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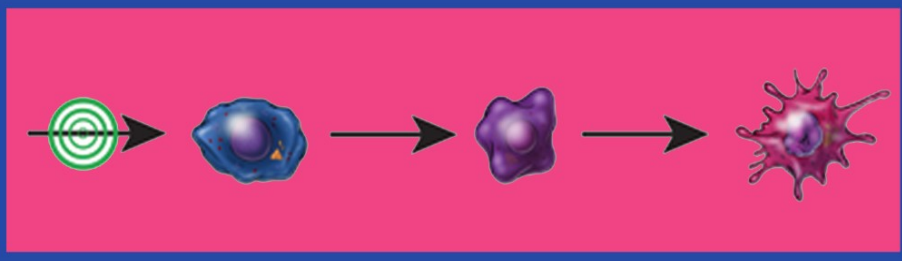

Origin of Cancers

Clinical Perspectives and Implications of a Stem-Cell Theory of Cancer

Springer

Cancer Treatment and Research

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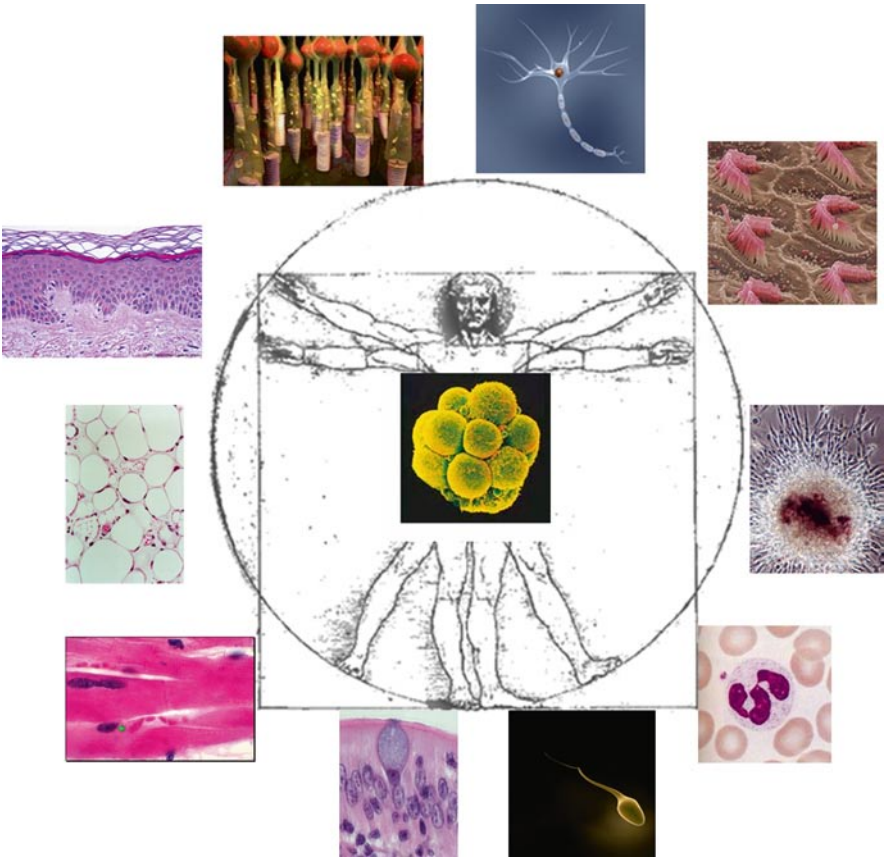
Preface

Précis

This book is a treatise about the origin of cancers. I would like to convince readers that the basic tenets of the theory of a stem-cell origin of cancers also constitute a *unified theory of cancer*.



Stem-cell origin of normal (and cancer) cells: Vitruvian version

Every truth passes through three stages before it is
recognized. In the first it is ridiculed, in the second,
it is opposed, in the third, it is regarded as self-evident.
– Arthur Schopenhauer

Every person has a unique story to tell. My story is about cancer. Cancer touches the lives of countless people. Often enough, it leaves indelible tracks. Many lives have been lost; others are forever changed. For those who confront this deadly scourge, there is a sense of urgency, if not of desperation. For those who face imminent death, life becomes even more precious and carries a special meaning. As an oncologist, I am touched daily by cancer. I feel its inception, evolution, and aftermath. It seems as though we are fighting an incessant war against cancer at the front line in the trenches. This is my story about cancer.

Some people are terrific storytellers. Others have incredible tales to tell. Occasionally, a terrific storyteller tells an incredible story. I do not pretend to be a great raconteur or promise that this story will grip your heart. However, I believe that this book carries an important message beyond just enlightenment or a revelation about cancer. The time has finally arrived for us to formulate a unified theory that may elucidate the many facets of cancer. Although our knowledge about cancer has increased by leaps and bounds, we are still quite ignorant about its exact origin and basic inner workings. But there is growing awareness that our conventional wisdom about cancer is stale and woefully inadequate. More than ever, we both desire and need to craft an updated theory about cancer's origins that may improve our current investigations, explanations, and therapies for cancer.

This book sees cancer through the eyes of a clinical oncologist. In many respects, this is a personal treatise about the origin of cancer. The opinions expressed in this book are entirely my own; they have not been approved by any institution or endorsed by any establishment, and I take full responsibility for them. I hope to convince the readers of this book that the theory of a stem-cell origin of cancers provides some unique alternative insights into cancer. One day, this theory may completely overhaul our perspectives on the nature of cancer and our conduct in the business of cancer.

I would like to demonstrate that the basic tenets of the theory of a stem-cell origin of cancers also constitute a unified theory of cancer. The idea that the stem cell participates in the malignant process may soon let the "cancer genie" out of the bottle. This idea will permeate every aspect of cancer: heterogeneity, metastasis, immunity, drug resistance, etc. In this book, I do not claim to know the missing links or any solutions to the mysteries of cancer. Neither does the book pay respect or offer consolation to the victims of cancer. But there is an emerging realization that cancer is an integral and intrinsic part of our very nature. It is like a shadow that is impossible for us to escape. We need to learn how to live with our shadow. Whether we like it or not, we must make peace with it.

I forewarn readers that this book is also not meant to be a vigorous academic exercise. Instead, I have elected to ruminate about the important subject of the clinical implications and perspectives of a stem-cell theory of cancer in the form of informal essays and discourses. For the sake of brevity, I have chosen not to include too much detail on several topics. To the experts, my narrative may seem too simple, superficial, and generalized. Some readers may believe that the discussions are too perfunctory and naive for such a complicated subject. Unfortunately for those readers, it was not my intention to write an encyclopedia. Undoubtedly, each topic in this book deserves a book in its own right. Nevertheless, I made every effort to examine the intricacies of cancer without losing sight of the big picture.

To the best of my ability, I have tried to acknowledge wherever and whenever possible in this book the innumerable pioneers and leaders who generated this stem-cell theory of cancer and laid the foundation for a unified theory of cancer. Most of us would credit Rudolf Virchow as the first person to espouse the idea of a stem-cell origin of cancers in 1863. And Barry Pierce performed many seminal experiments that ignited renewed interest in this field. More recently, the theory of a stem-cell origin of cancers gained a tremendous boost and momentum from work performed by John Dirk, Michael Clarke, and Irwin Weissman. Other prominent and distinguished investigators who have joined their ranks include Sean Morrison, David Scadden, Max Wicha, and Robert Weinberg. This list of illustrious researchers keeps getting longer. I apologize if I have omitted any deserving individuals in this chronicle.

This book was inspired by an article published in 1994 by Stewart Sell and Barry Pierce, "Maturation Arrest of Stem Cell Differentiation Is a Common Pathway for the Cellular Origin of Teratocarcinomas and Epithelial Cancers." At the time, I was a fledgling clinical instructor in the Department of Genitourinary Medical Oncology at The University of Texas M. D. Anderson Cancer Center in Houston. I still cherish the wonderful times during those formative years when I was learning about this fantastic idea of a stem-cell origin of cancers. It occurred to me that this might be the unified theory of cancer our forefathers have sought over the ages. Incredibly, this eye-opening idea could explain almost any clinical observations about cancer. Unfortunately, an article about this viewpoint that I submitted to the *Lancet* was rejected for publication in October 2000. Eventually, however, *Lancet Oncology* published the article, "Stem-Cell Origin of Metastasis and Heterogeneity in Solid Tumours," in August 2002. After having failed in my attempts to publish two additional articles related to this unified theory of cancer, it dawned on me in October 2004 that I should start writing about this important and fascinating subject in a more comprehensive fashion as a book.

The crux of my story rests on a very simple question: What is cancer? We have heard about it, read about it, and talked about it. Many individuals in the clinics and on the streets have asked the same question. Perhaps it would be easier to answer this global question if we reframe it as, What is the origin of cancer? Buried in this mundane inquiry are many profound unknowns: Why has cancer become so prevalent? Why is it so difficult for us to eradicate cancer? It seems as though when we vanquish one cancer, we confront two more. Who is more realistic, the optimist who believes that one day we will banish cancer from the face of the earth or the pessimist who perceives that cancer is forever linked to our very being, like an undesirable appendage? This book will perhaps answer some of our most burning questions about cancer. I hope that it will separate cancer facts from cancer fiction. I think that it may interest both soothsayers and naysayers about cancer.

Solving the mystery of cancer's origin cannot be a more monumental task. My motivation for this undertaking is twofold. First, it is a worthy cause that may change the face of a most dreaded and dreadful disease. And second, it is a great adventure that will take us into the unknown and the unimaginable. Understandably, many daring individuals may wish to join the expedition. Perhaps basic scientists

should be in the forefront for this mission. They have the acumen and expertise to design the critical experiments that may one day validate (or refute) this theory about a stem-cell origin of cancers. However, I believe that someone else, with broader interests and a wider background, may be in the best position to envision the big picture and the panorama of cancer…someone who is not bound by the narrow focus on his or her own research or bogged down by the heavy burden of his or her own career. I hope to contribute to a unified theory of cancer by elaborating on its clinical implications, which may clarify the intents and results of many experiments designed to validate or refute this theory.

At the end of the day, the ultimate goal of our quest is to find a cure for cancer. I believe that this objective is inseparable from our lofty goal of discovering a unified theory of the origin of cancers. We currently tend to regard cancer as a tabula rasa, thinking that its eventual form is determined in large part by the various mutations cells acquire over time. However, this mentality forces us to adhere to the prevailing doctrine that cancer acquires rather than inherits its many malignant properties during carcinogenesis. Nowadays, we find it unthinkable that a cancer is preloaded with some malevolent power. Inevitably, we assume that cancer mimics or contains stem cells rather than originates from stem cells. In this book, I would like to show that rather than merely mimicking or containing stem cells, cancer actually originates from stem cells or, more precisely, from stem cell–like cells. I believe that solving the mystery of cancer's origin is tantamount to conquering cancer.

More importantly, I believe the time has finally arrived for us to revisit some of our most cherished dogmas about cancer. For instance, if many of the malignant properties (e.g., dormancy, self-renewal, drug resistance, immunity, multipotentiality) associated with cancer cells already exist in stem cells, then additional mutations in the same pathways that regulate these vital functions could be redundant or unnecessary for the formation of cancer. Perhaps only a few critical mutations are actually pivotal during carcinogenesis and are sufficient to nudge a stem cell into the ways of a cancer cell. Therefore, many of our traditional assumptions and popular conceptions of cancer (e.g., multistep carcinogenesis) may need amendment. And many of our previous and current scientific objectives and methods may be missing the mark because the targets we have been aiming for are either wrong or irrelevant and because the hypotheses we have adopted have been misguided or misleading. Now is the time for an improved, updated theory on the origin of cancers that will alter the cancer landscape for good. I hope that this book is the first cheer rather than the last hurrah for a unified theory of cancer.

Finally, I emphasize that many critical and defining experiments supporting this unified theory of cancer have already been performed. To better appreciate the significance of these experiments and to fully understand the nature of cancer, we must first take a deep breath and adopt a new mind-set. Whenever possible in this book, I have reexamined the results of those studies, reconsidering them from an unconventional angle and interpreting them in an unorthodox light according to the theory of a stem-cell origin of cancers. Toward the end, it becomes glaringly apparent that until now, we have forgotten a very important scientific

principle at our own peril. We have forgotten that experimental models are, by their very nature, manipulated and limited in their scope and relevance. Indeed, it would be presumptuous and even preposterous for us to equate an experiment with the hypothesis itself. In my view, this misconstrued stance is currently and continuously being promoted under the guise of hypothesis-generating research. We need to keep reminding ourselves that an experiment is merely a tool used to test a given hypothesis. Otherwise, we delude ourselves and become trapped by the belief that the experiment itself is an end rather than the means to an end. It is disconcerting to realize how we are relying on the results of experiments even though we have betrayed one of the most fundamental principles of science in designing those experiments.

Sometimes ideas seem trivial or even frivolous. But when an idea completely changes the way we perceive our world and how we live in it, it is anything but trivial or frivolous. It is true that many ideas are recycled: old ideas evolve and continue to evolve through time. Therefore, not only is this unified theory of cancer potentially revolutionary, it is also evolutionary. This book attempts to put the past and future of cancer into a unique perspective. I hope that it will bridge our invaluable past clinical observations with improved future therapeutic strategies. The principal purpose of this endeavor is to convince readers that cancer may actually have its origin in stem cells or, more precisely, in progenitor cells with stemness features. Cancer is neither a deliberate nor an inadvertent freak of nature. It is rife with stem-cell features and properties. Indeed, how a malignant cell manages to hijack almost every characteristic of a stem cell is a genuinely stunning feat. If we choose to recognize it as arising from stem cells, the cancer cell has not changed its stripes after all.

Acknowledgments

Inspiration for this book came from the work of Stewart Sell and Barry Pierce. I am very fortunate to have the help and support of my wife, Shu-Fen Chiu, who endured many hours of my absent-mindedness and stubbornness. I thank my parents, Pei-Chun Tu, MD, and Yue-Fang Fan, who gave me confidence and encouragement. I am very grateful to my scientific manuscript editor, Karen Phillips, ELS, who helped me make the book a reality. I also owe a great deal to my colleagues and friends (Ana Aparicio, MD, Bradley Atkinson, PharmD, Paul Corn, MD, PhD, Eric Jonasch, MD, Jeri Kim, MD, Sue-Hwa Lin, PhD, Sendurai Mani, PhD, Padmanee Sharma, MD, PhD, Dean Tang, PhD, Nizar Tannir, MD, and Timothy Thompson, PhD), who took the time in spite of their busy schedules to review selected chapters of this book and provided me with invaluable comments and advice. Finally, I dedicate this book to my mentors, Christopher Logothetis, MD, and Sue-Hwa Lin, PhD, who provide an academic environment conducive to inquisitiveness and open-mindedness. Without their leadership, guidance, and assistance, this book would not have been possible.

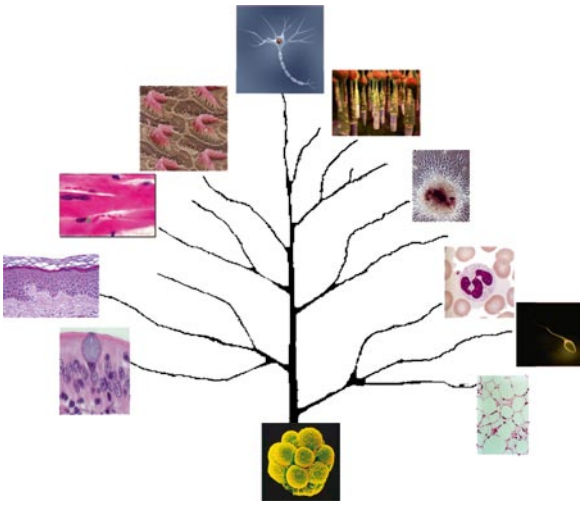
References

Sell S, Pierce GB (1994) Maturation arrest of stem cell differentiation is a common pathway for the cellular origin of teratocarcinomas and epithelial cancers. Lab Invest 70:6–22.

Tu S-M, Lin S-H (2002) Logothetis CJ. Stem-cell origin of metastasis and heterogeneity in solid tumours. Lancet Oncol 3:508–513.

Contents

Chapter 1
Introduction



Stem-cell origin of normal (and cancer) cells: root and stem version

The resistance to a new idea increases as the square of its importance.

– Bertrand Russell

Précis

Ironically, there appears to be malignant potential in every stem cell and stem-cell potential in every malignant cell. Thus, an imperfect stem cell manages to become a perfect cancer cell. Our challenge is to distinguish what is stemness from what is malignant.

Solid tumors (including lung, colorectal, breast, and prostate cancers) comprise more than 90% of human malignancies. Compared with hematologic malignancies, solid tumors are less accessible, making the study of their developmental origins more challenging. Furthermore, the complex interactions between a

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malignant cell and its neighboring cells at the primary or metastatic site render solid tumors immensely diverse and complicated in their biologic characteristics and clinical behavior. These are among the many factors that have hampered our efforts to solve the mystery of cancer and to cure a majority of human cancers today.

To successfully combat cancer, both doctors and patients need to better understand its basic nature. After all, knowing the weaknesses of our enemy will greatly improve our chances of conquering it. However, to tackle a formidable disease like cancer is particularly daunting and even overwhelming. Where do we begin this odyssey? How do we fight this perennial war? This is a crusade in which the reward is more than just conquest of a dreaded plague: It is an endeavor that may also enlighten us about our own potential and frailties. Although there is no easy answer, we may acquire invaluable clues about the basic nature of cancer by asking the right questions. A good place to start in our quest to improve our understanding and treatment of cancer is to reframe our question about its origins.

The theory of a stem-cell origin of cancers is neither groundbreaking nor earth-shattering. In fact, many prominent investigators have already established the foundation for this concept and performed the vital experiments to support its merit and validity. What I hope to contribute is to show how this idea may completely transform our current views and perspective about cancer. The purpose of this book is to show how the theory of a stem-cell origin of cancers poses a challenge to conventional wisdom and invites fresh interpretations of the many classic observations about the secrets and ravages of cancer. Eventually, I anticipate that the various ramifications of this theory will be embraced by both academicians and laymen and become ingrained in the daily conscience and routine of every cancer physician and his or her patients.

On the surface, it seems that a malignant cell and a stem cell are simply mirror images of each other. Ironically, there appears to be malignant potential in every stem cell and stem cell potential in every malignant cell. In other words, an imperfect stem cell manages to become a perfect cancer cell. This conflict between good and evil in the same cell reminds us of the yin–yang relationship in all matter or beings. Perhaps an appropriate symbolic representative of the stem-cell origin of cancers is Shiva, the Hindu god of both creation and destruction. He has the power of a progenitor, like a stem cell. Yet he is also capable of destroying everything he has created, like a malignant cell. Our challenge is to discern stemness from malignancy. We need to identify the trademarks that separate a stem cell from a cancer cell.

The immense heterogeneity of malignancy is pervasive. It baffles our desire to categorize the disease and confounds our strategies to treat it. Likewise, cancer immunity seems deeply rooted. It empowers the cancer with an uncanny ability to elude an otherwise intact immune system and foil many promising vaccine therapies. In addition, the intrinsic drug resistance of many cancers seems beyond mere coincidence. This cannot be just another clever ruse or devious ploy designed to thwart our efforts and hopes to cure cancer. A recurring theme in our efforts to solve

the mystery of cancer is that most if not all of these intriguing properties of malignancy can be traced to its stem-cell roots.

A stem-cell origin of cancers will fundamentally change our current stance on the prognostic implications of cancer (Chap. 10). The Holy Grail of cancer research is to find ways to distinguish rapidly lethal cancers from relatively indolent ones. Various prognostic factors have been used to serve this purpose so that we can appropriately treat a threatening cancer aggressively and an incidental cancer sparingly. In the end, many of these prognostic features such as tumor grade, clinical stage, and various molecular markers may be mere reflections of a hierarchic order in the stem-cell origin of a particular cancer. I believe that the unifying theory of a stem-cell origin of cancers offers an improved explanation of why some cancers are incredibly deadly and others strangely indolent. It provides a better prediction of why some cancers are so diversified and variable, yet others can be quite homogeneous and restricted. It gives a more plausible answer as to why some cancers are so intractable, while others are more susceptible to various treatments.

The theory of a stem-cell origin of cancers will also greatly alter our current understanding of cancer antigens (Chap. 11). After all, the success of any targeted therapy against cancer depends to a large extent on the pertinence of the targeted cancer antigens. With myriad candidate cancer antigens being discovered and investigated, it becomes imperative for us to validate these various targets for cancer treatment. Otherwise, it is inevitable that many of these targeted treatments will be found to be surprisingly ineffective and disappointing. Like the proverbial story of the elephant and the blind men, what we perceive may not reflect reality: A particular target may not be representative of the whole cancer cell. Perhaps it takes several relevant cancer targets together to constitute the basic blueprint of a cancer cell. Indeed, I predict that the germane cancer targets are likely to be closely associated with certain stem-cell properties.

Although the inherent heterogeneity of cancer is accepted as part and parcel of malignancy, its true nature has never been well explained. Several enigmatic terms have been used to describe this intrinsic heterogeneity of cancer, such as "transformed" to a more aggressive phenotype or "reprogrammed" or "dedifferentiated" to another pathologic entity. Like Proteus, both malignant and stem cells have many faces and may assume various shapes and forms. I propose that the innate heterogeneity of cancer is a revelation of its multipotentiality and its stem-cell origin (Chap. 12). Like the stem cell from which it originates, a malignant cell has the potential to display various phenotypes depending on its stem-cell origin, interaction with neighboring cells, and effects of selection through therapy. I hypothesize that rather than converting into an immature phenotype, a malignant cell is in fact derived from it and can display both immature and mature phenotypes.

In most instances, it is the metastasis that kills. Like a marauding predator, cancer invades and damages various vital organs. The basis for metastasis is believed by many investigators and clinicians alike to be the key to our understanding and treatment of cancer. Recent data suggest, surprisingly, that the seed for metastasis may have already been sown in the primary tumor from the outset (Chap. 13). One cannot help but notice that much of the same prowess that allows a metastatic malignant

cell to migrate, extravasate, invade, and thrive at distant sites is already ingrained within the stem cell from which it is derived. I postulate that the nature of the involved stem cell determines both the resultant malignant cell's predilection to metastasize and its pattern of metastasis.

Another mystery of cancer relates to its uncanny ability to evade an apparently intact immune system (Chap. 14). Like Hermes, stem cells wear a helmet of invisibility at will. Like Hades's agent, cancer cells put on a similar helmet. Not surprisingly, most malignant tumors are not easily recognized or detected by our body's surveillance mechanisms. After all, if a malignant cell does originate from a stem cell, it would be difficult for even a competent immune system to distinguish between the two cell types. Cancer cells appear to leave no tracks. They are insidious or subversive and are more than ready to disrupt the established normalcy of life. For these reasons, extraordinary effort is needed to devise vaccines to treat most cancers. I envision that the theory of a stem-cell origin of cancers will help us realize the plausibility of discovering alternative strategies and improving the design of an ultimate cancer vaccine.

It is a sobering thought that most malignant tumors either acquire drug resistance easily or are inherently drug resistant. Many oncologists have accepted the cruel reality that a vast majority of cancers cannot be cured with our current arsenal of cytotoxic therapies. Like Hydra, cancer seems invulnerable and indestructible. A Herculean effort is required to vanquish it. Again, it may not be a mere coincidence that stem cells are also intrinsically drug resistant. To ensure healthy progeny, stem cells need to protect themselves from any potential harm inflicted on them by various drugs or toxins. I hypothesize that this innate property of drug resistance is passed on from a stem cell to a malignant cell because the latter is derived from the former (Chap. 15).

One way to spark a new idea is to introduce a defining idea. Until recently, we have been quick to attribute all the malignant tricks of a cancer cell to its aberrant genetic changes or signal pathways because of the prevailing belief that they are operative and responsible for its Houdinian acts. In this book, I resist simply reporting a deluge of research data about these aberrant genetic changes or signal pathways. Instead, I will introduce an alternative notion of cancer according to its cellular origins and stem-cell roots. In other words, cancer is more like Lucifer, the fallen angel, than Houdini, the great magician. I anticipate that this change of viewpoint or mentality will substantially modify the future landscape, particularly the focus and emphasis of cancer research. Throughout this book, I highlight and elaborate on several defining ideas about cancer according to the theory of a stem-cell origin of cancers:

- The malignant phenotypes are determined as much by their cells of origin as by their genetic makeup or epigenetic changes. Hence, an aggressive (i.e., higher grade and stage) tumor is a distinct entity rather than a product of rapid transformation from an indolent (lower grade and stage) tumor.
- Many malignant phenotypes may not need to be acquired by a cancer because they are already there from the outset. Therefore, certain cancer targets that elaborate these malignant phenotypes may not be the prime driver of a cancer. As an effect rather than the cause of cancer, they may not even be pertinent, much less ideal cancer targets.

- Phenotypic complexity is greater for tumors arising earlier in a stem-cell hierarchy. Hence, mixed tumors tend to originate from earlier progenitor stem cells, while pure tumors tend to arise from later progenitor stem cells. A mixed tumor may express a pure phenotype under the right conditions but not vice versa.

Admittedly, the theory of a stem-cell origin of cancers will not provide us with an impenetrable truth, nor will it snatch us from the jaws of complacent ignorance. Nevertheless, I believe that it has the power and potential to change the face of cancer: our conception about it and our perception of it. To think that the theory will rock the world of science may be presumptuous. To dream that the hypothesis will have the same effect that Charles Darwin's theory of evolution had on the origin of species or Albert Einstein's theory of relativity on the origin of the universe may be preposterous. But I hope that it will shed some new light on our desire to understand cancer and provide a breath of fresh air that invigorates us in our battle against cancer.

Perhaps critics and skeptics will say that these ideas are trifles. There are already too many ideas and a mountain of data to sort through. However, I believe that the theory of a stem-cell origin of cancers is comprehensive, intuitive, and yet simple enough that it will clarify many of the oncologic conundrums of yesterday, today, and tomorrow. I hope that it will point in the right direction, pave a smooth path, and provide the mighty engine to drive us toward the conquest of cancer as soon as possible. A great idea is not trifling when it saves us priceless time, resources, and energy. It may reduce the pain, agony, frustration, and sacrifice many patients endure when they are diagnosed with cancer and when they receive ineffective treatments for it. Some great ideas build bridges, others, barriers. We definitely do not want to shoot ourselves in the foot when novel or creative ideas become buried in trivialities or hamstrung by detractions.

It may be a foregone conclusion that ideas are worthless without proof. A great idea should be able to withstand the tests of time and scrutiny. The theory of a stem-cell origin of cancers is likely to fling wide open many doors of opportunity in our fight against cancer. It will inspire more novel ideas and instigate even more innovative strategies to elucidate the basic nature of cancer. It will spur us to design more ingenious and relevant experiments. It will facilitate and expedite the institution of more improved and personalized therapies. The theory of a stem-cell origin of cancers may very well lead us into uncharted territory. Indeed, it will be a strange twist of fate as we strive to find the wellspring of cancer if we discover the fountain of stem cells instead. To some of us, the writing is already on the wall about the origin of cancers. I forecast that the theory of a stem-cell origin of cancers is poised to surpass and supplant current doctrines about cancer. It is destined to sow the seeds of a major breakthrough and a sweeping revolution in our understanding and treatment of cancer.

Chapter 2
Cancer Myths

Unicorn was obtained from Microsoft clip art; Pegasus is reproduced with permission from Michael Bossom, Arts Encaustic Ltd., Trem ar Daf, Glogue, Pembrokeshire, Wales, UK

For men are prone to go it blind
Along the calf-paths of the mind,
And work away from sun to sun
To do what other men have done.
They follow in the beaten track,
And out and in, and forth and back,
And still their devious course pursue,
To keep the path that others do.

– Sam Walter Foss, "The Calf-Path"

Précis

Without a correct hypothesis, even the most rigorous scientific methods and promising experimental results can lead us astray, generating and propagating more cancer myths.

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Introduction

A myth (derived from the Greek word *mythos*) is a traditional story, often about how the world and its creatures came into being. The active beings in myths are generally gods, heroes, and exotic creatures. Mythical stories are usually said to have taken place before recorded time. Nowadays, the word "myth" is commonly used to denote something that is widely believed but false. It tends to be a disparaging term used to label the religious (or nonreligious) beliefs and stories of other cultures as being incorrect or even ridiculous.

I consider a cancer myth to be a misconception about cancer that is patently wrong but widely accepted nonetheless. Without scientific proof, it is difficult to dispel these cancer myths. As a result, these myths hold great, almost magical power even today, and the word "myth" is not considered pejorative by any means. It is deeply ingrained in our consciousness and continues to govern our beliefs. This chapter will show that when viewed in a different light, some of our most cherished and revered beliefs about cancer might in fact be cancer myths. I believe that it is only a matter of time before these cancer myths are toppled from their lofty pedestals.

Stem-Cell Origin of Cancers

In a strict sense, any assertion that cancer arises from stem cells is misguided: After all, it is doubtful that cancer originates from actual stem cells, which by nature are dormant. We need to remember that by being dormant, real stem cells do not undergo self-renewal or differentiation. It is therefore hard to imagine that cancer can arise from such immutable cells. Hence, we consider a stem-cell origin of cancer to be a myth. Nevertheless, the truth may be buried in the semantics of stem cells, somewhere between stem cells and progenitor cells.

It is plausible that most malignancies arise not from stem cells themselves but from cancer-initiating cells that have certain stem cell–like properties: We may call them "progenitor stem cells." The exact identity and nature of these cells are still unknown and require further investigation. I hypothesize that there is a spectrum of these progenitor stem cells in the upper echelons of a stem-cell hierarchy, somewhere between stem cells and progenitor cells. When progenitor cells in the lower echelons of this stem-cell hierarchy acquire stem cell–like properties such as self-renewal and differentiation arrest, cancer may also arise. However, the cancer that develops in these cases tends to be relatively less aggressive and less heterogeneous than those that arise from the higher echelons of a stem-cell hierarchy because those in the lower echelons have more progenitor- than stem-cell characteristics.

The idea that cancer tends to occur in the immediate progeny or first generation of stem cells rather than in stem cells themselves is likely to disprove one of our most popular myths. I postulate that the cancer-initiating cells are stem cells that have been newly released from their "stem-cell niche" and are no longer dormant or immobilized. However, these cells still retain the capacity to self-renew and differentiate into multiple lineages. They are committed to undergoing asymmetric

division or have just passed this critical phase. Therefore, a cadre of cancer-initiating cells may not be stem cells as we know them but still retain stem-cell features and thus considered stem cell–like cells. Also, it should not be surprising that not all stem cells are created equal. I hypothesize that there are different types of stem cells just like there are different types of everything else. I propose the existence of a spectrum of cancer-initiating cells whose origin reflects their lineages in the stem-cell hierarchy and captures the real essence of the so-called cancer stem cells.

Therefore, I believe that the strict conception of cancer's arising from a stem cell is a myth. However, such a misconception is quite understandable, because the study of stem cells can be a tricky business. We are currently at a stage in which we do not know exactly what we are looking at or looking for. It is as though we are dealing with the law of uncertainty in biology. If we are not cognizant, the very act of examining a stem cell may transform it. I propose a guiding principle that may dispel the myth of a stem-cell origin of cancers: Cancer arises when self-renewal is uncoupled from differentiation within a progenitor stem cell as a result of defects occurring within that cell or its onco-niche.

Dedifferentiation of Cancer

It is astonishing how many of our most erudite cancer specialists still embrace the notion of dedifferentiation of cancer without any second thoughts. This idea is so pervasive partly because we have always assumed that any differentiated cells in the body can become mutated and cancerous. Furthermore, in comparison with somatic cells, cancer cells appear relatively undifferentiated. Therefore, when undifferentiated cells replace differentiated cells during carcinogenesis, we infer that dedifferentiation has occurred. It seems as though we have adopted the concept of dedifferentiation reflexively and accepted it as the mechanism of action underlying cancer formation without reservation.

Clearly, if our assumption that any cells can become mutated and cancerous is false or flawed, then the playing field will be quite different. Similarly, when the equations are changed, we have a new formula, and when themes are altered, we have a new story. According to the theory of a stem-cell origin of cancers, the conversion of a differentiated phenotype to an undifferentiated one during malignant transformation is ill conceived and misconstrued. Expression of the undifferentiated phenotype in a malignancy is a manifestation of its stem-cell origins. Today, dedifferentiation is perhaps one of the most preposterous yet pervasive cancer myths.

Magic Bullets

We still cling to the dream of finding a magic bullet to destroy cancer. Such a magic bullet may be considered a myth when we do not know the origins of cancer and a hoax once we begin to understand them. Until recently, we have relied on

the principles of the gene theory to explain many inner workings of the cell. Discrete genes produce discrete proteins, which elaborate discrete functions within the cell. It follows that when a mutated gene produces a defective protein, the aberrant protein will disrupt normal functions within that cell. Advanced cancers happen to develop and accumulate more mutated genes than early cancers do. We reason that mutated genes play a critical role in malignant transformation and that finding magic bullets to repair any damage caused by the mutated genes may lead to a cure for cancer.

Many of our current cancer models – and cancer myths – are founded on the gene theory. For example, Hahn and Weinberg [1] suggested that several discrete genetic mutations could account for the formation of cancer. On the basis of the laboratory criteria for malignancy (e.g., colony formation, anchorage independence), they manufactured cancer cells from normal cells by introducing a finite number of specific mutations (i.e., pRB, p53, ras, and telomerase). Unfortunately, other criteria for malignancy (e.g., invasion and metastasis) remain largely neglected in this laboratory model. Nevertheless, if the principles derived from the gene theory are true, a magic bullet that reverses or repairs the pertinent mutated gene should be able to counteract the malignant process.

Recently, findings from the human genome studies have challenged the established notions [2]. No longer can we regard the protein-coding component of genes as the only relevant protagonists in the functions of a cell. After all, they make up only a small fraction (1.5–2%) of the human genome. Evidence suggests that other parts of the genome, including vast amounts of the so-called junk DNA, which until now have been thought to be biologically inactive, also play important roles. The human genome is not composed of a few genes that determine function. Instead, it is composed of a vast interwoven network of genes and nongenes with very few unused DNA sequences. In this intricate system, genes are but one of the many types of DNA sequences that engender a particular function. Therefore, the idea of a magic bullet that targets a mutated gene in an effort to fix a malfunction is no longer tenable. Instead, we may need a "smart bomb" that targets an entire functional network to be able to accomplish this mission. Considering how complex cancer can be, I doubt that fixing a single genetic defect with a magic bullet will get the job done.

Therefore, the time has arrived for us to replace our traditional but obsolete gene theory with a brand-new network theory. Our previous assumptions about magic bullets based on the gene theory need a belated facelift. It is no longer surprising that so many biologic phenotypes, such as intelligence, weight, height – and cancer – are not straightforward, because they are governed by this network of genes and nongenes rather than by discrete genes. It is also no longer surprising why so many selected mutations turn out to be disappointing cancer targets because they are neither relevant nor valid. When laboratory results are obtained on the basis of a wrong theory and are interpreted in the wrong context, they become an experimental artifact rather than a scientific fact. In light of this new network theory, the existence of a magic bullet for cancer therapy is a cancer myth.

Multistep Carcinogenesis

When current doctrines are reexamined in a new light, many old dogmas turn out to be odd myths. For example, one of the proudest products of the gene theory is the idea of multistep carcinogenesis. We have always accepted without question that advanced tumors have more genetic mutations than early tumors have (Fig. 2.1). At first glance, this notion seems completely logical and makes perfect sense. But what if fewer mutations are required to form a full-fledged cancer in early stem cells because they are already endowed with many stem-cell or malignant properties? (Fig. 2.2). Conversely, late progenitor stem cells would need to acquire more mutations to become fully malignant because they have fewer stem-cell or malignant traits to begin with. It is astonishing how this idea is a complete reversal of our currently accepted cancer models!

In a similar vein, do all tumors follow the rule of evolution from low-grade to high-grade tumors and from low-stage to high-stage tumors? Are tumors at lower stages more amenable to surgery because there is a window of opportunity to resect them before they disseminate? Or is it because they are different, intrinsically more confined tumors in the first place and therefore more amenable to surgery? Do some tumors skip many of the steps in tumor grade and clinical stage? Do cancers acquire certain mutations that produce more aggressive phenotypes, or do they merely express more stem-cell features when the occasion arises? Also, should we attribute

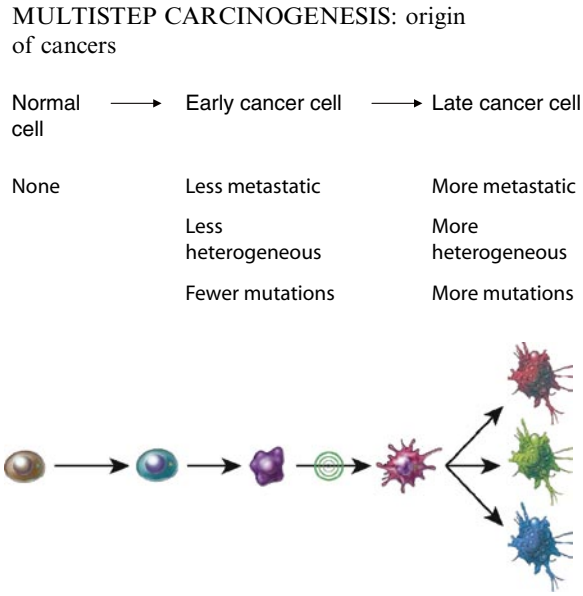


Fig. 2.1 The multistep model of carcinogenesis

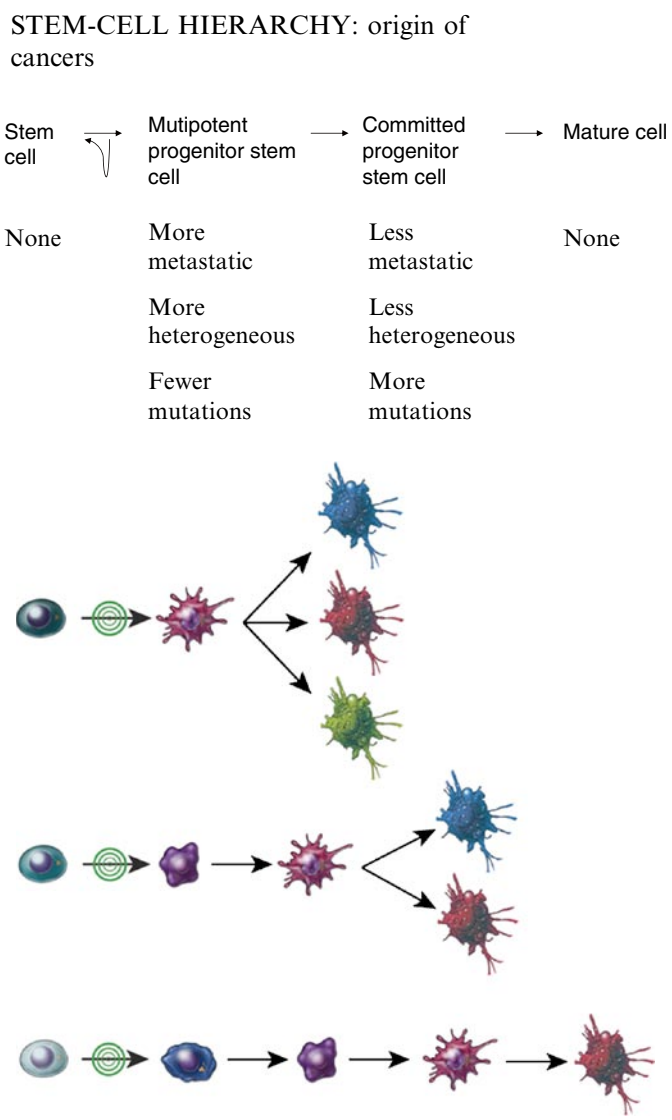


Fig. 2.2 In contrast to the multistep model of carcinogenesis, the stem-cell theory of cancer predicts that earlier stem cells require fewer mutations to become fulminantly malignant (i.e., more metastatic and heterogeneous) than later progenitor stem cells require to become just as malignant

many of the epigenetic changes that occur during carcinogenesis to malignant transformation or stem-cell manifestations? One cannot emphasize enough why and how our radical new theory (if it has any merit at all) may completely shatter the myth of multistep carcinogenesis and have profound ramifications on its many offshoots, including the prevention, screening, and early detection of cancer.

Cancer Signatures

There is every reason and considerable incentive for us to validate the individual genetic mutations and to credential the global molecular signatures of cancer. This objective takes into account the intricate networks that may be at play simultaneously in a cancer cell. Cancer signatures, which may be unique to an individual like our personal signatures, may help us reach the ultimate goal of personalized medicine and improve the diagnosis, prognostication, and treatment of cancer. But according to the theory of a stem-cell origin of cancers, is a cancer signature also a cancer myth?

There is reason to suspect that cancer signatures, as we have envisioned them, are an illusion. The notion that there is a static, stable cancer signature that helps us to categorize the disease, predict its behavior, and formulate its therapy is a myth. The technology and the statistics behind the signatures (e.g., microarray studies) may be "sexy," but in the end, do the cancer signatures really represent what we believe they represent? Without a proper understanding of the origin or fundamentals of cancer – without even the right perspective or framework – how do we tell whether a signature is counterfeit? For example, if the cancer stem cells are buried deep within a tumor, then the cancer signature from the bulk of the tumor may not be truly representative; i.e., the differentiated cancer cells may not reflect the hidden cancer stem cells. In addition, it is conceivable that a tumor's expressed signature may be dynamic, changing with the condition of the immediate microenvironment at a given time. If a signature is in constant flux without a common thread, trend, or trait, it will hardly be useful for the diagnosis, prognosis, or therapy of cancer.

Epithelial-to-Mesenchymal Transition

Another popular cancer myth is that a malignant cell starts as an epithelial cell and changes into a mesenchymal cell during the early phases of cancer metastasis. Because we have always assumed that carcinomas both start and end epithelially, epithelial-to-mesenchymal transition may be regarded as the magical and mysterious force that enables an epithelial cell to acquire mesenchymal features as it detaches and migrates from its site of origin. When a metastatic cell reaches its destination, an equally magical and mysterious force, namely mesenchymal-to-epithelial transition, enables the mesenchymal cell to become epithelial again.

But what if carcinomas do not start as epithelial cells, beginning instead as embryonic or stem cells, as Virchow first envisioned? Then it becomes apparent that a particular malignant cell can behave like a stem cell (instead of an epithelial cell), which is fully equipped and quite capable of displaying mesenchymal and other cellular features, including those of epithelial cells. When a metastatic stem cell reaches its destination, the local niche may influence its differentiation or conversion into various phenotypes, including the more common epithelial components. Therefore, the theory of a stem-cell origin of cancers exposes the fallacy of

an epithelial origin of cancers. It does account for the various guises of stromal–epithelial cellular interactions and for the ubiquitous manifestations of epithelial-to-mesenchymal transition or mesenchymal-to-epithelial transition in cancer. In the end, these different terms speak about the cancer cells' usurping the same developmental processes involving various embryonic or stem cells during embryogenesis to serve their own malignant purposes during carcinogenesis.

Gene Therapy

Gene therapy for cancer is not simple. For one, cancer possesses innumerable genetic mutations. We have already learned that individual tumors may accumulate as many as 90 mutated genes. Do we need to fix every one of them? How do we determine which mutations are pertinent and need to be fixed by gene therapy? It is true that only about 11 of the multitude of mutated genes contribute to the neoplastic process. Even if we need to fix only the critical ones, are they the same for a particular tumor type in different patients? As we learn more about the genetics of cancer, we also realize that gene therapy for cancer may be an illusion…and a myth.

Cancer Vaccine

The belief that a cancer vaccine is a cure-all is also a myth. If the origin of cancer has any stem-cell basis at all, then it is likely that most cancers will not respond to vaccines. We surmise that a majority of cancers possess innate immunity owing to their stem cell of origin, which defies vaccination. Indeed, this pessimism has so far been substantiated by the generally lackluster performance of cancer vaccines: a response rate of only about 3% [3].

This is not to say that cancer vaccine has no place at all in our battle against cancer, only that we need to define its proper role in an overall strategy to treat cancer and to dispel any myth about its cure-all potential. For example, cancer vaccines may be ideal in certain special tumors that express viral antigens (human papillomavirus in cervical cancers, hepatitis B and C viruses in hepatoma, Epstein–Barr virus in lymphoma). Although they have different mechanisms of action in the formation of cancer, these viruses may still involve the cancer-initiating cells or cancer stem cells. Another important clinical implication is that for a cancer vaccine to work at all, it must be used in the setting of low tumor burden or minimal residual disease for a low-grade or well-differentiated tumor (when patients with such tumors tend to fare better anyway). Hence, it is not surprising that the only FDA-approved vaccines to prevent cancer are those designed against virally induced malignancies (i.e., human papillomavirus and hepatitis B virus). The idiotypic protein vaccine (Id-KLH + GMCSF) by Kwak et al. [4] was designed for

low-grade follicular lymphoma, and the PR1-CTL vaccine by Molldrem (unpublished data) worked best in the setting of minimal residual disease in a patient with Inv16 acute myelogenous leukemia.

"Hypothesis-Generating" Research

In many respects, a myth is a product of our ignorance about and misunderstanding of the world around us. Out of genuine respect, curiosity, and awe, we have created some of the most fantastic ideas and stories to explain the many wonders and mysteries around us. Similarly, a cancer myth arises from our utter ignorance and misconceptions about cancer. Without a correct hypothesis, even the most rigorous of our scientific methods and experimental results can lead us astray, generating and propagating more cancer myths.

