J, Ottevanger PB, Thomas CM, Ten Kate-Booij MJ, van Herwaarden AE, et al. Trends in incidence for gestational trophoblastic disease over the last 20 years in a population-based study. Gynecol Oncol. 2016;140:70–5. doi:10.1016/j.ygyno.2015.11.014.
3. Froeling FE, Seckl MJ. Gestational trophoblastic tumours: an update for 2014. Curr Oncol Rep. 2014;16:408. doi:10.1007/s11912-014-0408-y.
4. Matsui H, Iitsuka Y, Yamazawa K, Tanaka N, Seki K, Sekiya S. Changes in the incidence of molar pregnancies. A population-based study in Chiba Prefecture and Japan between 1974 and 2000. Hum Reprod. 2003;18:172–5.
5. Berkowitz RS, Goldstein DP. Chorionic tumors. N Engl J Med. 1996;335:1740–8.
6. Berkowitz RS, Goldstein DP. Clinical practice. Molar pregnancy. N Engl J Med. 2009;360:1639–45.
7. Seckl MJ, Fisher RA, Salerno G, Rees H, Paradinas FJ, Foskett M, et al. Choriocarcinoma and partial hydatidiform moles. Lancet. 2000;356:36–9.
8. Fukunaga M, Katabuchi H, Nagasaka T, Mikami Y, Minamiguchi S, Lage JM. Interobserver and intraobserver variability in the diagnosis of hydatidiform mole. Am J Surg Pathol. 2005;29(7):942.
9. Buza N, Hui P. Immunohistochemistry and other ancillary techniques in the diagnosis of gestational trophoblastic diseases. Semin Diagn Pathol. 2014;31:223–32.
10. Banet N, DeScipio C, Murphy KM, Beierl K, Adams E, Vang R, et al. Characteristics of hydatidiform moles: analysis of a prospective series with p57 immunohistochemistry and molecular genotyping. Mod Pathol. 2014;27:238–54.
11. Buza N, Hui P. Partial hydatidiform mole: histologic parameters in correlation with DNA genotyping. Int J Gynecol Pathol. 2013;32:307–15.
12. Baergen RN, Kelly T, McGinniss MJ, Jones OW, Benirschke K. Complete hydatidiform mole with a coexistent embryo. Hum Pathol. 1996;27:731–4.
13. Piura B, Rabinovich A, Hershkovitz R, Maor E, Mazor M. Twin pregnancy with a complete hydatidiform mole and surviving co-existent fetus. Arch Gynecol Obstet. 2008;278:377–82. doi:10.1007/s00404-008-0591-x.
14. Keep D, Zaragoza MV, Hassold T, Redline RW. Very early complete hydatidiform mole. Hum Pathol. 1996;27:708–13.
15. Sebire NJ, Fisher RA, Rees HC. Histopathological diagnosis of partial and complete hydatidiform mole in the first trimester of pregnancy. Pediatr Dev Pathol. 2003;6:69–77.
16. Eagles N, Sebire NJ, Short D, Savage PM, Seckl MJ, Fisher RA. Risk of recurrent molar pregnancies following complete and partial hydatidiform moles. Hum Reprod. 2015;30:2055–63.
17. Sebire NJ, Fisher RA, Foskett M, Rees H, Seckl MJ, Newlands ES. Risk of recurrent hydatidiform mole and subsequent pregnancy outcome following complete or partial hydatidiform molar pregnancy. Br J Obstet Gynaecol. 2003;110:22–6.
18. Murdoch S, Djuric U, Mazhar B, Seoud M, Khan R, Kuick R, et al. Mutations in NALP7 cause recurrent hydatidiform moles and reproductive wastage in humans. Nat Genet. 2006;38:300–2.
19. Genest DR. Partial hydatidiform mole: clinicopathological features, differential diagnosis, ploidy and molecular studies, and gold standards for diagnosis. Int J Gynecol Pathol. 2001;20:315–22.
20. Scholz NB, Bolund L, Nyegaard M, Faaborg L, Jørgensen MW, Lund H, et al. Triploidy—observations in 154 diandric cases. PLoS One. 2015;10:e0142545. doi:10.1371/journal.pone.0142545.
21. Gaber LW, Redline RW, Mostoufi-Zadeh M, Driscoll SG. Invasive partial mole. Am J Clin Pathol. 1986;85(6):722–4.

22. Kukita Y, Yahara K, Tahira T, Higasa K, Sonoda M, Yamamoto K, et al. A definitive haplotype map as determined by genotyping duplicated haploid genomes finds a predominant haplotype preference at copy-number variation events. Am J Hum Genet. 2010;86:918–28. doi:10.1016/j.ajhg.2010.05.003.
23. Jiao L, Ghorani E, Sebire NJ, Seckl MJ. Intraplacental choriocarcinoma: systematic review and management guidance. Gynecol Oncol. 2016; doi:10.1016/j.ygyno.2016.03.026.
24. FIGO Oncology Committee. FIGO staging for gestational trophoblastic neoplasia 2000. FIGO Oncology Committee. Int J Gynaecol Obstet. 2002;77:285-7. Young RH, Scully RE. Placental-site trophoblastic tumor: current status. Clin Obstet Gynecol. 1984;27:248–58.
25. Baergen RN, Rutgers JL, Young RH, Osann K, Scully RE. Placental site trophoblastic tumor: a study of 55 cases and review of the literature emphasizing factors of prognostic significance. Gynecol Oncol. 2006;100:511–20.
26. Schmid P, Nagai Y, Agarwal R, Hancock B, Savage PM, Sebire NJ, et al. Prognostic markers and long-term outcome of placental-site trophoblastic tumours: a retrospective observational study. Lancet. 2009;374:48–55. doi:10.1016/S0140-6736(09)60618-8.
27. Hui P, Wang HL, Chu P, Yang B, Huang J, Baergen RN, et al. Absence of Y chromosome in human placental site trophoblastic tumor. Mod Pathol. 2007;20:1055–60.
28. IeM S. Trophogram, an immunohistochemistry-based algorithmic approach, in the differential diagnosis of trophoblastic tumors and tumorlike lesions. Ann Diagn Pathol. 2007;11:228–34.
29. Shih IM, Kurman RJ. Epithelioid trophoblastic tumor: a neoplasm distinct from choriocarcinoma and placental site trophoblastic tumor simulating carcinoma. Am J Surg Pathol. 1998;22:1393–403.
30. Fadare O, Parkash V, Carcangiu ML, Hui P. Epithelioid trophoblastic tumor: clinicopathological features with an emphasis on uterine cervical involvement. Mod Pathol. 2006;19:75–82.
31. Yap KL, Hafez MJ, Mao TL, Kurman RJ, Murphy KM, Shih IM. Lack of a y-chromosomal complement in the majority of gestational trophoblastic neoplasms. J Oncol. 2010; doi:10.1155/2010/364508.

Pathogenesis of Preeclampsia

14

Eiji Kondoh

Abstract

Preeclampsia is a leading cause of maternal and neonatal morbidity and mortality. Preeclampsia is typified by a systemic inflammatory state and impaired endothelial function. As a systemic inflammation increases with advancing gestation even in normal pregnancy, pregnancy is thought to be a "stress test for endothelial function" for women. The etiology of preeclampsia remains largely unknown, and a number of theories have been proposed to explain its cause. It has been postulated that poor placentation results in the release of factors that lead to excessive maternal inflammatory response and endothelial dysfunction. However, the heterogeneous clinical manifestations of the disease suggest multifactorial pathogenesis of preeclampsia, such as poor placentation, oxidative stress, inflammation, immune maladaptation, and angiogenic imbalance. This chapter covers recent progress in molecular studies of the pathogenesis of preeclampsia.

Keywords

Endothelial function • Pathogenesis • Placentation • Preeclampsia

14.1 Introduction

Preeclampsia is the most common hypertensive disorder of pregnancy, affecting 2–8% of pregnant women [1]. Preeclampsia is a syndrome of new-onset hypertension and proteinuria typically presenting after 20 weeks of gestation. Preeclampsia can lead to serious maternal complications, such as pulmonary edema, HELLP

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syndrome, placental abruption, eclampsia, and intracerebral hemorrhage. Preeclampsia is also associated with adverse fetal conditions such as growth restriction, oligohydramnios, and absent or reversed end-diastolic velocity in the umbilical artery. Consequently, preeclampsia is a leading cause of maternal and neonatal morbidity and mortality [1]. While it is well known that the placenta is essential to the development of preeclampsia, the heterogeneous clinical manifestations of the disease suggest multifactorial pathogenesis of preeclampsia. Because the pathogenesis has not been fully elucidated, prevention is not possible, and, even today, the fundamental therapy is to terminate the pregnancy. However, recent progress on genomic analyses using the placenta has gradually revealed molecular mechanisms underlying the pathogenesis of preeclampsia.

14.2 Two-Stage Hypothesis

The etiology of preeclampsia remains unknown, and a number of theories have been proposed to explain its cause. Zweifel described preeclampsia as a "disease of theories" [2]. Preeclampsia is clinically heterogeneous in its manifestation including the onset of the disease and the fetal growth. Abnormal trophoblast invasion with insufficient uterine spiral artery remodeling often occurs in early-onset preeclampsia and fetal growth restriction [3]. In contrast, normal spiral artery remodeling is usually observed in late-onset preeclampsia with normal fetal growth. So, Ness and Roberts hypothesized that there are distinct origins of preeclampsia, and both poor placentation (placental preeclampsia) and latent vascular dysfunction (maternal preeclampsia) are attributed to the development of preeclampsia [4]. Abnormal placentation is hypothesized to be associated with early-onset preeclampsia with fetal growth restriction. It has been postulated that poor placentation (stage 1) results in the release of factors that lead to excessive maternal inflammatory response and endothelial dysfunction (stage 2) [5, 6]. Thus, in early-onset preeclampsia, the sequence of abnormal trophoblast invasion to systemic endothelial dysfunction has been proposed to occur in two stages. On the other hand, preexisting maternal endothelial dysfunction (as with chronic hypertension, obesity, or diabetes mellitus) is thought to predispose to late-onset preeclampsia, which is more often linked to a normal placenta and normal fetal growth [5, 6]. As a systemic inflammation increases with advancing gestation even in normal pregnancy, pregnancy is a "stress test for endothelial function" for women. This burden to latent vascular dysfunction is enough to induce a severe systemic inflammatory response and subsequent endothelial dysfunction that is characteristic of preeclampsia [6].

14.3 Abnormal Trophoblast Invasion

Poor placentation is typified by insufficient remodeling of spiral arteries [7–9]. During normal placentation, extravillous trophoblasts (EVTs) invade into the decidua and the inner one third of the myometrium either interstitially or via spiral arteries. EVTs disrupt the endothelium and the smooth muscle layer and replace the

vascular wall. These conversions allow spiral arteries to get widely dilated independently of vasomotor control, thereby providing enough blood supply in intervillous space (IVS) to meet the requirements of the fetus [7–9]. On the other hand, in preeclampsia, endovascular trophoblast invasion is restricted to the decidual segments of the spiral arteries, resulting in less dilated vessels and intermittent hypoperfusion due to retention of vasoactive smooth muscle [7–9]. Burton proposed that the impaired vascular remodeling of the spiral arteries may not cause chronic placental hypoxia in itself, but that the retention of smooth muscle will increase the risk of spontaneous vasoconstriction and ischemia-reperfusion injury, leading to generation of oxidative stress [10]. Moreover, high-velocity and turbulent flow into the IVS will damage villous syncytiotrophoblast [10], which may enhance placentally derived stimuli, including pro-inflammatory cytokines, anti-angiogenic factors, and trophoblast microparticles.

14.4 Hypoxia

Early placental development occurs in a low-oxygen environment, which appears to prevent trophoblast differentiation. As maternal blood flow into the IVS is blocked by endovascular plugs of extravillous trophoblasts prior to 10 weeks' gestation, the oxygen concentration within the IVS is approximately 20 mmHg (2–3%) [11–13]. Once the plugs are displaced after 10–12 weeks' gestation, chorionic villi are bathed in maternal blood. As a result, the oxygen concentration rises to 40–80 mmHg (5–10%) in the second trimester [11–13]. The oxygen concentration in the IVS gradually decreases thereafter to the third trimester (40 mmHg) in response to increased fetal oxygen consumption [11–13]. In preeclamptic placenta, the oxygen concentrations in the IVS are generally thought to be low because of defective vascular remodeling of the spiral arteries. However, to date there has been no direct evidence to support this assumption. Burton proposed that there was a slight alteration of total blood flow in the IVS due to insufficient spiral artery remodeling [10]. Moreover, the impairment of villous syncytiotrophoblasts will result in reduced oxygen removal in the IVS, which leads to placental hyperoxia and fetal hypoxia. So, it is unclear whether preeclamptic placentas are actually hypoxic [12], and it is plausible that exaggerated oxidative stress in preeclampsia is secondary to intermittent hypoperfusion rather than chronic placental hypoxia.

14.5 Hypoxia-Inducible Factors

The hypoxia-inducible factors (HIFs) are central mediators of cellular adaptation to low oxygen and regulate placental development and maturation [12, 14, 15]. For instance, HIF-1 regulates TGF-beta3, which inhibits trophoblast differentiation and invasion and is overexpressed in preeclamptic placentas [14]. Transcription of HIF-1alpha and HIF-2alpha is differentially regulated under hypoxia in neuroblastoma [16]. HIF-1alpha protein is transiently stabilized under hypoxia (1% O_2), while HIF-2alpha protein gradually accumulates and controls prolonged hypoxic gene

activation. Moreover, HIF-2alpha is increased to a greater extent than HIF-1alpha under mild hypoxia (5% O_2). In the placenta, inconclusive results have been reported regarding HIF regulation in response to acute versus prolonged hypoxia [12]. Intriguingly, HIFs are activated through oxygen-independent factors including hormones (angiotensin II), cytokines (IL-1b, TNF-alpha, and NF-kB), and growth factors (TGF-b and IGF) [12, 15], and activation of HIFs during pregnancy is considered to play an important role in the pathogenesis of preeclampsia.

14.6 Oxidative Stress

Oxidative stress arises when the production of reactive oxygen species (ROS) exceeds the intrinsic antioxidant defenses [17]. ROS, such as superoxide anion, hydrogen peroxide, and hydroxyl radical, are chemically reactive molecules containing oxygen. ROS arise from the various sources such as mitochondria and cause cellular damage. The placenta is rich in mitochondria, is highly vascular, and is exposed to high maternal oxygen partial pressure, therefore resulting in increased production of ROS [18]. Indeed, increased production of lipid peroxides and oxidative stress are observed in normal pregnant women compared with nonpregnant women [19]. Moreover, preeclampsia is characterized by markedly decreased antioxidants such as glutathione or glutathione peroxidase activity, as well as increased oxidative stress [19, 20]. The higher production of oxidative stress in preeclampsia is probably due to insufficient remodeling of spiral arteries in the section of myometrial segments. Fluctuations in maternal blood flow to the placenta are thought to cause exaggerated oxidative stress. Consistently, labor, in which the placenta is exposed to repeated episodes of ischemia-reperfusion, produces high levels of oxidative stress [17, 21, 22].

Oxidative stress is one of many forms of cellular stress. ROS can activate redox-sensitive transcription factors (i.e., NF-kB, p53, AP1) and protein kinases (i.e., ERK1/2, SAPK/JNK, p38 MAPK) as physiological adaptive changes to alterations in the environment aimed at restoring homeostasis [17]. At higher levels, ROS lead to more extensive and irreparable cell damage, resulting in apoptosis or necrosis. ROS induce release of Ca^{2+} from the endoplasmic reticulum (ER) and are closely associated with mitochondrial and ER function [17]. Thus, oxidative stress rarely occurs in isolation, but is usually accompanied with other forms of cell responses, such as ER stress and unfolded protein responses [17, 23]. Placental oxidative stress causes diverse stress responses of syncytiotrophoblast including increased apoptosis and secretion of anti-angiogenic and pro-inflammatory products [6] and is thought to be an intermediary step in the pathogenesis of preeclampsia.