A common misconception is that experimentation is the final arbiter of truth. It may be true that experimentation is one way for us to separate fact from fiction. However, if we forget a basic principle of science and regard experimentation as the end rather than the means to an end, the results of that experiment may become a myth rather than the truth.

In principle, an experiment is designed to *test* a hypothesis, not to *formulate* one. By nature, an experiment involves controlling the various parameters and limiting the scope of its relevance. Results of an experiment may help us modify the hypothesis in question: Subsequent experiments then need to be performed to validate the revised hypothesis. Believing that the results of an experiment will advance a concept rather than merely support a hypothesis is a myth. Hence, we witness the advent of the so-called hypothesis-generating experiments. With a wrong mind-set and in an incorrect context, we must be careful not to turn a seminal experimental result into an egregious laboratory artifact and thus to propagate a cancer fallacy and cancer myth.

"Translational" Research

One cannot help but applaud the innumerable laboratory researchers for their ingenuity and perseverance. It seems there are no experiments they cannot do. The human urge to explore the unknown and test the unimaginable is insatiable. We are always ready to engage in interesting games and challenging tricks: They are both fulfilling and exhilarating. As long as we remember and understand that we are dealing with experiments and not reality, this is indeed a noble undertaking. However, as soon as we forget that we dwell in the virtual world of the laboratory and that we have manufactured artificial rules for our experiments, we are heading for a rude awakening. We love to play God. But we forget that creating life, stem cells, or cancer in the laboratory is only a gimmick. To think otherwise is to immerse ourselves in a grand delusion. Unless we constantly remind ourselves, we may

become entranced by nonsensical ideas and engage in worthless projects. A cardinal sin of experimental fallacy is to consider its meanings beyond its intentions: We forget that experiments are designed to test rather than to reflect reality. When we regard the results of an experiment literally and out of context, we are treading on forbidden if not treacherous ground.

For these reasons, it is a myth for us to think that cancer cures will come to the clinics by way of the laboratories. What we do in the laboratory is designed to uncover the basic mechanisms underlying cancer. Unfortunately, the results of laboratory research will not necessarily help us devise better treatments for cancer. Taking what happens in the laboratory directly to the clinic constitutes a gigantic leap of faith that is prone to failure and disappointment, especially when considered for the wrong disease types and patient populations. In each laboratory experiment, we dissect and test isolated events. In clinical studies, we examine and evaluate aggregate effects. Therefore, what happens in an experiment is not what happens in real patients. Without a clear understanding about the origin or nature of cancer, selection of the proper treatment for the right patients on the basis of experimental results can be quite haphazard and unreassuring.

Translational research thus means more than just moving forward with laboratory results to clinical trials. It means that we need to have better vision and rationale as well as greater relevancy regarding the cancer questions, issues, and problems. It means that conquering cancer has to begin in the clinics (where we really learn about these cancer questions, issues, and problems), make the transition into the laboratory (where we can elaborate on the mechanisms of action and signal pathways), and then move back into the clinics again. In the clinics, rather than in the laboratory, is where a correct hypothesis about the origins or nature of cancer is likely to be conceived and born.

Technologic vs. Scientific Breakthroughs

We are easily impressed and fascinated by technologic advances. But technologic advances do not necessarily guarantee scientific breakthroughs. A scientific breakthrough begins with the right hypothesis. Without the right hypothesis about the origin of cancer, many whose lives (rather than careers) depend on it will continue to suffer much more unnecessary agony, frustration, and disappointment. We need to realize that equating technologic advances with scientific breakthroughs is a cancer myth.

There is no denying that technologic advances enhance our knowledge base and our basic understanding of cancer. There is no dispute that our ever-improving bioinformatics systems, genomic and proteonomic studies, and so forth will continue to generate an inordinate amount of data. But an incredible amount of information may confound us just as much as it may enlighten us. Without the right hypothesis, it will be difficult for us to design the proper experiments, assimilate the results, comprehend their significance, or fulfill their utility. It is true that eventually we may still reach these goals by trial and error or by chance and luck. But

do we really need to take a more arduous and tortuous route to arrive at our destination? Do we really want to take a more time-consuming and resource-draining path to reach these goals?

Curing Cancer

We live in a historic time of cancer therapy. Never before have we had so much oncologic capital and so many dividends. Understandably, both the stakes and expectations of curing cancer are high. The bottom line is that many cancer patients have fared well from the application of novel and improved therapies. What has apparently escaped scrutiny, however, is that not every cancer patient benefits from all this therapeutic bounty. This observation begs a simple question: As long as our knowledge continues to accumulate and our treatments continue to improve, is it only a matter of time before we find a cure for cancer? This observation also stimulates an unsettling reservation: Is it possible that some of our current treatments provide clinical benefits because they happen to hit the right targets but for the wrong reasons? The former proposition predicts that we will eventually arrive at our destination of a cancer cure, whereas the latter assumes that some cancers simply cannot be cured (but can be conquered). It is the journey of cancer treatment rather than a destination of cancer cure that counts. In either case, we need to brace for a long and bumpy ride as we travel toward a nirvana of cancer cure.

Whom do we kid when we proclaim that one day in the near future all cancers will be cured? It is true that some cancers can be cured and that many cancer patients have been cured. But other cancers are blatantly lethal despite our best efforts to prevent, screen, diagnose, and treat them. These are the "real" cancers that still bear its ominous name and carry out its deadly deeds. They seem to have a manifest destiny. It seems that the best we can do with these "real" cancers is to accept the inevitable and to mitigate any suffering. After all, we can always treat a patient without curing the cancer. Indeed, some patients die *with* the cancer rather than *from* it. Although they were not *cured*, they did manage to *conquer* their cancer. Conquering cancer means that we aim for the best possible clinical outcome in a given case, provided that we recognize its exact origin and nature and prescribe the optimal and appropriate management for it.

Once the dust finally settles, we get a general sense that a sweeping proclamation about curing all cancers is rooted if not mired in myth.

The legendary king Canute the Great knew that certain forces are greater than we are. He demonstrated this with the simple example of our inability to turn away the tides on a shore. We similarly cannot change the course of many other aspects of nature. Once we understand the origins and nature of cancer and recognize not only our aspirations but also our limitations, it becomes a matter of accepting the calling of certain cancers. What pushes its buttons or pulls its strings? What are the collaborators and conspirators in its malevolent enterprises? Once we comprehend how a cancer runs its course, we are bound to become humbled about the reality of

curing it. No longer should we subject ourselves to the myth perpetuated by those who out of ignorance or cunning try to persuade us that we can cure all cancers.

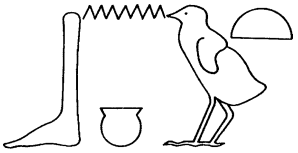
Conclusion

Everyone loves a great story. It seems that the human race learns better from parables and fables than from the rote recitation of dry facts. The fantastic stories passed down to us through the ages by our ancestors speak volumes and leave an indelible mark in our memories and a lasting impression on our minds. Cancer can be both a mystery and horror show. It is often a tragedy and a drama with more than its share of heroes and villains. As we revisit the vast unknowns of cancer, we are easily held hostage by dogmas or myths and become bombarded by hype or hoax. The theory of a stem-cell origin of cancers is a tale that needs to be told. It is a story worthy of being pursued and retold. Although on the surface its message sounds very obvious and familiar, its meaning has so far been just beyond our grasp and its implications still well over our reach. A benchmark for a great story is that it touches our mind and soul. It benefits both mankind and humanity. It is bigger than a myth.

References

1. Hahn WC, Weinberg RA (2002) Rules for making human tumor cells. N Eng J Med 347:1593–1603, Erratum in N Engl J Med 2003;348:674
2. The ENCODE Project Consortium (2007) Identification and analysis of functional elements in 1% of the human genome by the ENCODE pilot project. Nature 447:799–816
3. Rosenberg SA, Yang JC, Restifo NP (2004) Cancer immunotherapy: moving beyond current vaccines. Nat Med 10:909–915
4. Kwak LW, Young HA, Pennington RW, Weeks SD (1996) Vaccination with syngeneic, lymphoma-derived immunoglobulin idiotype combined with granulocyte/macrophage colony-stimulating factor primes mice for a protective T-cell response. Proc Natl Acad Sci USA 93:10972–10977

Chapter 3
History of Cancer



The hieroglyphic for the word *tumor* as described in the Edwin Smith papyrus. For details, refer to Breasted's translation of the document [1]. Reproduced with permission from Marcel Dekker, Inc., New York – Pitot HC (1986) Fundamentals of oncology, 3rd edn

From out of olde feldes, as men seyth,
Cometh al the newe corn, from yer to yere,
And out of olde bokes, in good feyth,
Cometh al this newe science that men lere.

– Chaucer, *Parliament of Foules*

Précis

Rudolf Virchow envisioned embryonal cells as having the capacity to generate different types of cancer, including those of an epithelial nature, even though they resided in the connective tissue of various organs.

Introduction

When we talk about any subject, knowing about its history somehow gives it more legitimacy and venerability. Knowing the history of a subject also provides us the opportunity to give credit and pay tribute to our pioneering forefathers who had the wisdom and courage to lay the cobblestones and plant the trees so that we can travel on our journeys and enjoy the bountiful fruits.

S.-M. Tu, *Origin of Cancers: Clinical Perspectives and Implications of a Stem-Cell Theory of Cancer*, Cancer Treatment and Research 154,
DOI 10.1007/978-1-4419-5968-3_3,

When we talk about the origin of cancer, it is difficult not to venture into the history of cancer. In some mysterious way, the origin of something always seems to intertwine with its history. Although the history of cancer deserves a whole book in its own right, this chapter highlights a few notable aspects of the many fascinating stories about cancer through the ages.

Paleo-oncology

Assuming that cancer is an intrinsic part of life, it can form during the lifetime of any living creature, and all creatures may develop cancer if they live long enough. Did the primeval dinosaurs, hominids, and other vertebrates also develop cancer and die from it? Hard evidence for the existence of cancer at the dawn of time may be found in fossilized bone since bone is the only organ that remains long after death. One may also confirm the presence of cancer in another relic, namely the human language, by words denoting "tumor" in ancient scrolls and historic texts.

Very few cancers have been found in the fossilized remains of long-extinct dinosaurs that roamed the earth between 230 and 65 million years ago. Most bone tumors found in dinosaurs probably resulted from injury, arthritis, or other nonmalignant diseases instead of cancer [2]. The first case of a malignancy might be a chondrosarcoma discovered in a fossilized theropod dinosaur (*Allosaurus fragilis*) found in the Late Jurassic Formation in Utah; it was a large mass of newly formed bone that had invaded the humerus [3]. Another fossilized bone fragment showing multiple lytic lesions with cortical bone invasion suggestive of metastatic cancer was discovered in a large terrestrial dinosaur found in the Jurassic Morrison Formation in Colorado [4].

To date, no definite case of cancer has been found in ancient humans, who first appeared on the evolutionary scene about 4 million years ago in Africa [5]. Louis Leakey was thought to have found the oldest hominid tumor in the fossilized jawbone of a Neolithic man living about 500,000 years ago in Kanam, Kenya, but although this tumor had features consistent with those of Burkitt lymphoma, it was later diagnosed as a benign bone callus of an infected fracture [6, 7]. Another interesting fossilized remnant belonged to a *Homo erectus*, or Java man, who lived along the Solo River in central Java about 500,000 years ago. He had a growth in the bone on the posterior aspect of his left femur near the insertion point of the adductor muscle [8]. For years, this lesion was thought to have been a bone sarcoma, but now it has been diagnosed as myositis ossificans, a nonmalignant lesion that occurs after injury and hemorrhage into the muscle, which subsequently becomes scarred and calcified [6, 8].

Thus, cancer appears to have been a rare entity in antiquity. Among the thousands of fossilized bones from Neanderthal men found in Europe, only one, the Stetten II parietal bone from Germany, dating to about 35,000 years ago, has been suggestive of the presence of a tumor, namely a meningioma [9]. And in

a review of thousands of ancient Egyptian mummies, Pahl [10] reported finding only 44 cases of neoplasm. Of these, seven were determined to be nasopharygeal carcinoma [11] and nine were interpreted as multiple myeloma [12]. Strouhal [13] also reported having identified 176 cases of malignant neoplasms in ancient European populations. It was only after the Middle Ages that the number of documented cases of cancer increased significantly. Hence, cancer was strikingly uncommon in prehistoric and ancient human populations.

Civilization vs. Age

Some say that cancer is characteristic of vertebrate animals. It would be impossible to detect cancer in invertebrates existing before the dawn of time because they left no skeletal remains. Perhaps cancer does not develop in invertebrates because of their shorter life span. Cancer is extremely rare in amphibians and birds and occurs only slightly more frequently in fish, reptiles, and the lower mammals. The earliest known case of a possible tumor was found in a *Dinichthys*, an armored fish from the Upper Devonian period (about 350 million years ago), that was found in Cleveland, Ohio [14]. The earliest known case of an unequivocal neoplastic tumor was found in the partial skeleton of a North American Lower Carboniferous (about 300 million years ago) fossil fish, *Phanerosteon mirabile* [15]. As a consequence of natural selection, sick animals were perhaps easy prey and had a slim chance of surviving any handicap and passing on their liability to their offspring.

The earliest examples of cancer in mammals – osteosarcoma – were found in the fossilized remains of a Pleistocene buffalo and a Holocene goat [16, 17]. It is remarkable that no neoplasm was identified among the millions of fossilized bones recovered and examined from a multitude of animals that were trapped and died in a Pleistocene freshwater lake that contained a tar seep in Rancho La Brea, California, more than 2 million years ago [2]. In light of the vast number of fossilized bones of reptiles, birds, and mammals that have been studied, neoplasms were indeed an exceedingly rare occurrence in prehistoric times.

One might argue that dinosaurs and prehistoric animals in their heyday as well as the ancient hominids did not live long enough to develop cancer. The odds of finding a rare cancer preserved in the fossilized remains of even an occasional individual with some longevity are infinitesimal. However, the numbers tell another story: Cancer incidence does not always correlate with life expectancy [2]. For example, the highest incidence of cancer occurs in countries (e.g., Hungary, Belgium) that do not have the highest healthy life expectancy (HALE) at birth. Several countries that have a high HALE (e.g., Japan, Switzerland, Sweden) have a low incidence of cancer. On the other hand, some countries that have disparate HALEs (e.g., United Arab Emirates, Ethiopia) have an equal incidence of cancer, and some countries with geographic proximity and similar HALEs (e.g., Paraguay, Uruguay) have considerable differences in cancer incidence [18].

It has therefore been hypothesized that the evolution of human civilization has played an important role in the evolution of cancer. In other words, changes in the environment (e.g., pollution), diet, and life style may have contributed to the increased prevalence of cancer in humans over the last centuries. It is interesting that the prevalence of cancer also happens to be much higher among animals that have been domesticated or are in captivity than it is among wild animals, although this observation could be a consequence of the longer life span of the former animals or our better surveillance of them. Perhaps this is just another price these animals have had to pay for living in our civilization and sharing with us our pollution, diet, and lifestyle.

Written Evidence

Human language preserves our heritage and is one of our greatest treasures. Although tumors were described in many ancient texts, it is apparent that people did not recognize cancer as a distinct disease entity until fairly recently. Diseases in ancient times were often associated with the presence and power of supernatural beings or evil spirits. Magic and exorcism were used to protect health and to repel the demons or fiends that caused disease. Among believers in reincarnation, sins committed in earlier lifetimes may be the cause of disease. In the Chinese philosophy of harmony in life, disruption of that harmony by natural forces in the universe can result in disease and death. And in the Hebrew tradition, health was given to and taken from man consistent with the monotheistic concept of Jehovah or God. Because disease could afflict humans as a consequence of their sins, prayer and repentance were necessary for healing and preventing recurrence.

The oldest known written description indicating that cancer afflicted our early ancestors was found in seven papyri dated around 1600 BC in Egypt, in which a hieroglyphic meaning “tumor” of the breast was depicted (see the figure at the beginning of this chapter). The Edwin Smith papyrus described a case of bulging tumors of the breast that was consistent with a diagnosis of breast cancer and also described surgery for cancer [1]. Similarly, the George Ebers papyrus [19] mentioned tumors of the god Chonsu, the descriptions of which were consistent with a diagnosis of osteolytic lesions of metastatic cancer, multiple myeloma, or malignant lymphoma and also outlined pharmaceutical, mechanical, and magical treatments for cancer.

During the Sumerian period (around 3000 BC) in Mesopotamia, Chaldean (Akkadian) cuneiform incantations contained words and phrases that suggested the presence of cancer. Lenormant’s [20] English translation of one of these ancient documents described “ulcers of a bad kind,” “ulcers which spread,” and “the nurse whose breast becomes ulcerated, and the nurse who dies of the ulceration of her breast.”

The Sanskrit health scriptures, known as the four Vedas, date back to 2000 BC. Disturbances in three humors (doshas) – the Váyu (wind), Pittam (bile), and Kapham

(phlegm) – were believed to cause imbalances in the Rakta (blood) and to lead to diseases. A treatise by Susruta, a royal physician to the king of Gandhara in 120–162 AD, described tumors called Gulmas inside the body. The Susruta Samhitã also mentioned tumors of the nose, rectal and urinary passages, vagina, and skin, and some that appeared as a chain in the neck [21]. Although many of those lesions could be attributable to an infectious process, some of them could be related to a malignancy. The Hindi word for cancer (the illness) is karka T rog (Fig. 3.1a), the Latin equivalent of which is "crab."

Written Chinese medical records began in early 2000 BC. The classic texts, including the Shu Ching (Classic History) and the Shih Ching (Book of Songs), were written not long after 1000 BC [5]. However, cancer was not recognized as a disease entity in these texts, and there was no evidence to support the idea that a diagnosis of cancer was made in ancient China [5]. The Chinese character for cancer, ái, is shown in Fig. 3.1b.

In the Bible, Titus is said to have experienced headaches for 7 years after he destroyed the temple in Jerusalem. At autopsy, a tumor was found in his brain. Given the relatively long course of his disease symptoms, one might speculate that

Fig. 3.1 (**a**) Karka T rog, the Hindi word for cancer (the illness) written in Hindi. Courtesy of Dr. Indranil Dutta, Center for the Study of Languages, Rice University, Houston, Texas. (**b**) Ái, the Chinese word for cancer. Courtesy of Dr. Pei-Chun Tu, Sacramento, California. (**c**) Karcinos, Greek for cancer. Courtesy of Dr. Dora C. Pozzi, Department of Modern and Classical Languages, University of Houston, Houston, Texas. The Hindi and Greek words both mean "crab"

he had a meningioma. Similarly, King Jehoram is said to have died 2 years after suffering from a bowel disease. It is possible that he had colorectal cancer [22]. Again, it appears that cancer was not recognized as a discrete disease entity in the Talmud, the Bible [5], or the Koran.

Brief History of Cancer

Hippocrates (460–370 BC), the great Greek physician, is considered by many to be the father of medicine and thought to have been the first person to clearly recognize a difference between benign and malignant tumors. During his time, there was a sense that little could be done for patients with cancer. It was concerning cancer that he applied one of the cardinal rules of medicine: *Primum non nocere* (i.e., first do no harm). He noticed that the blood vessels surrounding a malignant tumor looked liked the claws of crab. Hippocrates is thus the person who coined the word "karkinos" (Greek for "crab"; Fig. 3.1c) to describe these tumors [23]. This term translates to carcinos or carcinoma in English.

In the second century AD, Claudius Galen, another great physician, distinguished tumors "according to nature" from those "exceeding nature" and those "contrary to nature." Galen also suggested a similarity in gross outline between a crab and "tumors contrary to nature," which would be considered equivalent to the cancer as we know today. Hippocrates and Galen are credited with transforming the practice of medicine from superstition and magic to observation and logical reasoning. They tried to explain the cause of cancer as a result of excessive black bile produced by the spleen and stomach (i.e., the Humoral Doctrine [5]).

In 1761, Giovanni Morgagni [24] of Padua, a physician, pathologist, and teacher, published a report of about 700 autopsies, most of which he or his friends had performed, in a book titled *De Sedibus et Causis Morborum (The Seats and Causes of Disease)*. Morgagni described anatomic findings of cancer in organs and attempted to correlate these findings with clinical signs and symptoms. His study showed that cancer was a discrete entity that involved a single organ. The results of his work contradicted the prevailing Humoral Doctrine and laid the foundation for oncology, or the study of cancer [5].

It was not until the nineteenth century AD that physicians and scientists began to study cancer more systemically and intensively. Marie Francois-Xavier Bichat [25], a French surgeon–pathologist, described the anatomy of many neoplasms in humans and suggested in his *Traite des Membranes* that cancer was an "accidental formation" of membranes or tissues in an organ. Thus, Bichat further refined the idea that various tissue components made up parts of organs and that cancer could be made up of the same elements in a similar manner.

The existence of the microscope, invented by van Leeuwenhoek in the late seventeenth century, also added momentum to the quest to find the cause of cancer. Johannes Müller [26] extended the findings of Bichat and demonstrated, with the help of a microscope, that the cancer tissue was composed of cells; he

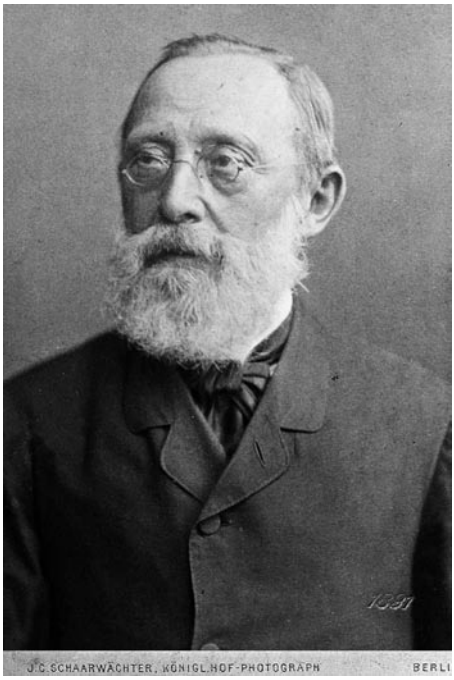


Fig. 3.2 Rudolf Ludwig Karl Virchow (1821–1902). Photographed by J. C. Schaarwächler in 1891. Reproduced with permission from the Wellcome Medical Museum, London, UK

reported his findings in a publication called *On the Finer Structure and Forms of Morbid Tumors*.

Rudolf Virchow (Fig. 3.2) is considered by many to be the founder of cellular pathology. He correlated the clinical course of illness with microscopic findings and provided the scientific basis for the modern pathologic study of cancer. He was the proponent of a famous doctrine, *Omnis cellula e cellula* (i.e., every cell from a cell). Just as an animal can spring only from an animal and a plant only from a plant, a cell must arise from a previously existing cell [27]. However, the question remains whether a cancer cell originates from a normal cell, and if so, what kind of normal cell.

History of the Stem-Cell Theory

Two general theories to account for the origin of cancer have persisted to this day. First, Laennec believed that normal cells could convert to cancer cells. He noted that many cancers resembled normal tissues of the body and that "there are as many varieties of these as there are kinds of normal tissues" [28]. In other words, *omni cellula e cellula ejusdem naturae* (every cell arises from a cell of the same kind). But Müller subscribed to an alternative concept: Cancer cells exist

from the embryonic stage of life but do not express themselves until later in the organism's existence.

In 1863, Virchow began a series of lectures about cancer's arising from a reservoir of undifferentiated cells in the connective tissue of various organs [29]. Virchow thought that these embryonal cells (i.e., stem cells!) were multipotent and had the capacity to generate different (e.g., epithelial) types of cancer even though they resided in the connective tissue of various organs. Despite his extraordinary foresight, Virchow's views about the origin of cancer were strongly opposed and eventually abandoned by the scientific community. The work of Carl Thiersch [30] and Wilhelm Waldeyer [31], who showed that epithelial cancers were derived from epithelial cells and not from cells within the connective tissue, helped to discredit Virchow's ideas about the stem-cell origin of cancer until recently.

In 1875, Cohnheim [32] published his theory of "embryonal rests" to explain the presence of displaced muscle germ cells in Wilms' tumor. Embryonal rests were defined as misplaced embryonal cells from development and the source of tumors that form later in life. He hypothesized that these displaced embryonal cells are incorporated into adult tissue and subsequently give rise to diverse malignant phenotypes, including the malignant muscle and kidney cells in a Wilms' tumor. However, other cells displaced during embryogenesis, such as APUD (amine precursor uptake and decarboxylation) cells in the adrenal medulla and thyroid cells along the thyroglossal duct, do not normally become malignant. These embryonal rests are not found in the earliest stages of cancer such as carcinoma in situ. Although a few cancers (e.g., extragonadal germ-cell tumors) may develop from embryonal rests, it is clear that most do not.

It is ironic that Virchow, the "pope of pathology," was probably the first person to hypothesize a stem-cell origin of cancer. Various versions of the embryonal rests theory are but offshoots from his ideas. Virchow believed in the existence of a reservoir of multipotent undifferentiated cells that are capable of generating many different types of cancer. He thought that these ubiquitous cells reside in the connective tissue and are capable of engendering epithelial tumors. Although, as noted earlier, this particular view of cancer's origin was discredited until recently, it was the first to have some semblance of the current theory of a stem-cell origin of cancers. Because of the strong opposition he encountered and the virtual impossibility of proving the presence of undifferentiated cells in connective tissue and their association with the origin of any cancers, Virchow never committed or could commit his belief to printed form [5].

Virchow continued to believe for the rest of his life that most carcinomas arise from precursor cells in the connective tissue [33], but unfortunately, his idea about a stem-cell origin of cancer was before its time. Even today, we have difficulty identifying the elusive stem cells. However, it seems only proper that we credit Virchow as the first person to advance the theory of a stem-cell origin of cancers. When we eventually prove that most cancers do arise from stem cells or stem cell–like cells and that these stealthy cells are indeed located in the connective tissues, Virchow's original vision will finally receive its due!

Saint Peregrine

There is no denying that history still governs many aspects of our lives. We physicians take the Hippocratic oath before starting our life's work. The principle of *primum non nocere* still governs our mind, our conscience, and our behavior when we are given the responsibility and privilege of treating all patients, particularly those with cancer.

Another crucial aspect of patient care is faith, especially when it concerns the care of patients with cancer. The power of faith is embodied by the patron saint of cancer (or of any serious illnesses), Saint Peregrine. Today, many cancer patients and their loved ones, health workers, and researchers seek strength and solace through special intermediaries like Saint Peregrine [34].

Peregrine Laziosi was born in 1265 in the town of Forli, Italy. He was the only son of a wealthy couple with a noble background. In his youth, Peregrine had strong opinions and was politically set against the Papacy. In 1283, Saint Philip Benizi was sent by Pope Martin IV to convince the people of Forli to return to full communion with the Church. Instead, he was driven out of Forli by an angry crowd. Peregrine heckled and struck Saint Philip. Remarkably, Saint Philip did not resist and offered his other cheek in response. Peregrine was deeply moved by this divine gesture. He confessed and promised to change his ways.

Peregrine began to channel his energy into good deeds. He showed great compassion and devoted his life to the sick and poor. He joined the Servants of Mary at Siena and was ordained a priest. Later, he returned to his hometown of Forli and started a Servite mission there. It was said that he performed many penances, one of which involved standing as long as it was not necessary for him to sit. Eventually, he developed varicose veins and a painful nonhealing cancer. The doctor who diagnosed his cancer, Paolo Salazio, decided that Peregrine's leg needed to be amputated. The night before surgery, Peregrine prayed before an image of the crucified Christ. He asked God for healing, but he also prayed to accept God's will for him. In his sleep, he had a vision that Jesus came down from the cross and touched his cancerous leg. Miraculously, when Peregrine woke up, his sores were healed and the cancer in his leg was completely cured. He lived another 20 years, until 1345, when he died at 80 years. Peregrine was canonized by Pope St. Benedict XIII in 1726 and has been named the patron saint of those suffering from cancer [34].

Prayers to Saint Peregrine can be soothing and inspiring to the healing and the healed alike. Here are some excerpts from prayers dedicated to St. Peregrine:

From a patient: "But if it should be God's holy will that I bear this sickness, obtain for me courage and strength to accept these trials from the loving hand of God with patience and resignation, because He knows what is best for the salvation of my soul."

For a patient: "Give him (her) patience in bearing suffering, and resignation to Your divine will. Give him (her) the consolation he (she) needs and especially a cure, if it be Your holy will."

For the doctors: "Fill with the vision of faith all doctors. Let them see in their patients the image of Your own sufferings and feel in their own hearts Your compassion that they may always reverence those for whom they care, knowing them to be children of Your Father."

For the researchers: "[Y]ou alone hold the keys to life and death. Yet, in Your divine love for us, You have gifted Your people with wondrous skill and intelligence in overcoming disease. Direct with Your wisdom our brothers and sisters who work in cancer research that they may be faithful in the service of Your children and successful in their efforts to combat this disease."

War on Cancer

In 1937, the United States Congress passed the National Cancer Institute Act and made the conquest of cancer a national goal. This Act created the National Cancer Institute, which was expected to break new ground by conducting its own research, promoting research in other institutions, and coordinating cancer-related projects and activities. In 1971, President Richard Nixon declared war on cancer and enacted the National Cancer Act, launching a national cancer program. Since then, many have asked, "Are we winning the war on cancer?"

It seems as though we have won at least some battles if not the war against cancer. Consider the facts: In 1946, the 5-year survival rate associated with all cancers combined was 35%. Today, it is 65%. In 1946, we treated cancer with surgery, x-rays, and radium. We also used Russian "life serum" or estrogen tablets for prostate cancer, Coley's toxin or streptococcal infections to induce tumor regression in sarcoma, and nitrogen mustard gas to treat lymphoma. Without doubt, we have made great strides in cancer treatment over the last decades. But there is still much room for improvement. We are still far from a complete victory or a cure of many, if not most, cancers in our war against cancer.

A most glaring weakness or failure in our war against cancer is that we still cannot explain the origin of cancer or pinpoint a cause for it. Our ideas are becoming stagnant. We need more innovative and breakthrough therapies. Although we can substantially reduce our risk of cancer by living a healthy lifestyle and eating a healthful diet, we cannot guarantee that such measures will eliminate cancer altogether. Although our treatments for cancer have improved by leaps and bounds, we still have trouble combating some of the more virulent and intractable types of cancer.

I believe that the theory of a stem-cell origin of cancers will provide us the requisite arsenal to win the war against cancer.

Conclusion

Some say we learn from history. Others say history repeats itself. One wonders whether there are lessons to be unlearned and stories to be revised in the history of cancer. After all, history is told and written by humans, sometimes in their infinite

wisdom but other times with their utmost bias. In the end, most of us like the status quo and will resist any change at all costs. In fact, many of our prominent or entrenched leaders are also likely to be the most vocal critics and strongest opponents of any change. There are very few true trailblazers among us.

Despite all the giant strides we have made in our knowledge and treatment of cancer, there is still a huge divide when it concerns a thorough understanding about cancer's origins. The jury is still out about whether the stem-cell theory of cancer will be a turning point in the history of cancer and whether it will revolutionize human thought and usher in another era in oncology. In many ways, the theory of a stem-cell origin of cancers has always had and will for some time have a Tinker Bell effect: It exists simply because we believe in it. Often enough, it takes considerable time before we can prove a theory; sometimes, it seems like an eternity.

References

1. Breasted JH (1930) The Edwin Smith surgical papyrus, published in facsimile and hieroglyphic transliteration with translation and commentary in two volumes. Univ. Chicago Press, Chicago, IL. Cited by: Fitzgerald PJ (2000) From demons and evil spirits to cancer genes. American Registry of Pathology, Washington, DC
2. Capasso LL (2005) Antiquity of cancer [review]. Int J Cancer 113:2–13
3. Stadtman K (1992) News from members. Soc Vert Paleont 155:45. Cited by: Capasso LL (2005) Antiquity of cancer [review]. Int J Cancer 113:2–13
4. Rothschild BM, Witzke BJ, Hershkovitz I (1999) Metastatic cancer in the Jurassic. Lancet 354:398. Cited by: Capasso LL (2005) Antiquity of cancer [review]. Int J Cancer 113:2–13
5. Fitzgerald PJ (2000) From demons and evil spirits to cancer genes. American Registry of Pathology, Washington, DC
6. Brothwell D (1967) The evidence for neoplasms. In: Brothwell D, Sandison AT (eds) Diseases in antiquity; a survey of the diseases, injuries, and surgery of early populations. Charles Thomas, Springfield, IL. Cited by: Fitzgerald PJ (2000) From demons and evil spirits to cancer genes. American Registry of Pathology, Washington, DC
7. Sandison AT (1975) Kanam mandible's tumor [letter]. Lancet 1:279. Cited by: Fitzgerald PJ (2000) From demons and evil spirits to cancer genes. American Registry of Pathology, Washington, DC
8. Ortner DJ, Putschar WG (1981) Identification of pathological conditions in human skeletal remains. Smithsonian contributions to anthropology, no. 28. Smithsonian Institution Press, Washington, DC. Cited by: Fitzgerald PJ (2000) From demons and evil spirits to cancer genes. American Registry of Pathology, Washington, DC
9. Czarnetzki A (1980) Pathological changes in the morphology of the young Paleolithic remain from Stetten (Southwest Germany). J Hum Evol 9:15–17. Cited by: Capasso LL (2005) Antiquity of cancer [review]. Int J Cancer 113:2–13
10. Pahl WM (1986) Tumors of bones and soft tissues in ancient Egypt and Nubia: a synopsis of the detected cases. Int J Anthropol 1:267–276. Cited by: Capasso LL (2005) Antiquity of cancer [review]. Int J Cancer 113:2–13
11. Strouhal E (1978) Ancient Egyptian case of carcinoma. Bull NY Acad Med 54:290–302. Cited by: Capasso LL (2005) Antiquity of cancer [review]. Int J Cancer 113:2–13
12. Strouhal E, Vyhnanek L (1981) New cases of malignant tumours from late periods cemeteries at Abusir and Saqqara (Egypt). Ossa 8:165–189. Cited by: Capasso LL (2005) Antiquity of cancer [review]. Int J Cancer 113:2–13
13. Strouhal E (2001) Malignant tumours in past populations in Middle Europe. In: La Verghetta M, Capasso L (eds) Proceedings of the XIIIth European Meeting of the Paleopathology

Association. Edigrafical, Teramo, pp 265–272. Cited by: Capasso LL (2005) Antiquity of cancer [review]. Int J Cancer 113:2–13
14. Scheele WE (1954) Prehistoric animal. World Publishing, Cleveland, pp 1–125. Cited by: Capasso LL (2005) Antiquity of cancer [review]. Int J Cancer 113:2–13
15. Moodie RL (1927) Tumors in the lower carboniferous. Science 66:540. Cited by: Capasso LL (2005) Antiquity of cancer [review]. Int J Cancer 113:2–13
16. Capasso L, Di Tota G (1996) The antiquity of osteosarcoma. Int J Osteoarcheol 6:512–514. Cited in: Capasso LL (2005) Antiquity of cancer [review]. Int J Cancer 113:2–13
17. Conkling S (1990) News from members. Soc Vert Paleont 150:41. Cited by: Capasso LL (2005) Antiquity of cancer [review]. Int J Cancer 113:2–13
18. World Health Organization. On the Web site of the Globocam 2000. Cited by: Capasso LL (2005) Antiquity of cancer [review]. Int J Cancer 113:2–13
19. Ebbell B (1937) The papyrus Ebers: the greatest Egyptian document. Levin and Monksgaare, Copenhagen. Cited by: Fitzgerald PJ (2000) From demons and evil spirits to cancer genes. American Registry of Pathology, Washington, DC
20. Lenormant F (1878) Chaldean magic and sorcery. Its origin and development. English Translation. Bagster, London, pp 4–5. Cited by: Fitzgerald PJ (2000) From demons and evil spirits to cancer genes. American Registry of Pathology, Washington, DC
21. Bhishagratna KL (1998) Susruta Samhitã, trans-ed. 3 vols. Chowkhamba Sanskrit Series Office, Varanasi, India. Cited by: Fitzgerald PJ (2000) From demons and evil spirits to cancer genes. American Registry of Pathology, Washington, DC
22. Preuss J, Rosner F (ed) (1978) Julius Preuss's biblical and talmudic medicine. Sanhedrin Press, New York. Cited by: Fitzgerald PJ (2000) From demons and evil spirits to cancer genes. American Registry of Pathology, Washington, DC
23. National Cancer Institute SEER training module. Cancer: a historic perspective. Available at: http://training.seer.cancer.gov/disease/history/. Accessed September 7, 2009
24. Morgagni GB (1983) De sedibus et causis morborum [The seats and causes of diseases] [Alexander B, Trans.]. Classics of Medicine Library, Birmingham, AL. Cited by: Fitzgerald PJ (2000) From demons and evil spirits to cancer genes. American Registry of Pathology, Washington, DC
25. Bichat MF-X (1813) Treatise of the membranes in general and on different membranes in particular [Husson M, Coffin JG, trans]. In: A new edition. Enlarged by an historical notice of the life and writings of the author. Cummings and Hilliard, Boston. Classics of Medicine Library (reprint), Birmingham, AL, 1987. Cited by: Fitzgerald PJ (2000) From demons and evil spirits to cancer genes. American Registry of Pathology, Washington, DC
26. Müller J (1838) Ueber den feineren bau und die formen der krankhaften geschwulste. G. Reimer, Berlin. Cited by: Fitzgerald PJ (2000) From demons and evil spirits to cancer genes. American Registry of Pathology, Washington, DC
27. Virchow R (1978) Cellular pathology as based upon physiological and pathological history [Chance F, Trans.]. Classics of Medicine Library, Birmingham, AL. Cited by: Fitzgerald PJ (2000) From demons and evil spirits to cancer genes. American Registry of Pathology, Washington, DC
28. Shimkin MB (1977) Contrary to nature. U.S. Department of Health, Education, and Welfare, Washington, DC. Cited by: Pitot HC, Loeb DD (2002) Fundamentals of oncology, 4th ed. Marcel Dekker, New York, NY
29. Virchow R (1863–1865) Die krankhaften geschwulste. 3 vols. A Hirschwald, Berlin. Cited by: Fitzgerald PJ (2000) From demons and evil spirits to cancer genes. American Registry of Pathology, Washington, DC
30. Thiersch C (1865) Der Epithelialkrebs namentlich der haut. Engelmann, Leipzig, Germany. Cited by: Fitzgerald PJ (2000) From demons and evil spirits to cancer genes. American Registry of Pathology, Washington, DC
31. Waldeyer W (1867) Die Entwickelung der Carcinoma. Arch Path Anat Phys Klin Med 41:470–523. Cited by: Fitzgerald PJ (2000) From demons and evil spirits to cancer genes. American Registry of Pathology, Washington, DC

32. Cohnheim J (1875) Congenitales querqestreiftes Muskelsarcom der Nieren. Arch Pathol Anat Phys Klin Med 65:64–69. Cited by: Fitzgerald PJ (2000) From demons and evil spirits to cancer genes. American Registry of Pathology, Washington, DC
33. Ackerknecht EH (1953) Rudolph Virchow. Doctor, statesman, anthropologist. Univ. Wisconsin Press, Madison, WI. Cited by: Fitzgerald PJ (2000) From demons and evil spirits to cancer genes. American Registry of Pathology, Washington, DC
34. Etling M (ed) (2003) Florentine. Lives of the saints. Saint Peregrine with prayers and devotions. Regina Press, Melville, NY

Chapter 4
Origin of Cancer

Crossroad sign obtained from Google Images
(www.pricoldclimate.org/.../sustainability films)

Many questions need to be answered, just as many answers need to be questioned.

– Anonymous

Précis

A useful road map of cancer is that all paths leading to the cancer cell go by the way of a stem cell.

Introduction

When it comes to asking a great question about anything, it is a safe bet that inquiring about its origin will often seize the moment. Be it a question about the origin of cancer, of humans, or of life itself, one cannot help but become overwhelmed by the mystical dimensions and epic implications of the question. Surely, we are not tampering with any secret or sacred codes. Discovering the origin of cancer may clarify our befuddlement about this disease and add meaning to what we already

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know about cancer, about humans with cancer, and maybe even about the subtleties of life itself. More importantly, it may offer us a glimmer of hope about conquering this dreaded – and dreadful – disease.

Right now, we have no easy answer for this ultimate question about the origin of cancer. In fact, our view about its origin relies as much on faith as on any scientific truth. This is so because many of our current hypotheses may be flawed and our experiments misguided. Consequently, the laboratory evidence derived from them seems circumstantial, incomplete, or even irrelevant. Clearly, we need to formulate an improved theory about the origin of cancer. We must abandon our comfort zone and familiar mind-set about the origin of cancer. We should be prepared to swim against the current rather than go with the flow. Otherwise, our chances of breaking the cancer code might be lower than those of winning a lottery.

Cancer Statistics

Before the twentieth century, cancer was a strikingly rare event for the human species. Most people died at a relatively young age from other causes, such as infections, accidents, and childbirth. However, over the last century, we have been witnessing a considerable boost in life expectancy as a result of improved health care. For instance, we performed an extreme makeover in the prevention and treatment of innumerable deadly infections with the advent and availability of vaccines and antibiotics. We hope to make similar substantial headway in the prevention and treatment of cardiovascular diseases (i.e., heart disease and stroke) in the near future. Cancer is already a considerable cause of morbidity and mortality in industrialized nations, and it is conceivable that it will become the major cause of morbidity and mortality for the entire human species before the end of the twenty-first century. Already, it is the biggest killer of those younger than 75 years. Among those between 45 and 64 years, cancer is responsible for more deaths than the next three causes (heart disease, accidents, and stroke) combined. It is also the leading cause of death of children, those in their 30s, and everyone in between. In the foreseeable future, it will become an even greater public health issue as life expectancy increases in developing countries. I believe that our ability to conquer cancer depends to a large extent on our ability to discover its inscrutable origins.

Origin of Cancer

One key to conquering cancer is to elucidate its origins. Many people may not realize that this platitude is perhaps one of the greatest understatements about cancer. Elucidating the origin of cancer implies that we will have a better

understanding about its underlying causes and basic mechanisms. It means that we will soon discover the best and perhaps the only ways of conquering cancer.

Until recently, our main focus on the origin of cancer has been on causative agents: putative genetic mutations and aberrant signal pathways. However, it is increasingly evident that the origin of cancer may very well lie beyond genetic mutations and aberrant signal pathways. We need an updated cancer theory to help us devise pertinent experiments and improved treatments. I hypothesize that the cells of origin within which these genetic mutations or aberrant pathways occur play an important role in the evolution and final manifestation of a malignant phenotype. The time has finally arrived for us to formulate a unified theory of cancer: that the cell of origin is just as pivotal in the formation of cancer as the genetic mutations and aberrant signal pathways within it are. When it concerns the origin of cancer, we believe that stem cells and stem cell–like cells steal the thunder.

Theoretical Oncologists

A theory is an organized concept. It allows us to reach beyond our most incredible imaginings. Otherwise, the vast unknowns of the universe and the intricate inner workings of matter seem utterly untouchable and indescribable. But theoretical physicists have explored these unfathomable worlds and conveyed to us a sense of awe and wonder about their existence through fancy theories and elaborate mathematics. Their ideas have allowed us to establish a conceptual framework on which we can build a virtual universe and virtual matter that can be tested and refined to reflect and perhaps represent the real universe and real matter.

Although cancer seems more tangible than the universe or matter, it is just as elusive and mysterious. Often, the surges of information about cancer that come our way seem to have no real bearing on it and may even lead us further astray from the truth. I therefore believe that we need a “theoretical oncologist” who can guide us toward the right path. Otherwise, we will continue to follow the herd, being oblivious of the fact that the blind could be leading the blind. We drift and sway in whichever wind of hope or hype that happens to be blowing at the moment. When it concerns cancer research and a cure for cancer, a theoretical oncologist is one who will wave a red flag rather than a white one.

It is true that many theories lack rigor or depth. Some theories are no more than a flight of fancy that focuses on only a modicum of reality. Without proof, a theory has no substance. But we should remember that sometimes a theory is simply before its time and thus is almost impossible to prove. A visionary who theorizes needs the audacity to challenge the norm. More often than not, he or she will be regarded as a heretic rather than a seer and an apostate rather than a savior. Therefore, all theorists must be prepared to sacrifice their pride and honor, because they may become a laughingstock in their profession.

Oncogenes

In many ways, the discovery of oncogenes provided the foundation for our current theories on the origin of cancer. Landmark studies by Peyton Rous laid the groundwork for this discovery. Rous showed that a sarcomatous tumor growing on a Plymouth Rock hen could be transferred to other hens [1]. He demonstrated that this adoptive tumor formation did not require passage of intact tumor cells but could be accomplished using cell-free filtrates that excluded bacteria [2]. Rous was one of the first scientists to report the existence of a tumor virus, now known as the Rous sarcoma virus (RSV). In 1958, Temin and Rubin [3] developed the focus assay in which RSV-infected cells are overlaid with agar to keep the progeny virus localized. This technique enabled them to isolate individual viruses and identify src (i.e., v-src) as the gene responsible for the transforming potential of the RSV. Eventually, their method led to the discovery of c-src, the first cellular proto-oncogene. Proto-oncogenes are dominant growth-stimulating genes that have a constitutive or housekeeping function in a normal cell; their abnormal counterparts are oncogenes or cancer-causing genes. Thus, a mutation that disrupts the normal function of a proto-oncogene in a cell contributes to the formation of cancer in that cell.