14.7 Inflammation

Inflammatory response is already well developed in normal pregnancy [24], and preeclampsia is a state of an exaggerated inflammatory response including abnormal cytokine production and neutrophil and endothelial cell activation [25]. Both normal

and preeclamptic pregnancies exhibit an increased inflammatory response with advancing gestation [26]. There is now ample evidence to support that increased inflammatory response is associated with clinical manifestations of preeclampsia in animal models. For instance, chronic administration of endotoxin [27], TNF-alpha [28], IL-6 [29], and LPS [30] into pregnant rats causes preeclampsia-like symptoms. Activated neutrophils could cause vascular damage with activation of coagulation and platelets [31]. In addition to neutrophils, other immune effectors that appear to play important roles in preeclampsia are cytokines, which are major initiators and mediators of inflammation and endothelial dysfunction [25, 32, 33]. Cytokines can be generally classified as pro- and anti-inflammatory. Cytokines such as IL-1, IL-2, IL-8, IL-18, TNF-alpha, and IFN-gamma are pro-inflammatory. The expression of IL-1, IL-2, IL-18, and TNF-alpha are elevated in the preeclamptic placentas [34–38]. The anti-inflammatory cytokines, IL-4 and IL-10, are also secreted by placental tissues. IL-10 is a potent suppressor of pro-inflammatory cytokines such as TNF-alpha and IFN-gamma, and its placental production is decreased in the preeclamptic placentas [39, 40]. Indeed, IL-10 knockout mice exhibit mild hypertension with fetal growth restriction [41], and hypoxia induces preeclampsia-like features in pregnant IL-10 knockout mice [42]. Although pro-inflammatory cytokines are produced by trophoblasts and also by macrophages and stromal cells of the placenta, the high pro-inflammatory cytokine levels seen in peripheral blood in preeclamptic pregnancies are believed to be caused in great part by monocytes [25, 43, 44]. It has been proposed that the syncytiotrophoblast microparticles (STBM) stimulate the production of the pro-inflammatory cytokines through the activation of monocytes [43, 44]. As markedly increased amounts of STBM are shed into the maternal circulation in preeclampsia [45], STBM impairs maternal vascular endothelial function [46]. Cytokines may contribute to an increased release of STBM by stimulating enhanced trophoblast apoptosis [47], bringing about a vicious cycle of an excess of pro-inflammatory cytokines and the increased shedding of STBM.

14.8 Immune Maladaptation

Maternal-fetal immune maladaptation is believed to be one of the underlying mechanisms that contribute to the development of preeclampsia.

14.8.1 Uterine Natural Killer Cells

Uterine natural killer (uNK) cells comprise approximately 70% of the decidual leukocyte population in early human pregnancy [48, 49]. uNK cells play a crucial role in trophoblast invasion and initiating spiral artery remodeling. uNK cells surround or infiltrate the vascular wall before trophoblast invasion, and the number of uNK cells is reduced in the second half of pregnancy [50]. uNK cells are a major source of IFN-gamma [51], which acts in an autocrine manner on uNK cells, stimulating further production of IFN-gamma, as well as angiogenic growth factors including

angiopoietin-1 (Ang-1), Ang-2, TGF-beta1, and VEGF-C [52]. IFN-gamma inhibits extravillous trophoblast cell invasion. uNK cell-derived factors, such as MMP-2, MMP-7, and MMP-9, urokinase plasminogen activator, and Ang-2 are thought to be involved in remodeling of spiral arteries, leading to extensive disorganization and apoptotic loss of vascular smooth muscle cells and endothelial cells [50, 53–55]. uNK cell supernatants from 12 to 14 weeks' gestation, but not 8–10 weeks, stimulate extravillous trophoblast invasion, suggesting that regulation of extravillous trophoblast invasion by uNK cells is dependent on gestational age [56].

14.8.2 Macrophages

Macrophages are the second most abundant leukocyte population in the decidua comprising approximately 20% of all decidual leukocytes [48]. The number of macrophages does not alter substantially with increasing gestational age. Decidual macrophages are thought to contribute not only to the process of apoptotic cell clearance but also to immune tolerance at the maternal-fetal interface. Decidual macrophages produce IL-10 and indoleamine 2,3-dioxygenase (IDO) [57, 58] and suppress IFN-gamma production by T cells in early pregnancy [59]. On the other hand, decidual macrophages secrete pro-inflammatory cytokines such as IL-6, IL-8, and TNF-alpha through HLA-G receptors [60]. Moreover, activated macrophages produce TNF-alpha and inhibit human cytotrophoblast invasiveness [61]. In preeclampsia, myometrial spiral arteries are surrounded by large numbers of macrophages in the absence of trophoblast invasion [62]. In contrast, in normal pregnancies, myometrial spiral arteries are surrounded by extravillous trophoblast cells with sparse macrophage infiltration. Because activated macrophages produce both TNF-alpha and IL-10, an imbalance of these opposing cytokines may be involved in the pathogenesis of preeclampsia [63].

14.8.3 Toll-like Receptors

Toll-like receptors (TLRs) play a key role in the innate immune system and appear to contribute to the development of preeclampsia. TLR4 expression is increased in interstitial trophoblasts of patients with preeclampsia [64]. In a rat model, TLR4 activation in the placenta is associated with poor placentation and preeclampsia-like syndrome [65], which are improved by inhibiting the TLR4 signaling [66]. In addition, TLR3 activation during pregnancy causes preeclampsia-like symptoms, which are exacerbated in pregnant IL-10 knockout mice [67].

14.8.4 The Complement System

The complement system plays a crucial role in innate immunity. The complement cascade is activated in normal pregnancy, and excessive complement activation is observed in preeclampsia [68–71]. It was reported that eculizumab, a humanized

monoclonal antibody against terminal complement protein C5, was an effective treatment for a woman presenting with severe preeclampsia/HELLP syndrome at 26 weeks' gestation [72], indicating that complement inhibition may be an effective treatment for severe preeclampsia/HELLP syndrome. Apart from adverse effects of complement activation, C1q is widely distributed in human decidual stroma and plays an important role in promoting trophoblast invasion [73]. C1q-deficient mice exhibit impaired trophoblast invasion and preeclampsia-like features [74].

14.9 Angiogenic Imbalance

Preeclampsia can be thought of as a two-stage disorder, and the second stage is typified by the placenta-derived soluble angiogenic and anti-angiogenic factors into the maternal circulation [75].

14.9.1 Placental Growth Factor

Placental growth factor (PlGF) is predominantly expressed by syncytiotrophoblast and exerts its biological function through the binding and activation of VEGF receptor 1 (also known as Flt-1), which is initially identified as a receptor for VEGF-A [76]. PlGF is expressed at a low level in endothelial cells and other organs including the heart, lung, thyroid, skeletal muscle, and adipose tissue [76]. In normal pregnancy, PlGF can be detected in the maternal circulation from 8 weeks' gestation. Serum PlGF rises steadily to 29–32 weeks and falls thereafter until delivery [77]. The PlGF levels are significantly lower in the women who later develop preeclampsia than in the controls [77]. Statins have been shown to reverse various pathophysiologic pathways associated with preeclampsia, such as angiogenic imbalance and oxidative stress [78–80]. Pravastatin induces PlGF and improves features of preeclampsia in a mouse model [80]. It was recently reported that treatment with recombinant PlGF ameliorates preeclampsia-like symptoms in a primate model, as well as a rat model of preeclampsia produced by reduced uterine perfusion pressure [81, 82].

14.9.2 Soluble Fms-like Tyrosine Kinase

Soluble Flt-1 (sFlt-1), an antagonist of VEGF and PlGF, is highly expressed in the placenta. The extraplacental sources of circulating sFLT-1 include endothelial cells, peripheral blood mononuclear cells, and adipose tissue [83, 84]. In normal pregnancy, the sFlt-1 levels are stable during the early and middle stages of gestation, and there is a slow and steady increase beginning at 33–36 weeks [77]. sFlt-1 is increased in the placenta and serum of women with preeclampsia [85]. Exogenous administration of sFlt-1 into pregnant rats induces major features of preeclampsia [85], and elevated levels of sFlt-1 and decreased levels of PlGF are observed in a rat

model of preeclampsia produced by reduced uterine perfusion pressure [86]. Extracorporeal removal of sFlt-1 contributes to a successful prolongation of pregnancy [87, 88]. The evidence suggests that excess circulating sFlt-1 is closely related to the pathogenesis of preeclampsia.

14.9.3 Soluble Endoglin

Endoglin (Eng) is a TGF-beta co-receptor that is expressed mainly on the surface of endothelial cells but is also expressed on placental syncytiotrophoblasts. Levels of soluble Eng (sEng), resulting from the cleavage of full-length Eng, are markedly elevated in preeclampsia [89]. sEng impairs binding of TGF-beta to its receptors and inhibits nitric oxide-dependent vasodilatation by the regulation of eNOS expression. Overexpression of sEng in pregnant rats induces preeclampsia-like syndrome, which is exacerbated by the coadministration of sFlt-1, leading to the development of HELLP syndrome and fetal growth restriction [89].

14.9.4 Angiogenic Biomarkers of Preeclampsia

An excess of sFlt-1 and sEng and lower PlGF are generally released into the circulation of preeclamptic patients. However, the altered levels of placenta-derived angiogenic markers are more pronounced in early-onset rather than in late-onset preeclampsia [90]. On the contrary, angiogenic biomarker profile may not differ among late-onset preeclampsia, gestational hypertension, and normotensive pregnancy [91]. These observations support that the angiogenic biomarkers can be used to predict early-onset preeclampsia, which develops differently from late-onset preeclampsia. With accumulating evidence that sFlt-1/PlGF ratio is a promising diagnostic and predictive biomarker, careful clinical examinations have been conducted to confirm their usefulness in the prediction of preeclampsia [92–94].

14.10 Autoantibodies Against Angiotensin Type 1 Receptor II

The renin-angiotensin system is implicated in preeclampsia. Not a few preeclamptic patients have an IgG autoantibody that binds to angiotensin II receptor, type 1, or AT1 receptor [95, 96]. The agonistic autoantibodies to AT1, termed AT1-AAs, induce preeclampsia in pregnant mice [97]. AT1-AAs are produced by CD19(+) CD5(+) cells, and the frequency of CD19(+)CD5(+) cells in peripheral blood of preeclamptic patients is strikingly increased compared with normal pregnant women [98]. AT1-AA stimulates sFlt-1 and sEng production in the placenta of pregnant mice through TNF-alpha pathways [99, 100]. As angiotensin II is known to increase vascular permeability and edema formation [101], AT-1-AA may be relevant to heterogeneity of clinical manifestation of preeclampsia.

References

1. Steegers EA, von Dadelszen P, Duvekot JJ, Pijnenborg R. Pre-eclampsia. Lancet. 2010;376:631–44.
2. Zweifel P. Eklampsie. In: Döderlein A, editor. Handbuch der Geburtshilfe. Wiesbaden: Bergmann; 1916. p. 672–723.
3. Khong TY, De Wolf F, Robertson WB, Brosens I. Inadequate maternal vascular response to placentation in pregnancies complicated by pre-eclampsia and by small-for-gestational age infants. Br J Obstet Gynaecol. 1986;93:1049–59.
4. Ness RB, Roberts JM. Heterogeneous causes constituting the single syndrome of preeclampsia: a hypothesis and its implications. Am J Obstet Gynecol. 1996;175:1365–70.
5. Roberts JM, Hubel CA. The two stage model of preeclampsia: variations on the theme. Placenta. 2009;30(Suppl A):S32–7.
6. Staff AC, Benton SJ, von Dadelszen P, Roberts JM, Taylor RN, Powers RW, Charnock-Jones DS, Redman CW. Redefining preeclampsia using placenta-derived biomarkers. Hypertension. 2013;61:932–42.
7. Brosens IA, Robertson WB, Dixon HG. The role of the spiral arteries in the pathogenesis of preeclampsia. Obstet Gynecol Annu. 1972;1:177–91.
8. Staff AC, Dechend R, Pijnenborg R. Learning from the placenta: acute atherosis and vascular remodeling in preeclampsia-novel aspects for atherosclerosis and future cardiovascular health. Hypertension. 2010;56:1026–34.
9. Brosens I, Pijnenborg R, Vercruysse L, Romero R. The "great obstetrical syndromes" are associated with disorders of deep placentation. Am J Obstet Gynecol. 2011;204:193–201.
10. Burton GJ, Woods AW, Jauniaux E, Kingdom JC. Rheological and physiological consequences of conversion of the maternal spiral arteries for uteroplacental blood flow during human pregnancy. Placenta. 2009;30:473–82.
11. James JL, Stone PR, Chamley LW. The regulation of trophoblast differentiation by oxygen in the first trimester of pregnancy. Hum Reprod Update. 2006;12:137–44.
12. Pringle KG, Kind KL, Sferruzzi-Perri AN, Thompson JG, Roberts CT. Beyond oxygen: complex regulation and activity of hypoxia inducible factors in pregnancy. Hum Reprod Update. 2010;16:415–31.
13. Tuuli MG, Longtine MS, Nelson DM. Review: oxygen and trophoblast biology—a source of controversy. Placenta. 2011;32(Suppl 2):S109–18.
14. Caniggia I, Winter JL. Adriana and Luisa Castellucci Award lecture 2001. Hypoxia inducible factor-1: oxygen regulation of trophoblast differentiation in normal and pre-eclamptic pregnancies—a review. Placenta. 2002;23(Suppl A):S47–57.
15. Patel J, Landers K, Mortimer RH, Richard K. Regulation of hypoxia inducible factors (HIF) in hypoxia and normoxia during placental development. Placenta. 2010;31:951–7.
16. Holmquist-Mengelbier L, Fredlund E, Löfstedt T, Noguera R, Navarro S, Nilsson H, Pietras A, Vallon-Christersson J, Borg A, Gradin K, Poellinger L, Påhlman S. Recruitment of HIF-1alpha and HIF-2alpha to common target genes is differentially regulated in neuroblastoma: HIF-2alpha promotes an aggressive phenotype. Cancer Cell. 2006;10:413–23.
17. Burton GJ, Jauniaux E. Oxidative stress. Best Pract Res Clin Obstet Gynaecol. 2011;25:287–99.
18. Casanueva E, Viteri FE. Iron and oxidative stress in pregnancy. J Nutr. 2003;133:1700S–8S.
19. Kaur G, Mishra S, Sehgal A, Prasad R. Alterations in lipid peroxidation and antioxidant status in pregnancy with preeclampsia. Mol Cell Biochem. 2008;313:37–44.
20. Siddiqui IA, Jaleel A, Tamimi W, Al Kadri HM. Role of oxidative stress in the pathogenesis of preeclampsia. Arch Gynecol Obstet. 2010;282:469–74.
21. Many A, Roberts JM. Increased xanthine oxidase during labour—implications for oxidative stress. Placenta. 1997;18:725–6.
22. Cindrova-Davies T, Yung HW, Johns J, Spasic-Boskovic O, Korolchuk S, Jauniaux E, Burton GJ, Charnock-Jones DS. Oxidative stress, gene expression, and protein changes induced in the human placenta during labor. Am J Pathol. 2007;171:1168–79.