The "Two-Hit" Hypothesis

Investigators later showed that defects in tumor-suppressor genes also lead to the development of cancer. In 1971, Knudson [4] formulated the "two-hit" hypothesis, which has greatly influenced our current view of cancer. He hypothesized that the gene responsible for retinoblastoma (Rb) is a tumor-suppressor gene. Tumor-suppressor genes also play a critical role in the normal function of a cell, and wild-type expression of Rb counteracts cancer formation by regulating cell proliferation and differentiation. However, both Rb alleles need to be inactivated within a single cell before that cell loses its protection from the growth-inhibitory effects of wild-type Rb. In a normal retinal cell, the first mutation (or "hit") at the Rb tumor-suppressor gene will not affect the cellular phenotype, but a second hit at the remaining wild-type allele will trigger the formation of cancer. For example, in familial retinoblastoma, the first Rb gene mutation is inherited through the germline and is therefore present in every cell. Consequently, cancer appears sooner in people with this mutation because only one additional mutation in the remaining wild-type allele is sufficient to initiate the formation of retinoblastoma. Therefore, as indicated above, both recessive tumor-suppressor genes in a cell need to be affected to cause the formation of cancer in that cell.

Initiation and Promotion

The idea of cancer initiation and promotion came from the models of chemical carcinogenesis. According to these models, the initiation event causes genetic damage in a premalignant cell, and the promotional factors stimulate the damaged

cell to become cancerous and proliferate. For instance, Yamagiwa and Ichikawa [5] demonstrated that skin tumors appeared on the ears of nearly all rabbits (after 100 days) when coal tar solution was painted onto their ears daily. However, it was not until 1955 that Kennaway [6] identified benzopyrene as the carcinogen in coal tar. In the meantime, Rous [7] and Berenblum [8] performed experiments that also supported the idea of initiation and promotion during carcinogenesis. They showed that the binding of benzopyrene to DNA in skin cells caused permanent genetic alterations (i.e., initiation) in these cells but that cancer would not form unless another stimulus, such as provided by croton oil (which contains methylcholanthrene, a phorbol ester), was applied to the affected skin (i.e., promotion) [9].

Multistep Carcinogenesis

In the early 1950s, Fisher and Hollomon [10] and then Nordling [11] proposed the concept of multistep carcinogenesis. However, this idea was not widely accepted until 1990, when Fearon and Vogelstein [12] championed and popularized it with the publication of an article on colorectal tumorigenesis. Undoubtedly, this idea made a profound impression on cancer researchers and practitioners: Today, most cancer biologists and oncologists still think about cancer according to the model of multistep carcinogenesis. Fearon and Vogelstein's model had the advantage of studying tumors that were easily visible during different stages of their development. In addition, those tumors at the different stages could be safely removed and their genetic constitution analyzed. What was observed in this model was presumed to also apply to other tumor types, which may not be as noticeable or accessible.

Fearon and Vogelstein found that the glysine at position 12 of the K-ras gene was mutated in 12% of the early adenomas, 42% of the intermediate adenomas, 57% of the advanced adenomas, and 50% of the carcinomas. A deletion in the long arm of chromosome 18 was associated with 13% of early adenomas, 11% of intermediate adenomas, 47% of late adenomas, and 73% of carcinomas. A gene whose loss may be responsible for cancer progression in this region of chromosome 18 is Smad-4, which is involved in transforming growth factor β signaling. Similarly, a deletion in the short arm of chromosome 17 was associated with 6% of early and intermediate adenomas, 24% of advanced adenomas, and 75% of carcinomas. A key gene in this region of chromosome 17 is the tumor-suppressor gene p53.

Although these genetic abnormalities can occur in any order, and not all are required to cause malignancy, the most likely sequence in which each successive step confers a growth advantage to the cell has been proposed: APC is the gatekeeper gene whose loss starts the initial step of converting normal colonic epithelium to an early adenoma and the whole cascade toward malignancy. The next step involves a Ras mutation, which may be responsible for the development of an intermediate adenoma. Then a deletion in 18q causes the formation of an advanced adenoma. And a p53 mutation will change the adenoma to a carcinoma. Finally, the development and accumulation of additional genetic changes contribute to a more aggressive and lethal carcinoma (Fig. 4.1).

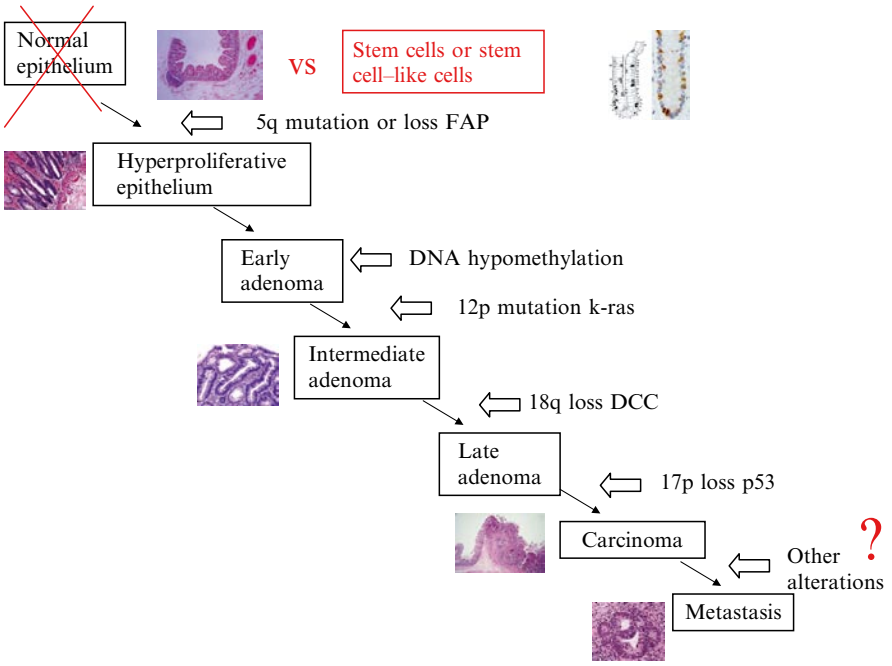

Fig. 4.1 Multistep carcinogenesis model of cancer. The involved cell of origin is in dispute: normal epithelial cells vs. stem cells or stem cell–like cells (in *red*). Also, many late malignant features, such as metastasis, may be present from the start in the stem cells or stem cell–like cells of origin (*question mark*)

Origins of Cancer, Revisited

It is tempting to try to fit all the possible genetic and epigenetic changes in a malignant cell into their rightful places. However, there seems to be insufficient room to accommodate all the putative oncogenes and tumor-suppressor genes in our current doctrine of initiation and promotion of carcinogenesis and multistep progression of malignancy. A crucial premise in our current view of cancer is that most, if not all, malignant phenotypes are gradually acquired through time. For example, hypermethylation in the promoter of certain genes and increased telomerase activity are observed in cancer cells but not in normal mature cells. Therefore, a cancer cell must have acquired these characteristics as it evolves from normalcy to malignancy. We assume that a neoplastic tumor acquires an increasingly more malignant phenotype as it marches down the pathway of multistep carcinogenesis.

But many of us have wondered whether our cherished doctrines about the acquisition of malignant phenotypes could in fact be misconceived or erroneous. For example, the multistep model of carcinogenesis considers the nature and number of

mutations to be paramount for the development of cancer. It completely ignores another possibility, which is that the type of cells in which these mutations occur also matters (Fig. 4.1). Perhaps it is time for us to look into an idea that is quite distinct and different from the one we have today: that many malignant phenotypes are already present or have always been present in evolving cancer cells rather than being acquired by nascent cancer cells.

In light of recent discoveries, this alternative view no longer seems farfetched. But any time a new and unconventional view comes along, our first reaction is to reject it and resist any change. To many, the idea that certain malignant phenotypes (e.g., metastatic potential, drug resistance) are preordained in a nascent cancer cell is preposterous. To others, the idea that this nascent cancer cell has already inscribed its malignant signature (e.g., hypermethylation) and bequeathed the gift of virtual immortality (e.g., upgraded telomerase activity) by a progenitor stem cell to its descendants is quite alien. However, I postulate that many features of malignant cells in fact reflect those of stem cells. And that the nature of a particular stem cell from which a cancer originates determines the degree and extent of its malignant phenotype as well as how and when it will eventually manifest.

Perhaps the writing has always been on the wall although nobody would heed or even read it. The first clue about an indigenous nature of cancer is glaringly evident from the discovery that for every oncogene, there is a matching proto-oncogene. Indeed, what extraordinary misfortune for a cell to keep a vital proto-oncogene that could easily convert into a risky, liable oncogene! Why would any cell sit on a ticking time bomb unless it also happens to serve some indispensable, critical functions? Another clue about the homegrown nature of cancer comes from the similar match between a malignant cell and its normal stem-cell counterpart. Just like a proto-oncogene, a stem cell serves many indispensable, critical functions. When a proto-oncogene becomes defective, an oncogene ensues; when a stem cell goes berserk, a cancer cell starts to implode. Therefore, I contend that just as every oncogene is derived from a misfit proto-oncogene, every cancer cell originates from a rogue stem cell.

Stem-Cell Origin of Cancers

Surprisingly, abundant proof for a stem-cell theory of cancer already exists. People may not realize that many already-completed experiments have provided results supporting this theory. It was impossible to realize the implications of those experiments beforehand, though, because they were designed to test a different hypothesis and were interpreted in that light, not in light of this hypothesis. After all, how can we find something when we do not really know what we are looking for?

For example, Roy et al. [13] implanted human embryonic cell (hES)-derived dopaminergic neurons in the neostrata of parkinsonian rats with 6-hydroxydopamine-induced lesions. The dopaminergic implants yielded substantial and durable

(10-week) restoration of motor function in these animals. Unfortunately, the authors reported that the grafts also contained tumors that had expanding cores of undifferentiated mitotic neuroepithelial cells. When they performed the same experiments using naive hES cells, all four rats developed overtly symptomatic neoplastic masses within 4 weeks. Therefore, as predicted by the stem-cell theory of cancer, this experimental system demonstrated that early hES cells produced highly neoplastic tumors, whereas later progenitor cells (SHH^+-, $FGF8^+$-, and hMAST-induced dopaminergic neuronal cells) induced slower expansion of a less-anaplastic neuroepithelial lineage. It is hard to imagine how we could have designed a better experiment to prove the theory of a stem-cell origin of cancers or demonstrated more clearly that different progenitor stem cells produce separate, distinct malignant tumor types.

Similarly, Kroon et al. [14] showed that hES cells implanted in mice efficiently formed glucose-responsive endocrine cells. The insulin-expressing cells that resulted after engraftment exhibited many properties of functional β cells, including the expression of critical β-cell transcriptional factors, the appropriate processing of proinsulin, and the presence of mature endocrine secretory granules. Unfortunately, of the 46 grafts (from 105 animals that had received the implanted cells) that were independently examined by board-certified histopathologists, 15% showed the presence of malignancy with immature or mature teratoma. There is no denying that the results of this seminal experiment supported the premise of hES as a renewable supply of human insulin-secreting β cells. But one should remember that the experiments also provided some of our most irrefutable proof of the theory of a stem-cell origin of cancers.

Defining Idea

One way to spark an idea is to create a defining idea. My defining idea is that many malignant features have an endogenous rather than acquired origin. This idea implies that many malignant phenotypes have a stem-cell origin and may not be ideal targets for therapy. I believe that this defining idea would completely change our current mind-set about cancer and channel our future resources away from a traditional mode of cancer research and therapy.

In the following chapters, I will demonstrate that the stem-cell theory of cancer strikes at the very core and not the mere fringes of cancer. I will show that many of the pertinent malignant targets, pathways, and signatures recapitulate their stem-cell origins. The ensuing facts and realities should eliminate any false notions that all malignant characteristics are acquired by the cancer through time. I will discuss how and why many intriguing and sometimes controversial aspects of cancer, such as heterogeneity, metastasis, immunity, and drug resistance, could be reconciled with the theory of a stem-cell origin of cancers. I will also address several fascinating topics in oncology, such as field defect, aneuploidy, epithelial-to-mesenchymal transition, and transdifferentiation, and will elucidate them in the context of the theory of a stem-cell origin of cancers.

Conclusion

As we trace the various paths toward a full understanding of cancer, we cannot help but realize that they all emanate from stem cells. Have we been on the wrong track the whole time? Have we been making wrong turns again and again? Are the many signs bearing epithelial, stromal, or endothelial targets; oncogenes and tumor-suppressor genes; signal pathways and molecular signatures misleading? Perhaps a useful road map of cancer is that all paths leading to the cancer cell go by the way of a stem cell. If our goal is to understand and conquer cancer, what and where is our ultimate destination? I contend that solving the origin of cancer is our key to finding and recognizing this destination. We are at a crucial crossroad in our quest. I believe that the theory of a stem-cell origin of cancers will lead us closer and closer to solving the origin of cancer so that we can conquer it at last.

References

1. Rous P (1911) A sarcoma of the fowl transmissible by an agent separable from tumor cells. J Exp Med 13:397–411
2. Rous P (1911) Transmission of a malignant new growth by means of a cell-free filtrate. JAMA 56:198
3. Temin HM, Rubin H (1958) Characteristics of an assay for Rous sarcoma virus and Rous sarcoma cells in tissue culture. Virology 6:669–688
4. Knudson AG Jr (1971) Mutation and cancer: statistical study of retinoblastoma. Proc Natl Acad Sci USA 68:820–823
5. Yamagiwa K, Ichikawa KJ (1918) Experimental study of the pathogenesis of cancer. J Cancer Res 3:1–29
6. Kennaway E (1955) Identification of a carcinogenic compound in coal tar. Br Med J 2:749–752
7. Rous P, Kidd JG (1942) Conditional neoplasms and subthreshold neoplastic states. A study of the tar tumors of rabbits. J Exp Med 73:365–390
8. Berenblum I, Shubik P (1947) The role of croton oil applications, associated with a single painting of a carcinogen, in tumour induction of the mouse's skin. Br J Cancer 1:379–382
9. Friedwald WF, Rous P (1944) The initiation and promoting elements in tumor production. An analysis of the effects of tar, benzpyrene and methylcholanthrene on rabbit skin. J Exp Med 80:101–126
10. Fisher JC, Hollomon JH (1951) A hypothesis for the origin of cancer foci. Cancer 4:916–918
11. Nordling CO (1953) A new theory on cancer-inducing mechanism. Br J Cancer 7:68–72
12. Fearon E, Vogelstein B (1990) A genetic model for colorectal tumorigenesis. Cell 61:759–767
13. Roy NS, Cleren C, Singh SK et al (2006) Functional engraftment of human ES cell-derived dopaminergic enriched by coculture with telomerase-immortalized midbrain astrocytes. Nat Med 12:1259–1268, Erratum in: Nat Med 13(3):385
14. Kroon E, Martinson LA, Kadoya K et al (2008) Pancreatic endoderm derived from human embryonic stem cells generates glucose-responsive insulin-secreting cells in vivo. Nat Biotechnol 26:443–452

Chapter 5
Stem Cells

"Chicken and egg" was obtained from Google Images (www.wilsonmotivational.com/images/chicken_and_egg_chapv4.jpg&imgrefurl)

But there are also unknown unknowns,
The ones we don't know we don't know.

– Donald Rumsfield, Feb. 12, 2002, Department of Defense news briefing

Précis

A stem cell is not only quiescent but also immortal, not only immortal but also pluripotent, and not only pluripotent but also homeostatic.

Introduction

In many ways, "stem cell" is a misnomer. It is true that all somatic cells stem or originate from a stem cell. But a stem cell may be more akin to a seed or the root of all cells. Stem cells are not an imaginary or putative entity any more; finding them no longer belongs to the realm of science fiction. Stem cells are the "magic" cells that repair tissues and regenerate organs. One day in the not-too-distant future, they may become the miracle cells that heal many of our irreparable conditions and irreversible illnesses. However, as long as stem cells have such incredible power

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(and power can corrupt), there is the possibility that a benevolent stem cell can turn into a malevolent cancer cell. Therefore, reaping the clinical benefit of stem cells remains an enigma and can become a dilemma. In this respect, stem-cell therapy still borders on sorcery rather than science.

There are three types of stem cells: germinal, embryonic, and adult. Germinal stem cells produce ova or spermatozoa. Embryonic stem cells are derived from the first eight divisions of a fertilized ovum. During this stage, each cell can potentially develop into a whole individual (as in fraternal siblings). Adult stem cells have more limited differentiation potential and produce cells that mature into the various somatic cells of an organ. When a stem cell passes from the embryonic to the adult stage, there is a shift in the expression of various stem-cell genes and profiles. As an embryo develops into an adult organism, demands for organogenesis decrease and risks for carcinogenesis increase.

Meristem Cells

Botanists studied stem cells in plants long before zoologists studied them in animals. In plants, "meristem cells" should be distinguished from cells in the actual stem. Meristem is derived from the Greek word *meristos*, meaning "divided." Like their animal counterparts, meristem cells exist in embryos and in the various organs of an adult plant. For example, roots have clusters of meristem cells at their tips. When these cells divide, the roots grow and spread into the soil. Shoots also have meristem cells at their tips. As these cells proliferate, the plant grows its branches and leaves. Floral meristem cells enable the flower to bloom, and cambial meristem cells enable the trunk to increase its girth. Meristem cells in the stalk enable grass and corn to elongate.

But unlike their counterpart in animals, the meristems in plants do not undergo cellular repositioning or redeployment during development. The meristems in plants do not disseminate, owing to the closed and discontinuous nature of the vascular channels (i.e., phloem and xylem). They are blocked by cell walls with small perforations at regular intervals. In contrast, a stem cell or malignant cell in animals has found ways to circumvent or surmount many obstructions that may impede its passage. The nature of meristems thus explains in part why cancers in plants, known as galls, tend to grow locally and do not migrate or metastasize from a primary tumor to distant sites. Therefore, the innate behavior of a tumor cell, be it in a plant or in an animal, may have already been imprinted within the stem cell from which it emanates.

Regeneration

For years, the phenomenon of regeneration in animals has fascinated naturalists. Missing parts of an animal – like the tail of a lizard, the claw of a crab, the eye of a salamander, and the antenna of a snail – have been observed to grow back like new. The leg of a salamander, for example, can be replaced many times over.

Abraham Trembley was the first to study the regenerative capability of an entire organism (aptly named a hydra) by performing scientific experiments on them in 1740 (as cited in Parson [1]). He discovered that when an old hydra was cut into several pieces, each part could grow into a full organism. It would take another 250 years before biologists finally determined that a hydra has ten different cell types that are organized into two layers. The embryo-like cells behave like stem cells and endow the hydra with this remarkable power of regeneration. For his work, many people consider Trembley to be the father of stem-cell research.

The Cell Concept

In 1838, Matthias Schleiden and Theodor Schwann were the first to clarify that the cell is the basic building block of tissues in both plants and animals. In 1855, Rudolf Virchow [2] made the observation that every cell comes from a cell. In the 1890s, Hans Driesch found that when he dissected a four-cell sea urchin embryo, each of the four cells formed an entire larva. He had expected that each cell would develop into only the part of the animal it was destined to become. Thus, cells from an early embryo were far more "plastic" than was previously thought. The term "stammzelle" (stem cell) started to surface in the German literature during the late nineteenth century (as cited in Fitzgerald [3]).

Unfortunately, the search for stem cells was often fruitless. In 1895, Francis Herrick noted that a grown lobster could lose a claw and easily replace it by growing a new one. However, a thorough "examination of serial sections through this part of the limb revealed nothing…. Embryonic cells might be present but were not discernible" (as quoted in Parson [1]). Until recently, this observation still rang true, especially within the nonhematopoietic tissues. The mystery of stem cells endures.

Finding Stem Cells

In 1909, Alexander Maximow envisioned the existence of a hematopoietic stem cell (HSC) that formed the various cell types (e.g., white cells, red cells, platelets) in the blood. His proposal explained why bone marrow transplanted after a lethal dose of radiation would miraculously rescue an animal from imminent death. The prevailing view at the time was that certain chemical factors in the transplanted marrow enabled the various blood cell types to home from the blood into the bone marrow. Not until 1956, when Charles Ford provided the first definitive proof, was it known that certain cells in the donor's marrow and not any chemical factors had actually resurrected a transplant recipient's blood-making machinery (see Parson [1]). Unfortunately, finding a stem cell remains elusive – finding one in mammals is like searching for a needle in a haystack or like the ultimate "Where's Waldo?" puzzle.

In 1959, Seldon Bernstein and Tibby Russell performed landmark experiments that alluded to the presence of stem cells by virtue of the ability of fetal liver cells to reverse anemia in mice. In 1961, Ernest McCulloch and James Till came close to capturing and identifying stem cells when they recognized that donor HSCs were trapped by and then formed colonies in the spleens of irradiated recipient mice. The study of stem cells received a boost in 1970 when Gail Martin and Martin Evans discovered an improved cell culture method that could be used to maintain stem cells in vitro for prolonged periods. They accomplished this feat by adding or withdrawing "feeder cells" and by controlling the aggregation of stem cells. Feeder cells produce proteins that nourish stem cells and permit them to proliferate without differentiating; aggregation stimulates stem cells to differentiate, whereas preventing their aggregation keeps them from differentiating (see Parson [1]).

In 1988, Irving Weissman developed colony assay techniques and performed cell culture work that would lay the groundwork for identifying and isolating putative stem cells in the hematopoietic system. He reached this milestone by using monoclonal antibodies and ingenious detective work: tracing early precursor cells backward from mature blood cells and demonstrating consistent production of a full spectrum of blood cell progenies – lymphocytes, monocytes, erythrocytes, and granulocytes. His trailblazing studies laid the foundation for the establishment of special stem cells that could be used for future research and perhaps therapy (see Parson [1]): embryonic stem cells derived from human blastocysts, known as ES cells [4,5], and from human fetal tissues, known as EG cells [6].

The Nature of Stem Cells

Stem cells possess the capacity to regenerate an entire tissue and yet maintain strict homeostasis. They possess three key characteristics that ensure maintenance of a delicate balance between regeneration and homeostasis: (1) they remain dormant or quiescent within a stem-cell niche, (2) they undergo self-renewal and asymmetric division, and (3) they maintain pluripotency or form differentiated progeny (Fig. 5.1). Therefore, a stem cell is not only quiescent but also immortal, not only immortal but also pluripotent, and not only pluripotent but also homeostatic. We have learned that the number of stem cells is tightly regulated within a stem-cell niche. For example, estimates are that the total number of HSCs in animals as disparate as cats and mice is about 1.1×10^4 per animal [7].

Stemness

It is debatable whether the stemness of a particular stem cell arises from activation of specific stem-cell genes and pathways or from activation of these same stem-cell genes and pathways but within distinct cells in a stem-cell hierarchy.

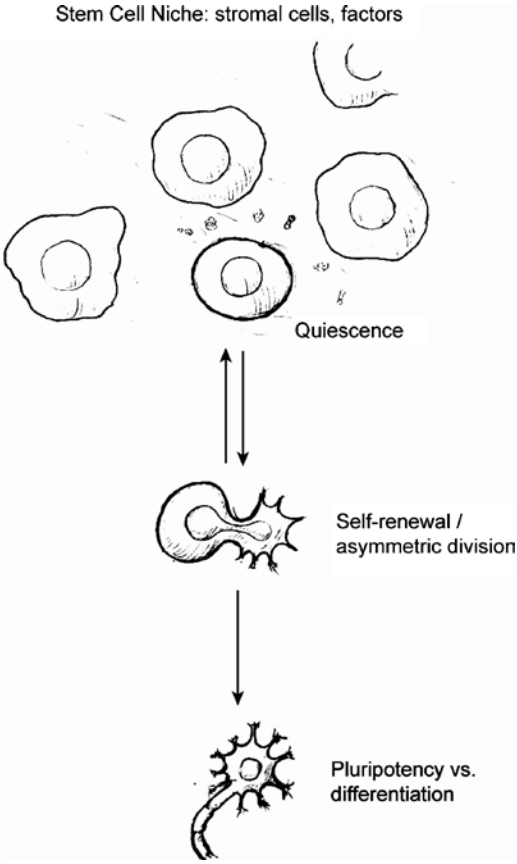

Fig. 5.1 Three characteristics of stem cells: (1) they remain dormant or quiescent within a stem-cell niche, (2) they undergo self-renewal and asymmetric division, and (3) they maintain pluripotency or form differentiated progeny

In other words, would activation of a particular stem-cell gene and pathway in different progenitor stem cells engender similar or different types of stem cells? Could the results obtained in progenitor stem cells also be obtained in somatic cells?

If we define a *stem-cell marker* as that which endows a cell with the ability to behave like a stem cell (such as to self-renew and differentiate), then various proteins may qualify as stem-cell markers. This should be distinguished from a particular *stem-cell profile* (of stem-cell markers), which defines a specific stem cell and its unique place in a stem-cell hierarchy. I speculate that insertion of a stem-cell marker into non–stem cells may render them "stem cell-like." However, insertion of a whole stem-cell profile into non–stem cells (if that is even possible) may reproduce a specific stem cell in a stem-cell hierarchy.

The Question of Stemness

The question of stemness is best illustrated by the case of primordial germ cells and germ cells derived from the later stages of germ-cell differentiation, which possess the capacity to produce ES cells. It is notable that ES cells can "differentiate" into germ cells as well as into many other adult stem-cell types. This is an example of the chicken-and-egg propositions that perpetually defy logic. It also echoes the famous phrase that the child is father of the man. Yet despite the apparent contradictions, there is some common sense. Stemness is more pervasive than one thinks.

Figure 5.2 illustrates how a continuum of stemness is preserved across generations of germ cells in a zygote, blastocyst, genital ridge, neonatal testis, and adult testis. Although stemness revolves in a cycle, it does so unidirectionally and irreversibly in this instance. At some stage between the gonadal germ cell in the genital ridge and the spermatid in the adult testis, there is a transition from stem cell to progenitor cell, when the cell no longer undergoes self-renewal or asymmetric division and when it loses its pluripotency and becomes differentiated. Hence, a spermatozoon is not a stem cell in the strict sense of the word, although it still retains stemness potential and could become a stem cell by virtue of its fusion with an ovum.

The Stemness Within

The epitome of stemness is the formation of a fertilized ovum, which gives rise to the prototypical ES cells. When two non–stem cells such as a spermatozoon and an ovum fuse, we witness a miracle in the making: the creation of a new cell, the fertilized ovum, which is the ultimate stem cell. Somehow, the fusion of two nonstem cells triggers an innate stemness. We should take a cue from this unique process of fertilization: Stemness is a property that may be bestowed on certain specialized cells and created in others. Perhaps the key to stemness may be found

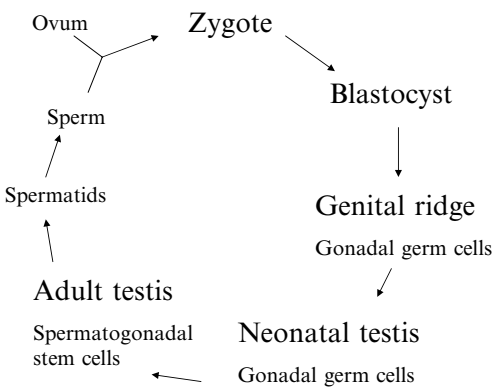

Fig. 5.2 A continuum of stemness is preserved across generations of germ cells in a zygote, blastocyst, genital ridge, neonatal testis, and adult testis

in the difference between an unfertilized and a fertilized ovum. I predict that the essence of stemness is ingrained within certain cell types and that the secret and mystery of stemness could be unraveled by studying the differences and similarities between a parthenogenetic and a fertilized ovum.

Parthenogenesis

Parthenogenesis (in Greek, parthenos means "virgin" and genesis, "birth") is a form of reproduction in which an ovum, an embryo, or a seed develops without fertilization by a male. Charles Bonnet discovered the phenomenon of parthenogenesis in the eighteenth century. In 1900, Jacques Loeb induced the first artificial parthenogenesis when he pricked unfertilized frog ova with a needle and found normal embryonic development in some pricked ova. Parthenogenesis occurs naturally in some species, including lower plants, invertebrates (e.g., aphids, some bees, ants), and lower animals (some reptiles and fish). Because parthenogenesis involves the inheritance and subsequent duplication of a single sex chromosome, the offspring will all be female if two like chromosomes determine the female sex but male if two unlike chromosomes determine the female sex. The parthenogenetic offspring are still capable of sexual reproduction if this mode exists in the species. As with all asexual reproduction, parthenogenesis has both disadvantages (reduced genetic diversity) and advantages (reproduction without the need for a male).

Whether it is the spermatozoon or the pinprick that triggers a potential stem cell (namely, an ovum) to become a definitive stem cell probably does not matter. I surmise that only certain cells are predestined or primed to become stem cells. The trigger of stem-cell formation may involve changes in the cell membrane. It is doubtful that transformation of a haploid to a diploid cell by itself is the critical event for the manifestation of stemness during parthenogenesis. There is something about the ovum that somehow defines stemness: its genetic and/or epigenetic expression, nuclear and/or cellular structure, cytoplasmic contents, and so forth. According to the phenomenon of parthenogenesis, *stemness is a cellular process* above and beyond the specific stemness characteristics.

Beyond Stemness

Stemness may also be considered a composite effect of the stem cell and its niche. Study in an invertebrate system (*Drosophila melanogaster*) showed that a stem-cell niche supports the proliferation of ectopic stem cells (follicle-cell progenitors) but not any regular somatic cells (inner germanium sheath cells) [8]. *Instead of making differentiated cells become undifferentiated*, the stem-cell niche keeps various stem cells (and potential cancer stem cells) from differentiation.

In this context, it is important to understand the limitations and challenges of the experiments for investigating stem cells. If we wish to expand the number of stem

cells for the purposes of studying them, we also need to expand the stem-cell niche. Otherwise, it will be a self-defeating endeavor; once we expand the stem cells without expanding the necessary or appropriate stem-cell niche, we are no longer dealing with the same stem cells. Therefore, the study of stem cells in solid organs cannot readily be performed without sufficient knowledge about their niche. Many of our established criteria for defining a stem cell in solid organs, such as its ability to grow after transplantation onto a nude mouse, its capability to retain dyes, its capacity to differentiate into cellular derivatives of all three germ layers, and so forth, may be grossly inadequate and inaccurate. Improved methods to define and identify stem cells are sorely needed.

Cloning

The idea that stemness is a cellular process also applies to cloning. When the nucleus of a somatic cell from the skin or intestine is inserted into the cytoplasm of an ovum, that ovum retains the capacity of an ES cell and can develop into a whole new organism after implantation into the uterus. Similarly, when a mature carrot or corn cell is placed in coconut milk, that carrot or corn cell can be manipulated to form a whole new carrot or corn plant. Does this mean that stemness is not a firm entity but is actually fluid-like? Does this imply that stemness is flexible or even malleable? We need to be reminded that even in these artificial settings, a differentiated nucleus is still being influenced by a "stem-cell milieu" derived from the ovum's cytoplasm, and a mature carrot or corn cell is being bathed in "stem-cell juice" obtained from the coconut. Conceivably, stemness in such cases may be determined to a large extent by certain stemness factors present in the ovum's cytoplasm or coconut juice.

Cloning is enshrouded by taboo. What happens after cloning in an animal is no longer anybody's guess. As expected, we play God poorly. We are witnessing more and more "uh-oh" than "ah-ha" moments in our forays into animal cloning. Estimates are that about 5% of the genes in a cloned animal are expressed incorrectly. Significant genomic alterations have been discovered to accumulate in human ES cells in culture [9]. The efficiency of cloning is still pitiful: Fewer than 5% of the ova that were used for cloning ended up as live animals. Dolly, the famous cloned sheep, was the sole survivor of 277 cloning attempts. Her cloned cells had telomeres that were closer in length to those of her 6-year-old biologic "mother" than to those of a naturally bred lamb. Perhaps it is not entirely surprising that Dolly developed lung cancer 7 years after her fateful birth in 1996.

Reprogramming

Recently there has been considerable enthusiasm and success in our attempts to reprogram somatic cells to become stem cells. The impact and implication of these ambitious endeavors cannot be more far-reaching. This would be a very

attractive and practical way for us to manufacture stem cells without encroaching on the ethics or morality of destroying human embryos. By manipulating cells derived from the same individual, this technology might also avert or circumvent problems related to immune incompatibility and tissue rejection. It is about time that mass production of stem cells for the regeneration of specific tissues and the treatment of many illnesses becomes a reality.

Takahashi et al. [10] induced the formation of pluripotent stem (iPS) cells from adult human skin fibroblasts by the transduction of four defined transcription factors (Oct3/4, Sox2, Klf4, and c-myc) using retroviruses. These human iPS cells resemble human ES cells in morphologic characteristics, proliferation, surface antigens, gene expression, epigenetic status of pluripotent cell-specific genes, and telomerase activity. They could also differentiate into cell types of the three primary germ layers in vitro and into teratomas. Yu et al. [11] also demonstrated that four factors (Oct4, Sox2, Nanog, and Lin28) were sufficient to reprogram human somatic cells from the foreskins of neonates to become iPS cells that exhibit the essential characteristics of ES cells. These iPS cells have normal karyotypes, express human ES cell-surface markers and genes, and possess the potential to differentiate into cellular derivatives of all three primary germ layers.

In many ways, these seminal experiments are reminiscent of those classic experiments by Hahn and Weinberg [12], who showed that discrete genetic mutations in somatic cells could induce the formation of cancer. On the basis of accepted laboratory criteria of malignancy (colony formation, anchorage independency, etc), they produced cancer cells from "normal" cells with four types of genetic mutations – pRB, p53, ras, and telomerase. The analogy of a somatic cell *reprogrammed* to form a stem cell using certain stem-cell factors and a normal cell *transformed* to form a cancer cell using certain oncogenes and tumor-suppressor genes is not without merit. After all, many aberrant stem-cell factors also happen to be oncogenes or tumor-suppressor genes. An aberrant stem cell behaves like a malignant cell.

It would be interesting to discover whether the experiments performed by Takahashi and Yu work only on mesenchymal cells or whether they would also work on epithelial cells. Did those experiments merely confirm that transduction of stem-cell factors (or markers) produced cells with stem-cell characteristics…a self-fulfilling prophecy? Or did they really prove that they had actually used a stem-cell profile and manufactured an ES cell (albeit an artificial one, on the basis of the accepted criteria and definitions of ES cells in the laboratory), which would be beyond the scope of the designed experiments (i.e., hypothesis-generating experiments)? (See Chap. 2.) In other words, a stem cell reprogrammed from a somatic cell in the laboratory may resemble but is not likely to be the same as one found in nature, just like a cancer cell transformed from a "normal" cell in the laboratory may resemble but is not likely to be the same as one found in nature. They are not meant to be the same. An experiment is an experiment, not reality. Experiments serve the wonderful purpose of allowing us to dissect the parts while ignoring the whole or examining a few snapshots while neglecting the complete movie. Scientific discipline should forbid our honest but insatiable urge to extrapolate the meanings of findings beyond the confines of an experiment. Otherwise, we will

continue to find ourselves on the wrong footing and take some nasty tumbles. For example, are the reprogrammed iPS cells really ES cells in the making, or could they be potential cancer cells lurking in the shadows?

Unknown Unknowns

There are undoubtedly still many unknown unknowns about stem cells. Is a stem cell a real cellular entity or a mere functional phenomenon? The latter is more in line with the idea that a stem cell is more fluid than concrete in nature; consequently, adult stem cells are not restricted in what they are destined to become but can morph into various cell lineages [13–17]. Could these results be mere experimental artifacts? Could there be some as-yet-unknown stem cells immersed somehow in tissue culture or hidden somewhere in stromal tissue that accounts for the tissue plasticity? I predict that many unknown, if not unimaginable, stem cells still remain to be discovered.

Recently, a rare population (about 0.02% of bone marrow mononuclear cells) of very small embryonic-like (VSEL) stem cells was discovered in adult bone marrow at the single-cell level [18]. These Sca-1$^+$, Lin$^-$, CD45$^-$ cells displayed morphologic features similar to those of early ES cells and also expressed markers characteristic of them such as SSEA-1, Oct-4, Nanog, and Rex-1. In vitro, VSEL cells are able to differentiate into all three primary germ-cell lineages. Does the presence of these VSELs contribute to the plasticity of HSCs and the phenomenon of transdifferentiation? Could it be the bone marrow–derived VSELs rather than the HSCs that regenerate the various lineages of different tissue or organs? Ironically, the identification of VSELs has rekindled Rudolf Virchow's concept of an embryonic origin of cancers, which laid the foundation for the current theory of a stem-cell origin of cancers (see Chap. 3).

Portrait of a Stem Cell

A pertinent question about the theory of a stem-cell origin of cancers is whether cancer is derived from stem cells or is driven by cells with stem cell–like properties. Put another way, when a progenitor stem cell gives rise to cancer, is this progenitor cell already primed to be abnormal in the first place because it possesses stem-cell features, or does it become abnormal after acquiring such stem-cell features? In the end, the answer depends on the nature or definition of a stem cell. Are we talking about the same stem cell? With our current experimental technology and expertise, can the real stem cell be isolated and studied the way we would like to?

Indeed, this complex problem is at hand when we study stem cells, which are by nature quiescent and pluripotent. Unlike non–stem cells, stem cells have the capacity to self-renew and to undergo asymmetric division. When a stem cell is released from its quiescent state and undergoes self-renewal or differentiation, it may already have crossed the Rubicon of stemness and become an entity that is entirely different from what we assume it is when it is in its primeval and pristine state, unless certain forces or factors from the so-called stem-cell niche are able to turn it back into its original stem-cell state. Therefore, as soon as a stem cell undergoes self-renewal and asymmetric division, which is coupled to differentiation, it starts to lose its very stemness. And as soon as it starts the differentiation process, it begins to lose its pluripotency. It may still be a stem cell, depending on the definition of a stem cell, but it is no longer the same stem cell.

If a cancer is derived from a stem cell and a stem cell is so ephemeral by nature, then it will be quite challenging if not almost impossible to prove the theory of a stem-cell origin of cancers. Inevitably, countless experiments will show contradictory and opposing evidence because we are not dealing with the real or the same stem cells. There will always be innumerable skeptics who will question and doubt (rightly so) the little indirect or circumstantial evidence supporting the merits of the theory of a stem-cell origin of cancers and for the very same reasons: We may not be dealing with the real or the same stem cells.

Indeed, it is almost as though stem cells obey a biologic Heisenberg's uncertainty principle: Once you detect and identify a stem cell that has been released from its quiescent state and has started self-renewal or differentiation, it is no longer the same stem cell. Somehow, its nascent stem-cell properties are already lost, perhaps forever. However, we may be able to detect traces of the original stem cell here and there or now and then, much like the Cheshire cat, which appears and disappears, leaving only a grin. But until we discover specific markers for the various stem cells and master the use of the various stem-cell niches, we will have difficulty proving the status of a stem cell because of its ever-changing fleeting nature, which deters any valiant attempts to investigate it.

Conclusion

The stem cell may be both a blessing and a curse. Although we continue to be captivated by its marvels, it is also the stem cell that issues a cancer cell's license to kill. And it is the stem cell's power that the cancer cell usurps for its devious ends. Elucidation of the stem-cell origin of solid tumors will improve our current understanding of malignancy. It will also galvanize the discovery of novel diagnostic tools, prognostic markers, and therapeutic targets in our battle against cancer. However, on the basis of the theory of a stem-cell origin of cancers, we need to remember and remain alert to the fact that although current research may unveil the untapped powers of stem cells, it may also unleash their potential hazards.

References

1. Parson AB (2004) The proteus effect: stem cells and their promise for medicine. Joseph Henry Press, Washington, DC. Chapters 1 and 3
2. Virchow R (1855) Archive fuer Pathologische [editorial]. Anat Physiol Klin Med 8:23–54
3. Fitzgerald PJ (2000) In: Thomas D (ed) From demons and evil spirits to cancer genes: the development of concepts concerning the causes of cancer and carcinogenesis. American Registry of Pathology, Washington, DC. Chapter 10
4. Bongso A, Fong CY, Ng SC, Ratnam S (1994) Isolation and culture of inner cell mass cells from human blastocysts. Hum Reprod 9:2110–2117
5. Thomson JA, Itskovitz-Eldor J, Shapiro SS et al (1998) Embryonic stem cell lines derived from human blastocysts. Science 282:1145–1147
6. Shamblott MJ, Axelman J, Wang S et al (1998) Derivation of pluripotent stem cells from cultured human primordial germ cells. Proc Natl Acad Sci USA 95:13726–13731
7. Abkowitz JL, Catlin SN, McCallie MT, Guttorp P (2002) Evidence that the number of hematopoietic stem cell per animal is conserved in mammals. Blood 100:2665–2667
8. Kai T, Spradling A (2003) An empty *Drosophila* stem cell niche reactivates the proliferation of ectopic cells. Proc Natl Acad Sci USA 100:4633–4638
9. Maitra A, Arking DE, Shivapurkar N et al (2005) Genomic alterations in cultured human embryonic stem cells. Nat Genet 37:1099–1103
10. Takahashi K, Tanabe K, Ohnuki M et al (2007) Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 131:861–872
11. Yu J, Vodyanik MA, Smuga-Otto K et al (2007) Induced pluripotent stem cell lines derived from human somatic cells. Science 318:1917–1920
12. Hahn WC, Weinberg RA (2002) Rules for making human tumor cells. N Engl J Med 347:1593–1603
13. Bjornson CR, Rietze RL, Reynolds BA, Magli MC, Vescovi AL (1999) Turning brain into blood: a hematopoietic fate adopted by adult neural stem cells in vivo. Science 283:534–537
14. Brazelton TR, Rossi FM, Keshet GI, Blau HM (2000) From marrow to brain: expression of neuronal phenotypes in adult mice. Science 290:1775–1779
15. Jiang Y, Jahagirdar BN, Reinhardt RL et al (2002) Pluripotency of mesenchymal stem cells derived from adult marrow. Nature 418:41–49
16. Krause DS, Theise ND, Collector MI et al (2001) Multi-organ, multi-lineage engraftment by a single bone marrow-derived stem cell. Cell 105:369–377
17. Mezey E, Chandross KJ, Harta G, Maki RA, McKercher SR (2000) Turning blood into brain: cells bearing neuronal antigens generated in vivo from bone marrow. Science 290:1779–1782
18. Kucia M, Reca R, Campbell FR et al (2006) A population of very small embryonic-like (VSEL) CXCR4(+)SSEA-1(+)Oct-4(+) stem cells identified in adult bone marrow. Leukemia 20:857–869

Chapter 6
Stem Cells and Cancer

"Lucifer," the fallen angel, was obtained from Google Images (www.picknettprince.com/.../lucifer.htm)

[T]he Lord gave, and the Lord hath taken away.

– Job 1:21

Précis

In principle, any cell with stem-cell properties is poised to become malignant given the right conditions and circumstances. The stem-cell characteristic is what gives cancer its free rein.

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Introduction

The resemblance between a stem cell and a cancer cell is astounding. Inevitably, this resemblance raises an all-important question: Could there be a relationship between the two cell types and, if so, what is that relationship?

So far, we are inclined to assume that many genetic or epigenetic aberrations associated with cancer also contribute to the formation of cancer, simply because they are found in cancer cells but not in normal ones. However, when we consider that many of the supposed aberrations in a cancer cell may actually have an equivalent function in a normal stem-cell counterpart, our entire perspective about cancer begins to change.

This chapter illustrates the considerable evidence that supports the theory of a stem-cell origin of cancers. The evidence indicates that cancer (1) contains stem-cell features and (2) emanates from stem cell–like cells or progenitor stem cells. When we consider that certain genetic or epigenetic aberrations are actually *engendered* by a stem cell rather than *acquired* by a cancer cell, many malignant phenotypes, such as increased telomerase activity and aneuploidy, begin to have a new and different meaning. We realize that perhaps cancer does not need to start from scratch after all.

A Tale of Two Cells

Nowadays, we commit vast resources to studying the most essential and relevant characteristics of cancer. Many of these cancer characteristics (e.g., oncogenes, angiogenesis, metastasis, drug resistance, cancer immunity) have become a whole discipline on their own. Looking at these and other properties (Fig. 6.1), we cannot help but notice a striking similarity between cancer cells and stem cells. Can it be just incredible coincidence that many of the same essential and relevant characteristics of a cancer cell that command and deserve such attention also happen to comprise the very basic features of a stem cell? It may no longer be tenable for us to deny the existence of a special link between cancer cells and stem cells. We should not keep burying our heads in the sand and pretending that these two cell types do not have any special relationship.