23. Redman CW, Sargent IL, Staff AC. IFPA senior award lecture: making sense of preeclampsia—two placental causes of preeclampsia? Placenta. 2014;35(Suppl):S20–5.
24. Redman CW, Sacks GP, Sargent IL. Preeclampsia: an excessive maternal inflammatory response to pregnancy. Am J Obstet Gynecol. 1999;180:499–506.
25. Raghupathy R. Cytokines as key players in the pathophysiology of preeclampsia. Med Princ Pract. 2013;22(Suppl 1):8–19.
26. Brewster JA, Orsi NM, Gopichandran N, McShane P, Ekbote UV, Walker JJ. Gestational effects on host inflammatory response in normal and pre-eclamptic pregnancies. Eur J Obstet Gynecol Reprod Biol. 2008;140:21–6.
27. Faas MM, Broekema M, Moes H, van der Schaaf G, Heineman MJ, de Vos P. Altered monocyte function in experimental preeclampsia in the rat. Am J Obstet Gynecol. 2004;191:1192–8.
28. LaMarca BB, Bennett WA, Alexander BT, Cockrell K, Granger JP. Hypertension produced by reductions in uterine perfusion in the pregnant rat: role of tumor necrosis factor-alpha. Hypertension. 2005;46:1022–5.
29. Gadonski G, LaMarca BB, Sullivan E, Bennett W, Chandler D, Granger JP. Hypertension produced by reductions in uterine perfusion in the pregnant rat: role of interleukin 6. Hypertension. 2006;48:711–6.
30. Cotechini T, Komisarenko M, Sperou A, Macdonald-Goodfellow S, Adams MA, Graham CH. Inflammation in rat pregnancy inhibits spiral artery remodeling leading to fetal growth restriction and features of preeclampsia. J Exp Med. 2014;13(211):165–79.
31. Clark P, Boswell F, Greer IA. The neutrophil and preeclampsia. Semin Reprod Endocrinol. 1998;16:57–64.
32. Conrad KP, Miles TM, Benyo DF. Circulating levels of immunoreactive cytokines in women with preeclampsia. Am J Reprod Immunol. 1998;40:102–11.
33. Szarka A, Rigó Jr J, Lázár L, Beko G, Molvarec A. Circulating cytokines, chemokines and adhesion molecules in normal pregnancy and preeclampsia determined by multiplex suspension array. BMC Immunol. 2010;2(11):59.
34. Wang Y, Walsh SW. TNF alpha concentrations and mRNA expression are increased in preeclamptic placentas. J Reprod Immunol. 1996;32:157–69.
35. Rinehart BK, Terrone DA, Lagoo-Deenadayalan S, Barber WH, Hale EA, Martin Jr JN, Bennett WA. Expression of the placental cytokines tumor necrosis factor alpha, interleukin 1beta, and interleukin 10 is increased in preeclampsia. Am J Obstet Gynecol. 1999;181:915–20.
36. Kawasaki K, Kondoh E, Chigusa Y, Ujita M, Murakami R, Mogami H, Brown JB, Okuno Y, Konishi I. Reliable pre-eclampsia pathways based on multiple independent microarray data sets. Mol Hum Reprod. 2015;21:217–24.
37. Huang X, Huang H, Dong M, Yao Q, Wang H. Serum and placental interleukin-18 are elevated in preeclampsia. J Reprod Immunol. 2005;65:77–87.
38. Arriaga-Pizano L, Jimenez-Zamudio L, Vadillo-Ortega F, Martinez-Flores A, Herrerias-Canedo T, Hernandez-Guerrero C. The predominant Th1 cytokine profile in maternal plasma of preeclamptic women is not reflected in the choriodecidual and fetal compartments. J Soc Gynecol Investig. 2005;12:335–42.
39. Hennessy A, Pilmore HL, Simmons LA, Painter DM. A deficiency of placental IL-10 in preeclampsia. J Immunol. 1999;163:3491–5.
40. Rein DT, Breidenbach M, Hönscheid B, Friebe-Hoffmann U, Engel H, Göhring UJ, Uekermann L, Kurbacher CM, Schöndorf T. Preeclamptic women are deficient of interleukin-10 as assessed by cytokine release of trophoblast cells in vitro. Cytokine. 2003;23:119–25.
41. White CA, Johansson M, Roberts CT, Ramsay AJ, Robertson SA. Effect of interleukin-10 null mutation on maternal immune response and reproductive outcome in mice. Biol Reprod. 2004;70:123–31.
42. Lai Z, Kalkunte S, Sharma S. A critical role of interleukin-10 in modulating hypoxia-induced preeclampsia-like disease in mice. Hypertension. 2011;57:505–14.
43. Messerli M, May K, Hansson SR, Schneider H, Holzgreve W, Hahn S, Rusterholz C. Feto-maternal interactions in pregnancies: placental microparticles activate peripheral blood monocytes. Placenta. 2010;31:106–12.

44. Germain SJ, Sacks GP, Sooranna SR, Sargent IL, Redman CW. Systemic inflammatory priming in normal pregnancy and preeclampsia: the role of circulating syncytiotrophoblast microparticles. J Immunol. 2007;178:5949–56.
45. Knight M, Redman CW, Linton EA, Sargent IL. Shedding of syncytiotrophoblast microvilli into the maternal circulation in pre-eclamptic pregnancies. Br J Obstet Gynaecol. 1998;105:632–40.
46. Cockell AP, Learmont JG, Smárason AK, Redman CW, Sargent IL, Poston L. Human placental syncytiotrophoblast microvillous membranes impair maternal vascular endothelial function. Br J Obstet Gynaecol. 1997;104:235–40.
47. Chen LM, Liu B, Zhao HB, Stone P, Chen Q, Chamley L. IL-6, TNFalpha and TGFbeta promote nonapoptotic trophoblast deportation and subsequently causes endothelial cell activation. Placenta. 2010;31:75–80.
48. Bulmer JN, Morrison L, Longfellow M, Ritson A, Pace D. Granulated lymphocytes in human endometrium: histochemical and immunohistochemical studies. Hum Reprod. 1991;6:791–8.
49. Lash GE, Robson SC, Bulmer JN. Review: functional role of uterine natural killer (uNK) cells in human early pregnancy decidua. Placenta. 2010;31(Suppl):S87–92.
50. Smith SD, Dunk CE, Aplin JD, Harris LK, Jones RL. Evidence for immune cell involvement in decidual spiral arteriole remodeling in early human pregnancy. Am J Pathol. 2009;174:1959–71.
51. Lash GE, Otun HA, Innes BA, Kirkley M, De Oliveira L, Searle RF, Robson SC, Bulmer JN. Interferon-gamma inhibits extravillous trophoblast cell invasion by a mechanism that involves both changes in apoptosis and protease levels. FASEB J. 2006;20:2512–8.
52. Lash GE, Schiessl B, Kirkley M, Innes BA, Cooper A, Searle RF, Robson SC, Bulmer JN. Expression of angiogenic growth factors by uterine natural killer cells during early pregnancy. J Leukoc Biol. 2006;80:572–80.
53. Naruse K, Lash GE, Innes BA, Otun HA, Searle RF, Robson SC, Bulmer JN. Localization of matrix metalloproteinase (MMP)-2, MMP-9 and tissue inhibitors for MMPs (TIMPs) in uterine natural killer cells in early human pregnancy. Hum Reprod. 2009;24:553–61.
54. Naruse K, Lash GE, Bulmer JN, Innes BA, Otun HA, Searle RF, Robson SC. The urokinase plasminogen activator (uPA) system in uterine natural killer cells in the placental bed during early pregnancy. Placenta. 2009;30:398–404.
55. Robson A, Harris LK, Innes BA, Lash GE, Aljunaidy MM, Aplin JD, Baker PN, Robson SC, Bulmer JN. Uterine natural killer cells initiate spiral artery remodeling in human pregnancy. FASEB J. 2012;26:4876–85.
56. Lash GE, Otun HA, Innes BA, Percival K, Searle RF, Robson SC, Bulmer JN. Regulation of extravillous trophoblast invasion by uterine natural killer cells is dependent on gestational age. Hum Reprod. 2010;25:1137–45.
57. Lidström C, Matthiesen L, Berg G, Sharma S, Ernerudh J, Ekerfelt C. Cytokine secretion patterns of NK cells and macrophages in early human pregnancy decidua and blood: implications for suppressor macrophages in decidua. Am J Reprod Immunol. 2003;50:444–52.
58. Heikkinen J, Möttönen M, Komi J, Alanen A, Lassila O. Phenotypic characterization of human decidual macrophages. Clin Exp Immunol. 2003;131:498–505.
59. Sayama S, Nagamatsu T, Schust DJ, Itaoka N, Ichikawa M, Kawana K, Yamashita T, Kozuma S, Fujii T. Human decidual macrophages suppress IFN-γ production by T cells through costimulatory B7-H1:PD-1 signaling in early pregnancy. J Reprod Immunol. 2013;100:109–17.
60. Li C, Houser BL, Nicotra ML, Strominger JL. HLA-G homodimer-induced cytokine secretion through HLA-G receptors on human decidual macrophages and natural killer cells. Proc Natl Acad Sci U S A. 2009;106:5767–72.
61. Renaud SJ, Postovit LM, Macdonald-Goodfellow SK, McDonald GT, Caldwell JD, Graham CH. Activated macrophages inhibit human cytotrophoblast invasiveness in vitro. Biol Reprod. 2005;73:237–43.
62. Reister F, Frank HG, Heyl W, Kosanke G, Huppertz B, Schröder W, Kaufmann P, Rath W. The distribution of macrophages in spiral arteries of the placental bed in pre-eclampsia differs from that in healthy patients. Placenta. 1999;20:229–33.

63. Nagamatsu T, Schust DJ. The immunomodulatory roles of macrophages at the maternal-fetal interface. Reprod Sci. 2010;17:209–18.
64. Kim YM, Romero R, Oh SY, Kim CJ, Kilburn BA, Armant DR, Nien JK, Gomez R, Mazor M, Saito S, Abrahams VM, Mor G. Toll-like receptor 4: a potential link between "danger signals," the innate immune system, and preeclampsia? Am J Obstet Gynecol. 2005;193:921–7.
65. Xue P, Zheng M, Gong P, Lin C, Zhou J, Li Y, Shen L, Diao Z, Yan G, Sun H, Hu Y. Single administration of ultra-low-dose lipopolysaccharide in rat early pregnancy induces TLR4 activation in the placenta contributing to preeclampsia. PLoS One. 2015;10:e0124001.
66. Gong P, Liu M, Hong G, Li Y, Xue P, Zheng M, Wu M, Shen L, Yang M, Diao Z, Hu Y. Curcumin improves LPS-induced preeclampsia-like phenotype in rat by inhibiting the TLR4 signaling pathway. Placenta. 2016;41:45–52.
67. Chatterjee P, Chiasson VL, Kopriva SE, Young KJ, Chatterjee V, Jones KA, Mitchell BM. Interleukin 10 deficiency exacerbates toll-like receptor 3-induced preeclampsia-like symptoms in mice. Hypertension. 2011;58:489–96.
68. Haeger M, Unander M, Bengtsson A. Enhanced anaphylatoxin and terminal C5b-9 complement complex formation in patients with the syndrome of hemolysis, elevated liver enzymes, and low platelet count. Obstet Gynecol. 1990;76:698–702.
69. Haeger M, Unander M, Bengtsson A. Complement activation in relation to development of preeclampsia. Obstet Gynecol. 1991;78:46–9.
70. Derzsy Z, Prohászka Z, Rigó Jr J, Füst G, Molvarec A. Activation of the complement system in normal pregnancy and preeclampsia. Mol Immunol. 2010;47:1500–6.
71. Minamiguchi S, Mikami Y, Nakajima N, Salah A, Kondoh E, Tatsumi K, Konishi I, Haga H. Complement split product C4d deposition in placenta in systemic lupus erythematosus and pregnancy-induced hypertension. Pathol Int. 2013;63:150–7.
72. Burwick RM, Feinberg BB. Eculizumab for the treatment of preeclampsia/HELLP syndrome. Placenta. 2013;34:201–3.
73. Agostinis C, Bulla R, Tripodo C, Gismondi A, Stabile H, Bossi F, Guarnotta C, Garlanda C, De Seta F, Spessotto P, Santoni A, Ghebrehiwet B, Girardi G, Tedesco F. An alternative role of C1q in cell migration and tissue remodeling: contribution to trophoblast invasion and placental development. J Immunol. 2010;185:4420–9.
74. Singh J, Ahmed A, Girardi G. Role of complement component C1q in the onset of preeclampsia in mice. Hypertension. 2011;58:716–24.
75. Maynard S, Epstein FH, Karumanchi SA. Preeclampsia and angiogenic imbalance. Annu Rev Med. 2008;59:61–78.
76. De Falco S. The discovery of placenta growth factor and its biological activity. Exp Mol Med. 2012;44:1–9.
77. Levine RJ, Maynard SE, Qian C, Lim KH, England LJ, Yu KF, Schisterman EF, Thadhani R, Sachs BP, Epstein FH, Sibai BM, Sukhatme VP, Karumanchi SA. Circulating angiogenic factors and the risk of preeclampsia. N Engl J Med. 2004;350:672–83.
78. Brownfoot FC, Tong S, Hannan NJ, Binder NK, Walker SP, Cannon P, Hastie R, Onda K, Kaitu'u-Lino TJ. Effects of pravastatin on human placenta, endothelium, and women with severe preeclampsia. Hypertension. 2015;66:687–97.
79. Chigusa Y, Kawasaki K, Kondoh E, Mogami H, Ujita M, Fujita K, Tatsumi K, Takeda S, Konishi I. Simvastatin inhibits oxidative stress via the activation of nuclear factor erythroid 2-related factor 2 signaling in trophoblast cells. J Obstet Gynaecol Res. 2016;42:36–43.
80. Kumasawa K, Ikawa M, Kidoya H, Hasuwa H, Saito-Fujita T, Morioka Y, Takakura N, Kimura T, Okabe M. Pravastatin induces placental growth factor (PGF) and ameliorates preeclampsia in a mouse model. Proc Natl Acad Sci U S A. 2011;108:1451–5.
81. Spradley FT, Tan AY, Joo WS, Daniels G, Kussie P, Karumanchi SA, Granger JP. Placental growth factor administration abolishes placental ischemia-induced hypertension. Hypertension. 2016;67:740–7.
82. Makris A, Yeung KR, Lim SM, Sunderland N, Heffernan S, Thompson JF, Iliopoulos J, Killingsworth MC, Yong J, Xu B, Ogle RF, Thadhani R, Karumanchi SA, Hennessy

A. Placental growth factor reduces blood pressure in a uteroplacental ischemia model of preeclampsia in nonhuman primates. Hypertension. 2016;67:1263–72.
83. Rajakumar A, Michael HM, Rajakumar PA, Shibata E, Hubel CA, Karumanchi SA, Thadhani R, Wolf M, Harger G, Markovic N. Extra-placental expression of vascular endothelial growth factor receptor-1, (Flt-1) and soluble Flt-1 (sFlt-1), by peripheral blood mononuclear cells (PBMCs) in normotensive and preeclamptic pregnant women. Placenta. 2005;26:563–73.
84. Herse F, Fain JN, Janke J, Engeli S, Kuhn C, Frey N, Weich HA, Bergmann A, Kappert K, Karumanchi SA, Luft FC, Muller DN, Staff AC, Dechend R. Adipose tissue-derived soluble fms-like tyrosine kinase 1 is an obesity-relevant endogenous paracrine adipokine. Hypertension. 2011;58:37–42.
85. Maynard SE, Min JY, Merchan J, Lim KH, Li J, Mondal S, Libermann TA, Morgan JP, Sellke FW, Stillman IE, Epstein FH, Sukhatme VP, Karumanchi SA. Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. J Clin Invest. 2003;111:649–58.
86. Gilbert JS, Babcock SA, Granger JP. Hypertension produced by reduced uterine perfusion in pregnant rats is associated with increased soluble fms-like tyrosine kinase-1 expression. Hypertension. 2007;50:1142–7.
87. Thadhani R, Kisner T, Hagmann H, Bossung V, Noack S, Schaarschmidt W, Jank A, Kribs A, Cornely OA, Kreyssig C, Hemphill L, Rigby AC, Khedkar S, Lindner TH, Mallmann P, Stepan H, Karumanchi SA, Benzing T. Pilot study of extracorporeal removal of soluble fms-like tyrosine kinase 1 in preeclampsia. Circulation. 2011;124:940–50.
88. Nakakita B, Mogami H, Kondoh E, Tsukamoto T, Yanagita M, Konishi I. Case of soluble fms-like tyrosine kinase 1 apheresis in severe pre-eclampsia developed at 15 weeks' gestation. J Obstet Gynaecol Res. 2015;41:1661–3.
89. Venkatesha S, Toporsian M, Lam C, Hanai J, Mammoto T, Kim YM, Bdolah Y, Lim KH, Yuan HT, Libermann TA, Stillman IE, Roberts D, D'Amore PA, Epstein FH, Sellke FW, Romero R, Sukhatme VP, Letarte M, Karumanchi SA. Soluble endoglin contributes to the pathogenesis of preeclampsia. Nat Med. 2006;12:642–9.
90. Wikström AK, Larsson A, Eriksson UJ, Nash P, Nordén-Lindeberg S, Olovsson M. Placental growth factor and soluble FMS-like tyrosine kinase-1 in early-onset and late-onset preeclampsia. Obstet Gynecol. 2007;109:1368–74.
91. Noori M, Donald AE, Angelakopoulou A, Hingorani AD, Williams DJ. Prospective study of placental angiogenic factors and maternal vascular function before and after preeclampsia and gestational hypertension. Circulation. 2010;122:478–87.
92. Verlohren S, Herraiz I, Lapaire O, Schlembach D, Zeisler H, Calda P, Sabria J, Markfeld-Erol F, Galindo A, Schoofs K, Denk B, Stepan H. New gestational phase-specific cutoff values for the use of the soluble fms-like tyrosine kinase-1/placental growth factor ratio as a diagnostic test for preeclampsia. Hypertension. 2014;63:346–52.
93. Liu Y, Zhao Y, Yu A, Zhao B, Gao Y, Niu H. Diagnostic accuracy of the soluble Fms-like tyrosine kinase-1/placental growth factor ratio for preeclampsia: a meta-analysis based on 20 studies. Arch Gynecol Obstet. 2015;292:507–18.
94. Zeisler H, Llurba E, Chantraine F, Vatish M, Staff AC, Sennström M, Olovsson M, Brennecke SP, Stepan H, Allegranza D, Dilba P, Schoedl M, Hund M, Verlohren S. Predictive value of the sFlt-1:PlGF ratio in women with suspected preeclampsia. N Engl J Med. 2016;374(1):13–22.
95. Wallukat G, Homuth V, Fischer T, Lindschau C, Horstkamp B, Jüpner A, Baur E, Nissen E, Vetter K, Neichel D, Dudenhausen JW, Haller H, Luft FC. Patients with preeclampsia develop agonistic autoantibodies against the angiotensin AT1 receptor. J Clin Invest. 1999; 103:945–52.
96. Siddiqui AH, Irani RA, Blackwell SC, Ramin SM, Kellems RE, Xia Y. Angiotensin receptor agonistic autoantibody is highly prevalent in preeclampsia: correlation with disease severity. Hypertension. 2010;55:386–93.
97. Zhou CC, Zhang Y, Irani RA, Zhang H, Mi T, Popek EJ, Hicks MJ, Ramin SM, Kellems RE, Xia Y. Angiotensin receptor agonistic autoantibodies induce pre-eclampsia in pregnant mice. Nat Med. 2008;14:855–62.

98. Jensen F, Wallukat G, Herse F, Budner O, El-Mousleh T, Costa SD, Dechend R, Zenclussen AC. CD19+CD5+ cells as indicators of preeclampsia. Hypertension. 2012;59:861–8.
99. Zhou CC, Irani RA, Zhang Y, Blackwell SC, Mi T, Wen J, Shelat H, Geng YJ, Ramin SM, Kellems RE, Xia Y. Angiotensin receptor agonistic autoantibody-mediated tumor necrosis factor-alpha induction contributes to increased soluble endoglin production in preeclampsia. Circulation. 2010;121:436–44.
100. Irani RA, Zhang Y, Zhou CC, Blackwell SC, Hicks MJ, Ramin SM, Kellems RE, Xia Y. Autoantibody-mediated angiotensin receptor activation contributes to preeclampsia through tumor necrosis factor-alpha signaling. Hypertension. 2010;55:1246–53.
101. Watanabe T, Barker TA, Berk BC. Angiotensin II and the endothelium: diverse signals and effects. Hypertension. 2005;45:163–9.

Molecular Mechanisms of Preterm Delivery

15

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Abstract

Preterm delivery occurs in approximately 10% of all pregnancies and it is a leading cause of infant morbidity and mortality, risking lifelong health problems in those who survive. Spontaneous preterm delivery and preterm premature rupture of membrane (pPROM) result from multiple causes, such as infection or inflammation, intrauterine bleeding, maternal stress and nutrition, and uterine overdistension. Infection is a leading cause of preterm delivery. Bacteria are recognized by pattern recognition receptors—such as toll-like receptors, which induces the release of inflammatory chemokines and cytokines. Chemokines and cytokines also result in decline of progesterone receptor (PR) function and initiate myometrial contraction, and part of PR function is regulated by microRNAs. Maternal stresses increase hypothalamic corticotropin-releasing hormone (CRH) and plasma glucocorticoid, which in turn stimulate the release of placental CRH as "placental clock," enhancing prostaglandin (PG) synthesis. Fetal fibronectin or thrombin increases matrix metalloproteinases and PGE2 synthesis in amnion mesenchymal cells, which lead to membrane rupture, cervical ripening, and myometrial contraction. Here, the current understanding of the molecular mechanisms of preterm delivery is summarized.

Keywords

Preterm delivery • Preterm premature rupture of membrane • Infection • Progesterone • Corticotropin-releasing hormone • Thrombin • Fetal fibronectin • Matrix metalloproteinase • Prostaglandin

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15.1 Introduction

Preterm delivery occurs in approximately 5% of all births in Japan and in 10% of births in the United States [1]. The preterm delivery rate in developed countries has recently been increasing due to advanced maternal age and multiple pregnancies by assisted reproductive techniques. There are several risk factors of preterm delivery: infection, low socioeconomic status, low body mass index, previous preterm delivery, obesity, cigarette smoking, maternal poor nutrition, young or advanced age, periodontal disease, poverty, and genital bleeding in the first and second trimester [1]. Adenomyosis is also considered as a risk factor of preterm delivery due to increased synthesis of prostaglandins [2]. Recent advances of molecular biology have revealed the new mechanism of preterm delivery, but vast majority still remains unknown. This is evident from the fact that there are no newly developed drugs that efficiently prevent preterm delivery for decades, so the continuing pursuit of basic research is necessary.

15.2 Stress-Associated Preterm Delivery

Maternal stresses such as depression, anxiety, and chronic stress are associated with preterm delivery [3]. Moreover, the risk of preterm delivery is increased in women who work long hours [1]. In the hypothalamus, glucocorticoids inhibit release of corticotropin-releasing hormone (CRH). This decreases expression of adrenal glucocorticoid to establish classic negative feedback. During pregnancy, in contrast, glucocorticoids stimulate release of CRH from placenta and fetal membrane, and increased CRH enhances production of prostaglandins from fetal membrane [4, 5], which play pivotal roles in human parturition by stimulating cervical ripening, myometrial contraction, and fetal membrane rupture [6] (Fig. 15.1). Increase of fetal CRH also upregulates secretion ACTH from fetal pituitary, which enhances production of cortisol and dehydroepiandrosterone (DHEA) sulfate by the fetal adrenal. DHEA sulfate is subsequently metabolized to DHEA and aromatized within the placenta to estrogens, which oppose the action of progesterone [7]. In addition, the rise in CRH expression also induces synthesis of surfactant protein, surfactant protein A (SP-A), by the fetal lung [8]. SP-A gene expression is also increased by proinflammatory stimuli such as interleukine-1 (IL-1) via activation of NF-κB [9]. Therefore, augmented surfactant production by the maturing fetal lung may serve as a fetal signal for the initiation of labor. Lockwood et al. observed an exponential increase in maternal levels of CRH during gestation, peaking at the time of delivery, and this maternal serum CRH is placental origin [5]. When maternal plasma CRH level around 16–20 weeks of gestation is high, women are destined to experience preterm delivery, whereas when maternal CRH is low, women go into post-term delivery [10]. Thus, placental secretion of CRH decides the timing of delivery, working as “placental clock” [10]. Increased adrenal release of glucocorticoids from maternal stress further releases placental CRH by positive feedback loop, which promotes preterm delivery. Maternal stress is derived from

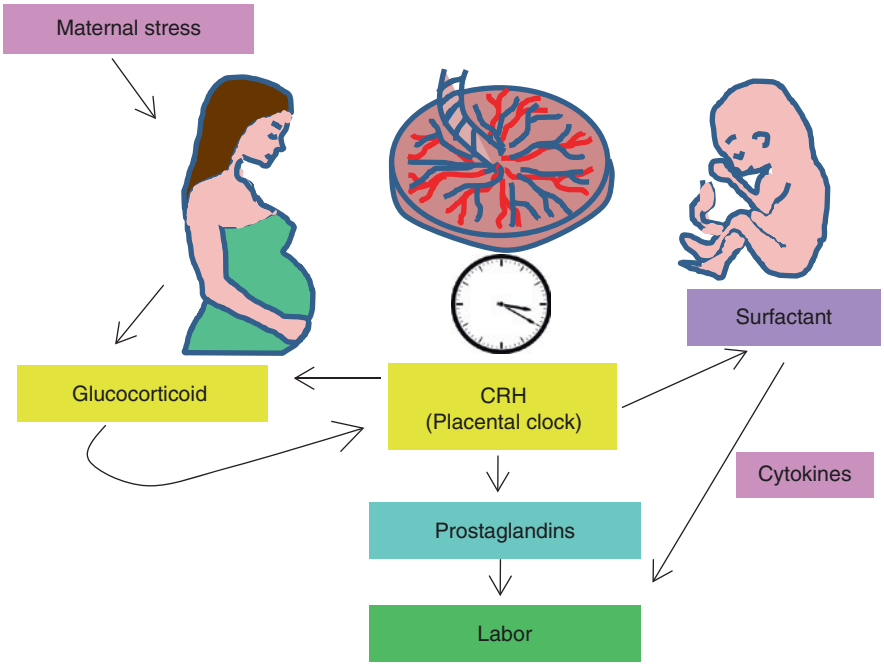

Fig. 15.1 Placental CRH decides the timing delivery as "placental clock". Maternal plasma CRH levels exponentially increase during pregnancy, peaking at term. This reflects the enhanced CRH synthesis in placenta. In women of preterm delivery, this increase is more rapid. Placental synthesis of CRH further increases glucocorticoid production in both mother and fetus in a manner of positive feed-forward loop. CRH stimulates prostaglandin production, which leads to cervical softening and myometrial contraction. The increase of CRH and glucocorticoid in fetus causes fetal lung maturation and surfactant synthesis. These surfactant proteins derived from fetus induce labor via upregulation of inflammatory cytokines

social factors, so it might be possible to decrease the preterm delivery rate by improving the environment of low socioeconomic individuals, reducing working hours for pregnant women, and providing psychological assistance to alleviate maternal mental stress.

15.3 Infection-Related Preterm Delivery and Vaginal Microbiome

Intrauterine infection is an important cause leading to preterm delivery, which occupies approximately 25% of preterm delivery cases [11]. Infection can occur between maternal decidua and fetal chorion (choriodecidual space) by bacteria ascending from vagina [12]. The most commonly identified bacteria are *Ureaplasma urealyticum*, *Mycoplasma hominis*, *Gardnerella vaginalis*, *Peptostreptococcus*, and *Bacteroides* species—all vaginal organisms of relatively low virulence [12].

Prevalence of a *Lactobacillus*-poor vaginal community is inversely correlated with gestational age at delivery [13]. Nearly one-third of women with *Lactobacillus*-poor vaginal community delivered very preterm infants. In contrast, at least three-quarters of women who carried their pregnancies to term had *Lactobacillus*-dominant vaginal microbiota. Risk for preterm birth was more prominent for subjects with elevated *Gardnerella* or *Ureaplasma* abundances [13]. Interestingly, hyaluronic acids play an important role in epithelial barrier protection of the lower reproductive tract from bacteria. Depletion of hyaluronic acids in the cervix and vagina resulted in increased epithelial and mucosal permeability of bacteria and increased preterm delivery rates in mice [14]. Thus, keeping healthy vaginal microbiota is a key for successful pregnancy.

The mechanisms by which intrauterine infections lead to preterm delivery are related to activation of the innate immune system (Fig. 15.2). Microorganisms are recognized by pattern recognition receptors—such as toll-like receptors (TLRs).

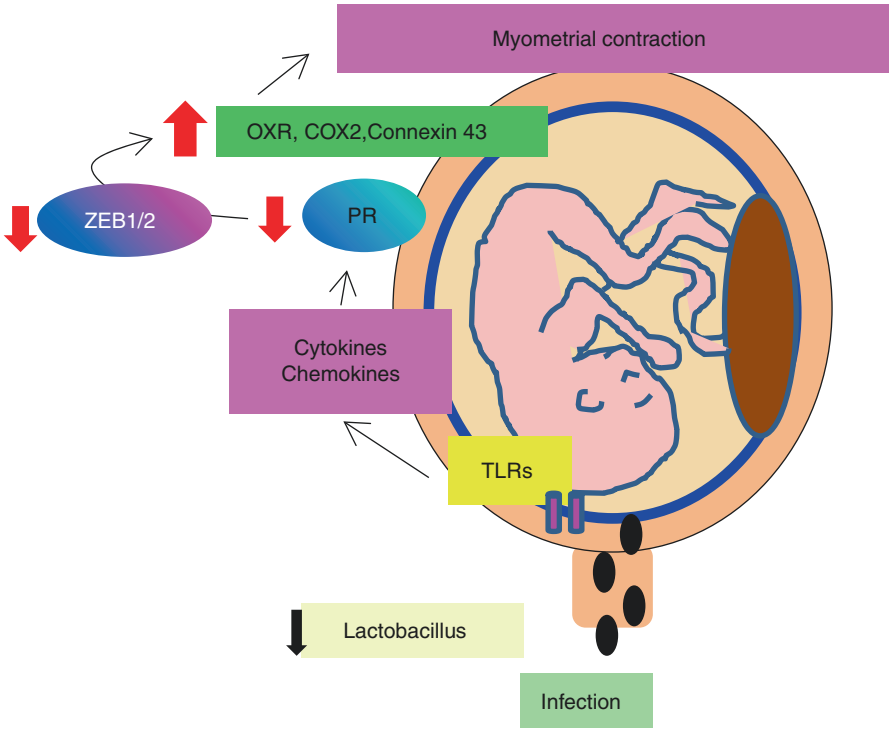

Fig. 15.2 Bacterial infections and myometrial contractions. Bacterial infections are recognized by pattern recognition receptors, such as toll-like receptors (TLRs). TLRs signaling activate inflammatory pathway as NFκB, which leads to induction of proinflammatory cytokines and chemokines. The inflammation induces decline of progesterone receptor (PR) and increases contraction-associated genes of uterus, oxytocin receptor, cylooxygenase-2 (COX2), and connexin 43 through downregulation of transcriptional factors, ZEB1 and ZEB2, which eventually leads to myometrial contraction and labor

TLRs are a family of transmembrane receptors that are involved in the regulation of the innate immune system [15]. TLR4 exists in both epithelial cells and mesenchymal cells of amnion [16]. TLR4-deficient mice are resistant to preterm delivery by intrauterine inoculation of heat-killed bacteria or LPS [17]. Activation of TLRs elicits the release of inflammatory chemokines and cytokines—such as IL-8, IL-1β, and tumor necrosis factor (TNF) α. Microbial endotoxins and proinflammatory cytokines stimulate the production of prostaglandins and matrix-degrading enzymes (matrix metalloproteinases, MMPs), which lead to preterm rupture of membrane. In the myometrium and cervix, proinflammatory cytokines activate inflammation-associated transcriptional factors such as NFκB and AP-1, inhibiting progesterone receptor (PR) function, which induces the expression of myometrial contractile genes [18]. In the cases of preterm delivery, concentrations of proinflammatory cytokines increase in amniotic fluid and migration of neutrophils and macrophages into the myometrium, cervix, and fetal membranes is observed [8, 19, 20]. Thus, inflammation plays an important role in both term and preterm delivery. In addition, intra-amniotic infection also attacks a fetus, causing a fetal systemic inflammatory response (FIRS). The concept of FIRS is determined by elevated fetal plasma IL-6 level [21]. FIRS is a risk factor for severe neonatal morbidity such as respiratory distress syndrome, neonatal sepsis, pneumonia, chronic lung disease, necrotizing enterocolitis, intraventriclular hemorrhage, and cerebral palsy [21]. Thus, although it is important to prevent intrauterine infection, immediate medical intervention to delivery is required once the sign of intra-amniotic infection appears to prevent a fetus from damage.

15.4 Myometrial Quiescence and Contraction

Throughout most of pregnancy, uterine quiescence is maintained by elevated progesterone acting through progesterone receptor (PR) [18]. In human, serum progesterone concentrations do not fall as labor approaches, so a decrease in local progesterone concentrations or number of receptors is a plausible mechanism of decline in PR function [22]. Progesterone antagonizes the inflammatory pathway such as NFκB and AP-1 by acting nuclear progesterone receptor (PR) and suppresses proinflammatory cytokines and chemokines. When pregnancy comes close to term, circulating estradiol-17β (E2) levels increases [23, 24], and enhanced estrogen receptor α (ERα) activity is enhanced [25, 26], which promote a proinflammatory cascade that contribute to the decline in PR function and initiate myometrial contraction (Fig. 15.2). Estrogens also induce an influx of macrophages and neutrophils into the uterus and further enhance proinflammatory event [27]. ERα activation facilitates myometrial contraction by enhancing transcription of the contraction-associated genes of the uterus, such as oxytocin receptor, connexin-43, and COX2 [25, 28–30]. The expressions of these contraction-associated genes are low throughout most of pregnancy but are highly upregulated at term.

A microRNA is a small noncoding RNA molecule (containing about 22 nucleotides) found in plants, animals, and some viruses, which functions in RNA silencing and posttranscriptional regulation of gene expression [31]. Recently, miR-200

family is found to be closely associated with labor. In both mouse and human uterus, miR-200 family (miR-200b and miR-429) is highly induced at term, whereas its target genes, ZEB1 and ZEB2, zinc finger E-box binding homeobox proteins, are downregulated [32]. ZEB1 and ZEB2 are transcriptional factors that are associated with epithelial mesenchymal transition. ZEB1 is directly upregulated by the action of P4/PR. ZEB1 and ZEB2 not only inhibit expression of the contraction-associated genes, oxytocin receptor and connexin-43, but also block oxytocin-induced contractility in human myometrial cells. The downregulation of ZEB1 and ZEB2 was observed in LPS- or RU486- induced mouse preterm delivery model. Thus, the miR-200 family and their targets, ZEB1 and ZEB2, are P4/PR-mediated regulators of uterine quiescence and contractility during pregnancy and labor.