Plight of the Tasmanian Devil

As far as we can tell, only select cell types can be safely and successfully transferred from one individual to another. In fact, the only cell type that can truly graft onto an appropriate tissue in another individual is a stem cell. It is true that ABO-matched packed red blood cells and single- or random-donor platelets can be transfused into immunocompetent individuals. But these differentiated cells have a

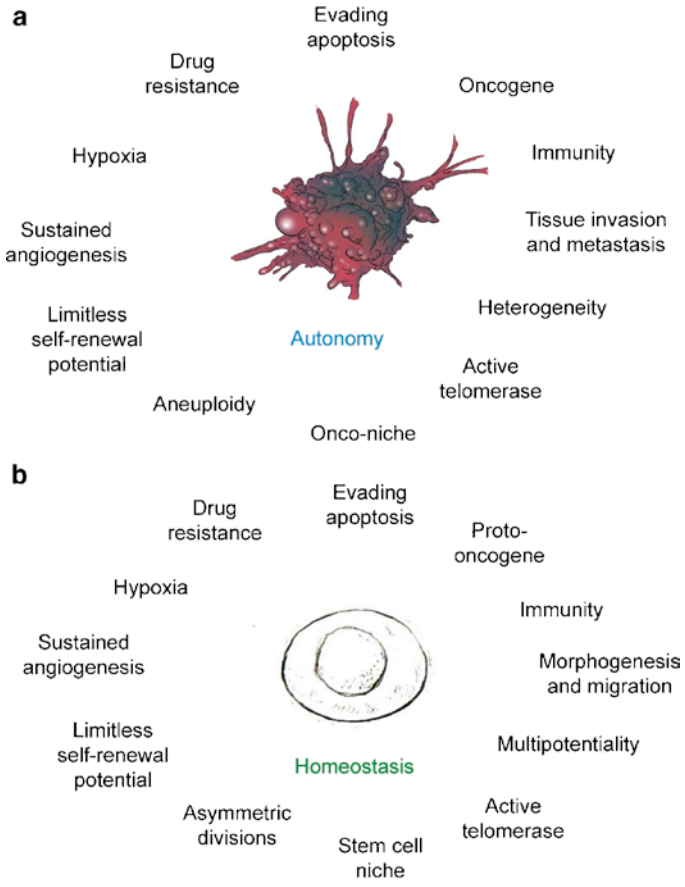

Fig. 6.1 Striking similarities between cancer cells and stem cells: many of the same essential and relevant characteristics of (**a**) a cancer cell also happen to comprise the very basic features of (**b**) a stem cell

limited life span and do not remain in the host for long. Coincidentally, the only other cell type that has the capacity to graft onto another individual is a cancer cell: Like stem cells, cancer cells can be transferred and maintained as xenografts. In particular, syngeneic or immunocompromised animals accept xenografts especially well. Allograft rejection in organ transplantation is minimized or prevented by tissue typing and immunosuppression. It is intriguing how alike stem cells and cancer cells can be in this and other respects. One wonders whether stem-cell grafts and tumor transplants use similar if not the same mechanisms. A possible link between the two cell types is that cancer cells are actually derived from stem cells.

Tasmanian devil facial tumor disease (DFTD) provides a graphic illustration of the phenomenon of tumor transplantation in nature. The Tasmanian devil (*Sarcophilus harrisii*), the largest living carnivorous marsupial, is indigenous to the

Australian island of Tasmania. Since 1996, when it first appeared, DFTD has caused a decline in the population of the Tasmanian devil by more than 60%. In 2007, McCallum et al [1]. projected that the species may become extinct within 5 years.

DFTD is believed to be an infectious cancer not involving any virus, bacterium, fungus, or parasite. Studies indicate that the infectious agent in DFTD is the cancer cell itself. Karyotypic and molecular genetic studies revealed that all DFTD tumors derive from a single clone of an individual animal's tumor [2, 3]. Furthermore, these tumors are genetically different from their host [3]. Therefore, DFTD is entirely host specific. The tumor cells spread directly between devils via the biting that frequently occurs during sexual encounters and their aggressive interactions over food [1]. Primary tumors occur on the face or in the oral cavity; metastases are common and can occur elsewhere [4]. Once clinical signs of the tumor are detected, the mortality rate reaches 100% within 6 months.

There is compelling evidence that DFTD spreads throughout the Tasmanian devil population because of a loss in major histocompatibility (MHC) diversity, as measured by microsatellites [5]. In particular, the MHC class I loci are so genetically similar that the animals in Eastern Tasmania are considered virtually syngeneic, with functionally identical MHC types [3]. Hence, even the competent immune system of a Tasmanian devil fails to recognize the tumors as foreign and does not reject them. It is astonishing how this phenomenon of cancer spread in a syngeneic population is almost identical to what happens with the development of cancer in an otherwise healthy person.

Therefore, this obscure phenomenon of DFTD has much to teach us about the underlying cause of cancer and, in many respects, supports, if not proves, the theory of a stem-cell origin of cancers. I speculate that DFTD is derived from a malignant clone that originates from an early stem cell and can be transferred and grafted in a syngeneic population. Because the DFTD cancer cells are selected for stemness on the basis of selfness, they are not easily recognized and can thrive in otherwise healthy individuals. I believe that DFTD is both an experiment of nature and living proof of the theory of a stem-cell origin of cancers.

Secondary Malignancy

Another experiment of nature that supports the theory of a stem-cell origin of cancers was evident in a study of secondary malignancy among patients who had undergone organ or bone marrow transplantation (BMT). Barsky et al. [6] demonstrated that 12% of solid cancers arising in non–sex matched transplant recipients (165 renal, 25 heart, 25 liver, 10 lung, and 55 bone marrow recipients) were of donor origin. This was a conservative estimate because some cancers of male origin might have spontaneously lost the Y chromosome. These solid cancers arose in various tissues, including the skin, lung, liver, kidney, and breast.

Barsky's study findings are significant because they relate to real people, not just to an animal model. They offer yet more evidence that cancer arises from stem cells or stem cell–like cells. Because only stem cells or stem cell–like cells can mobilize

from one individual and graft onto another individual, the results strongly suggest that the secondary malignancies arising in transplant recipients originate from stem cells or stem cell–like cells derived from either the donor or the donated transplanted organ. It is conceivable that a donor stem cell could migrate to and seed a recipient organ site where the conditions are more like those of an onco-niche than of a stem-cell niche and where they convert the donor stem cell into a secondary malignancy.

Stem-Cell Therapy

Reports of cancer development after stem-cell therapy also strengthen the theory of a stem-cell origin of cancers. In a mouse model, one third of animals that received corrective IL2RG gene therapy for severe combined immunodeficiency disease developed T-cell lymphoma [7]. Unfortunately, the use of a similar therapy devised for humans had to be halted in 2002 after four of 11 patients with severe combined immunodeficiency disease developed T-cell leukemia [8, 9]. Of note, the IL2RG gene therapy was delivered to the patients by way of a retrovirus carried by a $CD34^+$ stem cell. It was thought that the retrovirus might have integrated into the genome adjacent to a leukemogenic gene, which, on activation, could have instigated the malignant behavior of the transplanted $CD34^+$ stem cell. Therefore, defective stem cells were either unintentionally used or accidentally produced during such treatments. Thus, despite its immense therapeutic promise, stem-cell therapy may also entail hazards that need to be reckoned with.

Still another experiment of nature that supports the hypothesis of a stem-cell origin of cancer is the phenomenon of donor-cell leukemia (DCL). Estimates are that DCL accounts for about 5% of cases of recurrent leukemia after BMT [10]. Among 20 reported cases of patients with DCL, the diagnosis had been made from 2 months to 11 years after BMT [11, 12]. Conceivably, a stem cell transferred from donor to recipient during BMT is or becomes defective and causes the leukemia. Given that DCL has occurred after BMT from an unrelated donor, familial disposition alone does not appear to be responsible [12]. Since no donor in the reported cases of DCL subsequently developed leukemia, immune deficiencies or stromal defects in the recipient could have facilitated the development of malignancy [11].

Thus, it seems that stem-cell therapy has provided us another real-life experiment that supports, if not proves, the theory of a stem-cell origin of cancers.

Chronic Injury and Repair

The finding that chronic tissue injury is associated with cancer formation also supports the theory of a stem-cell origin of cancers [13]. Incessant tissue damage occurs during exposure to toxins (e.g., alcohol, cigarette smoke), infections (e.g., hepatitis, *Helicobacter pylori*), and inflammation (e.g., sclerosing cholangitis,

inflammatory bowel disease). This leads to a persistent state of tissue repair in which stem-cell pools are constantly being expanded and stem-cell pathways continuously activated. An expanded pool of activated stem cells over a prolonged period increases the risk of an oncogenic event and the eventual development of cancer. One cannot help but notice that the continuous activation of stem-cell pathways at play during chronic injury and repair is similar to that which is seen during carcinogenesis. In both conditions, deviation from the normal return to homeostasis and quiescence occurs after regeneration by the involved stem cells.

For example, the risk of heart disease associated with smoking reverts to that of a nonsmoker within 3 years of quitting smoking. However, the risk reduction for lung and other forms of cancer is negligible within that same period [14]. The differences between heart disease and cancer engendered by smoking may lie in the different origins of the diseases. When it concerns heart disease, smoking does not affect stem cells, but when it concerns cancer, smoking does affect stem cells. In the latter scenario, the damage and danger are more insidious and more difficult to repair or reverse once they have occurred in stem cells.

In 2004, Houghton et al. [15] performed a stunning experiment proving that stem cells were indeed the seeds of cancer sown after chronic injury, i.e., after induction of protracted infection, although not after acute injury, acute inflammation, or transient parietal cell loss in the stomach of C57BL/6 mice with *Helicobacter* infection. This study provided hard evidence that stem cells play a central role in carcinogenesis. An important implication of these findings was that in the formation of cancer it did not matter what kind of stem cell it was or where it came from, as long as it was a stem cell. Considering that the cancer formed was an epithelial malignancy and that it was derived from bone marrow–derived stem cells in this animal model, one should not underestimate the plasticity of stem cells and their ability to form diverse cancers throughout the body. It is amazing how the results of Houghton's laboratory experiments almost exactly replicated the results of Barsky's clinical study!

Cancer and Aging

How cancer may be so intricately intertwined with aging is yet another astonishing revelation of nature. Cancer takes many unexpected turns as it travels in the great unknown of aging, but in the end, one may say that the path to both cancer and aging must go by the way of stem cells. To appreciate a link between cancer and aging, we need to return to some basic principles of biology and certain tumor-suppressor genes.

An important aspect of aging is cellular senescence, which is a specialized form of growth arrest. Senescence is distinguished from other forms of growth arrest in that it is generally permanent and is associated with characteristic morphologic changes, chromatin alterations (e.g., SA-heterochromatic foci), and enzymatic activities (e.g., SA-β-galactosidase). We know that certain tumor-suppressor genes,

such as *p16^{INK4a}*, provide a fail-safe mechanism of protecting an organism from the risks of uncontrolled cellular proliferation: Impaired p16^{INK4a} cannot induce cell cycle arrest and thus fails to safeguard against uncontrolled cellular proliferation. Conversely, overactive p16^{INK4a} causes permanent cell cycle arrest and senescence. Hence, a negative consequence of p16^{INK4a} overdrive is limited self-renewal and restricted stem-cell proliferation, which promote senescence and accelerate aging.

During conditions of constant stress, such as chronic oxidative tension or inflammatory response, cellular proliferation and tissue regeneration increase. This creates an environment in which the chance of cellular damage and tissue injury is magnified. To protect a cell from permanent damage and serious injury, tumor-suppressor genes such as *p16^{INK4a}* are activated to minimize those dangers. Increased p16^{INK4a} activity puts a brake on stem-cell proliferation. Although increased p16^{INK4a} activity decreases the chance of cancer formation, it may inadvertently promote senescence and accelerate aging. Indeed, the results of experiments by Chang and coworkers at The University of Texas M. D. Anderson Cancer Center [16] indicated that increased senescence decreases the chance of tumor formation, but the animals do not experience any survival advantage because of accelerated aging.

Sean Morrison [17] pointed out that during fetal development, oncogenes are surging, whereas tumor-suppressor genes are waning. However, during aging, the opposite is true. He showed that self-renewal genes such as *bmi-1* shut down the tumor-suppressor gene *p16^{INK4a}*. Therefore, stemness genes turn off senescence genes. Although an individual may be protected from the risks of cancer by an active cadre of tumor-suppressor genes as he grows older, he becomes increasingly vulnerable to stem-cell senescence and is at the mercy of the aging process. Consequently, increased tumor suppressor–gene activity has a price tag: It may be an elixir against cancer, but it is also poisonous to aging.

The results of several studies have suggested that the benefit of longevity needs to be balanced against the risk of cancer development. Janzen et al. [18] showed that lack of p16^{INK4a} slowed the age-associated decline of hematopoietic stem cell (HSC) function. They found that p16$^{INK4a-/-}$ HSCs reconstituted the immune system better than wild-type HSCs in old but not young mice. Krishnamurthy et al. [19] demonstrated that older p16$^{INK4a-/-}$ mice had better islet-cell regeneration than did wild-type mice after toxin treatment. They explained that the age-associated rise in p16^{INK4a} impaired islet-cell regeneration and function, presumably by inhibiting the proliferation of β stem cells and/or their progenitors. Similarly, Molofsky et al. [20] reported that lack of p16^{INK4a} slowed the age-associated decline in the formation of neurons in the olfactory bulb of old but not young mice. One should note that although the older p16$^{INK4a-/-}$ mice had greater regenerative potential within the HSCs, islet cells, and olfactory stem cells in these studies, they actually had a shorter life span because they frequently succumbed to cancer at a younger age.

How do we reconcile this paradox of cancer and aging? If we could mitigate stress by eliminating continuous oxidative tension and eradicating chronic inflammation, then we would have obviated the need for enhanced cellular proliferation and tissue regeneration. In that case, there would be less need to protect a cell from serious insults or permanent impairments. One way to accomplish this lofty goal is

to adopt an active lifestyle and a healthy diet [21], which would lead to less need for the activation of certain tumor-suppressor genes (such as $p16^{INK4a}$) to fix any imminent problems. Hence, we would be able to both preserve stem-cell potential and delay senescence.

In short, the secret of Methuselah's longevity may lie in the trials and tribulations of stem cells.

Met: The Missing Link?

The *Met* proto-oncogene and oncogene are key regulators of physiologic and malignant pathologic growth, respectively. This is yet another instance in which the vital function of migratory growth belonging to a stem cell is usurped to become invasive growth in a cancer cell.

Recent data indicate that Met and its ligand, the hepatocyte growth factor, are important morphogenic factors that trigger the onset and progression of cancer. Expression of Met is a marker for liver stem cells because it is expressed only at low levels by mature hepatocytes. Purified stem cells that express Met can generate hepatocytes, cholangiocytes, pancreatic cells, and intestinal cells. Met is also a marker for candidate stem cells residing in the pancreas because it is barely expressed by differentiated pancreatic cells. Boccaccio and Comoglio [22] reported that a high percentage of tumors arising in the liver, pancreas, and intestines overexpress Met and that the overpression of Met correlates with metastatic potential and poor prognosis.

It is of interest that Met is a marker of the progeny of transformed stem cells that execute improper differentiation programs and yet are unable to forgo their stem-cell properties. This finding suggests that Met may be a missing link between malignant stem cells and differentiated cells. Met may finally expose the fallacy of dedifferentiation in cancer: Differentiated cancer cells do not dedifferentiate; instead, cancer contains stem cells as well as differentiated cells.

The beauty of the theory of a stem-cell origin of cancers is that the story continues to repeat, and different characters continue to rehearse the same roles. Who would have thought that Met is closely related to Wnt? As far as we know, Wnt signaling sustains stemness, and the Wnt pathway is vital to many stem cells. For instance, Wnt is switched off as the stem cell differentiates and migrates along the crypt–villus axis in the intestinal tract. Intriguingly, Met seems to play a role both upstream and downstream of the Wnt–β-catenin pathway.

Who could have imagined that Met also dances in a waltz of stemness with Notch, another important self-renewal partner? Evidence exists for reciprocity between Met and Notch, leading to a feedback loop. In this case, Notch activation blocks the signaling pathways of Met, thereby sustaining cellular stemness and clonogenic activity while stopping the migration of stem cells (and metastatic cells?) to a distant site.

Further, who could have guessed that Met is also intimately associated with hypoxia? Hypoxia favors both stemness and migration in tissues. During embryogenesis, hypoxia contributes to the establishment of the niches that maintain pluripotent cells. During carcinogenesis, hypoxia promotes self-renewal and an invasive phenotype. Hypoxia is also tied to other factors that affect the microenvironment, such as angiogenesis, which influences the emigration of differentiating cells to a designated favorable habitat. Not surprisingly, hypoxia affects numerous factors that influence the stem cell or its niche. Hypoxia-inducible factor 1 regulates the transcription of Met, CXCR4, and VEGF. In human tumors, Met expression correlates with hypoxia-inducible factor 1 expression and is usually higher in the inner tumor mass, where the oxygen tension is lower because oxygen diffusion is hampered.

Telomerase in Cancer and Stem Cells

The activity of telomerase, an enzyme that keeps telomeres (the ends of chromosomes) long and healthy, has been linked to cancer development. The length of the telomeres measures the life span of a cell like an hourglass measures time. Each time a cell divides, its telomere shortens. Hence, increased telomerase activity may keep the telomeres from shrinking and make a cell immortal. No wonder immortal cells such as cancer cells and cell lines have increased telomerase activity. Telomerase activity is normally repressed in somatic cells, but it is reactivated in more than 80% of human cancers [23]. Because telomerase activity increases in cancer cells but not in somatic cells, it is considered an important characteristic – maybe even a causal factor – in the formation of cancer.

However, one should remember that telomerase activity is also increased in another type of immortal cell, namely stem cells. In adults, telomerase remains active only in immature germ cells and stem cells. Telomerase activity is high during embryogenesis but suppressed in most somatic tissues after birth. Again, the resemblance between stem cells and cancer cells is striking and hard to miss. It begs many questions: Why would a cancer cell increase its telomerase activity when it could simply inherit such capability from its cell of origin, the stem cell? Does a cancer cell even need to reinvent the wheel of increased telomerase activity?

Flores et al. [24] performed experiments linking cancer cells and stem cells by showing that cancer cells may not even form without stem cells. They showed that telomerase-deficient ($Terc^{-/-}$) mice age prematurely but are resistant to tumor induction. The adult stem cells of these mice have decreased proliferative potential as a result of telomere shortening. It is as though the supposed stem cells with shortened telomere length or decreased telomerase activity no longer act like stem cells but act more like differentiated somatic cells. Because these $Terc^{-/-}$ stem cells with a limited life span behave like differentiated cells rather than stem cells, they no longer pose the threat of becoming cancer cells.

Therefore, many features of malignancy may not belong exclusively to malignancy after all when examined in a new light. This change of perspective, i.e., that maintenance of telomere length and increased telomerase activity is important for both stem cells and malignant cells, carries immense biologic and clinical implications. When we are unaware that there is a connection between the two cell types and the derivation of one from the other, we can easily attribute maintenance of telomere length and increased telomerase activity to be a cause of cancer or the exclusive property of cancer. Only when we realize that there is not much difference in telomere length and telomerase activity between cancer cells and stem cells do we finally realize that something is amiss. We begin to notice that many of the supposedly malignant targets may actually be bystanders rather than instigators of the formation of cancer. We need to be cognizant that without the right theory, certain putative malignant targets may actually be red herrings, giving us false leads again and again about the origin and nature of cancer.

Aneuploidy

Aneuploidy is the presence of an abnormal number of chromosomes in cells. It occurs commonly and is one of the most perplexing findings in human malignancy. Conceivably, any mistakes that occur during mitosis – the faithful pairing and segregation of chromosomes – can lead to imbalance and an abnormal number of chromosomes in the nascent cell. Under normal circumstances, these defective, unstable cells are eliminated by cell-cycle arrest and apoptotic cell death. This protective mechanism is provided by certain genes, such as the *p53* tumor-suppressor gene.

Therefore, it is not surprising that aneuploid tumors often contain inactivated *p53*, although it is still not entirely clear whether the inactivated *p53* is the cause or consequence of aneuploidy in malignant cells. Aneuploidy usually occurs early during tumorigenesis, whereas *p53* mutations are often late events. Nonmalignant cells isolated from $p53^{-/-}$ mice can also be aneuploid [25]. Currently, *p53* inactivation is believed not to be the primary cause of aneuploidy but is thought to facilitate chromosome instability by collaborating in other genetic events.

It is plausible that inactivation of *p53* provides a survival advantage for malignant cells in an otherwise lethal condition with unbalanced and unstable chromosomes. Altered polyploidy may be the cause of altered cell properties rather than an effect of aberrant cell-cycle control. Perhaps additional copies of complementing chromosomes could enhance the fitness of cells with chromosomal aberrations. Increased nuclear size and a reduced ratio of nuclear surface area to volume could affect the import and nuclear concentration of regulatory proteins and transcription factors. And the sudden union of redundant sets of genes could trigger widespread gene silencing, which would be advantageous when those genes happen to be deleterious [26]. Polyploidy is remarkably well tolerated in some cell types and even whole organisms: The selective advantage for polyploid cells in those conditions may also be applicable to malignant cells.

Again, it is tempting to associate aneuploidy with malignancy. Blaming aneuploidy as the cause rather than an effect of cancer is easy enough, but the whole playing field changes completely when we consider aneuploidy in light of the stem-cell theory of cancer. From that perspective, aneuploidy is no more than the product of the going awry of a fundamental stem-cell property, namely asymmetric division. A malignant stem cell undergoing aberrant asymmetric division is likely to produce polyploidy by failing to segregate and to form a nuclear membrane. Therefore, one of the most recognized insignia of cancer, aneuploidy, is also the consequence of an aberrant stem-cell property, asymmetric division. After all, only stem cells or progenitor cells with stemness features can undergo asymmetric division.

Therefore, aneuploidy offers more support for the theory of a stem-cell origin of cancers. This is not the first time that a particular stem-cell property has been used for a deviant malignant purpose: Aberrant asymmetric division leads to aneuploidy and genetic instability, which provide growth and survival advantage to a nascent cancer cell.

Conclusion

Sometimes, the line that separates a stem cell from a malignant cell seems blurred. I propose that certain malignant features, such as increased telomerase activity and aneuploidy, may actually be related to stem-cell phenotypes and can be traced to a stem-cell origin. I postulate that the potential vested in a cancer cell may be derived as much from its stem-cell origin as from the genetic and epigenetic changes that have occurred within it. Therefore, it would be fundamentally flawed and misguided to attribute certain stem-cell phenotypes to be essential malignant characteristics.

We accept that cancer is an incarnate evil. Ironically, cancer may also be an incarnation of stem cells. In our encounters with cancer, we are moving closer and closer toward uncovering its cells of origin, which may very well be stem cells or progenitor stem cells. In many ways, a cancer cell is the detestable product of a stem cell's "downfall," much like Lucifer, the Fallen Angel. What triggers the emergence of a cancer cell or the fall from grace of a stem cell remains a mystery.

References

1. McCallum H, Tompkins DM, Jones M et al (2007) Distribution and impacts of Tasmanian devil facial tumor disease. Ecohealth 4:318–325
2. Pearse A-M, Swift K (2006) Allograft theory: transmission of devil facial-tumour disease. Nature 439:549
3. Siddle HV, Kreiss A, Eldridge MD et al (2007) Transmission of a fatal clonal tumor by biting occurs due to depleted MHC diversity in a threatened carnivorous marsupial. Proc Natl Acad Sci USA 104:16221–16226

4. Loh R, Bergfeld J, Hayes D et al (2006) The pathology of devil facial tumor disease (DFTD) in Tasmanian devils (*Sarcophilus harrisii*). Vet Pathol 43:890–895
5. Jones ME, Paetkau D, Geffen E, Moritz C (2004) Genetic diversity and population structure of Tasmanian devils, the largest marsupial carnivore. Mol Ecol 13:2197–2209
6. Barsky SH, Ye Y, Xiao Y, Yearley K (2008) Insights into the stem cell origin of human cancers by studying a registry of bone marrow and other organ transplant recipients who later developed solid tumors [abstr]. Am Soc Clin Oncol 580 [Abstract 11010]
7. Woods NB, Bottero V, Schmidt M, von Kalle C, Verma IM (2006) Gene therapy: therapeutic gene causing lymphoma. Nature 440:1123
8. Hacein-Bey-Abina S, von Kalle C, Schmidt M et al (2003) A serious adverse event after successful gene therapy for X-linked severe combined immunodeficiency. N Engl J Med 348:255–256
9. Marshall E (2003) Second child in French trial is found to have leukemia. Science 299:320
10. Boyd CN, Ramberg RC, Thomas ED (1982) The incidence of recurrence of leukemia in donor cells after allogeneic bone marrow transplantation. Leuk Res 6:833–837
11. Cooley LD, Sears DA, Udden MM, Harrison WR, Baker KR (2000) Donor cell leukemia: report of a case occurring 11 years after allogeneic bone marrow transplantation and review of the literature. Am J Hematol 63:46–53
12. Hambach L, Eder M, Dammann E et al (2001) Donor cell-derived acute myeloid leukemia developing 14 months after matched unrelated bone marrow transplantation for chronic myeloid leukemia. Bone Marrow Transplant 28:705–707
13. Beachy PA, Karhadkar SS, Berman DM (2004) Tissue repair and stem cell renewal in carcinogenesis. Nature 432:324–331
14. Warner KE, Mackay JL (2008) Smoking cessation treatment in a public-health context [comment]. Lancet 371:1976–1978
15. Houghton J, Stoicov C, Nomura S et al (2004) Gastric cancer originating from bone marrow-derived cells. Science 306:1568–1571
16. Cosme-Blanco W, Shen MF, Lazar AJ et al (2007) Telomere dysfunction suppresses spontaneous tumorigenesis in vivo by initiating p53-dependent cellular senescence. EMBO Rep 8:497–503
17. Morrison S (2006) Stem cell self-renewal, cancer cell proliferation, and aging. In: 59th annual symposium on cancer research: stem cells in cancer and regenerative medicine, Houston, TX, October 27–29, p 37
18. Janzen V, Forkert R, Fleming H et al (2006) Stem cell aging modified by the cyclin dependent kinase inhibitor, $p16^{INK4a}$. Nature 443:421–426
19. Krishnamurthy J, Ransey M, Ligon K, Torrice C, Koh A, Bonner-Weir S, Sharpless NE (2006) $p16^{INK4a}$ induces age-dependent decline in islet regenerative potential [letter]. Nature 443:453–457
20. Molofsky AV, Slutsky SG, Joseph NM et al (2006) Increasing $p16^{Ink4a}$ expression reduces forebrain progenitor function and neurogenesis during aging. Nature 443:448–452
21. Ornish D, Lin J, Daubenmier J et al (2008) Increased telomerase activity and comprehensive lifestyle changes: a pilot study. Lancet Oncol 9:1048–1057, Erratum in Lancet Oncol 2008;9(12):1124
22. Boccaccio C, Comoglio PM (2006) Invasive growth: a *MET*-driven genetic programme for cancer and stem cells. Nat Rev Cancer 6:637–645
23. Ju Z, Rudolph KL (2006) Telomeres and telomerase in cancer stem cells. Eur J Cancer 42:1197–1203
24. Flores I, Cayuela ML, Blasco MA (2005) Effects of telomerase and telomere length on epidermal stem cell behavior. Science 309:1253–1256
25. Fukasawa K, Wiener F, Vande Woude GF, Mai S (1997) Genomic instability and apoptosis are frequent in p53 deficient young mice. Oncogene 15:1295–1302
26. Comai L (2000) Genetic and epigenetic interactions in allopolyploid plants. Plant Mol Biol 43:387–399

Chapter 7
Cancer Stem Cells

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If you carry a 00-number, it means you have license to kill, not get killed!

– From "Dr. No," Ian Fleming's James Bond series (film, 1962)

Précis

Different cancer types have different cancer stem cells owing to their different stem cells of origin.

Introduction

Great work in science is a gift from many individual geniuses, the culmination of countless inspirations and untold labor that ultimately lay the foundation of a masterpiece. Inevitably, someone else at the right time and right place emerges to put the finishing touches on the magnum opus.

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Often enough, we neglect to give credit where credit is due. Who had the road map that helped us find the origin of cancer? Who discovered the Rosetta Stone that translated our past knowledge into an improved understanding about the origin of cancer?

In this chapter, I acknowledge the many pioneers in cancer stem cell research. I also elaborate on how the presence of cancer stem cells can be explained by a stem-cell theory of cancer.

Matter and Energy

Consider the legacy of Albert Einstein's great achievement, $E=mc^2$ (1905). That equation unified important work already done by his predecessors on energy (E), mass (m), and the speed of light (c). Einstein managed to discern the relationship between what seemed like discrete physical entities. He had the ability to create this scientific masterpiece and the audacity to show us that even a speck of dust has a prodigious reservoir of untapped energy. In that magical stroke, he completely transformed our world and our view of it [1].

But Einstein's predecessors were outstanding scientists in their own right. Ole Roemer was one of them. In 1676, Roemer solved a great challenge of the time and predicted how long it would take the moon Io to orbit around Jupiter. He discovered that light traveled at a finite speed and calculated that speed to be 670,000,000 mph. Antoine-Laurent Lavoisier was another special scientist: In 1789, he proved that matter could neither be created nor destroyed and formulated the law of conservation of mass. And Michael Faraday discovered the power of electromagnetic force. In 1821, he performed landmark experiments that united electricity, magnetism, and motion and laid the foundation for our current concept of energy.

It is difficult to imagine the world of physical science before Einstein's time and even more so before his predecessors' time. Once established, an idea tends to be taken for granted and seems to acquire a life of its own. But an idea may become too big, too immutable, like a statue or monument. It becomes so entrenched in our minds and culture that it seems like dogma. Under such circumstances, we need not only an extraordinary occasion but also a superhuman effort to instigate a new idea and then, seemingly, an eternity to have the new idea accepted by cynical critics and an unknowledgeable populace.

Invention of the Telephone

It is true that Alexander Bell produced the first robust and functional telephone (1876). He also had the skills and personality to convince a skeptical public about its utility and potential. His invention transcended what seemed unimaginable at the

time and transformed our lives and our world. But to accomplish that, Bell did what all inventors do: He built on the combined wisdom of others, who had also built on the works of those before them [2].

Bell's first ideas about the telephone came from the work of Elisha Gray. Gray's "variable resistance pool" played an important role in the invention of the telephone, although it was inconvenient and impractical. Bell explored a different system, one that applied electromagnetism. This was in turn similar to the work done by Philipp Reis about a decade earlier, in 1860. Reis had transmitted sound by way of a diaphragm whose vibrations sent a continuous but variable flow of electricity to a second diaphragm, which translated the vibrations back into sound. Although Reis built a telephone that might be considered less functional than Bell's, his apparatus was nonetheless a prototype telephone. It is unclear how much his work influenced Bell's. However, it is possible that Reis's idea was influenced by that of Bourseul, who published a paper about a similar device in 1854.

There is no denying Bell's place as the inventor of the telephone. It is very simple and convenient for us to view the world this way but, unfortunately, our adoption of priority cheats all but one person of any deserved credit. Instead, we should thank *every* pioneer who contributed to the invention of the telephone – Bourseul, Reis, Gray, and Bell – for their immense ingenuity and wonderful gift to humankind. Like other scientific masterpieces, the telephone is the product of many people, not just one person.

Pioneers of Cancer Stem Cell Research

Leroy Stevens and Barry Pierce performed the first experiments that established a possible link between stem cells and cancer. They studied testicular tumors known as teratomas, which have fascinated humans since antiquity. *Teratos* means "monster" in Greek, and teratomas may take the form of grotesque, misshapen tissues and organs containing teeth, pieces of bone, muscles, skin, and hair. Stevens traced the origin of testicular teratomas to the genital ridge by tracking the path of deviant germ cells. However, when he transplanted fetal genital ridges that contained no germ cells into the testes of adult mice, no teratomas developed [3].

Kleinsmith and Pierce [4] first proved that a single pluripotent stem cell could give rise to a malignant tumor. They isolated a single cell from a teratoma and implanted it into the abdominal cavity of a mouse: The implanted cell duly grew and produced all the different cell types found in a teratoma. Earlier, Pierce and Dixon [5] had demonstrated that not every cell in a malignant teratoma is malignant. When stem cells differentiate, they lose their malignant potential. Despite their abnormal karyotypes, aberrant cells in a teratoma can develop into apparently normal tissues in the right microenvironment. By turning an

embryonic cell into a teratoma and vice versa, these investigators showed that pluripotent stem cells of early mouse embryos and those within teratomas were similar, if not identical, suggesting a close and possibly causal relationship between stem cells and cancer.

Cancer Stem Cells

The idea of cancer stem cells first originated from the work of Till and McCulloch in 1963 [6]. They demonstrated that self-renewing cells formed multilineage hematopoietic colonies (i.e., colony-forming units) in the spleen. In 1965, Brunschwig et al. [7] performed a controversial experiment showing that only a few certain tumor cells from patients with metastatic cancer actually had the capacity to initiate cancer when injected back into the same patients. He thus demonstrated for the first time that tumor cells might be organized in a hierarchical system. Subsequently, Hamburger and Salmon [8], using colony formation as a surrogate stem-cell assay, showed that only one in 1,000–5,000 cells from many human tumors was able to form a colony in soft agar. The results of these studies suggested that only certain cells could drive or sustain cancer growth in patients. However, until relatively recently, it was difficult to elucidate the malignant potential of these putative cancer stem cells without an in vivo model.

In 1997, Bonnet and Dick [9] first demonstrated the presence of cancer stem cells in animals. They found that only one in a million acute myeloid leukemia cells freshly harvested from patients reproduced the disease after injection into a nonobese diabetic mouse with severe combined immunodeficiency disease (i.e., NOD/SCID) mouse. Thus, an even smaller fraction of cells reproduced malignancy in a living animal than the fraction of those that could form colonies in culture. Because these rare cells expressed stem-cell markers (e.g., $CD34^+$, $CD38^-$), they resembled normal stem cells. Similar experiments were performed to show that cancer stem cells also existed in various solid tumors, including breast cancer [10], brain cancer [11], multiple myeloma [12], prostate cancer [13], and lung cancer [14].

The concept of cancer stem cells was formally introduced to the world by Irving Weissman in 2001 [15]. He and his group observed a striking resemblance between the self-renewal capability of malignant cells in a cancer and that of normal stem cells in an organ. They hypothesized that a small proportion of cancer stem cells in a malignant tumor possess this self-renewal potential and have the capacity to form a tumor and maintain its growth.

Origin of Cancer Stem Cells

The experiments showing that a single cancer cell could give rise to a new cancer and generate heterogeneous progeny provided evidence for a stem-cell origin of cancer. But a key question remains: Do cancer stem cells *originate* from stem

cells or do they merely *mimic* stem cells? If we assume that cancer stem cells exist, their existence raises several possibilities. To pose the question another way: Does a cancer cell display stemness features because (1) it arises from stem cells, (2) it behaves like stem cells, or (3) it associates with stem cells? Although the distinction between these possibilities may be subtle, it is not trivial: I believe that the difference carries enormous biologic ramifications and clinical implications.

In the first possibility, if a cancer stem cell is derived from a normal stem cell, it already arrives with a full stem-cell package – differentiation into various progenies, protection from immune surveillance, migration to distant sites, and so on. In other words, the intrinsic stem-cell features of a malignant cell are mostly inherited. And cancer develops when a stem cell has "gone bad." Hence, the pertinent cancer biomarkers, pathways, mechanisms, and phenotypes are largely determined by the stem cell of origin. The idea that stem-cell features of a cancer cell are born rather than bred is consistent with the theory of a stem-cell origin of cancers.

In the second possibility, if a cancer stem cell arises from any cell that acquires certain stem-cell features, it is equipped with a partial stem-cell package that makes it behave in a malignant manner. Thus, cancer develops when a good cell "learns the bad ways" of a stem cell. Theoretically, even a differentiated cell can become dedifferentiated and cancerous when it acquires the critical ingredient or essential component of a stem cell. The idea that stem-cell features in a cancer cell are bred rather than born is more compatible with current models of multistep carcinogenesis: Acquisition of mutations could disrupt normal cellular (and especially stem-cell) functions, making a progenitor cell or even a differentiated cell malignant.

Finally, in the third possibility, if a cancer contains merely displaced normal stem cells, it has a separate package. In this case, cancer develops when a "bad" cell associates with a "good" stem cell. In this scenario, a tumor attracts normal stem cells, which play a crucial role in the establishment and enhancement of the malignancy.

The Making of a Rogue Cell

Cancer stem cells capitalize on normal stem-cell characteristics. Therefore, many malignant phenotypes recapitulate stem-cell phenotypes. A hallmark of normal stem cells and their related cancer stem cells is their ability to self-renew and differentiate. We surmise that any disturbances in the self-renewal or differentiation of a normal stem cell could result in the development of a cancer stem cell. But other mechanisms also play an important role in the normal functioning and homeostasis of a stem cell. Hence, a disturbance in the specialized microenvironment known as the stem-cell niche or a disruption of asymmetric division could also contribute to the making of a cancer stem cell.

Within the stem-cell niche, a stem cell remains quiescent: It retains the ability to self-renew and not to differentiate. However, this stem-cell dormancy as afforded by the stem-cell niche is disrupted during carcinogenesis. A deleterious change may occur in a stem cell, rendering it no longer governable by the stem-cell niche, or an insalubrious change may occur in the stem-cell niche itself, which can then no longer govern the stem cell. Either way, an aberrant stem cell becomes a cancer stem cell. It starts to self-renew and differentiate improperly. Therefore, a global signature of cancer stem cells should include features of both cancer stem cells and their niche, just like a global signature of normal stem cells should reflect features of both normal stem cells and their niche.

There is also a close association between the various stem-cell characteristics and asymmetric division. Asymmetric division ensures homeostasis and preserves a pool of normal stem cells. Aberrant asymmetric division, on the other hand, disrupts this homeostasis and enriches a pool of cancer stem cells. When an aberrant stem cell transforms into a cancer stem cell, it is released from dormancy and undergoes aberrant asymmetric division. It begins to self-renew and differentiate improperly. Aberrant asymmetric division causes genetic instability and produces a whole spectrum of differentiated malignant cells, which constitute the bulk of the tumor and contribute to its heterogeneity. In many ways, these differentiated malignant cells mask the true identity and even hide the presence of cancer stem cells, making them particularly elusive and challenging to study.

Snags in the Stem-Cell Theory

Before we attribute the origin of cancer to stem cells, however, we need to recognize and reconcile some crucial shortcomings in the theory. Although a stem cell is quite capable of self-renewal and differentiation, it does not undergo either process under normal circumstances. Because cancer cells readily self-renew and often differentiate (albeit inappropriately), they seem to behave in a manner quite unlike that of stem cells. The fact that the two cell types have such diametrically opposite properties suggests that cancer cells cannot possibly arise from stem cells.

It is true that normal stem cells tend to remain quiescent unless there is a need to regenerate cellular components in a tissue. Only under those special circumstances are stem cells unleashed from the controls and confines of their niche. Cancer cells, interestingly enough, may also remain quiescent for prolonged periods before they become clinically evident. Certain conditions that cause the stem cell to become aberrant and/or alter the stem-cell niche may release the stem cell from its dormant state. Ironically, the conditions that affect the stem-cell niche (e.g., tissue repair and embryogenesis) might be very similar to those that promote malignant progression (e.g., chronic inflammation and carcinogenesis). The fact that both stem cells and cancer cells are essentially quiescent and yet quite capable of self-renewal and differentiation suggests that these cells are indeed related and that the latter could be derived from the former.

Another potential snag when we try to attribute the origin of cancer to stem cells is the realization that progenitor cells may also give rise to cancer. After all, progenitor cells by definition do not self-renew. Therefore, the assumption that cancer cells are derived from progenitor cells, which cannot self-renew, seems to run counter to the idea that cancer originates from stem cells and to refute the very foundation of the theory of a stem-cell origin of cancers.

However, it is not entirely clear whether certain progenitor cells (e.g., progenitor stem cells) possess (i.e., inherit or acquire) stem-cell properties that render them stem cell–like. I postulate that a gradient of self-renewal capacity irreversibly diminishes as the stem cell "moves" farther down the path of differentiation within a stem-cell hierarchy. In other words, there must be certain cells between stem cells and progenitor cells that still retain some stem-cell characteristics, such as self-renewal capability. We could thus consider these progenitor stem cells to be cancer-initiating stem cell–like cells because they do contain some stem-cell features.

In any case, I anticipate that cancer derived from progenitor stem cells with fewer stem-cell properties would be less threatening or aggressive than cancer derived from progenitor stem cells with more stem-cell properties. Even with the same genotypic aberrations, cancer stem cells arising from a late progenitor stem cell in a stem-cell hierarchy would display fewer malignant phenotypes. In fact, a tumor engendered from a progenitor cell that has no stem-cell features is likely to be indolent, if not altogether benign, i.e., hyperplastic. I speculate that different cancer types have different cancer stem cells owing to their unique stem cell of origin and that their stem cell of origin trumps their acquisition of unique genetic mutations. I also predict that it would require more genotypic aberrations for a late progenitor stem cell than it requires for an early one to become just as malignant.

Spontaneous Remissions

Evidence for the existence of cancer stem cells comes from unexpected quarters. Some such evidence may be found in the phenomenon of spontaneous regression of metastases. When it concerns cancer, spontaneous remission tends to be the exception rather than the rule. For example, in renal cell carcinoma, spontaneous regression of metastasis after nephrectomy occurs in up to 7% of patients [16]. Clearly, there must be an intricate relationship between the primary tumor and its metastatic deposits. In some instances, the primary tumor exerts a suppressive effect, whereas in others it exerts a stimulatory effect on its metastatic kin [17]. For example, it is hypothesized that some as-yet-unknown stimulatory factors produced by the primary tumor sustain the distant metastases. Postsurgically, with those stimulatory factors no longer present, the metastatic lesions regress.

However, I propose an alternative explanation for spontaneous remissions according to the theory of a stem-cell origin of cancers. I postulate that the primary

tumors shed circulating tumor cells (Chap. 17), some of which manage to become established at distant sites. The circulating tumor cells with the best chance of becoming metastatic are the so-called cancer stem cells. Spontaneous remission is less likely to occur if the cancer stem cells are derived from early stem cells in a stem-cell hierarchy. Conversely, cancer stem cells derived from late progenitor stem cells in that stem-cell hierarchy are inherently less metastatic. Metastatic loci established by primary tumors that originated from late progenitor stem cells are more likely to undergo spontaneous remission because they involve either weak cancer stem cells or no cancer stem cells at all. When the primary tumor is removed, the source of cancer stem cells is cut off, and the associated metastatic colonies cannot become self-sufficient or sustaining by themselves.

Succisa Virescit

The Latin phrase *sussisa virescit* means "to cut, only to regrow." It highlights a very important characteristic of cancer: It keeps growing back like a weed no matter how much and how hard we try to cut it out or uproot it. I suspect that whatever empowers a cancer must be cancer stem cells. Thus, to make any inroads against cancer, we need to discover the origin and nature of cancer stem cells by finding a litmus test that will distinguish cancer stem cells from stem cells.

The notion of distinct cancer stem cells with unique stem-cell origins suggests that certain cancer subtypes are amenable to surgery. In other words, we can afford to cut and *not* regrow (i.e., *sussisa non virescit*) certain cancer subtypes. I predict that various subtypes of prostate cancer, for example, have unique separate cellular origins and pursue distinctly different clinical courses. Hence, some tumors, such as pure ductal carcinoma, are intrinsically more indolent, whereas others, such as mixed ductal carcinoma, are inherently more lethal. Tumors derived from later (i.e., less pluripotent) progenitor stem cells in a stem-cell hierarchy tend to be homogeneous and exhibit a more nearly pure phenotype, whereas tumors derived from earlier (i.e., more pluripotent) progenitor stem cells tend to be heterogeneous and express a more mixed phenotype. This idea contradicts the traditional model of multistep carcinogenesis and views an aggressive tumor as a distinct entity rather than the product of rapid transformation from an indolent tumor; i.e., pure ductal carcinoma does not evolve to become mixed ductal carcinoma. This idea forecasts that a mixed tumor may be expressed as a pure tumor under the right conditions, but not vice versa. Therefore, certain prostate cancers (e.g., pure ductal carcinoma) tend to remain confined at the primary site for a prolonged period and may differentiate into drug-resistant "teratomatous" progenies. An effective way to treat such cancer types is to eradicate their cancer stem cells and the differentiated teratomatous components within the primary tumor by surgery [18].

Putative Progenitor Stem Cells

It is safe to say that the complete details of a stem-cell hierarchy in the hematopoietic or any tissue system remain to be elucidated. In other words, we do not know whether there are yet-to-be-discovered stem cells or progenitor stem cells that happen to retain or contain some of the stem-cell features in a full stem-cell hierarchy (Chap. 5). For instance, Gunsilius et al. [19] reported that the BCR-ABL gene was also detected in the endothelial cells of a patient with chronic myelogenous leukemia (CML). This discovery suggests that the BCR-ABL aberration must have occurred in an unknown stem cell downstream of the hematopoeitic stem cell (HSC) but upstream of the long-term HSC. Such a cell might be a hemangioblast, which has both hematopoietic and endothelial cell–differentiation potential but whose exact nature requires further confirmation.

Results of a study by Miyamoto et al. [20] also alluded to the existence of putative progenitor stem cells. They detected expression of the AML1-ETO fusion transcript in leukemia blasts, normal HSCs, and other bone marrow cells. Since normal AML1-ETO–expressing stem cells are not leukemic and can differentiate into normal progeny cells, it is surmised that the translocation must have occurred in normal HSCs and that additional mutations in a subset of these HSCs or their progenitor cells subsequently lead to the development of leukemia. Indeed, normal AML1-ETO–expressing HSCs are Lin^-, $CD34^+$, $CD38^-$, and $Thy\text{-}1^+$, whereas the leukemic stem cells are Lin^-, $CD34^+$, $CD38^-$, and $Thy\text{-}1^-$, suggesting that the transforming event may have occurred in downstream $Thy\text{-}1^-$ progenitor cells or a subset of $Thy\text{-}1^-$ HSCs.

The CML Model

CML is an ideal malignancy to use for elucidating the origin of cancer. I will illustrate how the theory of a stem-cell origin of cancers may cause a complete overhaul in our mind-set about the biologic and clinical implications of this cancer. What we learn from CML may also apply to other cancer types, including solid tumors.

Radich et al. [21] studied gene-expression changes in CML during disease progression and treatment response. Their results suggested that there are two phases of CML (chronic and accelerated/blast) rather than the conventional three phases (chronic, accelerated, and blast). Furthermore, they found that about 3,000 genes are significantly associated with the different phases of CML. If so many genes are significantly associated with the different phases of CML, then the chance that a single particular gene actually dictates the different phases of disease seems rather remote. On the other hand, if whole sets of genes dictate the different phases of CML, then there is a possibility that the distinct sets actually represent distinct cellular origins (rather than the acquisition of an inordinate number of genetic mutations in an evolving malignant cell) that ultimately determine the different types and phases of CML.

It is well known that there is a wide range of time (0.5–15 years) before the chronic phase (CP) of CML invariably and inexorably progresses to the accelerated (AP) and blastic (BP) phases of CML. Furthermore, most patients with CP CML respond well to therapy (e.g., imatinib). Radich et al. [21] demonstrated that patients who experienced a relapse after initial successful treatment with imatinib displayed a gene-expression pattern that was closely related to that of an advanced phase of the disease. Our current explanation for this phenomenon is that a steady accumulation of mutations eventually tips the balance and causes the transformation of CP CML to AP/BP CML. The time it takes for a CP CML to transform to a AP/BP CML depends on when in the timeline of the CML's progression the CP CML is first diagnosed (i.e., lead-time bias). The CML with the most mutations is also the one that is most resistant to therapy.

However, I propose an alternative view to explain this clinical observation. I hypothesize that there are at least two types of CML with distinct cellular origins (and consequently different cancer stem cells). One type (i.e., CPa CML) has greater malignant potential than the other because it is derived from an earlier stem cell and possesses more stem-cell features, including greater heterogeneity and increased drug resistance. The other type (i.e., CPb CML) has relatively less malignant potential because it is derived from a later progenitor stem cell. I predict that CPa CML likely progresses more rapidly to AP/BP CML than CPb CML does and also that it is inherently more resistant to therapy than CPb CML is, not because it acquires more mutations, but because it is a different disease altogether. In this scheme, fewer additional aberrations are required for CPa CML to transform into AP/BP CML, whereas more mutational events must occur on a separate timeline for CPb CML to progress into AP/BP CML (Fig. 7.1). An important aspect of this model is that CPa CML may have some similarities in phenotype to that of CPb CML. However, CPa CML may also express other phenotypes unique to earlier stem-cell pathways that are not found in CPb CML. In other words, CPa CML tends to express a more diverse or heterogeneous phenotype. For these reasons, and consistent with the results of Radich et al., CPa CML is expected to have a distinctly different molecular profile than CPb CML has.

Stem Cell vs. Progenitor Cell

At the end of the day, we need to address some pivotal questions about the origin of cancer and cancer stem cells: whether cancer arises from stem cells and whether it can also arise from progenitor cells. Although the assumption that cancer is a stem-cell disorder seems logical and intuitive, it is in dispute. The discovery that cancer may also arise from progenitor cells that have stem-cell properties conflicts with this assumption and puts the theory of a stem-cell origin of cancers in further dispute. Even if we accept the premise that cancer does originate from stem cells, there is still a crucial distinction depending on whether it is a stem-cell disorder or the acquisition of stem-cell characteristics. The question goes back to the perpetual

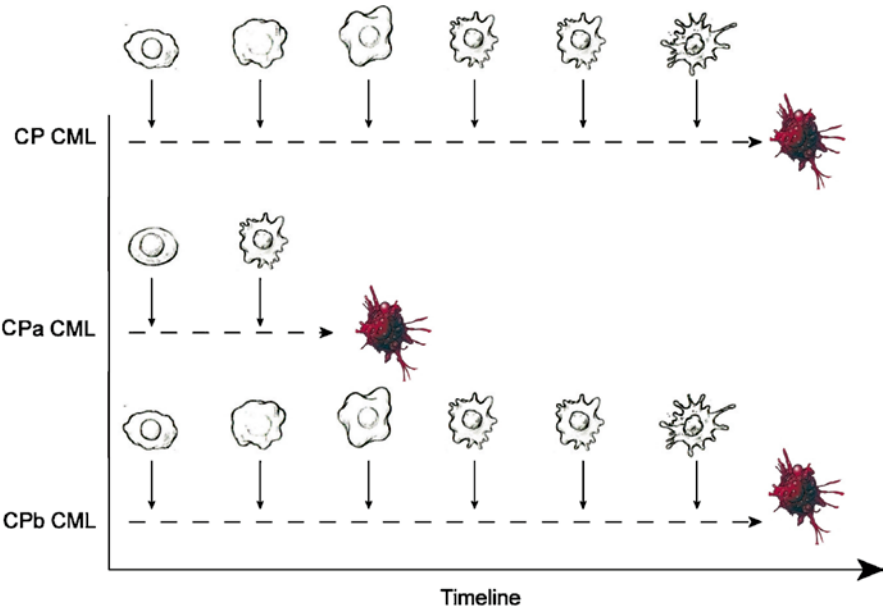


Fig. 7.1 Multistep vs. stem-cell models of chronic myelogenous leukemia (CML) progression from the chronic phase (CP) to the accelerated/blast phase (AP/BP). CPa and CPb are subsets of the CP

argument of whether progenitor cells or even differentiated cells can also become potentially cancerous by acquiring the all-important stem-cell characteristics.

Unlike other organ systems, the hematopoietic system offers us a relatively detailed, comprehensive glimpse into a hierarchy of cellular lineages. It shows us that there is a subset of long-term HSCs that can self-renew indefinitely. Another subset, short-term HSCs, retains the self-renewing capacity for only about 8 weeks before converting into multipotent progenitor cells, which can self-renew for only a brief period. These multipotent progenitor cells then give rise to several different lineages, including the common myeloid progenitor cells (CMPs), which can differentiate into granulocyte–macrophage progenitor cells (GMPs). Finally, GMPs can differentiate into either granulocytes or macrophages. As far as we know, differentiation proceeds through multiple maturation steps in an irreversible unidirectional manner.

But alas, experiments could always be done to suggest otherwise. For instance, results from a study by Cozzio et al. [22] seem to contradict a basic tenet of the stem-cell theory and suggest that cancer could arise from progenitor cells as well as stem cells. Those investigators obtained pure populations of both stem cells (i.e., HSCs) and progenitor cells (i.e., CMPs and GMPs). Transduction of a leukemogenic MLL-ENL fusion gene into these cells induced a similar leukemia in both the self-renewing stem cells and short-lived progenitor cells. Although the transformation efficiency was greater in the stem cells than it was in the progenitor

cells (i.e., HSC > CMP > GMP cells), the latency period for the onset of leukemia was similar for the different cell types. Furthermore, the immunophenotypes and expression profiles reflected maturation arrest at the late stage of myelomonocytic differentiation and appeared to be identical for the leukemias arising from stem cells and those from progenitor cells.

I contend that the results of this famous experiment still support the theory of a stem-cell origin of cancers. The possibility that fewer aberrations are needed for an HSC to become malignant, whereas additional mutations must occur in a progenitor cell before it becomes malignant, explains the differences in transformation efficiency in this study. It remains unknown whether those progenitor cells that transform have additional mutations or acquired mutations earlier during their evolution in a stem-cell hierarchy. Conceivably, the retrovirus used to transform cells would have selected for those progenitor cells that contained stem-cell features (e.g., contaminating HSCs), or perhaps some as-yet-unknown progenitor stem cells may be present in the purified pools. One could also argue that despite the obvious phenotypic similarities owing to the expression of the MLL-ENL fusion gene, the leukemias arising from different cells of origin are in fact biologically and clinically distinct. In other words, despite the expression of the same MLL-ENL fusion gene, the leukemias arising from HSCs ought to be more refractory to therapy, more prone to conversion to an increasingly heterogeneous or lethal phenotype, and more likely to confer an overall worse clinical outcome than the leukemias arising from GMPs are.

Bachoo et al. [23] also published important study results that suggested that both stem cells [e.g., neural stem cells (NSCs)] and differentiated cells (e.g., mature astrocytes) are converted to malignant cells. They used NSCs and mature astrocytes from the brains of newborn Ink4a^{-}/Arf^{-} mice. It is noteworthy that p16INK4a and p19ARF synergize to maintain terminal astrocyte differentiation. Therefore, whether Ink4a^{-}/Arf^{-} cells can actually differentiate into normal mature astrocytes is of interest. In fact, the Ink4a^{-}/Arf^{-} phenotype is a hallmark of NSCs. And if the Ink4a^{-}/Arf^{-} NSCs by nature do not differentiate, how does one obtain the Ink4a^{-}/Arf^{-} mature astrocytes in the first place? Ink4a^{-}/Arf^{-} mature astrocytes have a curiously and strikingly greater proliferative rate than wild-type astrocytes have. Furthermore, serially passaged Ink4a^{-}/Arf^{-} mature astrocytes exhibit immortal growth, unlike wild-type astrocytes.

One cannot help but become suspicious when mature astrocytes happen to behave like immortal Ink4a^{-}/Arf^{-} astrocytes. One wonders whether they are really pure mature astrocytes or they are contaminated with latent NSCs within the primary culture; serial passages tend to select for the latter cell type. It is noteworthy that the astrocyte culture must be maintained in serum to prevent in vitro dedifferentiation. I wonder whether in vitro dedifferentiation actually reflects the selection of NSCs or progenitor cells with stem-cell features in serum-free medium. Indeed, the treatment of Ink4a^{-}/Arf^{-} but not p53$^{-/-}$ mature astrocytes with epidermal growth factor induced dedifferentiation as well as transformation of these cells. After all, it is the Ink4a^{-}/Arf^{-} rather than the p53$^{-/-}$ phenotype that imposes stemness on astrocytes.

Stem-Cell Theory of Cancer

It is important to point out that the theory of a stem-cell origin of cancer goes beyond the idea of cancer stem cells. It explains a fundamental force behind cancer, namely aberrant self-renewal and cellular differentiation within the core of a tumor. However, it also embraces many other attributes of malignancy owing to its derivation from a stem cell – immortality, onco-niche, angiogenesis, immunity, drug resistance, heterogeneity, genomic instability, invasiveness, and metastasis.

A critical distinction between the two ideas is that one assumes cancer stem cells must originate from normal stem cells, whereas the other implies that the cancer stem cells merely mimic the normal stem cells. The first assumption allows less fluidity or reversibility in the stem-cell hierarchy. It predicts that once instigated in a stem cell, many malignant phenotypes are already in place rather than newly formed. Therefore, it challenges some very basic premises of modern oncology – the role of genetic mutations, the concept of genetic instability, the idea of dedifferentiation, and the model of multistep carcinogenesis. It forecasts that many cancer targets not involved in the stem-cell pathways may not be as relevant because they are an effect rather than a cause of carcinogenesis; i.e., they are a marker rather than a maker of cancer.

Perhaps the most surprising and paradoxic implication of our theory is that cancer actually does not originate from stem cells. Instead, it would be more correct to say that cancer is derived from cells that have varying degrees of stemness in a stem-cell hierarchy. Because stem cells are supposed to be quiescent by definition, it is less likely that many adverse events will happen to them as such. Only when they are released from their stem-cell niche and start to differentiate along a stem-cell hierarchy do the progenitor stem cells become vulnerable to a plethora of carcinogenic insults and injuries. Consequently, the type of cancer formed depends on the type of progenitor stem cells being affected. Because it is the degree of stemness within these cells that determines the type of malignancy being formed, I chose to keep the word “stem cell” in this book to emphasize this point.

Conclusion

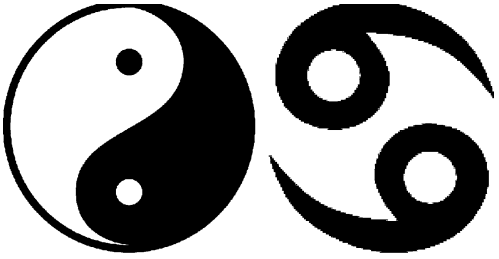
We have many questions and few answers about the origin of cancer. Although the body of a cancer cell seems obvious enough, the body of a stem cell is still quite obscure. One of the more salient features of a malignant tumor is that a core of cancer stem cells sustains it. Cancer is like a mob of rogue stem cells that have mastered the ways of normal stem cells and then mutinied. Words like “terrorist,” “insurgent,” and “militant” that have become so familiar to us in today’s world seem to describe cancer stem cells only too well. Other words that have become familiar these days, such as “avaricious,” “fraudulent,” and “predatory,” also seem to apply to cancer stem cells. Cancer stem cells are here to disturb our peace and threaten our wealth. Somehow, they have been issued a license to kill and steal. If a normal stem cell is the paragon of Opus Dei, then a cancer stem cell is the epitome of Opus Diaboli.

References

1. NOVA PBS science programming on air and online, October 11, 2005. Einstein's Big Idea. Ancestors of $E = mc^2$. Adapted from Bodanis D (2000) In: $E = mc^2$, biography of the world's most famous equation. Berkeley Publishing, New York
2. Lienhard JH (2005) Who invented the telephone? In: Engines of our ingenuity, episode 1098. The University of Houston's College of Engineering, Houston
3. Stevens LC (1964) Experimental production of testicular teratomas in mice. Proc Natl Acad Sci USA 52:654–661
4. Kleinsmith LJ, Pierce GB Jr (1964) Multipotentiality of single embryonal carcinoma cells. Cancer Res 24:1544–1551
5. Pierce GB, Dixon FJ Jr (1959) Testicular teratomas I. Demonstration of teratogenesis by metamorphosis of multipotential cells. Cancer 12:573–583
6. Becker AJ, McCulloch EA, Till JE (1963) Cytological demonstration of the clonal nature of spleen colonies derived from transplanted mouse marrow cells. Nature 197:452–454
7. Brunschwig A, Southam CM, Levin AG (1965) Host resistance to cancer. Clinical experiments by homotransplants, autotransplants and admixture of autologous leucocytes. Ann Surg 162:416–425
8. Hamburger AW, Salmon SE (1977) Primary bioassay of human tumor stem cells. Science 197:461–463
9. Bonnet D, Dick JE (1997) Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. Nat Med 3:730–737
10. Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF (2003) Prospective identification of tumorigenic breast cancer cells. Proc Natl Acad Sci USA 100:3983–3988, Erratum in Proc Natl Acad Sci USA 2003;100 (11):6890
11. Singh SK, Hawkins C, Clarke ID et al (2004) Identification of human brain tumour initiating cells [letter]. Nature 432:396–401
12. Matsui W, Huff CA, Wang Q et al (2004) Characterization of clonogenic multiple myeloma cells. Blood 103:2332–2336
13. Xin L, Lawson DA, Witte ON (2005) The Sca-1 cell surface marker enriches for a prostate-regenerating cell subpopulation that can initiate prostate tumorigenesis. Proc Natl Acad Sci USA 102:6942–6947
14. Kim CF, Jackson EL, Woolfenden AE et al (2005) Identification of bronchioalveolar stem cells in normal lung and lung cancer. Cell 121:823–835
15. Reya T, Morrison SJ, Clarke MF, Weissman IL (2001) Stem cells, cancer, and cancer stem cells [review]. Nature 414:105–111
16. Elhilali MM, Gleave M, Fradet Y et al; The Canadian Urologic Oncologic group (2000) Placebo-associated remissions in a multicentre, randomized, double-blind trial of interferon γ-1b for the treatment of metastatic renal cell carcinoma. BJU Int 86:613–618
17. Papac RJ (1998) Spontaneous regression of cancer: possible mechanisms [review]. In Vivo 12:571–578
18. Tu S-M, Lopez A, Leibovici D et al (2009) Ductal adenocarcinoma of the prostate: clinical features and implications after local therapy. Cancer 115:2872–2880
19. Gunsilius E, Duba H-C, Petzer AL et al (2000) Evidence from a leukaemia model for maintenance of vascular endothelium by bone-marrow derived endothelial cells. Lancet 355:1688–1691
20. Miyamoto T, Weissman IL, Akashi K et al (2000) AML1/ETO-expressing non-leukemic stem cells in acute myelogenous leukemia with 8;21 chromosomal translocation. Proc Natl Acad Sci USA 97:7521–7526

21. Radich JP, Dai H, Mao M et al (2006) Gene expression changes associated with progression and response in chronic myeloid leukemia. Proc Natl Acad Sci USA 103:2794–2799
22. Cozzio A, Passegué E, Ayton PM et al (2003) Similar MLL-associated leukemias arising from self-renewing stem cells and short-lived myeloid progenitors. Genes Dev 17:3029–3035
23. Bachoo RM, Maher EA, Ligon KL et al (2002) Epidermal growth factor receptor and Ink4a/Arf: convergent mechanisms governing terminal differentiation and transformation along the neural stem cell to astrocyte axis. Cancer Cell 1:269–277

Chapter 8
Cancer Niche



The Chinese symbol "yin and yang" mirrors the astrologic sign for Cancer, the crab

When the soil disappears, the soul disappears

– Ymber Delecto

Précis

Malignant transformation is not an entirely "cell-centric" event. It also involves *disrupted homeostasis between a perturbed cell and its disturbed niche*.

Introduction

A discussion about stem cells without mentioning the stem-cell niche does a great injustice to the subject: Something is missing from the discourse, as though one is trying to study the life of a creature without taking its habitat into consideration. The big picture of a stem cell is incomplete without its niche being included in the backdrop.

The niche keeps a stem cell as it is. In many ways, it is like the soil that preserves a cache of seeds in their pristine state. It protects the stem cell from excessive activity or premature exhaustion. When the occasion arises, the niche permits a stem cell to be released from its dormant state. The resultant stem cell then undergoes self-renewal

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and symmetric or asymmetric divisions. It differentiates into the respective appropriate progeny, which migrate to the assigned locations so that the right cell types and tissue components are regenerated.

The Dynamic Niche

In 1978, Schofield [1] first used the word *niche* to indicate a specialized microenvironment that houses stem cells. In architecture, a niche refers to a recess. In ecology, a niche is a habitat where an organism resides and reproduces. The study of germ cells in *Drosophila melanogaster* provided the first experimental evidence supporting the concept of a niche as a cellular microenvironment [2]. An important component of the stem-cell niche is the extracellular matrix. Examples of the extracellular matrix include β-1 integrin in the skin, tenascin in the nervous system, and osteopontin in the hematopoietic system. Specific cell types and paracrine factors are also important components of the stem-cell niche. Simple molecules, such as inorganic ions (e.g., Ca^{2+}) and reactive oxygen species, also contribute to the making of a stem-cell niche.

The niche is not a stagnant pool that bathes a stem cell or a cancer cell. Rather, the niche is a dynamic medium in constant flux that nurtures, regulates, and dictates the course of these cells. How the niche evolves along with the cells under its sphere of influence is open to conjecture and may be difficult to study. Surely, the niche must be quite different during embryologic development, when the hematopoietic stem cell undergoes differentiation and moves from the aorta–gonads–mesonephros to the placenta and then to the yolk sac, from what it is like when it subsequently migrates to the liver, the spleen, and finally to the bone marrow.

The Embryonic Niche

The niche determines the manifestation of a stem cell or a cancer cell. In a classic experiment, Leroy Stevens [3] demonstrated that normal stem cells obtained from a blastocyst formed a malignant teratoma when implanted in the vicinity of the testes. Conversely, Mintz and Illmensee [4] showed that malignant cells obtained from an embryonal carcinoma behaved like normal cells when inserted into the body of a blastocyst. Results from these and other experiments suggest that the embryonic microenvironment possesses antitumorigenic properties. For example, Rous sarcoma virus did not induce sarcomas in chicken embryos [5], and B16 murine melanoma cells failed to form tumors after exposure to embryonic niche factors derived from mouse skin [6].

It appears that certain cancer cells are quite capable of “speaking an embryonic language” and communicating with the endogenous embryonic cells. Topczewska et al. [7] showed that highly aggressive metastatic melanoma cells could organize the formation of an ectopic secondary dorsal axis complete with neuroectoderm, notochord, and nonaxial mesoderm when injected near the embryonic margin of a

zebrafish. Only the highly aggressive melanoma cells, not the indolent ones, secreted potent embryonic morphogens (e.g., Nodal) that initiated embryonic axis formation. Also, Postovit et al. [8] demonstrated that a three-dimensional microenvironment derived from human embryonic stem cells, but not their conditioned medium, reprogrammed aggressive melanoma cells to become less aggressive. These results thus allude to the possibility that embryonic elements derived from the embryonic niche could be harnessed to inhibit tumorigenesis and treat cancer.

Yin and Yang

Chinese philosophers during the Han Dynasty (207 BC–9 AD) formulated a principle known as yin and yang to account for the metaphysical workings of the entire universe. Yin and yang are two opposing forces, but yin can change into yang, and vice versa. This change occurs constantly, so neither one always dominates, and all conditions are subject to change into their opposites. The opposing forces and cyclic nature of yin and yang indicate that (1) all phenomena have within them the seeds of their opposite state, (2) all phenomena have the potential to change into their opposites, and (3) no phenomenon is completely devoid of its opposite state, since one produces the other, even though the opposite may not seem to be present. For example, health contains the seeds of sickness and sickness has the seeds of health. Therefore, one is never really completely healthy, since health does contain some inherent sickness.

Yin and yang are at work in stem-cell and cancer niches. Within a stem-cell niche, the inhibitory yin and stimulatory yang forces are in homeostasis. The inhibitory signals (e.g., BMP) are in constant balance with the stimulatory signals (e.g., Wnt). Hence, BMP activates Pten, which inhibits P13K, leading to decreased activity of Akt, which turns down Wnt. But when homeostasis is disrupted within an onco-niche, the stimulatory yang is favored over the inhibitory yin. Hence, a mutation in *Pten* may lead to a cascade of events that result in increased stimulatory signals, self-renewal, and cellular proliferation. When it concerns the stem cell or cancer cell and the stem-cell niche or onco-niche, there is no escape from the forces of yin and yang or "good" and "evil." In many respects, we already think this way: We converse more and more about positive and negative networks and about the intricate cross-talk in various biologic processes and oncologic mechanisms. No wonder the symbol of cancer mirrors that of yin and yang, as shown on the first page of this chapter.

Niche Matters

Thus, a discussion about the origin of cancer without mentioning the cancer niche is like prosecuting a criminal without investigating the crime scene. Normal healthy tissue has homeostasis – control, balance, and organization – of its cellular citizens.

However, when a malignancy arises, homeostasis is violated. The perpetrator could be a misfit stem cell, an unsavory niche, or both. So far, we have tended to indict only the wretched stem cell, which is no longer tethered within a strong niche. But we probably should also look into an unwholesome niche, which could have been the breeding ground for the rogue stem cell.

It is amazing how many seemingly benign factors in a stem-cell niche can become incriminating malignant elements during carcinogenesis. In an onco-niche, the instigating factors are more like a red tide than a fish out of water. In a red tide, the poisoned water causes an overgrowth of red algae that then suffocate not one but all fish. I postulate that the same benevolent niche that nurtures a stem cell becomes a malevolent niche when it begins to host a malignant cell. I suspect that most of the ingredients and components of an onco-niche are borrowed from a stem-cell niche. It does not need to reinvent a stem-cell "incubator" and repackage it into a cancer-cell ecosystem. The benign factors in a stem-cell niche could very well be in the wrong place for the wrong reasons at the wrong time and become the malignant elements in an onco-niche.

Donor-Cell Leukemia

The phenomenon of donor-cell leukemia (DCL) illustrates how a stem-cell niche becomes blurred with an onco-niche in real life. DCL is a rare but well-described disease entity that occurs when apparently normal donor hematopoietic cells transform and become leukemic in a recipient after hematopoietic-cell transplantation. Fialkow et al. [9] first reported DCL in 1971. Since then, reports of more than 50 cases have been published [10]. Recently, a large survey by the European Group for Blood and Marrow Transplantation revealed 14 cases of DCL that had arisen from 10,489 transplantations performed over a 21-year period [11]. The median time to the development of DCL was 17 months after hematopoietic-cell transplantation. Clearly, our ability to determine a donor origin of the leukemia affects the diagnosis of DCL. In the past, DCL was diagnosed mostly on the basis of morphologic differences, e.g., the presence of the donor Y chromosome in a female transplant recipient. In the future, however, DCL may be detected with improved analysis of donor–host chimerism by using, for example, short tandem-repeat analysis.

The observation that apparently healthy donor cells become leukemogenic shortly after transplantation supports the contention that host factors play an important role in the pathogenesis of DCL. In Hertenstein's study, donors from whom DCL developed did not seem to be at risk for and were not inclined to develop leukemia themselves [11]. Among the reported cases of DCL, no consistent pattern of cytogenetic abnormality has been found, and almost half have been associated with a normal karyotype. It seems that the host microenvironment in which the original malignancy developed could have triggered a similar oncogenic process within the donor cells.

The phenomenon of DCL thus supports the theory that the niche plays a critical role in the "Manifest Destiny" of a cancer cell. Malignant transformation is not an

entirely "cell-centric" event. It also involves disrupted homeostasis between a perturbed cell and a disturbed niche: It takes both the cancer cell and the onco-niche to make a cancer.

Stromal Factors

The stroma and certain stromal factors are well known to be important in epithelial carcinogenesis. For instance, many epithelial cells possess oncogene-activating mutations yet continue to function normally [12]. Radiation of the mammary gland induces irreversible changes in the stroma that contribute to carcinogenesis [13]. Mice with cell-specific ablation of the TGF-β receptor 2 in a subset of stromal fibroblasts develop carcinomas in the absence of any additional induced mutations in the epithelium [14]. And study of normal epithelial tissue adjacent to tumors has revealed similar patterns of mutations in both, suggesting that malignant cells can exist within normal tissues but are restrained by normal stromal cues [15].

Furthermore, during tumor progression, fibroblast-secreted protein 1 (Fsp1) is upregulated in carcinoma-associated fibroblasts (CAFs). Metastatic carcinoma cells injected into $Fsp1^{-/-}$ mice are less likely to form tumors and do not metastasize, whereas coinjection of $Fsp1^{+/+}$ fibroblasts with the tumor cells restores tumor development and metastasis [16]. Therefore, a logical and relevant question to ask about stromal cells and stromal factors is this: What is the origin and nature of the CAFs? The results of a study by Orimo et al. [17] suggested that the CAFs cannot evolve from cancer cells, i.e., they show no evidence of genetic alterations and undergo normal senescence in culture. Littlepage et al. [18] speculated that CAFs are a population of early developmental precursor cells that is initially present in the normal precancerous tissue and that expands in response to signals from cancer cells. Alternatively, malignant tumors might recruit CAFs from other sites, such as the bone marrow [19, 20], to accommodate a growing tumor with an enlarging onco-niche.

Epithelial–Stromal Interactions

The concept of epithelial–stromal cell interactions is compatible with the hypothesis of an onco-niche in which cancer cells interact with host cells and the microenvironment. A malignant cell, like its stem-cell counterpart, needs to interact with its neighbors. I postulate that if the host characteristics surrounding a cancer cell are similar to those surrounding a stem cell because of their common origin, then the two cell types may pick similar allies and hospitable neighborhoods.

Among cancer biologists, the realization is growing that in the complex game of cancer, the malignant epithelial cell is not the sole player. Other players (e.g., stromal and endothelial cells) are also important members of the team.

Although considered to be only role players, both stromal and endothelial cells are indispensable for the overall game plan and winning formula. Thus, to play well against an opposing team, we need to neutralize its principal player, which could very well be the malignant epithelial cell. But to beat the whole team, we also need to match well with each individual player, such as the stromal and endothelial cells. Hence, for the treatment of bone metastasis, we must neutralize not only the malignant epithelial cell that has spread to the bone but also contest the osteoblasts, osteoclasts, and endothelial cells in the bone that are assisting, blocking, and rebounding for it [21].

Perhaps we are confused by the definitions of stem cells. We are infatuated with the idea of targeting the epithelial, stromal, and endothelial compartments in a cancer with specific agents. For example, we are captivated by cytotoxic agents that eliminate the malignant epithelial cell, by stromal antagonists that target the mesenchymal element, and by vascular inhibitors that seek the endothelial component. Even now, we believe in treatments that interrupt certain malignant paracrine loops and various intricate pathways or cascades of epithelial–stromal interactions in a tumor. But what if all the malignant epithelial, stromal, and endothelial cells and all the epithelial–stromal interactions have a stem-cell origin? After all, we may have been trying the whole time to reinvent some treatments that actually target cancer stem cells and the onco-niche. This is the price we pay for not knowing what we are doing, for not understanding the true origin and nature of cancer.

Hypoxia

The stem-cell niche influences the function and differentiation of stem cells. Specific factors such as stromal-cell contacts, extracellular-matrix proteins, soluble factors, and hypoxia make up the immediate stem-cell niche. Hypoxia (3–5% O_2) promotes the survival of stem cells and maintains their pluripotency by activating HIF (hypoxia-inducible factor) 2a, which enhances Oct-4 expression [22]. In adults, Oct-4 is believed to be expressed exclusively by germ cells, although recent evidence suggests that Oct-4 is also present in adult stem-cell populations. Not surprisingly, ectopic Oct-4 expression also contributes to tumor growth. Thus, we again revisit a recurring theme in which a niche factor (e.g., hypoxia) appears to support stem cells and cancer cells similarly. It is ironic that both embryogenesis and carcinogenesis thrive in the same hypoxic microenvironment. In an incredible coincidence, hypoxia keeps both normal stem cells and cancer stem cells in a state of stemness and prevents them from differentiation.

An important property of normal stem cells is their ability to respond to a gradient of motomorphogens (i.e., chemokines) and to home to the appropriate sites during organogenesis and tissue repair. It is interesting that similar molecular mechanisms are at play when putative cancer stem cells migrate to selected sites during invasion and metastasis. Both normal and cancer stem cells express the seven transmembrane–spanning G protein–coupled receptor CXCR4 and respond to a gradient of its specific

chemotactic ligand, stromal-derived factor 1. And the expression of both CXCR4 and stromal-derived factor 1 is upregulated under hypoxia. Therefore, it appears that certain conditions, such as hypoxia, direct the homing of both normal and cancer stem cells to their particular stem-cell niche or onco-niche, respectively.

Niche as an Investigative Medium

In our investigation of the stem-cell niche, it is important for us to realize that many in vitro and in vivo conditions may not duplicate the natural conditions that maintain the stem cell as it is. In other words, unless the conditions of a stem-cell niche are kept just right, the stem cells may not be preserved and can easily change from one stem-cell state to another or to a more differentiated state. For an enlarging pool of normal or cancer stem cells, the corresponding stem-cell niche or onco-niche needs to expand as well to maintain them as stem cells. Therefore, an important caveat about the onco-niche is this: How can we investigate the precise nature of stem cells when we may not know how to maintain them as stem cells? How do we interpret the exact meaning of many experiments designed to study stem cells if they can be so ephemeral and mutable?

Conceivably, when we do not know enough about stem cells or how to keep them as stem cells, we may inadvertently alter their very being in the process of studying them. Thus, the results of an investigation may not answer the questions initially posed. Not understanding that we are dealing with a moving target is likely to produce more misconceptions and frustrations. Similarly, we may have unwittingly created an artificial, inappropriate, and misleading experimental system in the process of studying the onco-niche. For example, we like to use the aphorism, "cancer is like a wound that does not heal." But a cancer is not a nonhealing wound. Contrary to what one may think, in comparison with a noncancerous wound, a cancerous tumor shows relatively little regenerative activity or inflammatory reaction. Thus, an experiment that introduces exogenous cancer cells into an immunodeficient animal and produces local tissue injury can hardly represent the real events of carcinogenesis. We need to understand that what we observe in an experiment only remotely reflects what happens in reality. Often enough, what we see in an experiment is no more than an anomaly, an exception, or even a mischief of nature.

Niche as a Therapeutic Medium

The idea of an onco-niche is important partly because of its therapeutic implications. We need to remember that when we learn about the onco-niche, we must not neglect the stem-cell niche. Whether we like it or not, the two niches are intimately related. The stem-cell niche, which keeps stem cells quiescent, induces them to undergo asymmetric rather than symmetric division, mitigates self-renewal and promotes differentiation, and dissuades cellular invasion and migration, is also likely

to diminish the progression of cancer. It is conceivable that the same or similar stem-cell signal pathways that operate during normal embryonic development and tissue regeneration or repair become disrupted during carcinogenesis. The main difference between normal and cancer stem cells is in their response to homeostasis within their respective niches. Rather than fixing a particular target in a cancer cell or onco-niche, an effective therapy needs to modulate the cancer cell or onco-niche so that it behaves like a normal stem cell in a normal stem-cell niche. Hence, I postulate that certain drugs, such as statins (e.g., atorvastatin), hormones (e.g., phytoestrogens), angiogenesis inhibitors (e.g., bevacizumab), antiinflammatory agents (e.g., celecoxib), antioxidants (e.g., lycopenes), and vitamins (e.g., vitamin D) will be effective cancer chemopreventive agents or maintenance agents after cytoreductive therapy because they influence both the stem-cell niche and the onco-niche. I also predict that modulation of the onco-niche will become an important strategy for chemoprevention and for the maintenance treatment of cancer in the foreseeable future.

If a cancer cell were derived from a stem cell, then an onco-niche would keep a cancer cell, just as a stem-cell niche would keep a stem cell. Indeed, most people are quite willing to accept the idea that the cancer cell is like a "seed" that germinates during metastasis at a distant site where the appropriate conditions, or "soil," exist(s). It is not much of a leap to accept the idea that instead of keeping a cancer cell quiescent, an onco-niche may activate and drive it down the path of aberrant self-renewal, symmetric division, and mobilization: i.e., promoting carcinogenesis. Conversely, if we could manipulate the onco-niche and render it more akin to the stem-cell niche, then we might be able to keep cancer cells in check, making them more indolent, if not dormant. Therefore, one promising therapeutic maneuver is to modulate a cancer niche by inducing cancer cells to behave like or differentiate into more benign or indolent entities. The time will soon come when treatment of the onco-niche becomes an integral aspect of cancer therapy: It will be like managing the soil so that even if a malignant seed remains, it behaves in such a manner that it does not grow like a weed.

Conclusion

The theory of a stem-cell origin of cancers is not just about cancer stem cells, it is also about the onco-niche. Cancer is not a fluke of nature; there is a source, a cause, and a reason for its being. Whether a malignant tumor arises from a stem cell or a progenitor stem cell explains both its diverse origins and the diversity of cancer. Consequently, cancers and their respective onco-niches must go hand in hand with their corresponding stem cells or progenitor stem cells of origin and their corresponding stem-cell niches. This comprehensive view explains why some cancers are virulent and others are indolent, why some tumors are phenotypically more heterogeneous than others, why some tumors are more prone to metastasize and home to more variable sites, and why different tumors respond to different therapeutic modalities.

References

1. Schofield R (1978) The relationship between the spleen colony-forming cell and the hematopoietic stem cell. Blood Cells 4:7–25
2. Kai T, Spradling A (2003) An empty *Drosophila* stem cell niche reactivates the proliferation of ectopic cells. Proc Natl Acad Sci USA 100:4633–4638
3. Stevens LC (1964) Experimental production of testicular teratomas in mice. Proc Natl Acad Sci USA 52:654–661
4. Mintz B, Illmensee K (1975) Normal genetically mosaic mice produced from malignant teratocarcinoma cells. Proc Natl Acad Sci USA 72:3585–3589
5. Dolberg DS, Bissell MJ (1984) Inability of Rous sarcoma virus to cause sarcomas in avian embryo. Nature 309:552–556
6. Gershenson M, Graves K, Carson SD, Wells RS, Pierce GB (1986) Regulation of melanoma by the embryonic skin. Proc Natl Acad Sci USA 83:7307–7310
7. Topczewska JM, Postovit L-M, Margaryan NV et al (2006) Embryonic and tumorigenic pathways converge via Nodal signaling: role in melanoma aggressiveness. Nat Med 12:925–932
8. Postovit LM, Seftor EA, Seftor REB, Hendrix MJC (2006) A three-dimensional model to study the epigenetic effects induced by the microenvironment of human embryonic stem cells. Stem Cells 24:501–505
9. Fialkow PJ, Thomas ED, Bryant JI, Neiman PE (1971) Leukaemic transformation of engrafted human marrow cells in vivo. Lancet 1:251–255
10. Flynn CM, Kauffman DS (2007) Donor cell leukemia: insights into cancer stem cells and the stem cell niche. Blood 109:2688–2692
11. Hertenstein B, Hambach L, Bacigalupo A et al; Complications Subcommittee of the Chronic Leukaemia Working Party of the EBMT (2005) Development of leukemia in donor cells after allogeneic stem cells transplantation – a survey of the European Group for Blood and Marrow Transplantation (EBMT). Hematologica 90:969–975
12. Stoler DL, Chen N, Basik M et al (1999) The onset and extent of genomic instability in sporadic colorectal tumor progression. Proc Natl Acad Sci USA 96:15121–15126
13. Barcellos-Hoff MH, Ravani SA (2000) Irradiated mammary gland stroma promotes the expression of tumorigenic potential by unirradiated epithelial cells. Cancer Res 60:1254–1260
14. Bhowmick NA, Chytil A, Plieth D et al (2004) TGF-β signaling in fibroblasts modulates the oncogenic potential of adjacent epithelia. Science 303:848–851
15. Deng G, Lu Y, Zlotnikov G, Thor AD, Smith HS (1996) Loss of heterozygosity in normal tissue adjacent to breast carcinomas. Science 274:2057–2059
16. Grum-Schwensen B, Klingelhofer J, Berg CH et al (2005) Suppression of tumor development and metastasis formation in mice lacking the S100A4(mts1) gene. Cancer Res 65:3772–3780
17. Orimo A, Gupta PB, Sgroi DC et al (2005) Stromal fibroblasts present in invasive human breast carcinomas promote tumor growth and angiogenesis through elevated SDF-1/CXCL12 secretion. Cell 121:335–348
18. Littlepage LE, Egeblad M, Werb Z (2005) Coevolution of cancer and stromal cellular responses. Cancer Cell 7:499–500
19. Kaplan RN, Riba RD, Zacharoulis S et al (2005) VEGFR1-positive haematopoietic bone marrow progenitors initiate the pre-metastatic niche. Nature 438:820–827
20. McAllister SS, Gifford AM, Greiner AL et al (2008) Systemic endocrine instigation of indolent tumor growth requires osteopontin. Cell 133:994–1005
21. Tu S-M, Lin S-H (2008) Current trials using bone-targeting agents in prostate cancer [review]. Cancer J 14(1):35–39, Erratum in Cancer J 2008;14(3):199
22. Covello KL, Kehler J, Yu H et al (2006) HIF-2a regulates Oct-4: effects of hypoxia on stem cell function, embryonic development, and tumor growth. Genes Dev 20:557–570

Chapter 9
Ontogeny and Oncology

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Nature to be commanded must be obeyed.

– Francis Bacon

Précis

The expression *oncology recapitulates ontogeny* connotes that normality in one context may be anomaly in another.

Introduction

One cannot help but notice blatant similarities between the biologic portfolios of stem cells and malignant cells. The parallels between those two cell types constantly remind us about a possible link between them. For example, 34% of the modulated gene-expression profile of a leukemic cancer stem cell is shared by a hematopoietic stem cell [1]. Many biologic processes and clinical events can be explained by this special relationship between the two cell types without invoking other causes. It is tempting to speculate that the cancer cell has hijacked many if not most attributes

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of a stem cell: In due time, stem cells will teach us much about cancer, just as cancer will teach us much about stem cells.

According to the theory of a stem-cell origin of cancers, when a stem cell converts to a cancer cell, the cancer cell retains many stem-cell features. This is in stark contrast to the prevailing view that a mature cell transforms into an immature malignant phenotype by acquiring mutations. I postulate, however, that a malignant cell is derived from a stem cell, which has multipotentiality and can therefore manifest both immature and mature phenotypes. When we consider how rampant stem-cell features are manifested during malignant transformation and how innumerable embryologic markers, pathways, and processes resurface during malignant progression, then the whole story of a stem-cell origin of cancers begins to make more sense. Indeed, this is a genuine example of oncology's recapitulating ontogeny.

The Nature of the Beast

A phenomenon that keeps repeating is hard to ignore. One such phenomenon is oncology's recapitulation of ontogeny. How embryonic genes and factors keep resurfacing during carcinogenesis is yet another piece of the puzzle of cancer's arising from stem cells. It is as though many embryonic genes and factors behave like stem-cell genes and factors that become reactivated during carcinogenesis.

Indeed, nobody questions that primordial cells in a blastocyst have stem-cell qualities. But what about the rest of the embryonic cells during early development, when morphogenesis and organogenesis are in full swing? The coordinated network of embryonic genes and integrated cascade of embryonic factors proceed in space and time according to a master plan and predestined schedule. Where do we draw the line to mark when an embryonic cell no longer behaves like a stem cell? It is interesting that when the total number of cells increases exponentially in a growing organism, certain embryonic cells are sequestered in special niches and become adult stem cells. It is also ironic that this idea is actually a rebirth of the "embryonal rest" theory first proposed by Virchow and Cornheim more than a century ago (Chap. 3). How strange it is that great – and even mundane – ideas have a way of cycling back again and again!

The Power of Reiteration

One way to impress the mind is to reiterate. This chapter dwells on the recurring theme that carcinogenesis recapitulates embryogenesis and oncology recapitulates ontogeny. For example, *Twist-1* has been shown to function as an oncogene in many human cancers, including carcinoma, sarcoma, melanoma, and neuroblastoma. Recently, Twist-1 was found to be involved in breast cancer metastasis through the

regulation of epithelial-to-mesenchymal transition, or EMT [2]. But Twist-1 is also a transcription factor known to play a crucial role in morphogenesis during development [3]. And mounting evidence indicates that Twist-1 is an important negative regulator of apoptosis during embryogenesis [4, 5]. When oncology keeps knocking on ontogeny, it will eventually dawn on us that the root of cancer lies within aberrant stem cells.

The expression *oncology recapitulates ontogeny* connotes that normality in one context may be anomaly in another. Adoption of this perspective may render a conventional view untenable and an unconventional one acceptable. Sometimes, a simple idea may cause a radical departure from or even a complete reversal of our traditional views. For example, what is the role of hypermethylation in cancer? Hypermethylation of certain genes is considered aberrant in many malignant cells, but it is also a normal, prevalent process in stem cells: It keeps the stem cells relatively quiescent during normal development and embryogenesis. When demethylation occurs in certain promoters of various genes, the stem cells embark on a path of differentiation by assuming certain specialized phenotypes and preordained functions. Therefore, if cancer cells are derived from stem cells and retain many if not most of the stem-cell features, then hypermethylation could be considered an innate characteristic rather than an acquired property of malignant cells. It is as though a cancer cell bears the contours of, rather than wears the costume of, a stem cell.

Reactivation of Embryonic Genes

A recurring theme in cancer is that embryonic genes or pathways normally turned off in adult tissues are mistakenly turned back on in cancer. Why can we not just say that a stem cell that still retains the embryonic gene or pathway becomes aberrant and malignant, instead? Why do we need to resort to the idea of a more complex mechanism of having an embryonic gene or pathway reactivated in a differentiated cell? The former scenario makes more sense because the embryonic genes or pathways are already in place as part of a stem cell's whole genetic or epigenetic makeup, which happens to resemble that of its malignant cell counterpart. After all, a malignant cell arising from a stem cell is likely to retain many if not most of the stem cell's genotypic and phenotypic packages, including its putative embryonic genes or pathways.

An example of carcinogenesis' recapitulation of embryogenesis can be found in the case of *Hox* genes (Chap. 12). Multiple attempts have been made to implicate *Hox* genes as causing or contributing to the oncogenic process. But *Hox* genes are not oncogenes or tumor-suppressor genes in the classic sense. Their expression captures a time and space in embryogenesis. It is conceivable that *Hox* genes (and many other cancer targets) are mere passengers rather than the drivers of tumorigenesis. Indeed, they provide vital clues about the origin and nature of cancer, namely its cells of origin.

Lee et al. [6] demonstrated that distinct subtypes of hepatocellular carcinoma (HCC) might result from the transformation of different cellular lineages rather than from the activation of different oncogenic pathways. They showed that HCC-HB, a subtype of HCC, arises from bipotential hepatic progenitor cells rather than from hepatocytes. HCC-HB carries a distinct molecular signature (namely, increased Ap-1 complex activity) that reflects normal hepatogenesis during embryonic development. Again, carcinogenesis seems to resurrect preexisting but dormant signaling networks or pathways that had been active during embryogenesis. In that study, patients with HCC whose gene-expression profile resembled that of fetal hepatoblasts rather than hepatocytes had a worse prognosis (i.e., higher recurrence rate and shorter survival time).