15.5 Structure of Fetal Membrane and Preterm Premature Rupture of Membrane (pPROM)

Preterm premature rupture of membrane (pPROM) is associated with about one-third of preterm delivery cases and occurs in 1–3% of all pregnancies. The primary load-bearing structure of the fetal membranes is the amnion, which comprises a single layer of epithelial cells and an underlying layer of mesenchymal cells [33]. Mesenchymal cells are the primary source of collagen and matrix support in the amnion. Interstitial collagens (types I, III, and V) maintain the mechanical integrity of the amnion. Fetal membrane rupture is preceded by the degradation of collagen that is mediated primarily by matrix metalloproteinase (MMPs) in the amnion. Interstitial collagenase, MMP1, cleaves the triple helix of fibrillar collagen, which is then further degraded by the gelatinases, MMP2 and MMP9. pPROM and intera-uterine MMPs activity is closely correlated. MMP1 in amniotic fluid and MMP9 in amniotic membranes are elevated in women with pPROM [34–37]. Ehlers-Danlos syndrome, inheritable connective tissue disorder, is a risk factor of PROM by a defect in the structure, production, or processing of collagen or proteins that interact with collagen [38, 39].

15.6 Fibronectin

Fibronectin (FN) is a large extracellular glycoprotein that helps cells attach to the matrix. Fetal FN (fFN) is one of the FN proteins produced by fetal cells. It is diffusely distributed in the fetal membrane, from the amnion to decidua, providing structural support and adhesion of the fetal membranes to the uterine lining, and fFN in cervical and vaginal secretions has been used as a clinical marker of preterm delivery [40]. In vitro, fFN treatment results in increased expression of MMP1 and MMP9, mRNA, and enzymatic activity, as well as COX2 mRNA and PGE_2 synthesis in amnion mesenchymal cells, activating both NFκB and MAPK pathway [41] (Fig. 15.3). fFN has a unique alternatively spliced exon encoding extra domain-A (EDA) [42]. The treatment of amnion mesenchymal cells with recombinant EDA

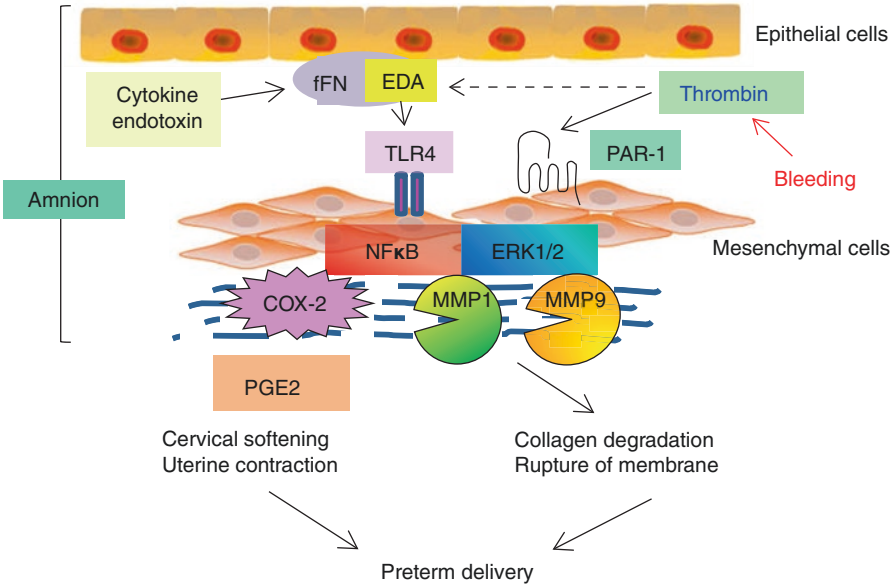

Fig. 15.3 fFN and thrombin signaling in the pathogenesis of pPROM and preterm delivery. LPS and proinflammatory cytokines such as TNF-α effect increased amounts of free fFN from amnion epithelial cells. Thrombin generated by intrauterine bleeding also increases free fFN in extracellular matrix of amnion. Released fFN activates TLR4 receptor on mesenchymal cells through its EDA. Activation of TLR4 leads to intracellular signaling through NFκB and ERK1/2 to induce expression of COX2 and MMPs, thereby leading to cervical ripening, uterine contractions, and collagenolytic degradation of the fetal membranes. Thrombin also directly activates PAR-1 signaling, which upregulates MMP9

also resulted in increases in MMP1 and MMP9 mRNA levels and enzymatic activity, as well as in the COX2 mRNA level and PGE_2 synthesis, indicating that EDA is a functional domain of fFN, and function of EDA was mediated via TLR4 [41]. Thus, neutralization of fFN-EDA domain or antagonism of TLR4 may have therapeutic potential for preterm delivery and pPROM.

A question is how fFN is increased and released in preterm delivery. Fibronectin-1 (FN1) protein and mRNA were dose-dependently increased by lipopolysaccharide (LPS) or TNF-α treatment in epithelial cells. This data show that epithelial cells of the amnion function as a sensor to harmful inflammatory stimuli and sends a "danger signal" by releasing fFN in the extracellular matrix. Then, mesenchymal cells receive the fFN danger signal from epithelial cells and begin producing MMPs and PGE_2. In other words, fFN "amplifies" the dangerous signal produced by endotoxins and proinflammatory cytokines in order to cause the rupture of the membrane or a preterm delivery via activation of MMPs and PGE2. This amplification of inflammation by fFN function would be evolutionally important because once infection has occurred, a fetus should be immediately released from harmful intrauterine inflammation by

delivery. Moreover, the delivery of an already infected fetus is the only way to protect a mother from fatal inflammation such as maternal sepsis.

15.7 Intrauterine Bleeding, Thrombin, and Risk of Preterm Delivery

Intrauterine bleeding or hematoma during early pregnancy is correlated with an increased risk for adverse maternal and neonatal complications. Nagy et al. reported a 2-fold increase in preterm delivery in the hematoma group [43]. Moreover, pregnancy-induced hypertension, preeclampsia, placental abruption, and fetal growth restriction were also frequent in this group. Similarly, Tuuli et al. reported that subchorionic hematoma was associated with a 1.5-fold increase in preterm delivery and pPROM, a 2-fold increase in spontaneous abortion and stillbirth, and a 5-fold increase in placental abruption [44]. These reports indicate that intrauterine bleeding during pregnancy is a strong risk factor of perinatal complications, especially of preterm delivery.

Thrombin is a trypsin-like serine proteinase, the most abundant enzyme associated with blood coagulation. In addition to its role in hemostasis, thrombin also influences normal and pathological processes, such as inflammation, tissue repair, embryogenesis, angiogenesis, and tumor invasion [45]. There is considerable clinical evidence pointing to a role of thrombin in preterm delivery. Thrombin-antithrombin complexes, markers of in vivo generation of thrombin, are increased in the plasma [46, 47] and amniotic fluid [46] of women in preterm labor or pPROM. Placental abruption-induced thrombin generation has been associated with fetal membrane weakening and pPROM [47, 48], and treatment of amnion explants with thrombin results in increased levels of MMP9 and mechanical weakening [49]. In an animal model, intrauterine administration of whole blood to pregnant rats stimulates myometrial contractility, whereas blood containing heparin or a thrombin inhibitor does not [50].

Thrombin activity was significantly increased in amniotic membranes from women who delivered preterm [51]. Considering that the decidua is the primary source of thrombin [52], increased thrombin activity is probably due to the bleeding from the decidua in the early stage of pregnancy, and thrombin activity would remain in the amnion for several months until the preterm delivery finally occurs. In primary amnion cells, thrombin treatment resulted in an increase of MMP1 and MMP9 mRNA and enzymatic activity, conversion of MMP2 to its active form, and COX2 mRNA and PGE_2 synthesis in amnion mesenchymal cells (Fig. 15.3). These activations were mediated by G protein-coupled thrombin receptor, protease-activated receptor-1 (PAR-1), and TLR4 [51]. When thrombin or PBS was locally injected into the uterus of pregnant mice, all thrombin-injected mice delivered preterm, whereas PBS did not [51]. In these mice, *collagenase-2 (MMP8)* and *collagenase-3 (MMP13)*, gelatinase *MMP9* mRNA as well as PGE_2 synthesis were all increased in fetal membranes. Thus, thrombin weakens the membrane by degrading collagen through upregulation of MMPs

and stimulates cervical ripening and myometrial contraction through the production of PGE_2.

Conclusion

Bacterial infection, presumably due to *Lactobacillus*-poor vaginal community, activates pattern recognition receptors, which induces the release of inflammatory chemokines and cytokines. Chemokines and cytokines result in decline of progesterone receptor (PR) function, and enhancement of estrogen receptor activity in uterus initiates myometrial contraction. Placental CRH exponentially increases during pregnancy, serving as a "placental clock," which is further increased by maternal and fetal glucocorticoid as positive feedback loop. CRH enhances prostaglandin synthesis. Fetal fibronectin or thrombin increases matrix metalloproteinases and PGE2 synthesis in amnion mesenchymal cells. Together, these molecular events converge to membrane rupture, cervical ripening, and myometrial contraction of preterm delivery and pPROM. Continuing basic research is necessary to reduce the preterm delivery.

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References

1. Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. Lancet. 2008;371(9606):75–84. doi:10.1016/S0140-6736(08)60074-4.
2. Juang CM, Chou P, Yen MS, Twu NF, Horng HC, Hsu WL. Adenomyosis and risk of preterm delivery. BJOG. 2007;114(2):165–9. doi:10.1111/j.1471-0528.2006.01186.x.
3. Hedegaard M, Henriksen TB, Sabroe S, Secher NJ. Psychological distress in pregnancy and preterm delivery. BMJ. 1993;307(6898):234–9.
4. Jones SA, Brooks AN, Challis JR. Steroids modulate corticotropin-releasing hormone production in human fetal membranes and placenta. J Clin Endocrinol Metab. 1989;68(4):825–30. doi:10.1210/jcem-68-4-825.
5. Lockwood CJ, Radunovic N, Nastic D, Petkovic S, Aigner S, Berkowitz GS. Corticotropin-releasing hormone and related pituitary-adrenal axis hormones in fetal and maternal blood during the second half of pregnancy. J Perinat Med. 1996;24(3):243–51.
6. Challis JR, Sloboda DM, Alfaidy N, Lye SJ, Gibb W, Patel FA, et al. Prostaglandins and mechanisms of preterm birth. Reproduction. 2002;124(1):1–17.
7. Mendelson CR. Minireview: fetal-maternal hormonal signaling in pregnancy and labor. Mol Endocrinol. 2009;23(7):947–54. doi:10.1210/me.2009-0016.
8. Condon JC, Jeyasuria P, Faust JM, Mendelson CR. Surfactant protein secreted by the maturing mouse fetal lung acts as a hormone that signals the initiation of parturition. Proc Natl Acad Sci U S A. 2004;101(14):4978–83. doi:10.1073/pnas.0401124101.
9. Islam KN, Mendelson CR. Potential role of nuclear factor kappaB and reactive oxygen species in cAMP and cytokine regulation of surfactant protein-A gene expression in lung type II cells. Mol Endocrinol. 2002;16(6):1428–40. doi:10.1210/mend.16.6.0856.
10. McLean M, Bisits A, Davies J, Woods R, Lowry P, Smith R. A placental clock controlling the length of human pregnancy. Nat Med. 1995;1(5):460–3.
11. Challis JR, Lockwood CJ, Myatt L, Norman JE, Strauss 3rd JF, Petraglia F. Inflammation and pregnancy. Reprod Sci. 2009;16(2):206–15. doi:10.1177/1933719108329095.

12. Goldenberg RL, Hauth JC, Andrews WW. Intrauterine infection and preterm delivery. N Engl J Med. 2000;342(20):1500–7. doi:10.1056/NEJM200005183422007.
13. DiGiulio DB, Callahan BJ, McMurdie PJ, Costello EK, Lyell DJ, Robaczewska A, et al. Temporal and spatial variation of the human microbiota during pregnancy. Proc Natl Acad Sci U S A. 2015;112(35):11060–5. doi:10.1073/pnas.1502875112.
14. Akgul Y, Word RA, Ensign LM, Yamaguchi Y, Lydon J, Hanes J, et al. Hyaluronan in cervical epithelia protects against infection-mediated preterm birth. J Clin Invest. 2014;124(12):5481–9. doi:10.1172/JCI78765.
15. Akira S, Takeda K. Toll-like receptor signalling. Nat Rev Immunol. 2004;4(7):499–511. doi:10.1038/nri1391.
16. Adams KM, Lucas J, Kapur RP, Stevens AM. LPS induces translocation of TLR4 in amniotic epithelium. Placenta. 2007;28(5–6):477–81. doi:10.1016/j.placenta.2006.08.004.
17. Wang H, Hirsch E. Bacterially-induced preterm labor and regulation of prostaglandin-metabolizing enzyme expression in mice: the role of toll-like receptor 4. Biol Reprod. 2003;69(6):1957–63. doi:10.1095/biolreprod.103.019620.
18. Renthal NE, Williams KC, Mendelson CR. MicroRNAs—mediators of myometrial contractility during pregnancy and labour. Nat Rev Endocrinol. 2013;9(7):391–401. doi:10.1038/nrendo.2013.96.
19. Osman I, Young A, Ledingham MA, Thomson AJ, Jordan F, Greer IA, et al. Leukocyte density and pro-inflammatory cytokine expression in human fetal membranes, decidua, cervix and myometrium before and during labour at term. Mol Hum Reprod. 2003;9(1):41–5.
20. Thomson AJ, Telfer JF, Young A, Campbell S, Stewart CJ, Cameron IT, et al. Leukocytes infiltrate the myometrium during human parturition: further evidence that labour is an inflammatory process. Hum Reprod. 1999;14(1):229–36.
21. Gomez R, Romero R, Ghezzi F, Yoon BH, Mazor M, Berry SM. The fetal inflammatory response syndrome. Am J Obstet Gynecol. 1998;179(1):194–202.
22. Romero R, Dey SK, Fisher SJ. Preterm labor: one syndrome, many causes. Science. 2014;345(6198):760–5. doi:10.1126/science.1251816.
23. Buster JE, Chang RJ, Preston DL, Elashoff RM, Cousins LM, Abraham GE, et al. Interrelationships of circulating maternal steroid concentrations in third trimester pregnancies. II. C18 and C19 steroids: estradiol, estriol, dehydroepiandrosterone, dehydroepiandrosterone sulfate, delta 5-androstenediol, delta 4-androstenedione, testosterone, and dihydrotestosterone. J Clin Endocrinol Metab. 1979;48(1):139–42. doi:10.1210/jcem-48-1-139.
24. Challis JR. Sharp increase in free circulating oestrogens immediately before parturition in sheep. Nature. 1971;229(5281):208.
25. Mesiano S, Chan EC, Fitter JT, Kwek K, Yeo G, Smith R. Progesterone withdrawal and estrogen activation in human parturition are coordinated by progesterone receptor A expression in the myometrium. J Clin Endocrinol Metab. 2002;87(6):2924–30. doi:10.1210/jcem.87.6.8609.
26. Welsh T, Johnson M, Yi L, Tan H, Rahman R, Merlino A, et al. Estrogen receptor (ER) expression and function in the pregnant human myometrium: estradiol via ERalpha activates ERK1/2 signaling in term myometrium. J Endocrinol. 2012;212(2):227–38. doi:10.1530/JOE-11-0358.
27. Tibbetts TA, Conneely OM, O'Malley BW. Progesterone via its receptor antagonizes the pro-inflammatory activity of estrogen in the mouse uterus. Biol Reprod. 1999;60(5):1158–65.
28. Murata T, Narita K, Honda K, Matsukawa S, Higuchi T. Differential regulation of estrogen receptor alpha and beta mRNAs in the rat uterus during pregnancy and labor: possible involvement of estrogen receptors in oxytocin receptor regulation. Endocr J. 2003;50(5):579–87.
29. Piersanti M, Lye SJ. Increase in messenger ribonucleic acid encoding the myometrial gap junction protein, connexin-43, requires protein synthesis and is associated with increased expression of the activator protein-1, c-fos. Endocrinology. 1995;136(8):3571–8. doi:10.1210/endo.136.8.7628395.
30. Tsuboi K, Sugimoto Y, Iwane A, Yamamoto K, Yamamoto S, Ichikawa A. Uterine expression of prostaglandin H2 synthase in late pregnancy and during parturition in prostaglandin F receptor-deficient mice. Endocrinology. 2000;141(1):315–24. doi:10.1210/endo.141.1.7236.

31. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell. 2004;116(2):281–97.
32. Renthal NE, Chen CC, Williams KC, Gerard RD, Prange-Kiel J, Mendelson CR. miR-200 family and targets, ZEB1 and ZEB2, modulate uterine quiescence and contractility during pregnancy and labor. Proc Natl Acad Sci U S A. 2010;107(48):20828–33. doi:10.1073/pnas.1008301107.
33. Parry S, Strauss 3rd JF. Premature rupture of the fetal membranes. N Engl J Med. 1998;338(10):663–70.
34. Athayde N, Edwin SS, Romero R, Gomez R, Maymon E, Pacora P, et al. A role for matrix metalloproteinase-9 in spontaneous rupture of the fetal membranes. Am J Obstet Gynecol. 1998;179(5):1248–53. doi:S0002937898701413 [pii].
35. Draper D, McGregor J, Hall J, Jones W, Beutz M, Heine RP, et al. Elevated protease activities in human amnion and chorion correlate with preterm premature rupture of membranes. Am J Obstet Gynecol. 1995;173(5):1506–12. doi:0002-9378(95)90640-1 [pii].
36. Maymon E, Romero R, Pacora P, Gervasi MT, Bianco K, Ghezzi F, et al. Evidence for the participation of interstitial collagenase (matrix metalloproteinase 1) in preterm premature rupture of membranes. Am J Obstet Gynecol. 2000;183(4):914–20. doi:10.1067/mob.2000.108879.
37. Vadillo-Ortega F, Gonzalez-Avila G, Furth EE, Lei H, Muschel RJ, Stetler-Stevenson WG, et al. 92-kd type IV collagenase (matrix metalloproteinase-9) activity in human amniochorion increases with labor. Am J Pathol. 1995;146(1):148–56.
38. Barabas AP. Ehlers-Danlos syndrome: associated with prematurity and premature rupture of foetal membranes; possible increase in incidence. Br Med J. 1966;2(5515):682–4.
39. Yen JL, Lin SP, Chen MR, Niu DM. Clinical features of Ehlers-Danlos syndrome. J Formos Med Assoc. 2006;105(6):475–80. doi:10.1016/S0929-6646(09)60187-X.
40. Lockwood CJ, Senyei AE, Dische MR, Casal D, Shah KD, Thung SN, et al. Fetal fibronectin in cervical and vaginal secretions as a predictor of preterm delivery. N Engl J Med. 1991;325(10):669–74. doi:10.1056/NEJM199109053251001.
41. Mogami H, Kishore AH, Shi H, Keller PW, Akgul Y, Word RA. Fetal fibronectin signaling induces matrix metalloproteases and cyclooxygenase-2 (COX-2) in amnion cells and preterm birth in mice. J Biol Chem. 2013;288(3):1953–66. doi:10.1074/jbc.M112.424366.
42. Kornblihtt AR, Vibe-Pedersen K, Baralle FE. Human fibronectin: molecular cloning evidence for two mRNA species differing by an internal segment coding for a structural domain. EMBO J. 1984;3(1):221–6.
43. Nagy S, Bush M, Stone J, Lapinski RH, Gardo S. Clinical significance of subchorionic and retroplacental hematomas detected in the first trimester of pregnancy. Obstet Gynecol. 2003;102(1):94–100.
44. Tuuli MG, Norman SM, Odibo AO, Macones GA, Cahill AG. Perinatal outcomes in women with subchorionic hematoma: a systematic review and meta-analysis. Obstet Gynecol. 2011;117(5):1205–12. doi:10.1097/AOG.0b013e31821568de.
45. Coughlin SR. Thrombin signalling and protease-activated receptors. Nature. 2000;407(6801):258–64. doi:10.1038/35025229.
46. Chaiworapongsa T, Espinoza J, Yoshimatsu J, Kim YM, Bujold E, Edwin S, et al. Activation of coagulation system in preterm labor and preterm premature rupture of membranes. J Matern Fetal Neonatal Med. 2002;11(6):368–73.
47. Rosen T, Kuczynski E, O'Neill LM, Funai EF, Lockwood CJ. Plasma levels of thrombin-antithrombin complexes predict preterm premature rupture of the fetal membranes. J Matern Fetal Med. 2001;10(5):297–300.
48. Mackenzie AP, Schatz F, Krikun G, Funai EF, Kadner S, Lockwood CJ. Mechanisms of abruption-induced premature rupture of the fetal membranes: Thrombin enhanced decidual matrix metalloproteinase-3 (stromelysin-1) expression. Am J Obstet Gynecol. 2004;191(6):1996–2001. doi:10.1016/j.ajog.2004.08.003.

49. Kumar D, Schatz F, Moore RM, Mercer BM, Rangaswamy N, Mansour JM, et al. The effects of thrombin and cytokines upon the biomechanics and remodeling of isolated amnion membrane, in vitro. Placenta. 2011;32(3):206–13. doi:10.1016/j.placenta.2011.01.006.
50. Balbin M, Fueyo A, Knauper V, Pendas AM, Lopez JM, Jimenez MG, et al. Collagenase 2 (MMP-8) expression in murine tissue-remodeling processes. Analysis of its potential role in postpartum involution of the uterus. J Biol Chem. 1998;273(37):23959–68.
51. Mogami H, Keller PW, Shi H, Word RA. Effect of thrombin on human amnion mesenchymal cells, mouse fetal membranes, and preterm birth. J Biol Chem. 2014;289(19):13295–307. doi:10.1074/jbc.M114.550541.
52. Lockwood CJ, Krikun G, Papp C, Toth-Pal E, Markiewicz L, Wang EY, et al. The role of progestationally regulated stromal cell tissue factor and type-1 plasminogen activator inhibitor (PAI-1) in endometrial hemostasis and menstruation. Ann N Y Acad Sci. 1994;734:57–79.

Developmental Origins of Health and Diseases (DOHaD): Perspective Toward Preemptive Medicine

16

Hiroaki Itoh and Naohiro Kanayama

Abstract

Noncommunicable diseases (NCDs) are chronic, noninfectious, and nontransmissible diseases. The World Health Organization (WHO) estimated that 63% of global deaths, approximately 36 million, were attributed to NCDs. The concept of the developmental origins of health and disease (DOHaD) revealed that undernourishment and overnourishment in utero are both causatively associated with the risk of NCDs in later life; a "U-shaped curve" was proposed for the relationship between nutritional conditions in utero and the risk of developing adult NCDs. The DOHaD concept of the "U-shaped curve" is assumed to explain, at least partly, why NCDs are becoming increasingly prevalent in both developing and developed countries because the former is expected to be related to undernourishment in utero and the latter overnourishment in utero. In this chapter, a possibility was discussed that supports the promising future contribution of perinatal and neonatal care to the establishment of "preemptive medicine" against the rapid spread of adult NCDs.

Keywords

Developmental origins of health and diseases (DOHaD) • Fetal origins of adult disease • Fetus • Pregnancy • Thrifty phenotype • Perinatal medicine • Neonate

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16.1 Introduction

Noncommunicable diseases (NCDs) are chronic, noninfectious, and nontransmissible diseases. The World Health Organization (WHO) has classified cardiovascular disorders (myocardial infarction or stroke), diabetes, chronic respiratory diseases, and malignancy as the four major disease types of NCDs. Moreover, some investigators regard mental health disorders as NCDs [1, 2]. The WHO estimated that 63% of global deaths, approximately 36 million, were attributed to NCDs and also that NCDs are expected to exceed communicable, maternal, perinatal, and nutritional diseases as the most common causes of death worldwide by 2030 [3, 4].

Increasing evidence from humans and animal models has demonstrated that environmental factors, such as nutritional conditions in the preconception, embryonic, fetal, neonatal, and/or infantile periods, affect the developmental process of specific organs as well as the regulation of their networks to maintain biological homeostasis and are involved in the development of risk factors for NCDs in adulthood [5–7]. The concept of the developmental origins of health and disease (DOHaD) was consequently proposed [8, 9]. Since epidemiological studies have revealed that undernourishment and overnourishment in utero are both causatively associated with the risk of NCDs in later life, a "U-shaped curve" was proposed for the relationship between nutritional conditions in utero and the risk of developing adult NCDs [10–16] (Fig. 16.1). The annual number of deaths due to NCDs continues to increase in developing and developed countries [3, 4, 15]. The DOHaD concept of the "U-shaped curve" is assumed to explain, at least partly, why NCDs are becoming increasingly prevalent in developing and developed countries because the former is expected to be related to undernourishment in utero and the latter overnourishment in utero [17] (Fig. 16.1).

The concept of "preemptive medicine" was recently proposed as a new preventive strategy for the current prevalence of NCDs, i.e., the identification of high-risk populations and early interventions during a latent period before the onset of apparent clinical symptoms [5, 18, 19]. The DOHaD concept, presumably associated with epigenetic modifications [20], highlights the promising future contribution of perinatal and neonatal care to the establishment of "preemptive medicine" against the rapid spread of adult NCDs [17]. In this chapter, a possibility was discussed that supports a nutritional imbalance in utero and/or in early postnatal life contributing to the recent epidemic of NCDs.

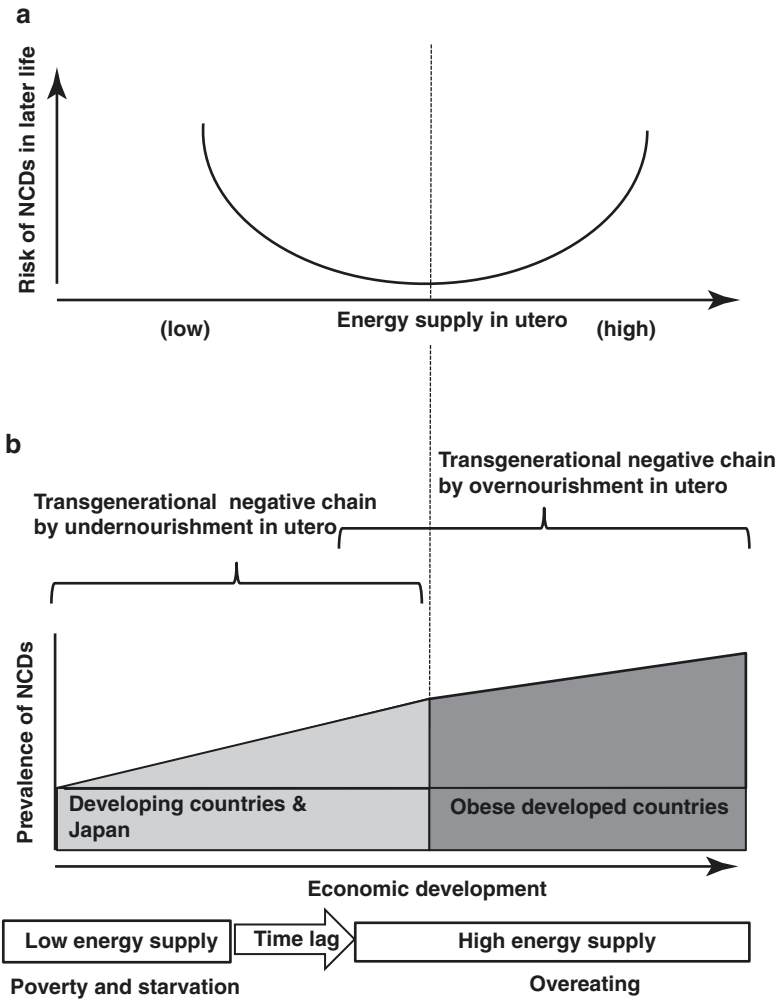

Fig. 16.1 Energy supply in utero and the risk of NCDs in later life (**a**). The prevalence of NCDs in developing and developed countries may be associated with differences in energy supply in utero (**b**) [17]

16.2 The DOHaD Theory

Three pioneering epidemiological studies, i.e., the Dutch famine during World War II [21–24], a British study in the county of Hertfordshire (Barker hypothesis) [25–27], and the Helsinki Birth Cohort [28–32], indicated that a relationship exists between environmental aggression in the early developmental period and the incidence of NCDs in later life. Subsequent epidemiological studies on different ethnic groups in different locations worldwide, together with excellent animal studies, revealed the novel concept of the early establishment of metabolic adjustments by interorgan communication networks that affect the morbidity of NCDs throughout life [33–35], leading to a new scientific theory, DOHaD [6, 8, 36].

16.3 Undernourishment In Utero and Developmental Risk of NCDs in Later Life

Two major parameters have been used in epidemiological studies to support the contribution of undernourishment in utero to the developmental risk of NCDs, i.e., maternal low-energy intake during pregnancy and a low birthweight less than 2500 g. Maternal low-energy intake means a low-energy supply for the maintenance of mothers and fetuses, while a low birthweight less than 2500 g is one of the parameters of anthropometry of newborns, with neither being identical to undernourishment in utero.

Epidemiological evidence to connect undernourishment in utero to the development of adult NCDs initially emerged in victims of the Dutch famine in 1944–1945 during World War II. The adult and/or elderly offspring of women exposed to the famine in gestation were predisposed to NCDs, such as schizophrenia, antisocial personalities, cognitive decline, coronary heart disease, hypertension, an atherogenic lipid profile, disturbed blood coagulation, obesity, impaired glucose tolerance, metabolic syndrome, increased stress responsiveness, obstructive airway disease, and decreased renal function [21–24]. Fetal exposure to the Chinese Famine in 1959–1961 also showed a similar trajectory toward a predisposition to NCDs [37–39]. The relationship between fetal exposure to the Chinese Famine and an increased risk of adult metabolic syndrome was stronger among subjects with a Western-style calorie-rich diet [40].

British studies by Barker et al. [25–27] and the Helsinki Birth Cohort study [28–32] together revealed that individuals born with a low birthweight less than 2500 g were predisposed to NCDs in adulthood, including coronary heart disease, impaired glucose tolerance, hypertension, metabolic syndrome, dyslipidemia, and cognitive decline. However, it is important to note that the concept of a low birthweight less than 2500 g is different from that of fetal growth restriction (FGR), intrauterine growth restriction (IUGR), or small for gestational age (SGA). In perinatal medicine, FGR or IUGR commonly means that the estimated fetal weight is less than the tenth percentile for gestational age as assessed through an ultrasound observation in

utero [41], whereas SGA generally means that a birthweight is less than the tenth percentile for gestational age [42]. Their reference charts differ among populations, localizations, and even generations. Mean birthweight is more than 3400 g in the United States [43] but is approximately 3000 g in Japan [44]. Therefore, caution is needed in the interpretation of etiological data based on a low birthweight less than 2500 g because the basic characteristics of individuals with a low birthweight may differ among the populations studied. Furthermore, most premature newborns are classified as having a low birthweight even if their birthweight is within the normal range for their gestational ages. Thus, small babies are not always simply a result of undernourishment in utero. Nevertheless, significant relationships have been reported between a low birthweight and elevated adiposity in children [45, 46] and adults [29, 47, 48].

16.4 The Thrifty Phenotype Hypothesis

Although large numbers of theoretical models have been proposed to explain the mechanistic basis underlying possible associations between undernourishment in utero and obesity-related metabolic disorders in later life [49], the *thrifty phenotype* hypothesis by Hales and Barker [50] is the most promising model [13, 14, 51]. They proposed the concept of an adaptive response to undernourishment in utero that is a *trade-off* between saving energy consumption in utero and downsizing the fetal body. Embryonic and/or fetal *predictive adaptive responses* may adjust the development of their own metabolic regulation systems in response to the environmental characteristics surrounding their mothers for the purpose of matching themselves to the predicted postnatal circumstances and improving survivability in life after birth [52] (Fig. 16.2). The optimization of fetal body growth in utero is hypothesized to lead to a distinct and permanent metabolic phenotype, the *thrifty phenotype*, of enhanced energy economy, similar to a hybrid electric vehicle, for the purpose of matching the predicted postnatal circumstances of long-lasting insufficient food supply and improving survivability through a life of incessant starvation [50, 51] (Fig. 16.2).

According to the "match" aspect of the *thrifty phenotype* hypothesis, a previous study reported that small babies in Gambia, including those born during a nutritionally debilitating hunger season, maintained healthy metabolic as well as cardiovascular conditions into adulthood with the complete absence of metabolic syndrome if they retained their frugal lifestyle in rural areas [53] (Fig. 16.2).

As for the *mismatch* feature of the *thrifty phenotype*, it was hypothesized that the *thrifty phenotype* may become disadvantageous for the survival of the fittest when nutrition is more abundant in the postnatal environment than had been expected from the prenatal environment of undernourishment because the enhanced energy economy of the *thrifty phenotype* may cause a *mismatch* to the excess energy supply associated with the modern lifestyle of overeating, thereby predisposing adults to NCDs, particularly those related to obesity and/or diabetes [12–14, 50, 54–56] (Fig. 16.2).