Liu et al. [7] demonstrated that lung cancer can also be seen through the lens of lung development. They showed that the basic genetic makeup and the molecular pathways during development provide a rational framework for the understanding of their roles during carcinogenesis. The association between lung development and lung cancer suggests an ontogenic basis for the diagnosis and prognosis of lung cancer. Hence, lung cancers associated with earlier stages of lung development confer a worse clinical outcome. Perhaps the malignant cell does not reinvent itself as much as it reveals its innate stem-cell self. And the best way for it to reveal its stem-cell self and origins would be through its embryonic roots.

Phillips et al. [8] reported similar findings and demonstrated that the aggressiveness of high-grade gliomas (HGG) is regulated by processes not unlike those involved in forebrain neurogenesis. The prognosis of the different subtypes of HGG paralleled the differentiation stages of the neural stem cells from which they originate. Specifically, the Mes subtype of HGG tended to show increased activity of PI3K/Akt signaling (e.g., Pten loss) and decreased expression of the Notch pathway elements (e.g., DLL3). Interestingly enough, both PTEN and Notch play critical roles during forebrain neurogenesis and maintain neural stem cells or progenitor cells in a proliferating undifferentiated state. Thus, the more aggressive behavior and worse prognosis conferred by Mes HGG may be largely governed by the same processes that also regulate the fate of stem cells during neurogenesis.

Resurgence vs. Reprogramming

It is tempting to think that many genes in cancer are damaged beyond repair. It is also tantalizing to think that many such genes are reprogrammed by mistake. In the process, we have adopted a view in which such malfunctioning genes cause havoc and disarray and create monstrous or “Frankenstein” cells in a malignant tumor.

But if we believe that a healthful system can become a sickly one, then it is easy to envision how a stem cell can convert into a cancer cell and how the cancer cell may use the abilities and functions of the stem cell in the wrong context and under false pretenses. In short, a cancer cell is a stem cell running amok.

Perhaps *reprogramming* is not the right word for our purposes. Reprogramming implies an intentional rather than a spontaneous action and suggests a deliberate manipulation of the genetic code or expression. It also betrays our ignorance about cancer's nebulous origins and sinister nature. It is true that when oncology recapitulates ontogeny, the trigger for action may be deliberate rather than random. But there is no need to invoke any genetic makeup or products when the same or similar stem-cell factors, pathways, and networks being used during embryologic development are also being used for malignant transformation. In those circumstances, *resurgence* may be a better word than *reprogramming*. Resurgence suggests incitement, whereas reprogramming suggests recalibration of a malignant process. Resurgence indicates that a local disturbance has developed into a riot, expanded into a rebellion, spread to become a revolution, and culminated in a coup d'état. Resurgence also alludes to the fact that all of the involved instigators and their supporters, together with their tools of communications and weapons of aggression, have always been available and accessible. Therefore, any perturbation – from within or without – can start a series of mutinous acts and destructive behaviors.

Asymmetric Division

In many ways, the question of symmetric versus asymmetric division captures the essence of oncology's recapitulation of ontogeny. Symmetric division permits the expansion of stem-cell number and the restoration of stem-cell pools depleted by injury or disease. The vast majority of embryologic stem cells divide symmetrically to generate sufficient cells during development. In contrast, adult stem cells divide asymmetrically to maintain homeostasis: Under steady-state conditions, an adult stem cell divides asymmetrically to generate one daughter cell that retains its stem-cell fate and one daughter cell that is destined to differentiate into somatic cells. Asymmetric division is influenced intrinsically by the partitioning of cell-fate determinants and extrinsically by the effects of microenvironmental cues. Conceivably, stem cells regularly switch from symmetric to asymmetric division and vice versa, depending on the physiologic conditions at the time. Perhaps by necessity, or perhaps by default, when asymmetric division is disrupted, uncontrolled symmetric division occurs and malignant transformation ensues. According to this notion, carcinogenesis can occur in any cell that has this special stem-cell feature, namely the capacity to undergo asymmetric division.

The idea that asymmetric division is an intrinsic, integral part of stem-cell biology and carcinogenesis implies that the machinery that regulates asymmetric division has the capacity to protect against cancer. Indeed, Morrison and Kimble [9] showed that genes regulating cell polarity [such as Partner of Inscuteable (*Pins*) and atypical protein kinase C (*aPKC*)], cell fate determinants (such as *Numb* and *Prospero*), and mitotic spindle alignment have tumor-suppressor functions. Disruption of asymmetric division and its associated machinery produces some of

the most enduring hallmarks of malignancy, such as genomic instability and aneuploidy. The concept that asymmetric division is pivotal for carcinogenesis is consistent with the observation that cellular aneuploidy is an early acquisition in many tumors. Duesberg et al. [10] showed that carcinogens such as asbestos and arsenic initially do not cause gene mutations but do produce aneuploid lesions. Consequently, normal cells may become aneuploid long before they display any of the other stigmata of cancer.

Polycomb Silencers

Support for the concept of oncology's recapitulating ontogeny may also be found in the study of polycomb silencers. An association between the Polycomb group (PcG) protein gene family and cancer formation arose from the study of mouse Bmi1, a homolog of the *Drosophila melanogaster* Psc [11]. PcGs are transcriptional repressors that silence specific sets of genes through chromatin modifications. Because they alter gene expression without affecting the primary DNA sequence, they are known as epigenetic regulators. PcG proteins were originally identified in the fruit fly as repressors of *Hox* genes. Hence, they are primarily known for their role in maintaining cell identity during the establishment of the body plan.

Recent studies demonstrated that PcG proteins regulate stem-cell pluripotency by maintaining the repression of alternative-lineage genes that are necessary for differentiation of stem cells into various tissue types [11]. In embryonic stem cells, a significant proportion of PcG-targeted genes are controlled by three transcription factors, OCT4, SOX2, and Nanog, which are essential for maintaining stem-cell pluripotency. Thus, PcG proteins directly repress a large group of developmental regulators whose activation would otherwise promote differentiation; i.e., the stem-cell state is determined by specific suppression of differentiation genes. Selective derepression of PcG targets and activation of a certain subset of genes follows commitment to a particular cell fate during cellular differentiation.

The transforming potential of PcG proteins might be linked to their function as a keeper of the stem-cell fate. Disruption of PcG function can result in malignant transformation. One should not forget that the stem-cell fate is rigorously regulated by and closely connected to its respective cellular microenvironment or niche, which also plays an integral role in the malignant process. This leads to a key question: During carcinogenesis, could aberrant PcG proteins cause a differentiated cell to become dedifferentiated and malignant? Alternatively, do PcG proteins keep the aberrant malignant cells in a stem-cell state and unable to differentiate (which makes more sense, according to the stem-cell theory of cancer)? This pivotal question keeps reminding us about the central controversy of whether cancers indeed arise from differentiated cells and, if so, do cancers arising from differentiated progenitor cells possess an altogether different phenotype from that in cancers arising from stem cells or progenitor stem cells, despite the same genetic and epigenetic changes?

Epithelial-to-Mesenchymal Transition

Additional support for the concept of oncology's recapitulating ontogeny comes in the form of EMT. This idea envisions a change from one differentiated cell lineage to another during carcinogenesis. However, phenotypic switching is already rampant for the modulation of morphogenesis in the developing embryo [12]. EMT permits an epithelial cell to lose its polarity and detach from neighboring cells and enables it to acquire mesenchymal properties and become mobile or migratory. EMT plays a critical role during gastrulation and the formation of various tissues and organs, such as the neural crest, heart, and craniofacial structures. Not surprisingly, malignant cells have again stolen this embryologic cue for their own purposes during tumor invasion and cancer metastasis.

It is amazing that the same complex and coordinated set of molecular, transcriptional, and histologic changes occurring in EMT during embryogenesis are also commonly associated with cancer formation and progression [13]. Both involve a cascade of growth factors (e.g., transforming growth factor-β, Wnt), transcription factors (e.g., snail, SMAD, LEF, nuclear β-catenin), adhesion molecules (e.g., cadherins, integrins), cytoskeletal modulators (e.g., rho family proteins), and extracellular proteases (e.g., matrix metalloproteinases, plasminogen activators). An important note is that only a very small proportion of tumor cells actually display the EMT signature. This is reminiscent of the fact that putative cancer stem cells also constitute a very small proportion of the entire tumor.

Perhaps we do not need to invoke a special phenomenon like EMT to explain embryologic development when the involved cells have stem-cell properties and the potential to express both epithelial and mesenchymal lineages. Similarly, we need not invoke any fancy mechanisms like EMT to explain the formation and progression of malignancy when they can be easily traced to a stem-cell origin of cancer. I postulate that rather than making a transition from an epithelial to a mesenchymal phenotype, a malignant cell may express one or both phenotypes because of its stem cell of origin. This obviates the need for malignant cells to devise another special mechanism just to revert from a mesenchymal to an epithelial phenotype (MET) after they have metastasized. Hence, a stem cell that gives rise to a carcinosarcoma can express either or both epithelial and stromal phenotypes in the primary tumor or at the metastatic sites without needing to resort to either EMT or MET.

Compartmentalization of Cancer

It is ironic that one way for us to come to understand the inner workings of a living thing is to kill and dissect it. Another way for us to appreciate the intricacies of an object is to destroy and disassemble it. By visualizing its parts and learning how the various components are interconnected, we can comprehend the whole much more easily. Therefore, when we investigate cancer, we like to divide a solid tumor

into several compartments such as the epithelium, the mesenchyme (or stroma), and the endothelium. Because carcinoma is epithelial, the epithelial compartment was once thought to be almost entirely responsible for the malignant behavior of a tumor. Nowadays, we realize that cells are not really isolated units but depend on one another to survive and thrive. The concept of cell–cell interactions indicates that the mesenchyme is also an integral component of the whole malignant body. Moreover, exciting research on angiogenesis has clarified that the endothelial compartment is also an important component of the whole tumor.

Although partitioning cancer into epithelial, mesenchymal, and endothelial components seems conceptually appealing and intellectually sound, it does not negate the possibility that the various compartments are still derived from a common origin. Just as in the argument about the nature of EMT, I contend that the compartmentalization of cancer has a stem-cell origin. After all, a stem cell can evolve and differentiate into any cell type. Although the various progeny cells may or may not share their genotypes or phenotypes, they do coexist. Together, they constitute a favorable onco-niche for malignant cells, much like a stem-cell niche for stem cells. For example, an integral component of the stem-cell and onco-niches is the endothelial compartment. Many tumor types that express certain angiogenesis markers, e.g., vascular endothelial growth factor and microvessel density, in various cancers [14, 15] display a more aggressive phenotype and confer a worse prognosis than the types that do not express them. Therefore, angiogenesis is just as vital for stem cells and organogenesis in a developing embryo as it is for cancer cells and carcinogenesis in a developing malignancy.

Clinical Implications

The concept of oncology's recapitulating ontogeny has important clinical implications. One cannot help but notice that the same biologic properties that facilitate embryologic development also favor malignant transformation. No wonder some agents that possess chemoprevention effects (such as the nonsteroidal anti-inflammatory drug ibuprofen) or antitumor activities (such as the angiogenesis inhibitor thalidomide) may cause birth defects when taken at a time when fetal development uses the same biologic mechanisms. In other words, many chemoprevention and cancer drugs that target the same biologic pathways do not discriminate between malignant transformation and embryologic development.

The concept of oncology's recapitulating ontogeny also has important therapeutic implications. Targeting the various components of a tumor for therapeutic purposes may be easier when we parcel it into different compartments. For example, targeting only the malignant epithelial cells and treating them with cytotoxic chemotherapeutic agents may be insufficient; the remaining cancer cells tend to recover immediately because they are sustained by neighboring mesenchymal and endothelial cells. Consequently, one should also target the stromal elements and make the microenvironment less hospitable for a tumor to settle into and prosper. One should also

attack the endothelial component, thereby disrupting any angiogenic vessels that can bring ample supplies of nutrients and oxygen to support and feed the tumor. This could be the reason why therapy using combined cytotoxic chemotherapy and angiogenesis inhibitors or other selected targeted agents provides improved clinical outcomes [16–20].

Conclusion

Good advice to keep in mind when learning difficult lessons is to reiterate the lessons. Another good piece of advice when solving difficult puzzles is to look for repeat patterns. On this account, the expression *oncology recapitulates ontogeny* beckons us toward the theory of a stem-cell origin of cancers. According to this concept, many genetic or epigenetic changes found in a cancer may not be responsible for its being: they are only the markers, not the makers of a malignancy. Consequently, we need to be aware that certain genetic and/or epigenetic changes may be superfluous and maybe not even relevant to the making of a cancer, as we once believed.

I postulate that many genetic or epigenetic aberrations in a cancer have an embryologic or stem-cell root. When it concerns our search for the origin of cancer, having the wrong theory and only foggy notions is not just disconcerting, it can be downright costly. Thus far, we have attempted to find the right hand to fit the glove rather than finding the right glove to fit the hand. It is as though we have been going backward instead of forward in our quest to find the origin of cancer. One might say that when we make the wrong ontogenic point, we have to pay a high oncologic price.

References

1. Gal H, Amariglio N, Trakhtenbrot L et al (2006) Gene expression profiles of AML derived stem cells; similarity to hematopoietic stem cells. Leukemia 20:2147–2154
2. Yang J, Mani SA, Donaher JL et al (2004) Twist, a master regulator of morphogenesis, plays an essential role in tumor metastasis. Cell 117:927–939
3. Chen Z-F, Behringer RR (1995) *twist* is required in head mesenchyme for cranial neural tube morphogenesis. Genes Dev 9:686–699
4. Puisieux A, Valsesia-Wittmann S, Ansieau S (2006) A twist for survival and cancer progression. Br J Cancer 94:13–17
5. Valsesia-Wittmann S, Magdeleine M, Dupasquier S et al (2004) Oncogenic cooperation between H-Twist and N-Myc overrides failsafe programs in cancer cells. Cancer Cell 6:625–630
6. Lee JS, Heo J, Libbrecht L et al (2006) A novel prognostic subtype of human hepatocellular carcinoma derived from hepatic progenitor cells. Nat Med 12:410–416
7. Liu H, Kho AT, Kohane IS et al (2006) Predicting survival within the lung cancer histopathological hierarchy using a multi-scale genomic model of development. PLoS Med 3:e232
8. Phillips HS, Kharbanda S, Chen R et al (2006) Molecular subclasses of high-grade glioma predict prognosis, delineate a pattern of disease progression, and resemble stages in neurogenesis. Cancer Cell 9:157–173

9. Morrison SJ, Kimble J (2006) Asymmetric and symmetric stem-cell divisions in development and cancer. Nature 441:1068–1074
10. Duesberg P, Fabarius A, Hehlmann R (2004) Aneuploidy, the primary cause of the multilateral genomic instability of neoplastic and preneoplastic cells. IUBMB Life 56:65–81
11. Sparmann A, van Lohuizen M (2006) Polycomb silencers control cell fate, development and cancer. Nat Rev Cancer 6:846–856
12. Tarin D, Thompson EW, Newgreen DF (2005) The fallacy of epithelial mesenchymal transition in neoplasia. Cancer Res 65:5996–6001
13. Thompson EW, Newgreen DF, Tarin D (2005) Carcinoma invasion and metastasis: a role for epithelial-mesenchymal transition? Cancer Res 65:5991–5995
14. Hasan J, Byers R, Jayson GC (2002) Intra-tumoural microvessel density in human solid tumours. Br J Cancer 86:1566–1577
15. Toi M, Matsumoto T, Bando H (2001) Vascular endothelial growth factor: its prognostic, predictive, and therapeutic implications [review]. Lancet Oncol 2:667–673
16. Giantonio BJ, Catalano PJ, Meropol NJ et al (2007) Bevacizumab in combination with oxaliplatin, fluorouracil, and leucovorin (FOLFOX4) for previously treated metastatic colorectal cancer: results from the Eastern Cooperative Oncology Group Study E3200. J Clin Oncol 25:1539–1544
17. Hurwitz H, Fehrenbacher L, Novotny W et al (2004) Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. N Engl J Med 350:2335–2342
18. Miller K, Wang M, Gralow J et al (2007) Paclitaxel plus bevacizumab versus paclitaxel alone for metastatic breast cancer. N Engl J Med 357:2666–2676
19. Sandler A, Gray R, Perry MC et al (2006) Paclitaxel-carboplatin alone or with bevacizumab for non-small-cell lung cancer. N Engl J Med 355:2542–2550, Erratum in N Engl J Med 2007;356(3):318
20. Vredenburgh JJ, Desjardins A, Herndon JE II et al (2007) Bevacizumab plus irinotecan in recurrent glioblastoma multiforme. J Clin Oncol 25:4722–4729

Chapter 10
Diagnosis and Prognosis

"Tower of Babel" appeared in the book, *A Dweller in Mesopotamia*, published by Donald Maxwell in 1921; obtained from Google Images (www.gutenberg.org/files/18031/18031-h/18031-h.html)

Let us build us a city and a tower whose top may reach into heaven; and let us make us a name.…

– Genesis 11:4

Précis

Without a right theory, technology would not help us solve the mysteries of cancer. It would only provide us with more stones to *build an oncologic Tower of Babel*.

Introduction

It was not long ago when the diagnosis of cancer meant certain death. However, there is no doubt that cancer carries a vastly different outlook today. We have become increasingly convinced that a minority of cancers can be cured and what may not be cured can be treated. For instance, current treatments can cure acute leukemias and manage chronic leukemias. As we gain more experience and knowledge, a peculiar picture of cancer begins to emerge: Cancer is a disease with many identities. As a matter of fact, more than 100 types of what we have summarily

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grouped together as "cancer" arise from all sorts of tissues. Some cancers may be viewed as an acute illness (like acute appendicitis and pneumonia) that is curable, whereas others behave more like a chronic disease (like hypertension and diabetes mellitus) that can be treated but not easily cured.

Not all malignancies are lethal. For example, basal cell carcinoma of the skin and carcinoid from various sites do not normally kill their hosts. Somehow, these tumors are intrinsically indolent. They rarely metastasize. Nevertheless, they are still considered malignant when left alone, because they can cause debilitating symptoms or ghastly complications. Among the elderly population with indolent tumors, it is the other competing and compelling health issues, not their tumors, that eventually dictate their clinical outcome. Hence, patients diagnosed with smoldering chronic lymphocytic leukemia can have a life expectancy similar to that of their age-matched healthy controls [1]. Likewise, patients with prostate cancer with a low Gleason score tumor have enjoyed prolonged survival time without any therapeutic intervention [2, 3].

One cannot help but realize, though, that certain malignancies at the other end of the spectrum are inevitably fatal. These are the archetypical cancers that continue to strike fear in our hearts. Despite promising advances in screening, diagnosis, and therapy, patients still die from such cancers. Our current therapies are surprisingly powerless in treating these malignancies. One wonders whether the dismal plight of patients afflicted with these aggressive cancers may be buried in statistics. For example, we are witnessing a decreased incidence of cervical and prostate cancers, but why is it that the mortality rates associated with these cancers remain relatively unchanged? We are constantly being reminded that statistics alone do not tell the whole story. If we focus on the so-called Big Four cancers (lung, colorectal, breast, and prostate), we find that although the 5-year survival rate has increased (up to 20%) since the 1970s for patients with localized disease, the 5-year survival rate has scarcely improved during the same time period for patients with distant metastasis [4].

Cancer 101

To master the art of cancer diagnosis and prognosis, we need to go back to the basics and take a prerequisite class about the origin of cancer. When it concerns one of the most important fundamentals of cancer, *its cell of origin*, it is surprising that we are still very much in the dark. Not knowing its cell of origin, we grope as we learn the various aspects of cancer. We have very rudimentary knowledge about the diagnosis of cancer and make only modest headway in its prognosis. To reach our goal of personalized care in oncology, we need to know more about the precise origin and nature of individual tumors. One way to reach this primary aim is by way of the theory of a stem-cell origin of cancers. Unfortunately, I believe that we are still a long way from discovering the "mastermind" behind the master plan of cancer.

Personalized Cancer

A primary goal of clinical oncology is to unravel the biologic basis of disparate clinical entities so that appropriate treatments can be tailored to the individual cancers. For instance, why do some patients experience an indolent clinical course and fare exceptionally well even with minimal or no treatment? Conversely, why do other patients experience such an inexorable clinical course that whatever actions taken seem inconsequential if not futile? We have used various scientific tools such as clinical staging, tumor grading, mathematical nomograms, and biologic markers to help us make the correct diagnosis and an appropriate prognosis for our patients. Unfortunately, these clinical devices seem crude for our purposes. But I believe that a fundamental yet revolutionary change is forthcoming in the way we conduct business in cancer.

If the malignant potential of a tumor is innate within the stem cell from which it originates, then a molecular signature of the originating stem cell should be present in that tumor from the beginning. Evidence in support of this notion includes findings that colon cancer cells and colon stem cells display similar gene-expression patterns [5] and that a metastatic signature is already present in many primary tumors from the start [6–8]. We thus speculate that both clinically indolent and lethal tumors [9] are inherently "branded" and can be reliably distinguished by their respective origin in a stem-cell hierarchy. I predict that a correct theory about the origin of cancers will alter the way we diagnose and prognosticate cancer in the near future. I sense that something big is about to happen in the world of cancer. Already, this sentiment is manifesting in various phrases such as personalized medicine and systems biology. Before long, it will dawn on us that a correct theory about the origin of cancers will radically transform what we know about cancer and how we deal with it.

Lethal vs. Indolent Ca.ncers

Some patients diagnosed with prostate cancer rapidly develop metastases and succumb to the disease, whereas others harbor apparently indolent disease and survive even with metastases. I predict that the clinically virulent prostate cancers will be shown to display stem-cell signatures reminiscent of their early stem-cell origins. In contrast, the clinically latent prostate cancers will be found to exhibit a distinctly different molecular profile reflective of their derivation from late progenitor stem cells in the stem-cell hierarchy. Both tumor types may express a common differentiated target (e.g., the androgen receptor and prostate-specific antigen in prostate cancer), and both may respond to treatments that target the differentiated phenotype (e.g., androgen ablation). However, I also believe that the clinically aggressive form will be shown to contain unique cancer targets that are consistent with its early stem-cell origins and that contribute to its ominous (i.e., androgen-independent) phenotype.

Similarly, certain superficial bladder tumors rapidly progress to muscle-invasive and metastatic bladder cancers, whereas others remain relatively dormant despite acquisition and accumulation of the same cancer targets [10]. I postulate that certain genetic mutations in an early stem cell of bladder lineage lead to the development of a fulminant lethal bladder cancer, whereas the same mutations in a daughter progenitor stem cell result in the formation of a relatively indolent bladder tumor. Therefore, superficial bladder tumors with disparate malignant potential should display distinctive molecular signatures. Specifically, I predict that lethal bladder tumors may be distinguished from indolent bladder tumors by the presence of unique early stem-cell markers. Thus, I believe that elucidating stem-cell markers will provide an opportunity for a breakthrough in the validation of relevant cancer targets for the diagnosis, prognosis, and treatment of cancer.

Stem-Cell Hierarchy

I propose the existence of a hierarchical order of cancer origins that can predict the clinical hallmarks of a malignancy. Aberrant stem cells early in the hierarchy tend to form tumors of higher histologic grade, more advanced clinical stage, increased metastatic potential, and worse prognosis. In contrast, defective progenitor stem cells later in the hierarchy give rise to tumors of lower histologic grade, less advanced clinical stage, decreased metastatic potential, and better prognosis. It should be emphasized that the *early stem-cell phenotype may also include the late stem-cell phenotype but not vice versa*. This schema provides a novel perspective on the origin of clinically lethal vs. indolent tumors. More importantly, it represents a paradigm shift that establishes the stem-cell origin as a critical determinant in the clinical implications of various malignancies [11].

As a general rule for this model of a stem-cell origin of cancers, aberrant stem cells early in the hierarchy tend to form tumors that are clinically “acute” (i.e., fast-growing or fulminant). In contrast, defective progenitor stem cells later in the hierarchy tend to form tumors that are “chronic” (i.e., slow-growing or indolent). I assume that the early stem cells may initially express late malignant phenotypes. Later on, expression of the early malignant phenotypes could be interpreted as transformation (e.g., Richter lymphoma, chronic myelogenous leukemia blast crisis, castrate-resistant prostate cancer). Therefore, according to this model of a stem-cell origin of cancers, some tumors derived from late progenitor stem cells should remain chronic and not transform to the acute phase of the disease. Finding cases of chronic lymphocytic leukemia, chronic myelogenous leukemia, breast cancer, and prostate cancer that remain chronic for decades would support the theory of a stem-cell origin of cancers.

Recently, the notion of cancer stem cells has been caught in a maelstrom of not only scientific curiosity but also controversy. I believe that the theory of a stem-cell origin of cancers espouses the basic principles of cancer stem cells but extends

beyond its underlying implications and expands into a new dimension. This theory explains why only a few cancer stem cells may be present in any given tumor [12–14]. Hence, stem cells that become aberrant late in the stem-cell hierarchy tend to differentiate more easily and sustain a smaller pool of cancer stem cells in a tumor. However, stem cells in which defects appear early in the stem-cell hierarchy differentiate less readily and maintain a larger proportion of cancer stem cells in a tumor. Consequently, tumors that contain a smaller fraction of cancer stem cells (and a larger proportion of differentiated cancer cells) are more likely to pursue an indolent clinical course, whereas tumors that contain a larger fraction of cancer stem cells (and a smaller proportion of differentiated cancer cells) are more likely to pursue an aggressive clinical course.

Selection vs. Evolution

As far as carcinogenesis is concerned, our current attitude favors evolution over selection of cancer. Indeed, it seems intuitive and logical that a small tumor grows to become a large tumor. We also assume that simple tumors (lower clinical stage) evolve to become complex tumors (higher clinical stage). In the same vein, we tacitly accept that over time, a relatively benign or innocuous tumor (low grade) gradually transforms to become a malignant or dangerous cancer (high grade). This conceptual stance inevitably leads to the unquestioned adoption of the multistep-carcinogenesis model of cancer: Cancer becomes bigger, more aggressive, and more lethal as it acquires and accumulates increasing numbers and types of genetic mutations. The acquisition and accumulation of these genetic events by the cancer cells confer on them a survival advantage. Accordingly, if selection of cancer occurs at all, it occurs *after* the evolution of cancer.

The idea of evolution vs. selection of cancer carries important clinical implications. If cancer evolves, it should be possible to stop it early in its tracks (e.g., by screening and prevention). It should also be possible to design selected therapies that target the various steps of carcinogenesis. Although we have enjoyed some notable successes with cancer screening, chemoprevention, and targeted therapy, we have also experienced some stunning failures. One cannot help but notice that some small tumors metastasize early and are rapidly lethal. These tumors seem so surprisingly aggressive from the very beginning that it is almost impossible to screen for or prevent them, let alone treat them. One wonders whether these tumors simply skipped the intermediate sizes, stages, and grades during the relatively short time of their evolution. If the most malignant cancers are predestined to do their evil deeds from the outset, then screening or prevention may be too little or too late, and many selected therapies may be picking the wrong targets. Without effective treatments, it may not even matter whether we diagnose such cancers early or late! In short, it is entirely plausible that such cancers are actually selected from the very beginning rather than having evolved through the multiple stages of cancer.

Disease Selection by Therapy

Another burning question in oncology concerns the biologic meaning of response to therapy. What is the basis for the common observation that the same treatment elicits quite variable results in what appear to be similar cancers? We assume that the reason for such disparity is the heterogeneity of these diseases. But what is the underlying basis for such heterogeneity? Often enough, we observe that a complete response to therapy confers a better clinical outcome. Sometimes, patients who respond seem to experience a survival advantage compared with those who do not respond to therapy. But is it really the treatment that provides the clinical benefit? Or does the treatment merely separate the different diseases, some of which happen to be intrinsically more susceptible to treatment and possess favorable prognostic features, whereas others are relatively invulnerable to therapeutic interventions and are inherently more deadly? In other words, could this be a phenomenon of *disease selection by therapy or therapy selection by disease*?

Perhaps the effect of many cancer treatments can be put into the right perspective if we focus on the cancer rather than the treatments. To frame this in another way, what does it really mean when we see a particular therapeutic intervention increasing the complete remission rate, prolonging the time of remission, or enhancing the overall survival time of patients with cancer? Does the treatment actually eliminate some "cancer personnel"? Or does the treatment merely reshuffle the cancer personnel? In other words, a specific therapy may target a particular subtype of cancer cells but not the whole cancer (Fig. 10.1). The product could be a selection of tumor subtypes from therapy. Hence, a treatment that eradicates the more differentiated cells and the least virulent constituents of a malignancy may improve the complete remission rate and the overall survival time. However, because this same treatment may not affect the cancer stem cells or the more virulent components of the malignancy, the cancer will remain life threatening if not lethal. For example, finasteride appears to be efficacious as a chemoprevention agent when used for low-grade prostate cancer, but its value as a chemopreventive agent for high-grade prostate cancer remains unknown [15]. Further, chemotherapy may prolong the time of remission in some patients with germ-cell tumors, but this is because what remains after therapy may be a residual teratoma, which is relatively indolent. I surmise that the ultimate clinical outcome depends just as much on the type of cancer being treated as on the type of cancer treatment being given.

Diagnosis and Prognosis

One cannot help but notice that a particular cancer has a wide spectrum of tumor subtypes. In the case of neuroendocrine tumors, for example, small-cell carcinoma is on top of the totem pole. Next is a gradient of high- to low-grade neuroendocrine carcinomas. On the bottom of the totem pole are the carcinoid tumors. Small-cell carcinoma is inherently aggressive: it grows rapidly and spreads widely. Patients

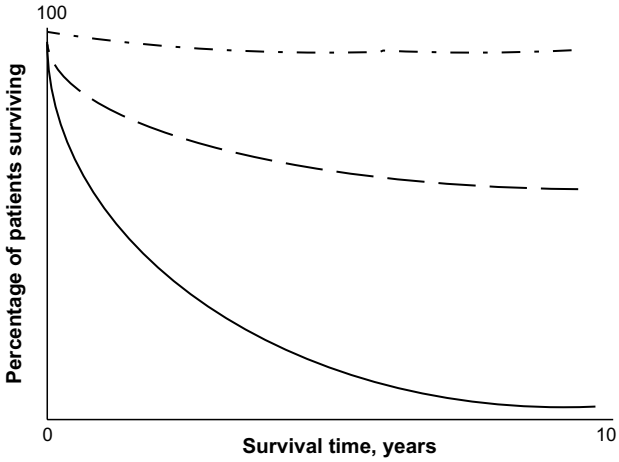


Fig. 10.1 A specific therapy may target a particular subtype of cancer cells but not the whole cancer. These *curves* depict the putative patterns of survival times for patients with cancers derived from early stem cells (*solid line*), intermediate stem cells (*dashed line*), and late progenitor stem cells (*dotted line*)

with small-cell carcinoma become ill in a hurry. They succumb to their disease quickly despite aggressive treatments. On the other hand, carcinoid tumors tend to be slow and indolent. Even when they metastasize, the patient may die with the disease rather than from it. I contend that this spectrum of tumor subtypes is the rule rather than the exception for most cancers. And I suspect that this gradient of tumor subtypes alludes to a hierarchical order in their respective stem cells of origin.

The notion that cancer cells recapture the essence of stem cells has already become evident in several microarray studies. For instance, certain molecular profiles found to be unique in malignant tumors actually recapitulate conserved genetic pathways normally activated in stem cells. Hence, Glinsky et al. [16] demonstrated that an 11-gene signature that embodied a stem cell–like expression profile (e.g., bmi-1 regulated) predicted the time to disease recurrence, distant metastasis, and death after therapy in diverse primary tumors (including epithelial malignancies, such as prostate, breast, lung, ovarian, and bladder cancers, and nonepithelial malignancies, such as lymphoma, mesothelioma, glioma, and acute myeloid leukemia). Data from studies like this support the hypothesis of a stem-cell origin of cancers, which predicts that the malignant potential of a particular tumor may be determined in large part by its place in a hierarchy of stem-cells during carcinogenesis.

As we strive to improve the diagnosis and prognosis of cancer, it is imperative that we distinguish whether the different cancer subtypes arise from different stem cells and progenitor stem cells or arise from an array of molecular alterations within these same stem cells and progenitor stem cells. Perhaps this distinction is a matter of semantics because alteration of a particular set of stem-cell elements may reflect its specific stem cell of origin. And perhaps this distinction is rather arbitrary and

artificial because one may be associated with or encompass the other. But what if this distinction is not only real but also crucial? This would indicate that different cancer subtypes arise because of their origins from different cell types rather than as a consequence of their different molecular alterations. It could produce a complete paradigm shift and a substantial overhaul of our current ways of diagnosing and prognosticating cancer.

Methods and Norms

To improve our future capabilities, reexamining our past ways of diagnosing and prognosticating cancer is informative. Eventually, the diagnosis and prognosis of a disease boils down to its pathologic characteristics, which are critical for the prediction of a tumor's clinical behavior and the selection of appropriate treatments. The time has finally arrived for pathology to catch up with the dawn of a new era, in which the theory of a stem-cell origin of cancers dictates its rules and principles.

Take the case of oncocytic tumors. We recognize the presence of oncocytes according to their microscopic appearance. Oncocytes are epithelial cells with abundant acidiphilic, granular cytoplasm that can be arranged in alveolar, tubular, trabecular, solid, or diffuse architectural patterns. Electron microscopy has demonstrated that the cytoplasm of oncocytes is packed with mitochondria. Not unexpectedly, oncocytic tumors consistently stain positive for the mitochondrial antigen mES-13. These tumors have been found in many organs, including the kidney, the salivary gland, and many endocrine organs, as well as in various other anatomic sites. Historically, we diagnosed oncocytic tumor on the basis of histologic criteria. However, the precise origin and exact nature of oncocytes are completely unknown. In short, "oncocyte" is a purely descriptive histologic entity.

Also traditionally, the diagnosis of an oncocytic tumor was closely linked to its prognosis by correlating its histologic features with clinical outcomes. Not knowing how to do any better, we have accepted this as the correct way of conducting business in oncology. Otherwise, how do we know whether an oncocytic tumor is benign, borderline, or malignant? We recognize that certain histologic features are associated with bad behavior of a tumor. However, we also realize that tumors tend not to be uniform and no universal rules govern their dispositions. One way to overcome such shortcomings is to use a set of convenient criteria to correlate histologic features with clinical outcomes. Hence, if an oncocytic tumor exhibits one *major* criterion of high mitotic rate, atypical mitoses, or venous invasion, it should be regarded as malignant and treated as such. If it has one *minor* criterion of large size, necrosis, or capsular invasion, it should be considered borderline. In other words, we are not sure what it really is, but it is not as dangerous as a malignant tumor. However, if the tumor does not possess any of either major or minor criteria, then it should be designated as benign, and we should leave it alone.

Thus, as this case illustrates, our current pathologic norms and methods for diagnosing and prognosticating cancer are crude to say the least. Their inadequacy

arises from a fundamental lack of understanding about the origin of cancer. Although these norms and methods may have served their purpose, they fail to address the crucial question of why, often enough, they do not work at all. They may have worked for this whole time partly because they are close enough to the "right answer" most of the time. After all, the histologic criteria being adopted do represent certain "stem-cell features" of cancer. For instance, the high mitotic rate, atypical mitosis, or vascular invasion could reflect self-renewal, loss of asymmetric division, and increased motility, capabilities inherent in aberrant stem cells. But no matter how clever or lucky we have been, these criteria do not conceal the fact that we are still just as ignorant as ever. Our current system to diagnose and prognosticate cancer is simply inadequate and obsolete. The time has arrived for us to consider a major paradigm shift. I propose that one way to start this radical makeover is to adopt the novel unified theory of a stem-cell origin of cancers.

Rosetta Stone or Tower of Babel?

It is important to point out that as long as we do not know the origin or nature of cancer, we will continue to be disappointed and bewildered in the face of advanced technology. For example, microarray technology has generated a great deal of hope and expectation about potential breakthroughs in the diagnosis and prognosis of cancer. But without the right theory about the origin of cancers, we should not be surprised that technology alone does not help us decipher the secrets of cancer. Without the correct code to the basic biology of cancer, we should not be shocked that microarray technology has not turned out to be the Rosetta stone of cancer. Instead, technology will only provide more stones for building an oncologic Tower of Babel, which could fall and be abandoned at any moment.

A potential pitfall of technologic advances like microarray analysis is that the results depend very much on the context in which the cells are being used or compared. Otherwise, how do we explain the finding that there is very little overlap in a multitude of gene-expression studies even within the same samples? Conceivably, we are detecting different gene expressions under different conditions or circumstances. Therefore, we are always at risk of comparing apples with oranges or even with different apples. Often enough, we do not even know the reason, cause, meaning, or significance of the statistical differences detected in the comparisons from such studies.

Another drawback when dealing with cancer is that the cells within a tumor are heterogeneous even under the best circumstances. If malignant cells were homogeneous, then of course we would be able to interpret the data more easily. But what if the root of malignancy in a tumor belongs to a fraction of the so-called cancer stem cells, whereas most of the tumor burden comprises differentiated cancer cells? When we add another layer to the complexity of cancer, we realize that many microarray experiments may be missing the boat because of our assumption that the genetic profile or molecular signature of the differentiated cancer cells is similar to that of the

cancer stem cells; i.e., they bear a similar insignia and are acceptable representatives of the cancer stem cells. Obviously, whether this assumption that the differentiated cancer cells still retain, capture, or reflect many if not all hallmarks of the quintessential cancer stem cells is correct has tremendous biologic and clinical implications.

Finally, we need to realize that most data obtained from microarray analyses (and most other experiments) are static "snapshots" rather than a dynamic "movie" of the many complex events and interactions occurring in real life. This is especially true in the study of genomic profiles of patients whose tissue samples tend to be limited. Just imagine how difficult it is to make sense of or interpret the whole story of a movie from only a few snapshots from it! It is like trying to know the identity of a person by looking at his shadow or to understand the content of a book by reading a few random pages. Claiming that we know the whole movie, person, or book in such instances is preposterous.

A Modest Proposal

It would be foolhardy to declare that I know the answer to the origins of cancer. I do not pretend to speak to a greater truth. However, in our continuing drama to solve the mysteries of cancer, we should not ignore what may seem like a trivial observation: Why is it that one cancer only maims and another invariably kills? In one case, a patient easily responds to various treatments and lives 30 more years. In another seemingly similar case, a patient scarcely responds to any treatment and survives barely 3 years. When it concerns the origins of cancer, I propose that selection determines the evolution of cancer rather than evolution's determining the selection of cancer.

It is important to remind ourselves that our mind-set may actually be confined or limited by scientific research. We can never see what happens to each colonic tumor as it evolves in a particular individual. We make inferences from available data that are convenient and practical for us to obtain. It is never easy, and it becomes even more difficult when we can only peek at a snapshot rather than view the whole movie of carcinogenesis. I worry that if we continue to operate with an incorrect theory and many wrong assumptions, we will be continuing to design the wrong experiments and interpreting the available data incorrectly. I speculate that the reason why one treatment is effective for a high-grade or advanced-stage tumor but not in a low-grade or early-stage tumor is not because of their different genetic makeup but because they are different diseases with different origins.

Conclusion

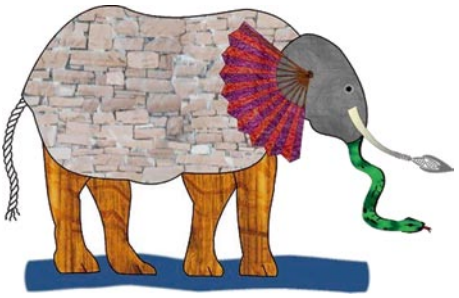
It is ironic how the deformity of a cancer cell reflects the beauty of a stem cell, and vice versa. Considering how little and rudimentary our knowledge is about cancer, one should not be surprised to find that our past and current means of diagnosing and

prognosticating cancer are truly very primitive. The way in which we have always administered our cancer treatment seems somewhat inhuman and will be inexcusable and unacceptable if we must continue similar treatments for the foreseeable future. One wonders whether human-rights advocates will at some point express outrage about our conduct in the diagnosis, prognosis, and treatment of cancer. Without a proper understanding of cancer's origins, we are often limited to guesswork. Instead of having a sound scientific basis for what we do, we often need to resort to trial and error. When it comes to conquering cancer, we need to rethink our current approaches rather than simply continuing to use our hit-or-miss methods.

References

1. Montserrat E, Rozman C (1993) Chronic lymphocytic leukaemia: prognostic factors and natural history. Baillieres Clin Haematol 6:849–866
2. Chodak GW, Thisted R, Gerber GS et al (1994) Results of conservative management of clinically localized prostate cancer. N Engl J Med 330:242–248
3. Johansson JE, Adami HO, Andersson SO et al (1992) High 10-year survival rate in patients with early, untreated prostatic cancer. JAMA 267:2191–2196
4. Leaf C (2004) Why we're losing the war on cancer [and how to win it] [Avastin, Erbitux, Gleevec...The new wonder drugs might make you think we're finally beating this dreaded scourge. We're not. Here's how to turn the fight around]. Fortune Magazine March 22, 2004, pp 77–92
5. van de Wetering M, Sancho E, Verweij C et al (2002) The β-catenin/TCF-4 complex imposes a crypt progenitor phenotype on colorectal cancer cells. Cell 111:241–250
6. Perou CM, Sørlie T, Eisen MB et al (2000) Molecular portraits of human breast tumours. Nature 406:747–752
7. Ramaswamy S, Ross KN, Lander ES et al (2003) A molecular signature of metastasis in primary solid tumors. Nat Genet 33:49–54
8. Ye Q-H, Qin L-X, Forgues M et al (2003) Predicting hepatitis B virus-positive metastatic hepatocellular carcinomas using gene expression profiling and supervised machine learning. Nat Med 9:416–423
9. van't Veer LJ, Dai H, van de Vijver MJ et al (2002) Gene expression profiling predicts clinical outcome of breast cancer [letter]. Nature 415:530–536
10. Spruck CH III, Ohneseit PF, Gonzalez-Zulueta M et al (1994) Two molecular pathways to transitional cell carcinoma of the bladder. Cancer Res 54:784–788
11. Tu S-M, Lin S-H, Logothetis CJ (2002) Stem-cell origin of metastasis and heterogeneity in solid tumours. Lancet Oncol 3:508–513
12. Al-Hajj M, Wicha MS, Benito-Hernandez A et al (2003) Prospective identification of tumorigenic breast cancer cells. Proc Natl Acad Sci USA 100:3983–3988, Erratum in Proc Natl Acad Sci USA 2003;100(11):6890
13. Lapidot T, Sirard C, Vormoor J et al (1994) A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. Nature 367:645–648
14. Singh SK, Clarke ID, Terasaki M et al (2003) Identification of a cancer stem cell in human brain tumors. Cancer Res 63:5821–5828
15. Thompson IM, Goodman PJ, Tangen CM et al (2003) The influence of finasteride on the development of prostate cancer. N Engl J Med 349:215–224
16. Glinsky GV, Berezovska O, Glinskii AB (2005) Microarray analysis identifies a death-from-cancer signature predicting therapy failure in patients with multiple types of cancer. J Clin Invest 115:1503–1521

Chapter 11
Cancer Targets



"The Elephant and the Blind Men" reproduced with permission from Jason Hunt, Portland, OR. The Natural Child Project (http://www.naturalchild.org)

The disputants, I ween,
Rail on in utter ignorance
Of what each other mean,
And prate about an Elephant
Not one of them has seen!

– John Godfrey Saxe

Précis

What ultimately determines the malignant phenotype depends not only on the nature of the cancer targets but also on the type of stem cells in which the cancer targets develop.

"The Blind Men and the Elephant." In: *The Poems of John Godfrey Saxe*. Complete Edition. James R. Osgood and Co, Boston, 1873, pp 135–136.

S.-M. Tu, *Origin of Cancers: Clinical Perspectives and Implications of a Stem-Cell Theory of Cancer*, Cancer Treatment and Research 154,
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Introduction

One marvels at the seemingly infinite list of putative cancer targets being discovered and attributed to the pathogenesis of malignancy. A skeptic may wonder how many of these targets are truly relevant biologically or clinically. A critic may worry that valuable time and vast resources have been wasted on the wrong targets for the treatment of a multitude of desperate cancer patients. Currently, we have no credible methods to validate the myriad candidate cancer targets: We need a fundamental shift in our paradigm of cancer to resolve this quandary.

Target du Jour

With powerful modern technology at our disposal, we have acquired a staggering amount of data at breathtaking speed. But increased knowledge does not always promise increased enlightenment. Perhaps our technology has raced way ahead of our theory. We urgently need to establish an intellectual platform to assimilate the vast amount of data collected. It is imperative that we discover a theoretical formula that will empower us to venture beyond mundane genetic mutations, signal pathways, and molecular signatures. The time has finally arrived for us to create a novel paradigm of cancer so we can improve our current interpretation of data and our design of future experiments – and unlock the mystery and secrets of cancer.

Before creating a new paradigm, though, should we not know the problem with the current one? Perhaps our current scientific method has produced many false cancer targets because of some erroneous premises. For example, it may be incorrect for us to assume that just because a genetic mutation is present in cancer cells but not in normal cells, it is automatically a cancer target. Likewise, we need to realize that just because such a genetic mutation is associated with cancer, it is not automatically the cause (as opposed to an effect) or a real mover (rather than a mere marker) of cancer. To admit that our current scientific method is refutable or fallible provides little consolation or confidence in our reservations. To say that these disclaimers carry tremendous biologic and clinical implications cannot be a greater understatement.