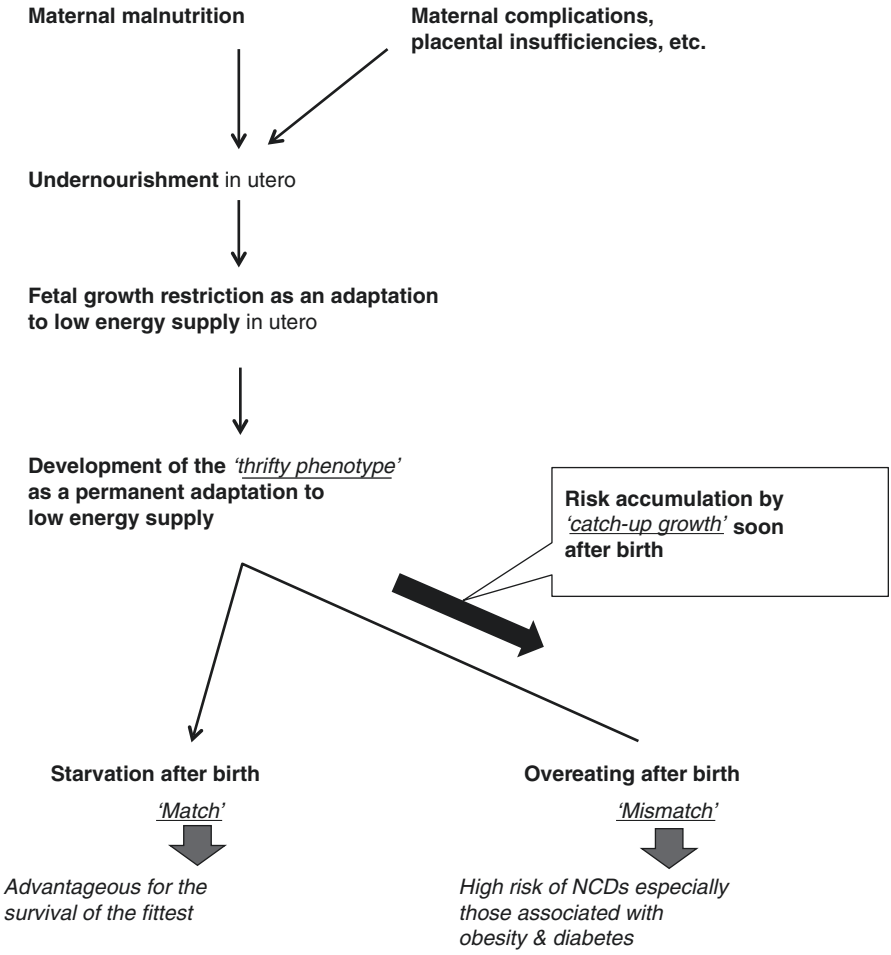

Fig. 16.2 Schematic illustration of undernourishment in utero, the *thrifty phenotype*, *catch-up growth*, and the risk of NCDs [17, 27, 50]

16.5 Mismatch in Developing Countries and Japan

Developing countries have been undergoing rapid and prominent economic improvements over the past few decades, and generations that experienced a low-energy supply during fetal life due to maternal poverty and/or political turmoil have now shifted to a life with an obesogenic diet [17] (Fig. 16.1). Therefore, individuals expected to have acquired the *thrifty phenotype* in utero encounter a *mismatch* to the excess energy supply provided by a calorie-rich obesogenic diet and develop a risk of NCDs, particularly those related to obesity and/or type 2 diabetes [54] (Figs. 16.1 and 16.2). The prevalence of diabetes has been rapidly increasing in developing countries, such as China, India, and Malaysia [57–59].

In Japan, the prevalence of obesity or being overweight has consistently increased among adult males as well as mature and elderly women, whereas

undernourishment is common among young women of childbearing age because of their strong desire to be thin [60]. A decrease in the body mass index of young Japanese women has been followed by an increase in low birthweight neonates as well as a decrease in mean birthweight [56, 61, 62]. The mean total caloric intake of the Japanese population has continuously decreased since 1970 [56, 61], suggesting that the incidence of obesity has increased despite a reduced energy supply. This paradoxical shift toward a possible obesity-prone phenotype in a relatively short period, less than half a century, in middle-aged and elderly Japanese populations argues against the major contribution of a Western lifestyle with a calorie-rich diet and insufficient exercise due to the widespread use of cars in favor of a presumed increase in the number of individuals with the *thrifty phenotype* due to undernourishment in utero. Kubota et al. reported that mean energy intake in pregnant Japanese women was less than 1600 kcal/day through pregnancy, 30% (second trimester) and 37% (third trimester) below the recommendations of the Ministry of Health, Labour, and Welfare in Japan [63], which suggests large numbers of relatively undernourished fetuses due to insufficient maternal energy intake. Thus, it is plausible that a nutritional imbalance in pregnant Japanese women may have established the *thrifty phenotype* in a large proportion of the next generation, thereby contributing, at least partly, to the development of obesity and/or type 2 diabetes with less energy intake [56, 62].

16.6 Risk Accumulation of NCDs by Catch-Up Growth

A systematic review revealed that small babies were more predisposed to adult obesity if they showed rapid *catch-up growth* soon after birth [64] (Fig. 16.2). The interaction between a prenatal low-energy supply and subsequent rapid *catch-up growth* soon after birth, presumably being equal to a rapid encounter with a postnatal high-energy supply, i.e., immediate and drastic *mismatch*, appeared to increase the risk of obesity and its associated metabolic disorders [10, 12, 65–68]. However, controversy surrounds the critical window or period of *catch-up growth*. Settler et al. suggested the importance of the first few weeks of postnatal life [69, 70]. Botton et al. showed that neonates with a faster weight gain velocity during the first 3 months showed a greater weight gain velocity after 3 years of age, leading to a larger fat mass in adolescence [71]. Ong et al. demonstrated the importance of growth until 2 years of age [64]. In contrast, several studies have reported that low birthweight children who grew excessively in later childhood were also at a higher risk of adult obesity [29, 72].

16.7 Overnourishment In Utero and the Risk of NCDs in Later Life

Obesity has prevailed in developed countries, particularly in North America, over the past several decades as a result of the oversupply of nutrients relative to the amount required to meet normal metabolic demands, and this has mainly been attributed to lifestyle such as the excess consumption of energy-rich meals

and declines in physical activity. However, the DOHaD theory proposes an alternative explanation for the increasing prevalence of obesity, i.e., a transgenerational negative chain by overnourishment in utero and/or in the early postnatal period, namely, fetuses and/or infants who experienced an early environment of excessive nutrients are predisposed to obesity and associated metabolic disorders in later life [9]. Salsberry et al. showed that maternal prepregnancy obesity was a significant risk factor for overweight adolescent offspring [73]. Maternal prepregnancy obesity and excessive weight gain during pregnancy have been causatively associated with the incidence of large-for-gestational-age infants [74–77] who are at high risk of childhood and adolescent obesity [74, 77, 78]. On the other hand, fetal exposure to diabetes or gestational diabetes during pregnancy, which may be linked to fetal exposure to high glucose levels, has been reported to increase the risk of childhood and adult obesity, diabetes, metabolic syndrome, and cardiovascular diseases [79, 80]. Human and animal studies revealed that an intrauterine high-energy supply consistently elevated the risk of NCDs in later life [81–83].

Recent human and animal studies have suggested that paternal obesity also induces the programming of offspring phenotypes related to the risk of NCDs via genetic and/or epigenetic changes in spermatozoa [84, 85].

Since not only undernourishment in utero but also overnourishment in utero have been causatively associated with the risk of NCDs in later life, a "U-shaped curve" has been proposed for the relationship between nutritional conditions in utero and the risk of developing adult NCDs [10–16] (Fig. 16.1). In developed countries, particularly those in which obesity is prevalent, the transgenerational risk of early exposure to an excess energy supply in the pre-contraceptive period and/or intrauterine period has been proposed to play a crucial role in increasing the risk of NCDs in addition to the simple lifestyle of an obesogenic diet and reduced physical activity [17] (Fig. 16.1). The rate of increases in the number of NCD patients in developing countries is distinctly higher than that in developed countries [15, 16]; therefore, the sequence of prenatal undernourishment and subsequent postnatal overnourishment may be a stronger risk factor for the development of adult NCDs in neonates undernourished in utero than continuous exposure to overnourishment throughout the entire life including the fetal period. Humans have struggled to adapt to starvation for millions of years; however, those in developed countries now need to adjust to an opposite environment, i.e., excess energy supply, throughout life even before birth.

Pathophysiological theories have been proposed for undernourishment in utero, such as a *predictive adaptive response*, the *thrifty phenotype*, and a *mismatch*; however, a pathophysiological theory has not yet been well established explaining the contribution of overnourishment in utero to the prevalence of NCDs in adulthood. It has not yet been fully clarified whether permanent phenotypic changes in response to overnourishment in utero are advantageous for the survival of the fittest in later life with an excess energy intake.

16.8 Perspective Toward Preemptive Medicine from Perinatal Care

The core concepts of preemptive medicine are the early identification of high-risk populations and early interventions during a latent period without symptoms or notable abnormalities in routine laboratory and physical examinations [5, 18, 19]. Evidence for the protective efficiency of such early interventions against the incidence of NCDs as well as medical economic cost performance is extremely limited. We propose the following candidates as realistic early interventions: (1) preparation of home and school educational programs on lifestyle based on scientific evidence, particularly lifelong benefits including the next generation [86]; (2) educational interventions before conception concerning nutritional aspects for fertile women, including the transgenerational negative chain according to the DOHaD theory [10–16, 87]; (3) nutritional interventions for lean and obese pregnant women, if effective [88, 89]; and (4) providing appropriate management of gestational diabetes [79, 80].

Since *catch-up growth* has been reported to increase the risk of NCDs [10, 12, 29, 64–67, 69–72], nutritional interventions for nursing women and/or bottle feeding neonates may be candidates for early interventions. Nevertheless, a previous study reported that children born small for their gestational age without *catch-up growth* were at high risk of a short stature in adulthood and need to be referred for growth hormone treatment [90]. Therefore, establishing standard methods to achieve optimal growth in neonates and/or infants is challenging because numerous factors, including genetic and epigenetic backgrounds, may be involved in their growth patterns.

One of the important concepts of preemptive medicine is identifying high-risk individuals in early life [5, 18, 19]. A simple assessment of birthweight is not sufficiently specific to clearly identify high-risk individuals. Extensive efforts have been made over the past few decades to establish effective biomarkers for the use in clinical practice with the ability to identify individuals at high risk of developing NCD [91–96]. The application of "omics" technologies has generated hundreds to thousands of biomarker candidates; however, very few of these have been translated into clinical care [95, 96]. The identification of useful biomarkers, their authorization, and governmental approval followed by translation into clinical settings will be long and difficult. Nevertheless, the rewards will be fruitful not only for individuals but also for socioeconomic contributions.

Conclusions

The rapid prevalence of NCDs has markedly increased healthcare and social security costs. In view of the pathogenesis of NCDs from the DOHaD theory, the perinatal care of fetuses as well as neonates has an important impact on the risk of NCDs in adulthood. An investigation of interventions from the viewpoint of perinatal care may provide useful insights for the development of preemptive medicine that targets NCDs.

References

1. Heitzinger K, Montano SM, Hawes SE, Alarcon JO, Zunt JR. A community-based cluster randomized survey of noncommunicable disease and risk factors in a peri-urban shantytown in Lima, Peru. BMC Int Health Hum Rights. 2014;14:19. doi:10.1186/1472-698X-14-19.
2. Berg Brigham K, Darlington M, Wright JS, Lewison G, Kanavos P, Durand-Zaleski I, et al. Mapping research activity on mental health disorders in Europe: study protocol for the Mapping_NCD project. Health Res Policy Syst. 2016;14(1):39. doi:10.1186/s12961-016-0111-6.
3. WHO. Global status report on noncommunicable diseases 2010. Description of the global burden of NCDs, their risk factors and determinants. 2010. http://www.who.int/nmh/publications/ncd_report2010/en/.
4. WHO. Noncommunicable disease. 2013. http://www.who.int/mediacentre/factsheets/fs355/en/.
5. Imura H. Life course health care and preemptive approach to non-communicable diseases. Proc Jpn Acad Ser B Phys Biol Sci. 2013;89(10):462–73.
6. Hanson MA, Gluckman PD. Developmental origins of health and disease—global public health implications. Best Pract Res Clin Obstet Gynaecol. 2014. doi:10.1016/j.bpobgyn.2014.06.007.
7. Hanson MA, Gluckman PD. Early developmental conditioning of later health and disease: physiology or pathophysiology? Physiol Rev. 2014;94(4):1027–76. doi:10.1152/physrev.00029.2013.
8. Gluckman PD, Hanson MA. Living with the past: evolution, development, and patterns of disease. Science. 2004;305(5691):1733–6.
9. Gluckman PD, Hanson MA. Developmental origins of health and disease. Cambridge: Cambridge University Press; 2006.
10. Taylor PD, Poston L. Developmental programming of obesity in mammals. Exp Physiol. 2007;92(2):287–98. expphysiol.2005.032854 [pii]. doi:10.1113/expphysiol.2005.032854.
11. Curhan GC, Willett WC, Rimm EB, Spiegelman D, Ascherio AL, Stampfer MJ. Birth weight and adult hypertension, diabetes mellitus, and obesity in US men. Circulation. 1996;94(12):3246–50.
12. Fernandez-Twinn DS, Ozanne SE. Mechanisms by which poor early growth programs type-2 diabetes, obesity and the metabolic syndrome. Physiol Behav. 2006;88(3):234–43. S0031-9384(06)00239-3 [pii]. doi:10.1016/j.physbeh.2006.05.039.
13. Prentice AM, Rayco-Solon P, Moore SE. Insights from the developing world: thrifty genotypes and thrifty phenotypes. Proc Nutr Soc. 2005;64(2):153–61. doi:S0029665105000194 [pii].
14. Prentice AM, Moore SE. Early programming of adult diseases in resource poor countries. Arch Dis Child. 2005;90(4):429–32. doi:10.1136/adc.2004.059030.
15. Katz AR. Noncommunicable diseases: global health priority or market opportunity? An illustration of the World Health Organization at its worst and at its best. Int J Health Serv Plan Adm Eval. 2013;43(3):437–58.
16. Prentice AM. Nutrition and chronic disease: lessons from the developing and developed world. Nestle Nutr Inst Workshop Ser. 2014;78:155–60. doi:10.1159/000354957.
17. Itoh H, Kanayama N. Nutritional conditions in early life and risk of non-communicable diseases (NCDs); the perspective of preemptive medicine in perinatal care. Hypertens Res Pregnancy. 2015;3:1–12. doi:10.14390/jsshp.3.1.
18. NIoH (NIH). Strategic vision for the future—from curative to pre-emptive medicine. 2011. http://www.nih.gov/strategicvision.htm.
19. Agboola SO, Ball M, Kvedar JC, Jethwani K. The future of connected health in preventive medicine. QJM. 2013;106(9):791–4. doi:10.1093/qjmed/hct088.
20. Kubota T, Miyake K, Hariya N, Mochizuki K. Epigenomic-basis of preemptive medicine for neurodevelopmental disorders. Curr Genomics. 2015;16(3):175–82. doi:10.2174/1389202916666150216221312.