Paradigm Shift

According to the theory of a stem-cell origin of malignancy, carcinogenesis is a protracted process requiring multiple steps to complete. Therefore, detecting cancer targets in a stem-cell derivative (e.g., a malignant cell) is relatively easy. However, finding such targets in a differentiated somatic cell, which has a limited life span, may be futile. Consequently, it would be inappropriate if not misleading to compare the presence of cancer targets between malignant and normal cells or to declare that certain targets are unique to a particular cancer on the basis of such comparisons.

Therefore, comparing malignant cells with their normal counterparts is at least not ideal, if not actually improper. Microarray and proteomic studies comparing malignant with normal tissues are likely to yield false positive or negative results. Since a particular cancer target would not be preserved in a normal differentiated cell, as it would be in a stem cell that could develop into a malignant cell, declaring such a target as unique or specific to the malignancy is inherently erroneous (i.e., falsely positive results). Furthermore, microarray and proteonomic studies may miss the malignant cells of interest, namely the cancer stem cells, which may comprise only a small fraction of the entire tumor. Consequently, such studies are likely to underrepresent or misrepresent the relevant cancer targets (i.e., falsely negative results). I postulate that the relevance and specificity of the cancer targets will be enhanced when we compare a malignant cell with its normal stem cell of origin in such experiments.

Stem-Cell Theory

We hypothesize that malignant cells originate from aberrant stem cells [1]. Certain cancer targets may initiate the abnormal process. The aberrancy itself causes further accumulation of cancer targets. Since *many tumor types may possess the same cancer targets*, it is less likely that these cancer targets themselves determine the uniqueness of a particular malignancy. *I postulate that the eventual phenotype of a malignancy depends more on its stem-cell origin than on its cancer targets*. This view contradicts a popular notion [2–4] that specific cancer targets define a malignancy and that the accumulation of additional targets determines the final malignant phenotype.

For the theory of a stem-cell origin of cancers to stand, it needs to answer all the fundamental questions about cancer. Among the many questions regarding heterogeneity, metastasis, drug resistance, immunity, and so forth, one question stands out: What is the difference between "stem-ness" in a cancer stem cell and that of a normal stem cell (from which it may be derived)? In another words, how do we ascribe a stem-cell phenotype to a cancer cell when that phenotype is also prevalent in a normal stem cell? Although it is easy enough to distinguish a malignant phenotype from a normal phenotype simply by comparing a cancer cell with a somatic cell, it will be a challenge to discern a malignant phenotype from its stem-cell counterpart. I anticipate that many hallmarks of normal stem cells are preserved in cancer cells. For this reason, finding a cancer stem cell's Achilles' heel may prove invaluable if not indispensable for the design of improved cancer therapies in the future.

Death by a Thousand "Hits"

Awareness is growing that our ever-expanding universe of putative cancer targets makes a mockery of any attempts to understand its significance. We have more questions than answers. Many if not most of these putative targets do not seem

relevant or even specific enough, as far as the cause of cancer is concerned. Simply said, the numbers do not add up. Recently, Sjöblom et al. [5] identified 189 genes that were mutated at a significant frequency in breast and colorectal cancers. Individual tumors accumulated an average of about 90 mutated genes, but only a subset (about 11 per tumor) contributed to the neoplastic process. Moreover, mutations in the breast tumors differed substantially from those in the colorectal tumors. Further, in a comprehensive genetic analysis of 24 pancreatic cancers, Jones et al. [6] found that those cancers contained an average of 63 genetic alterations involving a core set of 12 cellular signaling pathways and processes in more than 1,000 genes. Similarly, Parsons et al. [7] found an average of 47 mutations involving more than 750 genes in an integrated analysis of 22 human glioblastoma multiforme tumors.

Indeed, the number of mutational events that develop during carcinogenesis is likely to be even higher. For example, the Sjöblom study did not include mutations in genes that had not yet been sequenced, could not be sequenced successfully, or were not yet included in the CCDS database (www.ncbi.nlm.nih.gov/CCDS/). In addition, some genetic mutations would not be detected by the method used, including those in noncoding genes, those in noncoding regions of coding genes, and relatively large deletions or insertions, amplifications, and translocations. Furthermore, some mutations selected during tumorigenesis are altered at a lower frequency and therefore were not included in the analysis according to the criteria used in the study. Finally, that study overlooked the effect of epigenetics, which induces carcinogenesis by causing aberrations in DNA methylation, chromatin structure, or chromosome segregation without affecting the DNA itself.

The Problem with the Gene Theory

Our dilemma with cancer targets can be traced to our ambivalence about the gene theory. Ever since the discovery of insulin in the 1960s, we have embraced the principle of "one gene, one protein." We accept that a gene that produces one type of protein in an organism may produce a remarkably similar protein in another organism. This notion has become dogma, dictating our scientific behavior and guiding our experimental rules. It has also laid the foundation for our conviction that genetic mutations play a central role during carcinogenesis. Hence, it is only logical for us to assume that the responsible genetic mutations are also the relevant cancer targets. There is every reason to believe that these cancer targets are also ideal candidates for therapeutic interventions.

Alas, things are never quite as simple as they seem! It is disconcerting for many of us to realize that our long-held "one gene, one protein" principle may not be as simple or straightforward as it seems. Already, there is ample evidence that many biologic phenotypes (e.g., height, obesity, intelligence) are the composite result of a meshwork of genes and a complex interplay of their products. For instance, the genetic makeup of a virulent form of malaria involves interactions among as many as 500 genes. Conceivably, cancer, in its infinite forms and varieties, is just as complicated,

if not more so. The implication of this realization is astounding: No longer will it be sufficient for us to focus on just one gene or one pathway in our battle against cancer. With an incredible stroke of luck, the concept of "one gene, one phenotype" may apply to a minority of cases in a modest way, but unfortunately, the time has arrived for us to adopt a new mind-set and to adapt to a new reality of "a network of genes and an array of proteins."

Necessity or Redundancy?

If certain properties of or processes in cancer stem cells had already existed in the normal stem cells from which they arose, then conceivably these same properties or processes could be redundant and not causative of the formation of cancer. Alternatively, certain mutations that affect these vital properties or processes in normal stem cells could be necessary if not indispensable for the formation of malignancy. The key is, How do we separate a redundant cancer target from a necessary cancer target? And the challenge is, How do we determine whether the malignant cell has merely inherited a redundant cancer target from its normal stem cell of origin or a malignant cell is actually taking advantage of a necessary cancer target in a different way under a different circumstance for a different purpose?

There is a subtle yet profound difference between saying a tumor *contains* cancer stem cells and saying that it *is derived from* a defective stem cell. The difference may allude to the distinct origin of disparate cancers. For example, normal stem cells express high levels of β-catenin. Is it merely coincidence (i.e., necessity or redundancy?) that genes coding for β-catenin are also frequently mutated in colon cancer [8]? Similarly, normal stem cells express Bmi-1, a polycomb group protein that represses expression of the *INK4* locus genes $p16^{INK4A}$ and $p14^{ARF}$. Of interest is that many human cancers also harbor inactivating mutations of $p16^{INK4A}$ and $p14^{ARF}$ [9]. Therefore, when we say a tumor *contains* cancer stem cells, do we mean that certain cancer cells happen to mimic normal stem cells because they have usurped their "stem-cell machinery" and acquired the capacity to express diverse "stem-cell" phenotypes? Or, when we say a tumor *is derived* from a defective stem cell, do we imply an alternative possibility – that this very same "stem-cell machinery" is already and has always been in place and that the many additional mutational targets found in the tumor are largely redundant, mostly inconsequential, and could very well be red herrings?

Credentialing Cancer Targets

When we do not have a firm grasp on the exact origin or nature of cancer, credentialing cancer targets is a fishing expedition – we are constantly at the mercy of chance. In a cancer cell riddled with genetic mutations, which ones actually drive

the cancer and which only take a ride? *I predict that some of the most relevant cancer targets must also be stem-cell targets. Invariably, these cancer targets are intimately related to the basic mechanisms of stemness, such as self-renewal, differentiation, asymmetric division, and mobilization*. Genetic mutations that act like stem-cell targets are likely to be the cause rather than an effect of cancer. They tend to be the real movers rather than mere markers of cancer. Therefore, I suspect that most of the other putative cancer targets are pretenders rather than contenders. It is only a matter of time before they will be found to be poor diagnostic markers, weak prognostic factors, or worthless therapeutic targets.

We will encounter other trip-ups and traps when it concerns the credentialing of cancer targets. Given that a tumor is composed of a spectrum and variable proportions of undifferentiated and differentiated cells, random sampling of the tumor for the purpose of a research study may yield only a small piece of the whole puzzle. Inevitably, a kernel of truth will remain hidden in the body or buried in the depth of a tumor. Suppose that the cancer stem cells within a tumor bear the insignia of its flagship. Do the rest of the differentiated cancer cells also carry the same insignia? Unless the molecular signature of differentiated cancer cells reflects that of the cancer stem cells in the same tumor, it will not be as meaningful or useful. And unless the expression profile of differentiated cancer cells represents that of the cancer stem cells and is not at the whims of a given condition or time, it could be misleading if not bogus. Only if cancer stem cells make up the bulk of a tumor will random sampling of a tumor capture its real essence in a research study.

The Making of Human Cancer Cells

Ironically, the results of various experiments already performed in both the laboratory and the clinic have suggested that it is the type of stem cell rather than the genetic defects within it that ultimately determines a malignant phenotype. For example, insertion of a desired gene product into stem cells may be carcinogenic: The use of such stem cells in gene therapy has resulted in leukemia in several patients [10, 11]. Transfection of putative cancer targets into a cell line that is by nature “immortal” and possesses intrinsic stem-cell properties is sufficient to produce malignancy.

However, inducing malignancy in somatic cells (i.e., primary culture cells) is extremely difficult. Welm et al. [12] showed that expression of c-Met and c-Myc failed to produce carcinomas, but when the same genes were transduced into putative stem cells, they produced mammary carcinomas. Attempts to transform cultured normal human cells into tumor cells by introducing various candidate cancer targets (e.g., ras, myc, other oncogenes) invariably failed. Not until these cells were rendered immortal (with hTERT) and certain key regulatory pathways (e.g., ras, p53, pRB) were disrupted did neoplastic transformation occur [13]. Indeed, we have successfully “manufactured” a cell that behaves like a stem cell (or a cancer cell) by using certain stem-cell factors (e.g., twist, snail) [14]. Under those conditions,

the cultured normal cells may have been reprogrammed to assume some semblance of a stem cell (or a cancer cell). But even in the best of circumstances, the "manufactured" stem cell and cancer cell were only so in laboratory terms; they lacked other important stem-cell traits (e.g., formation of a whole tissue, organ, or organism) or cancer-cell traits (e.g., metastatic tendency) that characterize real stem cells and cancer cells.

Field Defect

On several occasions, cancer targets have been detected in normal or nonmalignant cells. Paradoxically, such cases strengthen the hypothesis of a stem-cell origin of malignancy. An example of cancer targets occurring in normal-appearing tissues is the phenomenon of field defect. According to the prevalent view, the presence of cancer targets in field defects represents early carcinogenic events and as such is a potential marker of cancer susceptibility [15, 16]. An alternative explanation is that the presence of cancer targets in a differentiated cell merely reflects its common lineage with a neighboring neoplastic cell, both of which originated from an aberrant ancestral stem cell [17].

One clue to a stem-cell origin of cancer may be found in the MMTV-wnt1 transgenic mouse model. These mice spontaneously develop mammary tumors. Keratin 6, which is normally expressed in embryonic tissue that gives rise to the mammary epithelium (but not in wild-type adult cells), was found to be widely expressed in the mammary tissue of these animals when they are adults. An intriguing finding was that the keratin 6 protein was expressed in both myoepithelial and luminal cells, with both cell lineages showing a loss of heterozygosity (LOH) of the *PTEN* tumor-suppressor gene. This result is consistent with the phenomenon of field defect and suggests that cancer cells are derived from a progenitor cell that gives rise to multiple cell lineages, including both malignant and nonmalignant cells [18].

Cancer Targets in Nonmalignant Cells

Examples of finding genetic or molecular hallmarks of cancer in nonmalignant cells are abundant. The discovery that oncogenic alterations can also be found in differentiated cells, which are otherwise not malignant, strengthens the hypothesis of a stem-cell origin of malignancy. Otherwise, how do we explain the finding that endothelial cells isolated from patients with chronic myelogenous leukemia express the same oncogenic characteristics (i.e., BCR-ABL expression) as their malignant counterparts, the myelogenous leukemia cells [19]? Likewise, the BCR-ABL fusion transcript was identified in normal mature blood cells [20, 21]. In addition, the oncogenic fusion transcript AML-ETO [from a balanced t(8:21) translocation] was found in many nonleukemic cells [22].

Other evidence supporting the contention that it takes more than just cancer targets themselves to cause malignancy can be found in nonmalignant pathologic entities. For example, patients afflicted by rheumatoid arthritis possess abnormal synoviocytes, which contain p53 mutations [23] and PTEN irregularities [24]. However, these abnormal synoviocytes are not truly transformed: They ultimately become senescent when grown in culture, and they do not metastasize to other organs in vivo. Therefore, in addition to the cancer targets, the cells within which these cancer targets develop matter.

These results defy and challenge the prevailing view that certain genetic aberrations are the cause of cancer. Because certain oncogenic alterations are also present in apparently normal cells, it seems likely that these cancer targets are insufficient by themselves to form cancers when found in the wrong cell types, such as differentiated cells. We cannot just sweep these conflicting data under the rug. Rather, the time has finally arrived for us to reexamine and reformulate our theory about the origin of cancers. I postulate that instead of certain genetic aberrations in any cells, certain cells (i.e., stem cells) that harbor the genetic aberrations are also complicit in causing cancer.

Benign Prostatic Hypertrophy and Prostate Cancer

Benign prostatic hypertrophy (BPH) is an example of a setting in which cancer targets are found in otherwise normal-appearing tissues. As the name implies, BPH is considered to be a benign disease. About 75% of men older than 75 years have BPH. Thus, BPH tends to occur in the same population of men who develop prostate cancer. It is unclear whether some of the same etiologic factors that contribute to the formation of prostate cancer, such as androgens, family history, and diet, may also cause BPH.

BPH arises primarily in the transition zone of the prostate, whereas prostate cancer arises predominantly in the peripheral zone. Although the cellular constitution and epithelial–stromal interactions at these sites may be distinctly dissimilar, the genetic alterations found in BPH have surprisingly been found to be more alike than unlike those found in prostate cancer. Werely et al. [25] detected genetic mutations in 38% of BPH specimens and in 77% of prostate cancer specimens. In that study, the use of probes for identifying certain genetic mutations could not distinguish BPH from prostate cancer because these genetic mutations were present in both cell types. Using DNA microarray technology, Shah and Getzenberg [26] demonstrated genetic fingerprinting that was similar in BPH and prostate cancer: Alterations in the growth-regulatory, immunologic, stroma-associated, and transcriptional factor–cell signaling genes occurred in both BPH and prostate cancer.

Despite the differences in the location of BPH and cancer in the prostate and the prevailing view that BPH and prostate cancer are separate entities with unique origins, it is tempting to link the two diseases. The fact that they display a similar genetic makeup suggests that they must have common or parallel pathways of disease onset

or progression. How do we reconcile these perpetually conflicting and confusing views? I believe that these and other available data support the hypothesis of a stem-cell origin of cancers and surmise that a specific genetic mutation that causes neoplasia in a stem cell may instead cause hyperplasia in a differentiated cell. *What ultimately determines the malignant phenotype depends not only on the nature of cancer targets but also on the type of stem cells in which these cancer targets develop*. Consequently, the molecular portfolios of BPH and prostate cancer may more closely resemble each other than not. I forecast that the subtle differences or similarities between a benign entity and its malignant counterpart allude to their disparate cellular origins and can be explained by the theory of a stem-cell origin of cancers.

Endometriosis and Ovarian Cancer

Endometriosis, the implantation of endometrium-like glandular and stromal cells into places other than their normal location in the uterus, is considered to be a benign disorder. Endometriosis is often diagnosed in women who experience infertility, pelvic pain, dyspareunia (pain on intercourse), and dysmenorrhea (painful menstrual periods). The idea that endometriosis might engender malignant transformation was proposed as long ago as 1925 [27]. According to Varma et al. [28], the risk of ovarian cancer is increased by a factor of 4.2 in the presence of endometriosis. Similarly, the incidence of endometriosis is higher in women with ovarian cancer (8–30%) than it is in women of reproductive age (7–15%) or postmenopausal women (<2%) with a background incidence of endometriosis. It is interesting that ovarian cancer associated with endometriosis, relative to that without endometriosis, is often diagnosed at a less-advanced stage and with a lower grade and carries a better prognosis. An important note is that ovarian cancer adjacent to tissue affected by or arising from endometriosis shows genetic LOH alterations (9p, 11q, 22q) similar to those in endometriosis [28]. Furthermore, mutations of PTEN and p53 have been found in both ovarian cancers and endometriosis.

Does this common genetic background indicate that one disease is derived from the other? Current opinions favor the notion that endometriosis is a premalignant lesion and plays a role in the sequential progression of malignant transformation from endometriosis to ovarian cancer. But as is true in the case of many other clinical observations when there are striking and sometimes irrefutable molecular similarities between a relatively benign entity and a malignant process, there may be another explanation. *According to the theory of a stem-cell origin of cancers, it is not only the genetic changes but also the cell types in which these genetic changes occur that ultimately determine a malignant phenotype*. Hence, I postulate that genetic changes in a late progenitor or differentiated endometrial cell cause endometriosis, whereas the same changes in a more proximal stem cell predispose the cell to undergo malignant transformation. I further postulate that both ovarian cancer and endometriosis cells display a proteomic profile or molecular signature that reflects their respective cells of origin.

Et Tu, Stromatogenesis?

The question of whether the stromal component in a developing tumor is itself neoplastic or simply a reaction of the host to the tumor remains unresolved. Neoplastic cells may produce and release stromatogenic growth factors that affect the local microenvironment and induce normal host stromal cells to respond in a manner conducive to the metastatic processes [29]. Hence, genetic alterations in the stroma by themselves may not cause malignancy but may contribute to the potential for malignancy by virtue of their effects on epithelial–stromal interactions in the onco-niche. Alternatively, results from recent studies suggest that stromatogenesis may not be indigenous: Instead, exogenous bone marrow–derived cells [30] or mesenchymal stem cells [31] could contribute to the stromal elements in a metastatic nidus.

The finding of similarities in LOH in the epithelium and the surrounding stroma of a malignancy supports the theory of a stem-cell origin of cancers. Kurose et al. [32] detected a LOH in both neoplastic breast epithelial cells (25–69%) and the surrounding benign stromal cells (17–61%). The eight most common markers of LOH in the stromal compartment were also present in the epithelial compartment. Genetic alterations (e.g., LOH) in epithelial cells tended to precede similar changes in stromal cells. Moinfar et al. [33, 34] also found LOH in both epithelial and stromal components of both infiltrating and noninfiltrating breast carcinomas and of small-cell carcinoma of the uterine cervix. However, the genetic alterations in several cases either were encountered exclusively in the stromal cells or the stromal genetic changes preceded those occurring in the malignant epithelium. These results suggest that genetic alterations in a stem cell manifest in both epithelial and stromal progeny cells and support the theory of a stem-cell origin of cancers.

Targeted Therapy

Finally, what exactly is the role of targeted therapy in our arsenal for treating cancer? Targeted therapy may not provide a cure, but it could improve survival time. Although any clinical benefit from targeted therapy seems rather modest, it is still relevant. However, it might take hundreds if not thousands of patients to be treated in an effort to detect a survival advantage of just a few months! Nevertheless, targeted therapy is here to stay. Considering the big picture of cancer targets described in this chapter, we still do not really understand the basis for their apparent therapeutic efficacy. How do targeted therapies work, and why do they even work at all?

Take the tyrosine-kinase receptor inhibitors as an example [35]. Although tyrosine kinases (e.g., fibroblast, epidermal, platelet-derived, and vascular endothelial growth factors) are better known as growth factors in adult organisms, they also have important roles as morphogens (i.e., molecules that act by forming concentration gradients through a tissue) during development. The tyrosine kinase families are evolutionarily conserved from fruit fly to human and serve as master regulators

of position-dependent cell fate during embryogenesis. The importance of these morphogenetic pathways in the maintenance of epithelial homeostasis is underscored by the fact that mutations that disrupt them are instrumental in the initiation of innumerable cancer cascades. In other words, the signaling pathways that pattern a developing organism (morphogenesis) are equally important for maintaining tissue homeostasis (morphostasis) after birth and for preventing carcinogenesis.

It is interesting that many targeted therapies, especially those that are a single agent with a single target, provide only limited clinical activity or benefit. In fact, the best results from targeted therapy have come from combination treatments (e.g., with chemotherapy) or when the targeting agents have multiple targets (e.g., sunitinib for the treatment of renal cell carcinoma). There is no doubt that successful targeted therapy provides meaningful effects and favorable outcomes because it affects relevant cancer targets. I suspect that these relevant cancer targets also happen to be or must be relevant stem-cell targets.

I postulate that other targeted therapies are beneficial because they favorably affect the stem cell- or onco-niche. It is true that most targeted agents are cytostatic rather than cytotoxic; they do not eradicate the cancer stem cells. Yet patients who receive such treatments may experience improved overall survival (OS) time [even without prolongation of the progression-free survival (PFS) time]. Somehow, such treatments may attenuate the onco-niche without eliminating the cancer stem cells (see Chap. 8).

Clinical Implications

Assuming we have a particular targeted therapy that selectively affects cancer stem cells or the onco-niche, how do we detect and measure its efficacy? It is evident that our current clinical tools and methods (e.g., the Response Evaluation Criteria in Solid Tumors to measure response, PFS, and OS) are woefully inadequate for meeting this challenge. Therefore, if we wish to successfully design targeted therapies against cancer stem cells or the onco-niche, we need to discover novel secondary end points (e.g., biomarkers) and adopt innovative clinical strategies.

Perhaps the old standards of assessment could still be used or just interpreted differently. For instance, if a targeted therapy improves the response rate and the PFS time but not the OS time, it may have eliminated the differentiated cancer cells but not affected the relevant cancer stem cells. I postulate that if the treatment improves both PFS and OS times, it must have favorably affected both the relevant cancer stem cells and the onco-niche. However, if the treatment improves the OS but not the PFS time, it may have favorably affected the onco-niche but not necessarily the cancer stem cells.

I propose the use of an alternative strategy to validate targeted therapy and assess its therapeutic effects on cancer stem cells and/or the onco-niche: to provide *PROOF of benefit, mechanism, and concept*. Hence, it is acceptable to proceed with or continue treatment when we can establish proof of benefit (e.g., improved symptom

control) and proof of mechanism (e.g., positive correlative studies). A cancer treatment may be considered clinically beneficial even if it only stabilizes the disease without producing any objective response (e.g., measurable or biomarker). What is currently missing with targeted therapy is proof of concept. Once we also secure this proof of concept (e.g., according to the theory of a stem-cell origin of cancers), then it should be more than appropriate to embrace and use a particular targeted therapy because the ultimate proof of clinical benefit, i.e., increased OS time, will eventually become evident (provided that toxicity, cost, and other practical issues have been resolved).

Conclusion

I hypothesize that what ultimately determines the malignant phenotype depends more on the type of stem cell in which the genetic targets are found than on the battery of genetic targets themselves. But stem cells are not all created equal. Therefore, I predict that various stem-cell markers that distinguish unique, distinct stem cells or progenitor stem cells will one day be validated as ideal targets for the diagnosis, prognosis, and treatment of the clinically pertinent cancers.

The moral of the cancer-targets story is that when we want to understand the whole elephant, we should not be obsessed with only its body, trunk, tusks, or tail. We need to know *all* the pertinent and unique parts that will help us identify the animal. Otherwise, we will all be left to envision or imagine the whole animal with only a few parts of it at our disposal, like the poor blind men. Concerning the relevance of cancer targets, perhaps the American historian and author Daniel Boorstin said it best: "The greatest obstacle to discovery is not ignorance – it is the illusion of knowledge."

References

1. Tu S-M, Lin S-H, Logothetis CJ (2002) Stem-cell origin of metastasis and heterogeneity in solid tumours. Lancet Oncol 3:508–513
2. Knudson AG Jr (1977) Mutation and cancer in man. Cancer 39(4 Suppl):1882–1886
3. Poste G, Fidler IJ (1980) The pathogenesis of cancer metastasis. Nature 283:139–146
4. Fearon ER, Vogelstein B (1990) A genetic model for colorectal tumorigenesis. Cell 61: 759–767
5. Sjöblom T, Jones S, Wood LD et al (2006) The consensus coding sequences of human breast and colorectal cancers. Science 314:268–274
6. Jones S, Zhang X, Parsons DW et al (2008) Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. Science 321:1801–1806
7. Parsons DW, Jones S, Zhang X et al (2008) An integrated genomic analysis of human glioblastoma multiforme. Science 321:1807–1812
8. Reya T, Clevers H (2005) Wnt signalling in stem cells and cancer. Nature 434:843–850
9. Sherr CJ (2001) The *INK4a/ARF* network in tumour suppression. Nat Rev Mol Cell Biol 2:731–737

10. Hacein-Bey-Abina S, von Kalle C, Schmidt M et al (2003) A serious adverse event after successful gene therapy for X-linked severe combined immunodeficiency. N Engl J Med 348:255–256
11. Marshall E (2003) Gene therapy: second child in French trial is found to have leukemia [News of the Week]. Science 299:320
12. Welm AL, Kim S, Welm BE et al (2005) *MET* and *MYC* cooperate in mammary tumorigenesis. Proc Natl Acad Sci USA 102:4324–4329
13. Hahn WC, Weinberg RA (2002) Rules for making human tumor cells. N Engl J Med 347:1593–1603, Erratum in N Engl J Med 2003;348(7):674
14. Mani SA, Guo W, Liao MJ et al (2008) The epithelial-mesenchymal transition generates cells with properties of stem cells. Cell 133:704–715
15. Sidransky D, Frost P, Von Eschenbach A et al (1992) Clonal origin bladder cancer. N Engl J Med 326:737–740
16. Yu YP, Landsittel D, Jing L et al (2004) Gene expression alterations in prostate cancer predicting tumor aggression and preceding development of malignancy. J Clin Oncol 22:2790–2799
17. Tsai YC, Simoneau AR, Spruck CH III et al (1995) Mosaicism in human epithelium: macroscopic monoclonal patches cover the urothelium. J Urol 153:1697–1700
18. Li Y, Welm B, Podsypanina K et al (2003) Evidence that transgenes encoding components of the Wnt signaling pathway preferentially induce mammary cancers from progenitor cells. Proc Natl Acad Sci USA 100:15853–15858
19. Gunsilius E, Duba H-C, Petzer AL et al (2000) Evidence from a leukaemia model for maintenance of vascular endothelium by bone-marrow-derived endothelial cells. Lancet 355:1688–1691
20. Biernaux C, Loos M, Sels A et al (1995) Detection of major bcr-abl gene expression at a very low level in blood cells of some healthy individuals. Blood 86:3118–3122
21. Bose S, Deininger M, Gora-Tybor J et al (1998) The presence of typical and atypical BCR-ABL fusion genes in leukocytes of normal individuals: biologic significance and implications for the assessment of minimal residual disease. Blood 92:3362–3367
22. Miyamoto T, Weissman IL, Akashi K (2000) AML1/ETO-expressing nonleukemic stem cells in acute myelogenous leukemia with 8;21 chromosomal translocation. Proc Natl Acad Sci USA 97:7521–7526
23. Firestein GS, Echeverri F, Yeo M et al (1997) Somatic mutations in the p53 tumor suppressor gene in rheumatoid arthritis synovium. Proc Natl Acad Sci USA 94:10895–10900
24. Pap T, Franz JK, Hummel KM et al (2000) Activation of synovial fibroblasts in rheumatoid arthritis: lack of expression of the tumour suppressor PTEN at sites of invasive growth and destruction. Arthritis Res 2:59–64
25. Werely CJ, Heyns CF, van Velden DJ et al (1996) DNA fingerprint detection of somatic mutations in benign prostatic hyperplasia and prostatic adenocarcinoma. Genes Chromosomes Cancer 17:31–36
26. Shah US, Getzenberg RH (2004) Fingerprinting the diseased prostate: associations between BPH and prostate cancer. J Cell Biochem 91:161–169
27. Sampson JA (1925) Endometrial carcinoma of the ovary, arising in endometrial tissue in that organ. Arch Surg 10:1–72
28. Varma R, Rollason T, Gupta JK et al (2004) Endometriosis and the neoplastic process. Reproduction 127:293–304
29. Kaplan RN, Riba RD, Zacharoulis S et al (2005) VEGFR1-positive haematopoietic bone marrow progenitors initiate the pre-metastatic niche. Nature 438:820–827
30. McAllister SS, Gifford AM, Greiner AL et al (2008) Systemic endocrine instigation of indolent tumor growth requires osteopontin. Cell 133:994–1005
31. Karnoub AE, Dash AB, Vo AP et al (2007) Mesenchymal stem cells within tumor stroma promote breast cancer metastasis. Nature 449:557–563
32. Kurose K, Hoshaw-Woodard S, Adeyinka A et al (2001) Genetic model of multi-step breast carcinogenesis involving the epithelium and stroma: clues to tumour-microenvironment interactions. Hum Mol Genet 10:1907–1913

33. Man Y, Mannion C, Kuhls E et al (2001) Allelic losses at 3p and 11p are detected in both epithelial and stromal components of cervical small-cell neuroendocrine carcinoma. Appl Immunohistochem Mol Morphol 9:340–345
34. Moinfar F, Man YG, Arnould L et al (2000) Concurrent and independent genetic alterations in the stromal and epithelial cells of mammary carcinoma: implications for tumorigenesis. Cancer Res 60:2562–2566
35. van den Brink GR, Offerhaus GJ (2007) The morphogenetic code and colon cancer development [review]. Cancer Cell 11:109–117

Chapter 12
Heterogeneity of Cancer

Diversity makes for a rich tapestry…all the threads of the tapestry are equal in value no matter what their color.

– Maya Angelou

Précis

Cancer comprises many diseases with diverse phenotypes.

Introduction

One of the most fascinating but frustrating aspects of cancer is its innate heterogeneity. It is as though we are looking through a kaleidoscope at the disease, and treating it is like dealing with a chameleon. To complicate matters, some tumors are intrinsically more heterogeneous than others, and it is not always easy to tell which ones they are. The biologic basis of cancer's heterogeneity is enigmatic: Clinical observations indicate that a solid tumor can change into an entirely different histologic type during the course of the disease or therapy. For example, prostate adenocarcinoma may reemerge as small-cell carcinoma, squamous carcinoma,

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or carcinosarcoma after androgen-ablation therapy or radiotherapy. Furthermore, germ-cell tumors may undergo somatic transformation to form non–germ cell elements, including carcinomas, neuroectodermal tumors, sarcomas, and hematologic malignancies.

This intrinsic heterogeneity of malignancy continues to baffle us and hamper our ability to treat it. Although the pathologic and clinical heterogeneity of cancer is well established and easily recognized, the basic biologic mechanisms underlying such heterogeneity still remain surprisingly unfathomable. However, I suspect that this inherent characteristic is a crucial key to unlocking the mystery of cancer's origins. This chapter discusses how the theory of a stem-cell origin of cancers may help us solve this mystery.

Apples and Oranges

Cancer's heterogeneity means that it comprises many diseases with many identities. By grouping these different diseases into one entity, we inevitably run into problems. Some cancers also display diverse phenotypes. Thus, at one level, we are comparing apples with oranges, but at another level, we are comparing different types of apples. Perhaps we have been naive in thinking that cancer is a simple problem of bad apples: Could it be that the problem runs deeper and may actually involve a rotten seed or a blighted tree? I believe that much of the complexity about this heterogeneity will be simplified by solving the origin and nature of various cancers, and many of our long-standing questions will finally be answered.

The Good, the Bad, and the Ugly

When it concerns heterogeneity, we wonder, Does cancer consist of all sorts of bad cells or is it, instead, a mix of good, bad, and ugly cells? The stem-cell theory of cancer suggests that a stem cell has both good and bad properties, with mostly the latter being manifest in a cancer cell. It is as though a cancer cell is the demonic alter ego of a stem cell, sort of a Dr. Jekyll and Mr. Hyde case. Alternatively, it could be that cancer occurs when a good stem cell falls from grace and becomes a bad cancer cell.

Stem-Cell Theory

According to Sell and Pierce [1], the initiation and promotion of malignancy arise from blocked differentiation of stem cells rather than from dedifferentiation of mature cells. Because it takes many months or even years for the promotion of

cancer to occur, its initiation has to take place in stem cells or progenitor stem cells because they are the only cells that can survive for a prolonged period. Hence, stem cells are prime targets for an initiation event or a "first-hit" mutation. Mutations occurring in somatic cells do not result in cancer because mature cells tend to have a shorter life span, normally perishing long before promotion events or "secondary hits" have a chance to occur in them.

However, it is still controversial whether stem cells even exist. Some authorities say that "a stem cell" is actually a function, not an entity [2]. But for the purposes of the stem-cell theory of cancer, it may not even matter whether a malignancy originates from a functional or a definitive stem cell, as long as it is derived from a cell with stemness features. As the following sections illustrate, this notion of stemness has some unexpected ramifications and paradoxic implications when it concerns carcinogenesis and cancer heterogeneity. Although stem cells live long enough to acquire the necessary mutations to become malignant, they actually need fewer mutations and take less time than somatic cells do to become malignant because they already possess an inherent capacity or the necessary ingredients to become malignant.

Origin of Heterogeneity

The origin and nature of cancer's heterogeneity boil down to a couple of simple questions. Do the various mutations that develop during the course of carcinogenesis cause heterogeneity? Or does the stem cell or progenitor stem cell of origin, with its intrinsic pluripotency, play a role in the manifestation of cancer heterogeneity? To pose the questions another way: Would different mutations in the same stem cell or the same mutation in different stem cells cause different malignancies?

Thus, it remains to be elucidated whether the heterogeneity of a particular malignancy arises from activation of different genetic (i.e., oncogenic) pathways or is determined by different cellular (i.e., stem-cell) targets in which the genetic changes occur. In the latter scenario, there would be some order and pattern, whereas in the former, there would be utter chaos and randomness. Perhaps both possibilities occur, depending on whether the mutation in question happens to involve certain stem-cell genes and pathways or stem cells themselves.

HOX Genes

Hox genes are members of the *HOX* family of homeobox genes. They encode transcriptional factors that control developmental patterning along the anterior–posterior axis in a spatial and temporal sequence within a tightly coupled network of signaling pathways. Originally identified in the fruit fly (*Drosophila*), *Hox* genes have been found to determine the "segmental identity" of higher vertebrates. During develop-

ment, expression of *Hox* genes becomes spatially restricted, correlating with their relative position in a gene cluster in the adult tissues.

Aberrant expression of *Hox* genes is associated with malignancy. Histologic heterogeneity arises when overexpression of a *Hox* gene promotes differentiation of one cell type in a unique pathway but blocks the expansion of another cell type into other pathways. This mechanism of action may account for some of the lineage infidelity and tumor heterogeneity in epithelial cancers. For example, specific *Hox* genes induced diverse phenotypes in an aberrant ovarian progenitor cell that contained the same genetic mutations [3]. Although simple ovarian surface epithelium is normally devoid of müllerian duct features, an ovarian carcinoma acquired fallopian tube characteristics with a serous phenotype through the aberrant expression of *Hoxa9*, uterine characteristics with an endometrioid phenotype through *Hoxa10*, and cervical features with a mucinous phenotype through *Hoxa13*. Conversely, Hingorani et al. [4] demonstrated that combinations of additional mutations in critical tumor-suppressor gene pathways did not lead to heterogeneity.

These results strongly suggest that the same mutation in different stem cells causes different malignancies. They do not favor the alternative but more popular view that different mutations in the same cell cause different malignancies.

Stem Cells and Heterogeneity

I postulate that the seed of many intriguing properties of cancer, including its heterogeneity, is sown much earlier than and beyond those of genetic factors or epigenetic mechanisms. I propose that it is the type of stem cell from which a neoplasm arises rather than the activation of specific pathways that determines the phenotypic diversity of a particular neoplasm. This idea assumes that malignancy is endowed with some stem-cell traits as a result of its derivation from a stem cell. Considering that an embryonic cell could potentially grow into more than 200 tissue types, one should not be surprised that a malignant cell, being derived from its stem-cell counterpart, could also manifest diverse phenotypes. This idea suggests that underlying the heterogeneity of cancer is some predictability and pattern rather than complete chaos and randomness. Thus, the theory of a stem-cell origin of cancers agrees more with an orderly model rather than a stochastic model of carcinogenesis.

I speculate that cancer heterogeneity arises from various cellular lineages and different cell types in a stem-cell hierarchy. When we consider that tumors originate from various stem-cell lineages, we are comparing apples with oranges, e.g., clear-cell vs. chromophobe carcinoma of the kidney. However, when we consider that tumors arise from different stem cells along a stem-cell hierarchy, we are dealing with different types of apples, e.g., clear-cell vs. sarcomatoid carcinoma of the kidney. Thus, at one level, heterogeneity depends on different stem-cell lineages: Tumor formation in different parts of a particular tissue from different individuals could account for this type of heterogeneity. At another level, heterogeneity depends on different stem cells of origin in a stem-cell hierarchy: The pluripotency of the stem cell of origin could drive this type of heterogeneity.

Pluripotency

A crucial aspect of heterogeneity in a tumor involves its potential to differentiate into various cellular types. Tumors derived from stem cells or early progenitor stem cells have a greater capacity to develop a more heterogeneous phenotype. Therefore, maturation arrest of earlier stem cells may yield tumors that contain cell types from all three germ layers (e.g., germ-cell tumors); of intermediate progenitor stem cells, tumors that contain cells of the endocrine, ductal, or exocrine types (e.g., respiratory and gastrointestinal cancers); and of later progenitor stem cells, tumors of a single cell type (e.g., basal cell carcinoma of the skin). Because the heart does not regenerate very well and may not contain stem cells, it is not as susceptible to the development of cancer as the other organs are. As a consequence, primary cardiac tumors are exceedingly rare (<0.1%). Most primary cardiac tumors are benign (e.g., myxomas), and cardiac carcinoma is almost nonexistent.

I propose that tumors derived from more pluripotent stem cells are potentially more heterogeneous because they have the potential to display both undifferentiated (i.e., high-grade or poorly differentiated) and differentiated phenotypes (as in mixed tumors). However, tumors derived from less pluripotent stem cells tend to be less heterogeneous and contain mostly differentiated cancer cells (i.e., low-grade or well-differentiated tumors). The presence and nature of differentiated cancer cells in a tumor have important clinical and therapeutic implications; such tumors tend to pursue a relatively indolent clinical course and may or may not respond to therapy. For instance, most differentiated prostate cancer cells are relatively sensitive to hormonal ablative therapy, whereas the differentiated cancer cells in a teratoma are completely refractory to cytotoxic treatments and need to be surgically resected. I propose that the inherent pluripotency of the stem cells of origin could account for the diverse phenotypes and intratumoral heterogeneity of many cancers.

Genetic Instability

A gigantic roadblock in our quest to understand (let alone cure) cancer is its innate ability to alter its many identities. How do we recognize the “real” cancer when it shows so many faces? How do we know what a cancer does when it wears so many hats? No wonder a normal healthy immune system fails to detect or track most cancers! And it is no surprise that many so-called targeted therapies entirely miss their mark and make scarcely a dent in the tumor. The fact remains that the inherent heterogeneity of cancer is a big reason for its protean manifestation.

Nowadays, one of our best explanations for the inscrutable heterogeneity of cancer is genetic instability. According to Lengauer et al. [5], certain mutational events confer genetic instability during early tumor formation. The ensuing genetic instability drives tumor progression by augmenting the rate of mutations in oncogenes and tumor-suppressor genes. These mutant genes provide a particular cancer cell with a selective growth advantage and spearhead the clonal expansion of the tumor.

However, something seems amiss with this current version of genetic instability. Genetic instability implies randomness during the formation of cancer. But in the midst of disorder and instability, there also seems to be some order and stability about cancer. Many traits of malignancy are selected and passed on from one generation of cancer cells to the next, like clockwork. It is no accident that many "unique" traits of malignancy also happen to be "essential" traits of stem cells.

The mystery of cancer heterogeneity and the secret of genetic instability could be unraveled by the theory of a stem-cell origin of cancers, which suggests that aberrant asymmetric divisions in a stem cell or progenitor stem cell could trigger genetic instability. Thus, genetic instability occurs only in a stem cell or progenitor stem cell, not in any other types of cells. The notion that genetic instability is the result of a deviant stem-cell trait has profound clinical implications. It connects the unpredictable clinical course of cancer to its stem-cell origins and links the genetic instability to a crucial stem-cell dysfunction, namely aberrant asymmetric division.

Clinical and Biologic Implications

A stem-cell origin of heterogeneity is evident in germ-cell tumors. Such tumors, consisting of choriocarcinoma, embryonal carcinoma, yolk sac tumor, teratoma, and seminoma, have distinguishable histologic features. One prevailing theory envisions clonal evolution of germ-cell tumor from seminoma to nonseminoma (Fig. 12.1a) [6]. Another envisions a malignant precursor that develops into either a seminoma or a nonseminoma (Fig. 12.1b) [7]. I propose an alternative model, in which discrete precursor cells in a stem-cell hierarchy give rise to either a mixed seminoma and nonseminoma or a pure seminoma (Fig. 12.1c) [8]. Because nonseminomatous germ-cell tumors originate from earlier gonadal stem cells, they tend to express a more heterogeneous phenotype than do pure seminomas, which are derived from later gonadal stem cells. We have found that atypical seminomas, because of their disparate stem-cell origins, have a distinct molecular signature that is different from those of their conventional seminoma counterparts (unpublished

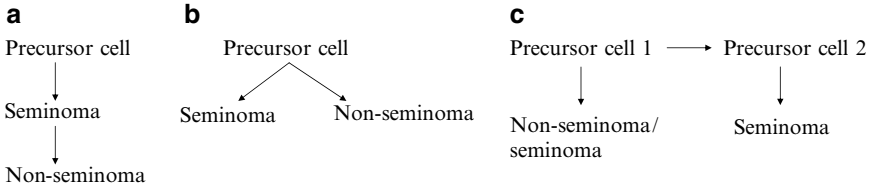

Fig. 12.1 (**a**) A prevailing theory envisions the clonal evolution of germ-cell tumor from seminoma to nonseminoma. (**b**) Another envisions a malignant precursor that develops into either a seminoma or a nonseminoma. I propose an alternative model (**c**), in which discrete precursor cells in a stem-cell hierarchy give rise to either a mixed seminoma plus nonseminoma or a pure seminoma

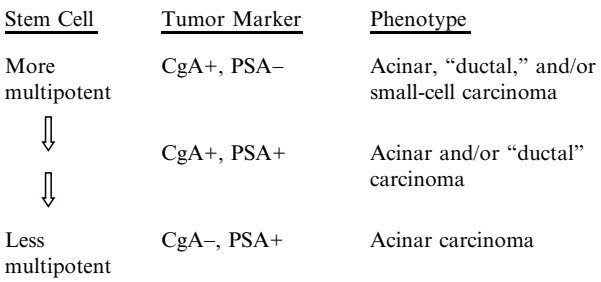

Fig. 12.2 Prostate cancers derived from earlier stem cells exhibit a more diverse phenotype and express chromogranin A (CgA) but not prostate-specific antigen (PSA). Those derived from later stem cells display a more restricted acinar carcinoma phenotype and express PSA but not CgA. There may be another subtype of prostate cancer that originates from intermediate progenitor stem cells and expresses both CgA and PSA

data). Indeed, Hofer et al. [9], after using expression tissue microarrays, concluded that there might be unique subtypes of seminomas.

The stem-cell theory of malignancy also predicts the existence of distinct subtypes of prostate cancer. Prostate cancer offers a unique opportunity to demonstrate such entities because of the availability of differentiation markers [e.g., prostate-specific antigen (PSA)] and the evidence of neuroendocrine precursors. For example, prostate cancers derived from earlier stem cells would exhibit a more diverse phenotype (including small-cell carcinoma) and express neuroendocrine markers such as chromogranin A (CgA) but not PSA. In contrast, prostate cancers derived from later stem cells would display a more restricted acinar carcinoma phenotype and express PSA but not CgA. And, according to the theory of a stem-cell origin of cancers, there must be another subtype of prostate cancer that originates from intermediate progenitor stem cells and expresses both CgA and PSA (Fig. 12.2) [8]. Study of these unique subtypes of prostate cancer and evaluation of their tumor-antigen profiles would be invaluable and should lend support to the theory of a stem-cell origin of heterogeneity in solid tumors.

Conclusion

I hypothesize that clonal evolution of cancer originates from distinct stem cells and that the type of these stem cells affects the phenotypic manifestation of the cancer. Tumors derived from earlier stem cells or progenitor stem cells in a stem-cell hierarchy have a more heterogeneous phenotype, whereas those derived from later stem cells or progenitor stem cells have a more homogeneous phenotype. This stem-cell theory embraces the concept of a clonal origin of cancer, with its underlying capacity for heterogeneity, and encompasses the principle of asymmetric division, which engenders genetic instability. I anticipate that elucidation of the stem-cell origin of

solid tumors will enhance our current understanding of the various aspects of malignancy, including cancer heterogeneity, and expedite the discovery of novel diagnostic tools, prognostic markers, and therapeutic targets in our battle against cancer.