21. Hoek HW, Susser E, Buck KA, Lumey LH, Lin SP, Gorman JM. Schizoid personality disorder after prenatal exposure to famine. Am J Psychiatry. 1996;153(12):1637–9. doi:10.1176/ajp.153.12.1637.
22. Ravelli AC, van Der Meulen JH, Osmond C, Barker DJ, Bleker OP. Obesity at the age of 50 y in men and women exposed to famine prenatally. Am J Clin Nutr. 1999;70(5):811–6.
23. Roseboom T, de Rooij S, Painter R. The Dutch famine and its long-term consequences for adult health. Early Hum Dev. 2006;82(8):485–91. doi:10.1016/j.earlhumdev.2006.07.001.
24. Kyle UG, Pichard C. The Dutch Famine of 1944–1945: a pathophysiological model of long-term consequences of wasting disease. Curr Opin Clin Nutr Metab Care. 2006;9(4):388–94. doi:10.1097/01.mco.0000232898.74415.42.
25. Barker DJ, Osmond C. Infant mortality, childhood nutrition, and ischaemic heart disease in England and Wales. Lancet. 1986;1(8489):1077–81.
26. Barker DJ, Winter PD, Osmond C, Margetts B, Simmonds SJ. Weight in infancy and death from ischaemic heart disease. Lancet. 1989;2(8663):577–80.
27. Hales CN, Barker DJ. Type 2 (non-insulin-dependent) diabetes mellitus: the thrifty phenotype hypothesis. Diabetologia. 1992;35(7):595–601.
28. Eriksson J, Forsen T, Tuomilehto J, Osmond C, Barker D. Fetal and childhood growth and hypertension in adult life. Hypertension. 2000;36(5):790–4.
29. Eriksson J, Forsen T, Tuomilehto J, Osmond C, Barker D. Size at birth, childhood growth and obesity in adult life. Int J Obes Relat Metab Disord. 2001;25(5):735–40.
30. Eriksson JG, Forsen T, Tuomilehto J, Osmond C, Barker DJ. Early growth and coronary heart disease in later life: longitudinal study. BMJ. 2001;322(7292):949–53.
31. Forsen T, Eriksson J, Tuomilehto J, Reunanen A, Osmond C, Barker D. The fetal and childhood growth of persons who develop type 2 diabetes. Ann Intern Med. 2000;133(3):176–82.
32. Osmond C, Kajantie E, Forsen TJ, Eriksson JG, Barker DJ. Infant growth and stroke in adult life: the Helsinki birth cohort study. Stroke. 2007;38(2):264–70. doi:10.1161/01.STR.0000254471.72186.03.
33. Hanson M, Godfrey KM, Lillycrop KA, Burdge GC, Gluckman PD. Developmental plasticity and developmental origins of non-communicable disease: theoretical considerations and epigenetic mechanisms. Prog Biophys Mol Biol. 2011;106(1):272–80. doi:10.1016/j.pbiomolbio.2010.12.008.
34. Barouki R, Gluckman PD, Grandjean P, Hanson M, Heindel JJ. Developmental origins of non-communicable disease: implications for research and public health. Environ Health Glob Access Sci Source. 2012;11:42. doi:10.1186/1476-069X-11-42.
35. Haugen AC, Schug TT, Collman G, Heindel JJ. Evolution of DOHaD: the impact of environmental health sciences. J Dev Orig Health Dis. 2015;6(2):55–64. doi:10.1017/S2040174414000580.
36. Silveira PP, Portella AK, Goldani MZ, Barbieri MA. Developmental origins of health and disease (DOHaD). J Pediatr. 2007;83(6):494–504. doi:10.2223/JPED.1728.
37. Song S, Wang W, Hu P. Famine, death, and madness: schizophrenia in early adulthood after prenatal exposure to the Chinese Great Leap Forward Famine. Soc Sci Med. 2009;68(7):1315–21. doi:10.1016/j.socscimed.2009.01.027.
38. Li Y, He Y, Qi L, Jaddoe VW, Feskens EJ, Yang X, et al. Exposure to the Chinese famine in early life and the risk of hyperglycemia and type 2 diabetes in adulthood. Diabetes. 2010;59(10):2400–6. doi:10.2337/db10-0385.
39. Zheng X, Wang Y, Ren W, Luo R, Zhang S, Zhang JH, et al. Risk of metabolic syndrome in adults exposed to the great Chinese famine during the fetal life and early childhood. Eur J Clin Nutr. 2012;66(2):231–6. doi:10.1038/ejcn.2011.161.
40. Li Y, Jaddoe VW, Qi L, He Y, Wang D, Lai J, et al. Exposure to the Chinese famine in early life and the risk of metabolic syndrome in adulthood. Diabetes Care. 2011;34(4):1014–8. doi:10.2337/dc10-2039.
41. Cunningham FG, Leveno KJ, Bloom SL, Spong CY, Dashe JS, Hoffman BL, et al., editors. Fetal-growth disorders. 24th ed. Williams obstetrics. New York: McGraw-Hill; 2014.

42. Lee PA, Chernausek SD, Hokken-Koelega AC, Czernichow P, International Small for Gestational Age Advisory B. International Small for Gestational Age Advisory Board consensus development conference statement: management of short children born small for gestational age, April 24–October 1, 2001. Pediatrics. 2003;111(6 Pt 1):1253–61.
43. Donahue SM, Kleinman KP, Gillman MW, Oken E. Trends in birth weight and gestational length among singleton term births in the United States: 1990–2005. Obstet Gynecol. 2010;115(2 Pt 1):357–64. doi:10.1097/AOG.0b013e3181cbd5f5.
44. Takimoto H, Yokoyama T, Yoshiike N, Fukuoka H. Increase in low-birth-weight infants in Japan and associated risk factors, 1980–2000. J Obstet Gynaecol Res. 2005;31(4):314–22. doi:10.1111/j.1447-0756.2005.00294.x.
45. Malina RM, Katzmarzyk PT, Beunen G. Birth weight and its relationship to size attained and relative fat distribution at 7 to 12 years of age. Obes Res. 1996;4(4):385–90.
46. Okosun IS, Liao Y, Rotimi CN, Dever GE, Cooper RS. Impact of birth weight on ethnic variations in subcutaneous and central adiposity in American children aged 5–11 years. A study from the Third National Health and Nutrition Examination Survey. Int J Obes Relat Metab Disord. 2000;24(4):479–84.
47. Law CM, Barker DJ, Osmond C, Fall CH, Simmonds SJ. Early growth and abdominal fatness in adult life. J Epidemiol Community Health. 1992;46(3):184–6.
48. Kensara OA, Wootton SA, Phillips DI, Patel M, Jackson AA, Elia M. Fetal programming of body composition: relation between birth weight and body composition measured with dual-energy X-ray absorptiometry and anthropometric methods in older Englishmen. Am J Clin Nutr. 2005;82(5):980–7. 82/5/980 [pii].
49. Muhlhausler BS, Adam CL, McMillen IC. Maternal nutrition and the programming of obesity: the brain. Organogenesis. 2008;4(3):144–52.
50. Hales CN, Barker DJ. The thrifty phenotype hypothesis. Br Med Bull. 2001;60:5–20.
51. Wells JC. The thrifty phenotype: an adaptation in growth or metabolism? Am J Hum Biol. 2011;23(1):65–75. doi:10.1002/ajhb.21100.
52. Gluckman PD, Hanson MA. The fetal matrix: evolution, development and disease. Cambridge: Cambridge University Press; 2005.
53. Moore SE, Halsall I, Howarth D, Poskitt EM, Prentice AM. Glucose, insulin and lipid metabolism in rural Gambians exposed to early malnutrition. Diabet Med. 2001;18(8):646–53. doi:565 [pii].
54. Gluckman PD, Hanson MA. Mismatch. Oxford: Oxford University Press; 2006.
55. Gluckman PD, Hanson MA. Developmental and epigenetic pathways to obesity: an evolutionary-developmental perspective. Int J Obes (Lond). 2008;32(Suppl 7):S62–71. ijo2008240 [pii]. doi:10.1038/ijo.2008.240.
56. Itoh H, Kanayama N. Low birth weight and risk of obesity—a potential problem of Japanese people. Curr Women's Health Rev. 2009;5:212–9.
57. Chan JC, Ng MC, Critchley JA, Lee SC, Cockram CS. Diabetes mellitus—a special medical challenge from a Chinese perspective. Diabetes Res Clin Pract. 2001;54(Suppl 1):S19–27.
58. Unnikrishnan R, Anjana RM, Mohan V. Diabetes mellitus and its complications in India. Nat Rev Endocrinol. 2016;12(6):357–70. doi:10.1038/nrendo.2016.53.
59. Hussein Z, Taher SW, Gilcharan Singh HK, Chee Siew Swee W. Diabetes care in Malaysia: problems, new models, and solutions. Ann Glob Health. 2015;81(6):851–62. doi:10.1016/j.aogh.2015.12.016.
60. Hayashi F, Takimoto H, Yoshita K, Yoshiike N. Perceived body size and desire for thinness of young Japanese women: a population-based survey. Br J Nutr. 2006;96(6):1154–62. doi:S0007114507207603 [pii].
61. Goto Y. Diseases in the 21st century. J Jpn Soc Study of Obes (Jpn). 2006;12:1–2.
62. Gluckman PD, Seng CY, Fukuoka H, Beedle AS, Hanson MA. Low birthweight and subsequent obesity in Japan. Lancet. 2007;369(9567):1081–2. S0140-6736(07)60524-8 [pii]. doi:10.1016/S0140-6736(07)60524-8.

63. Kubota K, Itoh H, Tasaka M, Naito H, Fukuoka Y, Muramatsu Kato K, et al. Changes of maternal dietary intake, bodyweight and fetal growth throughout pregnancy in pregnant Japanese women. J Obstet Gynaecol Res. 2013;39(9):1383–90. doi:10.1111/jog.12070.
64. Ong KK, Loos RJ. Rapid infancy weight gain and subsequent obesity: systematic reviews and hopeful suggestions. Acta Paediatr. 2006;95(8):904–8. L4702X6Q32U64K33 [pii]. doi:10.1080/08035250600719754.
65. Berends LM, Fernandez-Twinn DS, Martin-Gronert MS, Cripps RL, Ozanne SE. Catch-up growth following intra-uterine growth-restriction programmes an insulin-resistant phenotype in adipose tissue. Int J Obes (Lond). 2013;37(8):1051–7. doi:10.1038/ijo.2012.196.
66. Bol VV, Delattre AI, Reusens B, Raes M, Remacle C. Forced catch-up growth after fetal protein restriction alters the adipose tissue gene expression program leading to obesity in adult mice. Am J Physiol Regul Integr Comp Physiol. 2009;297(2):R291–9. doi:10.1152/ajpregu.90497.2008.
67. Gonzalez-Bulnes A, Ovilo C, Lopez-Bote CJ, Astiz S, Ayuso M, Perez-Solana ML, et al. Gender-specific early postnatal catch-up growth after intrauterine growth retardation by food restriction in swine with obesity/leptin resistance. Reproduction. 2012;144(2):269–78. doi:10.1530/REP-12-0105.
68. Dulloo AG, Jacquet J, Seydoux J, Montani JP. The thrifty 'catch-up fat' phenotype: its impact on insulin sensitivity during growth trajectories to obesity and metabolic syndrome. Int J Obes (Lond). 2006;30(Suppl 4):S23–35. doi:10.1038/sj.ijo.0803516.
69. Stettler N, Kumanyika SK, Katz SH, Zemel BS, Stallings VA. Rapid weight gain during infancy and obesity in young adulthood in a cohort of African Americans. Am J Clin Nutr. 2003;77(6):1374–8.
70. Stettler N, Stallings VA, Troxel AB, Zhao J, Schinnar R, Nelson SE, et al. Weight gain in the first week of life and overweight in adulthood: a cohort study of European American subjects fed infant formula. Circulation. 2005;111(15):1897–903. 111/15/1897 [pii]. doi:10.1161/01.CIR.0000161797.67671.A7.
71. Botton J, Heude B, Maccario J, Ducimetiere P, Charles MA. Postnatal weight and height growth velocities at different ages between birth and 5 y and body composition in adolescent boys and girls. Am J Clin Nutr. 2008;87(6):1760–8. 87/6/1760 [pii].
72. Rolland-Cachera MF, Deheeger M, Bellisle F, Sempe M, Guilloud-Bataille M, Patois E. Adiposity rebound in children: a simple indicator for predicting obesity. Am J Clin Nutr. 1984;39(1):129–35.
73. Salsberry PJ, Reagan PB. Taking the long view: the prenatal environment and early adolescent overweight. Res Nurs Health. 2007;30(3):297–307. doi:10.1002/nur.20215.
74. ACOG. ACOG Committee opinion no. 549: obesity in pregnancy. Obstet Gynecol. 2013;121(1):213–7. doi:10.1097/01.AOG.0000425667.10377.60.
75. Cedergren MI. Maternal morbid obesity and the risk of adverse pregnancy outcome. Obstet Gynecol. 2004;103(2):219–24. doi:10.1097/01.AOG.0000107291.46159.00.
76. Rode L, Nilas L, Wojdemann K, Tabor A. Obesity-related complications in Danish single cephalic term pregnancies. Obstet Gynecol. 2005;105(3):537–42. doi:10.1097/01.AOG.0000152304.39492.1c.
77. Hediger ML, Overpeck MD, McGlynn A, Kuczmarski RJ, Maurer KR, Davis WW. Growth and fatness at three to six years of age of children born small- or large-for-gestational age. Pediatrics. 1999;104(3):e33.
78. Sebire NJ, Jolly M, Harris JP, Wadsworth J, Joffe M, Beard RW, et al. Maternal obesity and pregnancy outcome: a study of 287,213 pregnancies in London. Int J Obes Relat Metab Disord. 2001;25(8):1175–82. doi:10.1038/sj.ijo.0801670.
79. Clausen TD, Mathiesen ER, Hansen T, Pedersen O, Jensen DM, Lauenborg J, et al. Overweight and the metabolic syndrome in adult offspring of women with diet-treated gestational diabetes mellitus or type 1 diabetes. J Clin Endocrinol Metab. 2009;94(7):2464–70. doi:10.1210/jc.2009-0305.

80. Moore TR. Fetal exposure to gestational diabetes contributes to subsequent adult metabolic syndrome. Am J Obstet Gynecol. 2010;202(6):643–9. doi:10.1016/j.ajog.2010.02.059.
81. Armitage JA, Poston L, Taylor PD. Developmental origins of obesity and the metabolic syndrome: the role of maternal obesity. Front Horm Res. 2008;36:73–84. doi:10.1159/0000115355.
82. Dyer JS, Rosenfeld CR. Metabolic imprinting by prenatal, perinatal, and postnatal overnutrition: a review. Semin Reprod Med. 2011;29(3):266–76. doi:10.1055/s-0031-1275521.
83. Li M, Sloboda DM, Vickers MH. Maternal obesity and developmental programming of metabolic disorders in offspring: evidence from animal models. Exp Diabetes Res. 2011;2011:592408. doi:10.1155/2011/592408.
84. McPherson NO, Fullston T, Aitken RJ, Lane M. Paternal obesity, interventions, and mechanistic pathways to impaired health in offspring. Ann Nutr Metab. 2014;64(3–4):231–8. doi:10.1159/000365026.
85. Chen Q, Yan M, Cao Z, Li X, Zhang Y, Shi J, et al. Sperm tsRNAs contribute to intergenerational inheritance of an acquired metabolic disorder. Science. 2016;351(6271):397–400. doi:10.1126/science.aad7977.
86. Bay JL, Mora HA, Sloboda DM, Morton SM, Vickers MH, Gluckman PD. Adolescent understanding of DOHaD concepts: a school-based intervention to support knowledge translation and behaviour change. J Dev Orig Health Dis. 2012;3(6):469–82. doi:10.1017/S2040174412000505.
87. Hanson MA, Bardsley A, De-Regil LM, Moore SE, Oken E, Poston L, et al. The International Federation of Gynecology and Obstetrics (FIGO) recommendations on adolescent, preconception, and maternal nutrition: "Think Nutrition First". Int J Gynaecol Obstet. 2015;131(Suppl 4):S213–53. doi:10.1016/S0020-7292(15)30023-0.
88. Kulkarni B, Kuper H, Radhakrishna KV, Hills AP, Byrne NM, Taylor A, et al. The association of early life supplemental nutrition with lean body mass and grip strength in adulthood: evidence from APCAPS. Am J Epidemiol. 2014;179(6):700–9. doi:10.1093/aje/kwt332.
89. Tanvig M. Offspring body size and metabolic profile—effects of lifestyle intervention in obese pregnant women. Dan Med J. 2014;61(7):B4893.
90. Houk CP, Lee PA. Early diagnosis and treatment referral of children born small for gestational age without catch-up growth are critical for optimal growth outcomes. Int J Pediatr Endocrinol. 2012;2012(1):11. doi:10.1186/1687-9856-2012-11.
91. Paulovich AG, Whiteaker JR, Hoofnagle AN, Wang P. The interface between biomarker discovery and clinical validation: the tar pit of the protein biomarker pipeline. Proteomics Clin Appl. 2008;2(10–11):1386–402. doi:10.1002/prca.200780174.
92. Zhang Z, Chan DW. The road from discovery to clinical diagnostics: lessons learned from the first FDA-cleared in vitro diagnostic multivariate index assay of proteomic biomarkers. Cancer Epidemiol Biomarkers Prev. 2010;19(12):2995–9. doi:10.1158/1055-9965.EPI-10-0580.
93. Michiels S, Kramar A, Koscielny S. Multidimensionality of microarrays: statistical challenges and (im)possible solutions. Mol Oncol. 2011;5(2):190–6. doi:10.1016/j.molonc.2011.01.002.
94. Fuzery AK, Levin J, Chan MM, Chan DW. Translation of proteomic biomarkers into FDA approved cancer diagnostics: issues and challenges. Clin Proteomics. 2013;10(1):13. doi:10.1186/1559-0275-10-13.
95. Gomez-Cabrero D, Abugessaisa I, Maier D, Teschendorff A, Merkenschlager M, Gisel A, et al. Data integration in the era of omics: current and future challenges. BMC Syst Biol. 2014;8(Suppl 2):I1. doi:10.1186/1752-0509-8-S2-I1.
96. Gupta S, Venkatesh A, Ray S, Srivastava S. Challenges and prospects for biomarker research: a current perspective from the developing world. Biochim Biophys Acta. 2014;1844(5):899–908. doi:10.1016/j.bbapap.2013.12.020.