References

1. Sell S, Pierce GB (1994) Maturation arrest of stem cell differentiation is a common pathway for the cellular origin of teratocarcinomas and epithelial cancers. Lab Invest 70:6–22
2. Blau HM, Brazelton TR, Weimann JM (2001) The evolving concept of a stem cell: entity or function? Cell 105:829–841
3. Cheng W, Liu J, Yoshida H et al (2005) Lineage infidelity of epithelial ovarian cancers is controlled by *HOX* genes that specify regional identity in the reproductive tract. Nat Med 11:531–537
4. Hingorani SR, Wang L, Multani AS et al (2005) $Trp53^{R172H}$ and $Kras^{G12D}$ cooperate to promote chromosomal instability and widely metastatic pancreatic ductal adenocarcinoma in mice. Cancer Cell 7:469–483
5. Lengauer C, Kinzler KW, Vogelstein B (1997) Genetic instability in colorectal cancers. Nature 386:623–627
6. Friedman NB (1951) The comparative morphogenesis of extragenital and gonadal teratoid tumors. Cancer 4:265–276
7. Pierce GB, Abell MR (1970) Embryonal carcinoma of the testis. Pathol Annu 5:27–60
8. Tu S-M, Lin S-H, Logothetis CJ (2002) Stem-cell origin of metastasis and heterogeneity in solid tumours. Lancet Oncol 3:508–513
9. Hofer MD, Browne TJ, He L et al (2005) Identification of two molecular groups of seminomas by using expression and tissue microarrays. Clin Cancer Res 11:5722–5729

Chapter 13
Metastasis

"Columbus Landing at Guanahani," by Theodore de Bry, 1594 (www.hdg.de/eurovisionen/html_eng/br2_4.html)

This island's mine, by Sycorax my mother,
Which thou takest from me.

– William Shakespeare (The Tempest, 1:2:333–4)

Précis

The metastatic potential of a tumor may be programmed from the start. The versatility and "ingenuity" of a metastatic cell can be matched only by those of a stem cell.

Introduction

We have been taught many times that the three cardinal rules of cancer prognosis are metastasis, metastasis, and metastasis. Because it is often the metastasis rather than the primary tumor that ultimately kills, solving the enigma of metastasis is tantamount to defeating cancer. Expanding our knowledge about the origin of

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metastasis will expedite the discovery of pertinent prognostic markers and the development of novel therapeutic targets. A key question remains, though: Do we have the audacity to cut the Gordian knot of metastasis?

Cell Migration and Tissue Repair

It may be obvious that hematopoietic stem cells migrate widely. But it may not be as apparent that stem cells within solid organs also migrate easily. Indeed, these latter cells are not deadlocked but exist in a cellular flux. For example, stem cells in the basal layer of the skin migrate toward the surface as differentiation occurs. Stem cells also migrate from the base toward the surface in colonic crypts as they differentiate into the various cell types (i.e., absorptive enterocytes, hormone-secreting endocrine cells, mucus-producing goblet cells, antigen-transporting M cells, and Paneth cells). Notably, the stem cells "round up" as they detach from the extracellular matrix during the migratory process. In contrast, when differentiated epithelial cells detach under the same physiologic conditions, they undergo apoptosis.

During tissue repair, it is generally assumed that stem cells become activated and mobilized. But without adequate stem-cell markers, identifying which cells actually detach and rapidly migrate to the denuded area of injury within hours in vivo or in situ is no simple matter. Beachy et al. [1] proposed that during tissue repair, stem-cell behavior recapitulates cancer metastasis and that specific signal pathways (e.g., those involving Hh or Wnt) are linked to both stem-cell functions and metastatic activities.

A Perfect Storm

A cascade of events needs to occur before metastasis becomes established. First, the malignant cell must detach from the primary tumor. Next, it must sneak into the circulatory system and survive there. Then, as it travels all over the body, it must select a preferred site to call home and settle down in. Finally, this metastatic cell must learn how to thrive in its new environment where not only does it need to be accepted by its neighbors but it also needs to find ways to eventually overpower and dominate them. The metastatic cell is like an intimidating Nazi cell, armed and dangerous. How the metastatic cell takes on this life of its own and achieves a position of hegemony is one of the most enduring mysteries of cancer biology.

One cannot help but notice that the versatility and "ingenuity" of a metastatic cell can be matched only by those of a stem cell. Both cell types contain the cytoskeletal machinery that allows them to mobilize easily. Both are equipped with an array of proteolytic enzymes that permit them to disembark at various destinations seemingly at will. They possess a Spartan knack of surviving in harsh, hostile environments. And they are endowed with the enviable ability to pick the right places to colonize, where there are sufficient supplies and support. By either force

or guile, these immigrating cells manage not to antagonize but instead to collaborate with the indigenous cells. Indeed, the parallels between metastatic and stem cells cannot be more striking: It is ironic that the quintessential metastatic cell appears to mimic a stem cell in every detail.

For example, the proteolytic enzyme metalloproteinase 9 (MMP-9) belongs to the family of MMPs that play an important role in cancer cell invasion. But MMP-9 is also involved in the migration and "homing" of hematopoietic stem cells [2]. Similarly, breast cancer and neuroblastoma cells express CXCR4 receptors that interact with a chemokine, CXCL12/SDF-1a, which is produced by stromal cells in the bone marrow [3, 4]. This accounts for the propensity of breast cancer and neuroblastoma to metastasize to bone. It may not be a coincidence that the migration of certain stem cells is also regulated by the same chemokines and receptors: CXCR4 is implicated in the homing and repopulation of human stem cells in the bone marrow of mice with severe combined immunodeficiency [5]. It is conceivable that the chemokine-receptor profile of a particular tumor belies its stem-cell origin and predicts its metastatic potential.

The Metastatic Journey

The metastatic journey is a matter of survival of the fittest. Even if normal and malignant cells were being shed into the hematogenous or lymphatic circulations, most of the cells would perish in their odyssey. It is neither easy nor common for even malignant cells to become established at a distant metastatic site. I postulate that only malignant cells with certain stem-cell features are able to successfully metastasize.

In fact, it is not an ordinary feat for any cell to detach from its designated place in a tissue. It takes even more skill for it to migrate into a vessel, sneaking into the circulation, where there are innumerable ways for it to perish – for example, by facing the unforgiving rigors of the capillary systems in the kidneys and lungs and the unrelenting scrutiny of the immune system in the lymph nodes and spleen. It is expected that only a "few, the proud" – i.e., the "Marines" among those cells – can meet this challenge. Only these special "chosen" cells survive the ordeal, because they know how to run the gauntlet of metastasis with their stem-cell embodiment. Other regular differentiated cells, which either dare or happen to venture into the circulation, will simply and inevitably bite the dust. I therefore postulate that only malignant cells with certain stem-cell features are able to successfully metastasize.

A Modern "Seed-and-Soil" Theory

Joshua Fidler [6] invoked a modern version of Paget's [7] "seed-and-soil" theory of metastasis. He explained that three principles could account for the various complexities of metastasis: (1) heterogeneity – cancer contains a heterogeneous population of cells with variable angiogenic, invasive, and metastatic potential, (2)

selection – cells that survive the journey to distant sites are selected for their metastatic potential, and (3) homing – success of the metastatic process depends on the ability of cells to survive and thrive in a new microenvironment. This newer version of the classical seed-and-soil theory easily accommodates many aspects of metastasis. However, I believe that the seed-and-soil theory of metastasis is due for another revision. In the latest edition, the theory would also take into account a stem-cell origin of cancers and the so-called cancer stem cells. In a manner that is more complete and comprehensive than ever, it would elucidate the generation of heterogeneity, the drive to metastasis, and the capacity to home to certain types of tissues.

Stem Cells and Metastasis

In their seminal review on the stem-cell origin of solid tumors, Sell and Pierce [8] theorized that malignancy arises from the maturation arrest of stem cells rather than from the dedifferentiation of somatic cells. They proposed that the initiation and promotion stages of malignancy occur in stem cells rather than somatic cells: Neoplasia develops in stem cells, whereas hyperplasia develops in somatic cells. I extend this view and hypothesize that the basic mechanisms of metastasis in solid tumors are also intimately related to a stem-cell origin of malignancy. I further postulate that the type of stem cell from which a neoplasm arises influences the metastatic potential of that neoplasm.

Clinical observations indicate that some tumors readily metastasize, whereas others seldom do. I theorize that tumors derived from an earlier stem cell (or progenitor stem cell) have relatively greater metastatic potential. According to this view, maturation arrest of an earlier stem cell would produce a tumor type that frequently metastasizes to various organs (e.g., small-cell carcinoma), whereas maturation arrest of a later stem cell would produce a tumor type that rarely if ever metastasizes (e.g., basal cell carcinoma of the skin). Tumors not derived from stem cells (e.g., hyperplastic lesions) do not metastasize.

This new hypothesis about a stem-cell origin of metastasis still embraces Paget's and Fidler's seed-and-soil theories of metastasis. A stem cell or malignant cell travels to a favorable environment and interacts with appropriate local growth factors during its course of metastasis, just like a seed spreads and then germinates after it lands on hospitable soil. Our view also accommodates the "adhesion molecule" theory of metastasis [9, 10], in which an aberrant stem cell or malignant cell reaches its destination by interacting with specific adhesion molecules expressed on the endothelial cells of targeted organs. Furthermore, this notion is compatible with the "homing" theory of metastasis [11], in which organ-specific chemotactic factors enter the circulation and entice an abnormal stem cell or malignant cell to invade into the walls of the blood vessels, follow a gradient of the chemotactic factors, and become established in selected organs.

Final Fury

An important aspect of the theory of a stem-cell origin of malignancy is its prediction that the metastatic potential of a tumor is programmed from the start. The results of recent studies have provided a substantial boost to this idea: Using microarray gene-expression profiling, several investigators found that the gene-expression signature was indistinguishable between primary neoplasms and their corresponding metastases. However, those primary neoplasms without metastases possessed a gene-expression signature altogether distinct from that of the primary neoplasms with metastases [12–14]. Comparisons of paired gene-expression arrays of primary tumors and autologous metastatic lesions often revealed striking similarities over a wide range of parameters, including a repertoire of genetic mutations [15–17], expression of epigenetically controlled genes [18], and overall transcriptional profile [19, 20]. Notably, the potential for invasion and metastasis of the primary tumor is encoded not only early in the development of the tumor but also throughout the bulk of the tumor, including the stroma [21, 22].

These results were somewhat shocking because they contradicted one of our most cherished cancer doctrines: Successive genetic mutations occur and accumulate as a neoplasm grows. Our traditional view predicates that *metastasis is a late phenomenon*: Certain specific genetic mutations enable a few selected tumor cells to metastasize during the later if not last stages of carcinogenesis. Metastasis is likened to Darwininan evolution, in which certain rare individual cells within the primary tumor acquire progressive genetic changes and are selected for metastasis [23]. However, one should not ignore these recent results, which challenge our conventional wisdom and refute our current notion that the metastatic potential represents the final fury of a cancer. An obvious implication of these results is that primary tumors obtained from definitive surgery or neoadjuvant therapy are invaluable for learning about the origin of cancers and their potential for metastasis.

Heterogeneity of Metastasis

At the center of the debate on the origin of metastasis is a nagging riddle, that even in the same patient, metastasis can be surprisingly varied morphologically, immunophenotypically, and genetically [24]. The heterogeneity of metastasis mirrors the heterogeneity of cancer. I postulate that a continuum of heterogeneity in the metastasis, as in the primary tumor, reflects the spectrum of multipotentiality inherent in its stem cell of origin in a stem-cell hierarchy.

Heterogeneity can manifest in two ways. On a *cellular* level, tumor arising from early stem cells may display a more diverse metastatic phenotype that includes both early (less differentiated) and late (more differentiated) stem-cell properties, whereas tumors originating from late progenitor stem cells in the hierarchy ought to exhibit

a more limited metastatic phenotype that involves mainly late (more differentiated) stem-cell features. On an *organic* level, cells that retain early stem-cell features (i.e., the cancer stem cells) tend to display more angiogenic, invasive, and metastatic characteristics within a tumor, whereas differentiated cancer cells are less angiogenic, invasive, and metastatic. The latter cells are more vulnerable and thus easier to eradicate with treatments or may even perish spontaneously without treatment.

Host–Cell Interactions

For the longest time, we have focused on metastatic cells in the study of metastasis. But the time has arrived for us to realize that host cells and the microenvironment are also implicated in the metastatic process. To a large extent, whether an immigrating metastatic cell becomes established in a foreign tissue depends on favorable interactions between the metastatic and host cells. Lyden et al. [25] showed that highly malignant cells failed to "take" and did not grow efficiently in mice with impaired host-dependent angiogenesis factors. Park et al. [26] demonstrated that certain loci in the mouse genome substantially influenced the metastatic efficiency of mammary tumors. I postulate that the biologic predilection of a malignant cell to interact with host cells during metastasis reflects that of a stem cell with its neighboring cells during development. In other words, a metastatic cell's ability to interact with certain host cells in the adult tissue may have already been imprinted in its stem cell of origin and is a recapitulation of that particular stem cell's tendency to interact with various stromal cells during embryogenesis.

It is not beyond reason to suspect that the metastatic nidus, with its unique stromal cells (e.g., smooth muscle cells, endothelial cells) and stromal factors (e.g., growth factors, angiogenesis factors), closely parallels a particular stem-cell niche, within which a stem cell has transformed into a malignant cell. The same ingredients that promote stem-cell proliferation, mobilization, and migration are now being used in malignant tumor growth, invasion, and metastasis. During organogenesis, embryonic cells hustle and bustle as they form various tissues and organs; proteolytic enzymes and invasion molecules operate at maximal speed; and autocrine, paracrine, and angiogenic factors work at full throttle. Only during carcinogenesis do these same functions and activities begin to become more sinister – every process seems disruptive and deliberate, every product appears disfigured and dysfunctional. What should have been a normal migration has become a malignant stampede.

Stem-Cell Niche vs. Onco-Niche

According to the theory of a stem-cell origin of cancers, what supports a stem cell may also sustain a cancer cell, because the two cell types have a common lineage if not a close kinship. A stem-cell niche is the base to which stem cells home and

the facility that maintains their stemness. This stem-cell niche determines when it is time for self-renewal and when for differentiation. A particular stem cell has its own unique niche with distinct stromal cells and paracrine factors. Presumably, a malignant cell recognizes the stem-cell niche to which it owes its stem cell of origin, except that now, in a different context, the stem-cell niche has become an onco-niche. Therefore, a cancer cell's interaction with its neighboring cells reflects that of its stem cell of origin. Similarly, its interaction with host cells and the local milieu determines its preferred sites of metastasis.

It is conceivable that cancer stem cells would seek the same niche that normal stem cells do. If cancer stem cells were derived from normal stem cells, they might have similar needs and use the same resources. When cancer stem cells colonize and displace what once belonged to normal stem cells at the primary site and in distant locations, one may regard this behavior as being parasitic. It is as though the parasitic malignant cells take advantage of the host stem cells, robbing them of their belongings and sapping all their goods. However, a true parasitic relationship involves two parties that are not phylogenetically related. Therefore, if cancer and normal stem cells were phylogenetically related (i.e., one being derived from the other), then parasitism would be considered an incorrect term. This is yet another example of natural selection, in which a cancer cell successfully evolves from its parental stem cell, outcompetes all contenders, and outright wins the ownership of a particular niche. In their mysterious ways, the origins of species and of cancers strike a similar chord.

Stem cells depend on stem-cell factors that comprise the stem-cell niche and maintain the stem-cell state. Separation of stem cells from this stem-cell niche (i.e., decreased stromal cell support and communication and decreased ligand concentration for stem-cell dependence receptors) leads to differentiation. In contrast, somatic cells need differentiation factors that keep them in a differentiated state. Separation of somatic cells from this niche results in apoptosis. Because they are derived from stem cells, malignant cells may also be predisposed to differentiation (as opposed to apoptosis) under conditions in which the stem-cell niche is altered (e.g., at the metastatic site, after therapy). Because the metastatic nidus offers a favorable onco-niche that resembles a corresponding stem-cell niche, I postulate that metastatic cells, especially cancer stem cells, are attracted to specific sites where such a stem-cell niche is present, just as metals are drawn to a magnet or insects to a pheromone.

Because different stem cells share certain features and properties that constitute their stemness, they may also have the same or similar stem-cell niches. Conversely, being derived from their respective normal stem cells and having inherited this resilient predisposition, the cancer stem cells may also acclimatize to various onco-niches. This laxity means that certain stem cells have an inherited capacity to migrate and thrive in various sites. Similarly, certain cancer stem cells have an inherited capability to metastasize to disparate sites. I postulate that cancer stem cells tend to metastasize to normal stem-cell niches that happen to resemble the niche of their stem cell of origin. In other words, the stem-cell niches and onco-niches could be similar if not identical. This idea implicates the predilection of a particular cancer to metastasize and to home to its sites of metastasis. It also

implies that targeting a normal stem-cell niche may affect an equivalent cancer stem-cell niche as well. This notion could provide us a meaningful and invaluable strategy to improve treatment of metastasis in particular and of cancer in general.

Clinical and Biologic Implications

The experience and experiments involving transplantation have intriguingly produced some of the most convincing data supporting a link between stem cells and metastasis. During bone marrow transplantation, for example, donor cells (i.e., hematopoietic stem cells) migrate to various nonhematopoietic tissues, including the liver, lung, brain, skeletal muscle, and bone [27–32]. Human stem cells derived from an engrafted kidney could migrate to the skin and undergo malignant transformation as a result of aberrant host factors, genetic mutations, or unfavorable cellular fusions [33]. Recently, Barsky et al. [34] epitomized this observation and proved that cancer does indeed have a stem-cell origin. They demonstrated that 12% of the solid cancers arising in non–sex matched transplant recipients were of donor origin. Their results support the notion that various stem cells are quite capable of migrating to different tissue sites, where they undergo malignant transformation and form various distinct types of primary cancer.

The observation that a particular treatment is beneficial for patients without metastases rather than for those with metastases poses some interesting questions. For example, why does an alternating chemotherapy regimen containing ifosfamide and etoposide plus doxorubicin, cyclophosphamide, and actinomycin C improve the survival of patients with Ewing sarcoma or primitive neuroectodermal tumor without metastases but not the survival of those with metastases [35]? Does this mean that it is effective for the treatment of this malignancy at earlier but not later stages of the disease? Or, alternatively, could the two patient populations – those who responded and those who did not – actually represent different disease entities arising from distinct stem cells in a stem-cell hierarchy? I surmise that patients whose cancer arises from a later progenitor stem cell tend not to have metastases and to respond better to the therapy, whereas patients whose cancer arises from an earlier stem cell tend to develop metastases and not respond as well to the same treatment.

Conclusion

When we contemplate a stem-cell origin of metastasis, we venture beyond normal boundaries, peer into unfathomable depths, and taste forbidden fruit. We confess to seeing the traditional views and understanding the conventional meanings of metastasis – heterogeneity, selection, and homing – from an entirely different angle and under a new light. A conceptual overhaul on cancer metastasis is in order. It rests on a new principle, that cancer originates from distinct stem cells that determine its

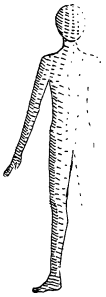
many phenotypic manifestations, including metastasis. It explains why tumors derived from an earlier stem cell readily metastasize, whereas tumors derived from a later progenitor stem cell seldom do so. It reminds us that to successfully treat cancer in general and metastasis in particular, we must return to their stem-cell roots (or seed) and take care of their stem-cell or onco-niche (or soil).

References

1. Beachy PA, Karhadkar SS, Berman DM (2004) Tissue repair and stem cell renewal in carcinogenesis. Nature 432:324–331
2. Heissig B, Hattori K, Dias S et al (2002) Recruitment of stem and progenitor cells from the bone marrow niche requires MMP-9 mediated release of Kit-ligand. Cell 109:625–637
3. Geminder H, Sagi-Assif O, Goldberg L et al (2001) A possible role for CXCR4 and its ligand, the CXC chemokine stromal cell-derived factor-1, in the development of bone marrow metastases in neuroblastoma. J Immunol 167:4747–4757
4. Müller A, Homey B, Soto H et al (2001) Involvement of chemokine receptors in breast cancer metastasis. Nature 410:50–56
5. Peled A, Petit I, Kollet O et al (1999) Dependence of human stem cell engraftment and repopulation of NOD/SCID mice on CXCR4. Science 283:845–848
6. Fidler IJ (2003) The pathogenesis of cancer metastasis: the 'seed and soil' hypothesis revisited. Nat Rev Cancer 3:453–458
7. Paget S (1889) The distribution of secondary growths in cancer of the breast. Lancet 1:571–573
8. Sell S, Pierce GB (1994) Maturation arrest of stem cell differentiation is a common pathway for the cellular origin of teratocarcinomas and epithelial cancers. Lab Invest 70:6–22
9. Kramer RH, Nicolson GL (1979) Interactions of tumor cells with vascular endothelial cell monolayers: a model for metastatic invasion. Proc Natl Acad Sci USA 76:5704–5708
10. Liotta LA, Wewer U, Rao NC et al (1988) Biochemical mechanisms of tumor invasion and metastases. Prog Clin Biol Res 256:3–16
11. Kohn EC, Francis EA, Liotta LA et al (1990) Heterogeneity of the motility responses in malignant tumor cells: a biological basis for the diversity and homing of metastatic cells. Int J Cancer 46:287–292
12. Perou CM, Sørlie T, Eisen MB et al (2000) Molecular portraits of human breast tumours [letter]. Nature 406:747–752
13. van de Vijver MJ, He YD, van 't Veer LJ et al (2002) A gene-expression signature as a predictor of survival in breast cancer. N Engl J Med 347:1999–2009
14. Ye Q-H, Qin L-X, Forgues M et al (2003) Predicting hepatitis B virus-positive metastatic hepatocellular carcinomas using gene expression profiling and supervised machine learning. Nat Med 9:416–423
15. Khan ZA, Jonas SK, Le-Marer N et al (2000) *p53* mutations in primary and metastatic tumors and circulating tumor cells from colorectal carcinoma patients. Clin Cancer Res 6:3499–3504
16. Losi L, Benhattar J, Costa J (1992) Stability of K-ras mutations throughout the natural history of human colorectal cancer. Eur J Cancer 28A(6–7):1115–1120
17. Zauber P, Sabbath-Solitare M, Marotta SP et al (2003) Molecular changes in the Ki-ras and APC genes in primary colorectal carcinoma and synchronous metastases compared with the findings in accompanying adenomas. Mol Pathol 56:137–140
18. Dalerba P, Ricci A, Russo V et al (1998) High homogeneity of MAGE, BAGE, GAGE, tyrosinase and Melan-A/MART-1 gene expression in clusters of multiple simultaneous metastases of human melanoma: implications for protocol design of therapeutic antigen-specific vaccination strategies. Int J Cancer 77:200–204
19. D'Arrigo A, Belluco C, Ambrosi A et al (2005) Metastatic transcriptional pattern revealed by gene expression profiling in primary colorectal carcinoma. Int J Cancer 115:256–262

20. Weigelt B, Glas AM, Wessels LF et al (2003) Gene expression profiles of primary breast tumors maintained in distant metastases. Proc Natl Acad Sci USA 100:15901–15905
21. Ramaswamy S, Ross KN, Lander ES et al (2003) A molecular signature of metastasis in primary solid tumors [letter]. Nat Genet 33:49–54
22. van't Veer LJ, Dai H, van de Vijver MJ et al (2002) Gene expression profiling predicts clinical outcome of breast cancer. Nature 415:530–536
23. Bernards R, Weinberg RA (2002) A progression puzzle. Nature 418:823
24. Shah RB, Mehra R, Chinnaiyan AM et al (2004) Androgen-independent prostate cancer is a heterogeneous group of diseases: lessons from a rapid autopsy program. Cancer Res 64:9209–9216
25. Lyden D, Young AZ, Zagzag D et al (1999) Id1 and Id3 are required for neurogenesis, angiogenesis and vascularization of tumour xenografts. Nature 401:670–677
26. Park Y-G, Zhao X, Lesueur F et al (2005) *Sipa1* is a candidate for underlying the metastasis efficiency modifier locus *Mtes1*. Nat Genet 37:1055–1062
27. Eglitis MA, Mezey E (1997) Hematopoietic cells differentiate into both microglia and macroglia in the brains of adult mice. Proc Natl Acad Sci USA 94:4080–4085
28. Ferrari G, Cusella-De Angelis G, Coletta M et al (1998) Muscle regeneration by bone marrow-derived myogenic progenitors. Science 279:1528–1530, Erratum in Science 1998;281(5379):923
29. Gussoni E, Soneoka Y, Strickland CD et al (1999) Dystrophin expression in the mdx mouse restored by stem cell transplantation. Nature 401:390–394
30. Horwitz EM, Prockop DJ, Fitzpatrick LA et al (1999) Transplantability and therapeutic effects of bone marrow-derived mesenchymal cells in children with osteogenesis imperfecta. Nat Med 5:309–313
31. Krause DS, Theise ND, Collector MI et al (2001) Multi-organ, multi-lineage engraftment by a single bone marrow-derived stem cell. Cell 105:369–377
32. Petersen BE, Bowen WC, Patrene KD et al (1999) Bone marrow as a potential source of hepatic oval cells. Science 284:1168–1170
33. Aractingi S, Kanitakis J, Euvrard S et al (2005) Skin carcinoma arising from donor cells in a kidney transplant recipient. Cancer Res 65:1755–1760
34. Barsky SH, Ye Y, Xiao Y et al (2008) Insights into the stem cell origin of human cancers by studying a registry of bone marrow and other organ transplant recipients who later developed solid tumors [abstract]. J Clin Oncol 26(May 20 suppl) [Abstr 11010]
35. Grier HE, Krailo MD, Tarbell NJ et al (2003) Addition of ifosfamide and etoposide to standard chemotherapy for Ewing's sarcoma and primitive neuroectodermal tumor of bone. N Engl J Med 348:694–701

Chapter 14
Cancer Immunity



Is then death nothing in the unseen,
The omnipresent unseen in the seen,
When the face has gone in the seen,
& now is inscrutable in the unseen?

– Robin Ouzman Hislop, in "Invisible Story"

Précis

The theory of a stem-cell origin of cancer and cancer immunity implies that certain tumor antigens must also be stem-cell antigens.

Introduction

The basic nature of cancer immunity is still shrouded in mystery. It is very difficult for even an otherwise intact immune system to distinguish a cancerous from a normal cell. Why is that? The malignant cell is well hidden, seemingly wearing

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a cloak of invisibility and poised to pounce on its unsuspecting host. Is cancer immunity simply a matter of clever camouflage or a case of brilliant espionage? Somehow, the cancer cell manages to infiltrate and subvert the entire organism for its own evil purposes. There is no doubt that the basic nature of cancer immunity continues to tickle our imagination.

According to the theory of a stem-cell origin of cancers, cancer cells are derived from and may mimic stem cells [1]. The idea that malignant cells and stem cells have a common origin presents the possibility that some tumor-associated antigens must also be stem-cell antigens. This idea is consistent with the observation that tumor-associated antigens tend to be weakly immunogenic or functionally nonimmunogenic. The thought that cancer may be inborn and not at all foreign to the host has profound implications on how we view cancer in general and cancer immunity in particular.

This chapter illustrates how the theory of a stem-cell origin of cancers relates to cancer immunity and how this novel idea may revolutionize the future design of anticancer vaccines and immunotherapies.

Immune Surveillance

Harnessing the immune system to fight cancer seems counterintuitive to some of us; the idea of developing a vaccine against cancer seems inherently flawed and potentially futile. Why should we need to prime or boost the immune system when it is already quite healthy and robust? After all, our immune system is capable of fighting bacterial and viral infections and fending off other foreign agents. How do we immunize against an entity that already exists in our body rather than being forthcoming? Perhaps the real problem is not an incompetent immune system but an insidious malignant process, instead. To unravel the mystery of cancer immunity, we need to further investigate its biologic origins. Thinking outside the box may enable us to view cancer immunity in a new light and expedite the discovery of novel cancer markers and the design of improved therapeutic targets.

Except for lymphomas and skin cancers, most cancers do not occur more frequently in immunocompromised patients than in those with intact immune systems [2]. Patients who undergo organ transplantation and receive immunosuppressive drugs do not experience a higher incidence of the most common cancers (i.e., prostate, breast, and lung carcinomas) than the rest of the population does [3]. This observation suggests that immune surveillance plays an essential role in keeping potential malignant cells in check but that it is not at all responsible for the development of malignancy. This is consistent with the view that cancer development occurs in spite of a competent immune system and that cancer growth may accelerate in the presence of an impaired immune system. Ultimately, the malignant cells that arise from stem cells resemble stem cells, and both are destined to elude immune surveillance.

Immunotolerance

The immune system is designed to tolerate stem cells and their tissue derivatives. Consequently, it is likely to overlook cancer cells that are also stem-cell derivatives. A prime example of immunotolerance is the acceptance of a semiallogeneic fetus by its mother. In many respects, a fetus resembles a neoplasm because, like a malignant tumor, the fetus is derived from and composed of stem cells. I suspect that fetal and malignant cells may use similar mechanisms to achieve immunotolerance; certain factors that mediate immunotolerance are present in both fetal and stem cells and in malignant cells. Hence, certain fetal antigens present on stem cells may be considered equivalent if not identical to tumor-associated antigens. In other words, these fetal, stem-cell, and tumor-associated antigens are in fact one and the same and play a pivotal role in the immunotolerance of their respective cells.

The phenomenon of fetal "microchimerism," or persistence of fetal cells in the mother after delivery, also indicates that stem cells are intimately involved in immunotolerance. Fetal microchimerism was first reported by Schröder et al. [4] and subsequently confirmed by other investigators [5, 6]. Liégeois et al. [7] first proposed the term microchimerism to describe the apparently stable long-term survival of allogeneic mouse fetal cells in the maternal mouse without induction of graft-versus-host disease. Fetal progenitor $CD34^+$ cells have been shown to persist in maternal blood for as long as 27 years postpartum [8]. Chimerism could also result from widespread seeding of donor hematopoietic cells arising from kidney, liver, or intestine transplants [9]. It seems that whether a fetus, a tumor, or a graft is accepted by the mother, host, or recipient depends on some unique properties of the stem cell. I anticipate that cancer cells, like fetal and stem cells, are endowed with certain biologic properties that prevent them from being recognized and attacked by a normal immune system.

Human Leukocyte Antigen Expression

There are two types of major histocompatibility (MHC) antigens, known as class I (MHC-I) and class II (MHC-II) molecules. They are encoded by the human leukocyte antigen (HLA) class I and class II genes, respectively. HLA-I molecules are expressed in almost all cell types (except for stem cells and cancer cells), whereas HLA-II molecules are expressed mainly on antigen-presenting cells (APCs), such as B cells, monocytes, and dendritic cells. Together with β_2-microglobulin (β_2M), MHC-I antigen presents peptides to $CD8^+$ cytotoxic T lymphocytes (CTLs), and MHC-II antigen presents peptides to $CD4^+$ helper T cells to initiate a specific immune response. In addition, T-cell activation requires the presence of both MHC and costimulatory molecules. Without a costimulatory signal, partial T-cell activation leads to anergy or a state of long-lasting immune unresponsiveness.

A crucial function of MHC-I antigen is to identify and designate which cells in the body constitute "self." Foreign cells or donor cells with a different MHC-I profile are vigorously attacked and rejected by the host's army of CTLs. Another vital function of the MHC-I antigen is to defend against potentially serious and lethal viral infections. When a cell is infested by viruses and tagged with viral antigens, certain CTLs recognize the specific viral peptide bound to the MHC-1 molecule, become activated, and are recruited in time to eliminate the damaged cell.

A clue that neoplastic cells are derived from abnormal stem cells is the finding that malignant and stem cells have similar HLA profiles. For instance, stem cells express very low levels of MHC-I molecules on their cell surfaces [10]. During differentiation of stem cells, the expression of MHC-I molecules increases moderately. Coincidentally, it is common to find decreased expression of MHC-I molecules on malignant cells. Therefore, one could account for the low expression of the MHC-I antigens on neoplastic cells simply by considering their stem-cell origin without invoking any downregulation or alteration of the MHC antigens. According to the theory of a stem-cell origin of cancer and cancer immunity, both stem cells and malignant cells express lower levels of MHC-I antigens and have a loss of costimulatory or gain of coinhibitory molecules that enables them to avoid being attacked by the CTLs.

HLA-G and Immunotolerance

In the setting of lost, altered, or absent MHC-I molecules, another layer of defense against foreign agents involves natural killer (NK) cells. The question is, How do stem cells and cancer cells, which tend to express low levels MHC-I antigens, elude the vigilance and wrath of NK cells?

Recently, we have learned more about the role of HLA-G and other coinhibitory molecules in immunotolerance. HLA-G is a nonclassical HLA-I antigen found on the extravillous cytotrophoblast at the fetal–maternal interface. Its presence at a known immunologically "privileged" site suggests that it functions as the protector of the fetus from maternal allorecognition and rejection. The finding that HLA-G is able to establish and maintain immunotolerance by suppressing the action of multiple immunocompetent cells supports this view [11]. For example, binding of HLA-G to inhibitory receptors such as the immunoglobulin-like transcript-2 (CD85j) in CTLs suppresses CTL-mediated cytolysis. Similarly, the binding of HLA-G to immunoglobulin-like transcript-4 (CD85d) in APCs suppresses the T-cell proliferative response, whereas the binding to KIR2DL4/p49 (CD158d) in NK cells suppresses NK cell–mediated cytolysis. Therefore, the HLA-G protein can bind to various receptors on a variety of immunocompetent cells (i.e., B cells, T cells, APCs, and NKs) and exert its immunotolerance functions at different stages of an immune response.

HLA-G mediates immunotolerance and is present on both fetal and malignant cells [12]. It is interesting that HLA-G molecules have been detected in embryonic tissues from oocyte to blastocyst. Whether HLA-G is present in stem cells in general

and adult stem cells in particular remains unknown. In the adult, HLA-G antigens have been detected in corneal cells. HLA-G antigens have also been found in a variety of malignant cells [11]. Focal expression of HLA-G in these tumors is consistent with the presence of so-called cancer stem cells. I propose that the aberrant expression of HLA-G by tumor cells can be explained by their origin from stem cells.

Both immune cells and tumor cells produce soluble HLA-G in vivo. Like other soluble HLA-I antigens, HLA-G antigen is present in the serum. Presumably, embryonic cells and stem cells also produce soluble HLA-G that can be measured in the amniotic fluid during early development. An increased HLA-G concentration is associated with downmodulation of the immune response [11] and with improved allograft acceptance [13, 14]. Coincidentally, an increased serum HLA-G concentration has been found to correlate with advanced cancer stage and increased tumor load [15].

The Meaning of β_2M

The nonglycosylated protein β_2M is synthesized by all nucleated cells and forms complexes with the heavy chain of MHC-I antigen through noncovalent linkages on the cell surface. It is believed that the downregulation of both classical and nonclassical MHC-I expression in stem cells and cancer cells contributes to their immune evasiveness and allows their escape from elimination by the attacking CTLs and NK cells, respectively. What happens to its β_2M partner during downregulation of the MHC-I molecule is a matter of conjecture.

A known mitogen, β_2M has been shown to promote the growth of stromal cells, including osteoblasts [16]. I wonder whether an increased concentration of soluble β_2M is prevalent during embryogenesis, when stem cells are activated, and during oncogenesis, when cancer cells are activated. I speculate that increased soluble β_2M concentrations help to establish the stem-cell and cancer-cell niches: An elevated β_2M level is associated with low MHC-I expression, and both of these conditions manage to keep immune surveillance in check on the resident stem cells and cancer cells, respectively. As a corollary, decreased soluble β_2M levels, which occur when MHC-I expression increases, promote the opposite effect and result in the differentiation of stem cells and cancer cells. The theory of a stem-cell origin of cancer and cancer immunity explains why certain aggressive malignancies are associated with relatively elevated serum β_2M levels.

I postulate that tumors derived from early progenitor stem cells express lower levels of MHC-I antigens and higher β_2M levels, whereas tumors derived from later progenitor stem cells express higher levels of MHC-I antigens and lower β_2M levels. Hence, tumors originating from earlier progenitor stem cells are less immunogenic and more resistant to immunotherapy; in contrast, tumors originating from later progenitor stem cells may be more amenable to immunomodulation. I further hypothesize that some melanomas and renal-cell carcinomas are relatively more responsive to interleukin-2 (IL-2) and interferon-alfa treatments because they originate from later

progenitor stem cells. Thus, the theory of a stem-cell origin of cancer immunity invokes a novel conceptual platform for explaining the relative immunogenicity of diverse tumors and for designing improved immunotherapies in the future.

Immune Activation

Despite subtle biologic differences between malignant cells and stem cells, some important differences may exist that can be exploited for therapeutic purposes. A fundamental property of cancer cells, genomic instability, is believed to be the cause of failure of many cancer therapies. Genomic instability creates heterogeneity and promotes drug resistance. It is the source of cancer's incredible powers, but it may also be the cause of its only weakness. By producing neoantigens through genetic instability, a cancer may expose its vulnerabilities and become susceptible to immune activation.

Important lessons can be learned from the cure of several malignancies, such as testicular cancer and acute leukemias. When chemotherapy is curative in these tumors, it induces massive tumor-cell death. Increased tumor-antigen release may effectively recruit APCs (e.g., dendritic cells and macrophages) and successfully modulate the immunogenicity of the cancers being treated [17]. Unfortunately, most cancers do not respond sufficiently well to chemotherapy and cannot be cured in this manner. One way to overcome insufficient tumor-antigen release is to enhance immune activation through accentuation of positive checkpoints or elimination of negative checkpoints. In principle, one could enhance tumor-antigen release and improve the therapeutic window of chemotherapy, radiotherapy, or targeted therapy by combining such treatments with agents that reverse coinhibitory effects or activate specific CTLs [18].

Immunosupportive therapy offers hope that one day we may discover ways to distinguish cancer cells from stem cells and improve or even revolutionize our current treatment of cancer. How does this idea withstand the scrutiny of our theory of a stem-cell origin of cancers? It is still unknown whether genetic instability produces abundant neoantigens. It is also unclear whether early stem cells release fewer neoantigens than late progenitor stem cells do. And it remains to be seen whether neoantigens from early stem cells are less immunogenic than those from late progenitor stem cells. After all, very few cells would be considered to be more "self" than our own stem cells early in a stem-cell hierarchy.

Minimal Residual Disease

Although an acute leukemia may be cured according to molecular or genetic criteria, minimal residual disease still exists but is clinically inconsequential [19]. What is the basis for this cure in the face of continued, persistent disease? How do we reconcile a cure of minimal residual cancer with immunotolerance?

Successful treatments of any cancers need to reduce the tumor burden in such a manner that the immune system can easily engage the few remaining cancer cells, and a homeostatic niche can better manage the minimal residual cancer cells. For example, a curative treatment could induce the minimal residual cancer cells to assume a more differentiated phenotype that is handled more easily by the immune system or cause them to revert to an original stem-cell phenotype that is more amenable to homeostatic regulation by a stem-cell niche (see Chap. 8).

Cancer Vaccines

After 40 years of intense research, the clinical benefit achieved with cancer vaccines seems rather modest. For example, certain melanomas and renal-cell carcinomas may express relatively high concentrations of HLA-I antigens (because they originate from late progenitor stem cells), which render them more amenable to vaccine therapy or immunotherapy in an adjuvant setting. Certain cervical and prostate carcinomas express viral antigens (because of a viral origin), which contribute to their heightened immunogenicity. Considering how like rather than how unlike the cells are with each other, I anticipate that it is an immense task for the immune system to distinguish a cancer cell from its respective stem cell of origin and for us to design an effective cancer vaccine on the basis of such differences. This could very well be the reason why the promise of an ultimate cancer vaccine remains unfulfilled.

Melanoma

Despite some progress, randomized trials using cancer vaccine for melanoma have been largely disappointing to date. For example, Canvaxin (CancerVax Corp., Carlsbad, CA) is a melanoma vaccine prepared from allogeneic melanoma cell lines grown in culture. Since 1984, Canvaxin has been tested and clearly shown to elicit immunogenicity in clinical trials. The results of one nonrandomized trial suggested clinical benefit for patients who underwent surgery for stage IV melanoma followed by adjuvant vaccine therapy [20]. The 5-year overall survival rate was 39% for the vaccine-treated patients and 19% for a computer-matched control group ($P = 0.0001$). Unfortunately, subsequent randomized phase III trials using the same vaccine in patients with stage III and resected stage IV melanoma demonstrated no clinical benefit, and the whole Canvaxin project was abandoned in 2007.

Another randomized phase III trial (EORTC 18961) of postoperative GM2-KLH21 vaccine for patients with stage II node-negative melanoma was prematurely closed in 2007 when it was found not only to be ineffective but also potentially detrimental. According to Eggermont et al. [21], “the outcomes of the three to four largest adjuvant vaccine trials involving 3,000–3,800 patients with stages II, III, and IV melanoma were worse, worse, worse, and worse.”

Renal-Cell Carcinoma

Currently, even less evidence supports the effectiveness of vaccine for the treatment of renal-cell carcinoma. A prospective randomized study using autologous tumor cells combined with bacillus Calmette–Guèrin revealed no improvement in disease-free or overall survival time after 5 years of follow-up [22]. Whether other innovative immunotherapeutic approaches, including the autologous tumor-derived vitespen vaccine [23] and allogeneic stem-cell transplantation [24], may provide clinical benefit awaits confirmation.

Cervical Carcinoma

It seems intuitive that tumors with a viral origin that express viral antigens may be more immunogenic than those without a viral origin. Presumably, the virus infected a stem cell and initiated carcinogenesis by affecting key oncogenetic factors within the stem cell. This may be the reason that Gardasil [human papillomavirus quadrivalent (types 6, 11, 16, and 18) vaccine, recombinant; Merck & Co., Inc., Whitehouse Station, NJ], a vaccine designed to target four types of human papillomavirus (HPV), has been shown to decrease the occurrence of high-grade cervical intraepithelial neoplasia related to HPV-16 or HPV-18 [25]. The vaccine is tailored for incipient tumors and therefore is most beneficial in those cases in which the tumors are only starting to form and when there is minimal tumor burden. Gardasil may also be effective for the treatment of other tumor types (e.g., bladder, penile) in which HPV is presumed to play a role in carcinogenesis.

Prostate Carcinoma

The idea that tumors arising from late progenitor stem cells are more susceptible to immunomodulation than those arising from early stem cells suggests that certain prostate cancers might also be amenable to treatments with cancer vaccines. I speculate that tumors derived from late prostate progenitor stem cells express prostate differentiation antigens (e.g., prostate-specific antigen) and have relatively low Gleason scores. These more-differentiated tumors express higher levels of MHC-I antigens and are inherently more immunogenic than the less-differentiated tumors. Consequently, they are likely to be more amenable to immunotherapy. For example, sipuleucel-T (Provenge; Dendreon Corp., Seattle, WA; autologous APCs activated against prostatic acid phosphatase–granulocyte–macrophage colony-stimulating factor) provided a survival benefit (median survival, 25.9 years for sipuleucel-T vs. 21.4 years for placebo) in patients with advanced but asymptomatic

prostate cancer and a Gleason score of 7 or lower [26]. However, no evidence that sipuleucel-T provides clinical benefit for those patients whose Gleason score is higher than 7 exists so far. Of interest, a randomized phase III trial (VITAL-2) using GVAX immunotherapy (Cell Genesys, Inc., South San Francisco, CA; whole cells derived from two prostate cancer cell lines modified to secrete granulocyte–macrophage colony-stimulating factor) revealed no evidence of clinical benefit for patients with advanced symptomatic prostate cancer and was prematurely terminated in 2008.

There is a twist in the saga of prostate cancer vaccine: Recent data suggest that some prostate cancers have an infectious cause. Using a DNA Virochip that contains genetic sequences derived from all known viruses, Urisman et al. [27] detected the presence of a probable retrovirus in half of the prostate cancer samples from patients who were homozygous for a hereditary prostate cancer mutation in one gene, R462Q. This gene has been mapped to an antiviral protein, ribonuclease L (RNASEL), that prevents viral infection by triggering apoptosis and the elimination of virus-infected cells. Urisman and colleagues also discovered that the presence of a xenotropic murine-like retrovirus (XMRV) was about 30 times more common in men with the R462Q variant than it was in those without the variant. They speculated that these men are more susceptible to infection with the XMRV virus or have a decreased ability to fight the virus once it is acquired. XMRV might have jumped from the mouse to the human genome many millennia ago. A causal relationship between XMRV and prostate cancer remains to be proven. Conceivably, a viral origin renders certain prostate tumors more immunogenic and more amenable to treatment with cancer vaccines.

Autoimmunity

The association between neoplasms and autoimmune diseases is strong [28, 29]. Indeed, autoimmunity often arises after successful immunotherapy of some cancers. For example, a positive thyroid autoantibody titer is highly correlated with increased survival in patients with renal-cell carcinoma who received IL-2 and interferon alfa-2 therapy [30]. Most patients who experienced substantial regression of their metastatic melanoma after treatment with tumor-infiltrating lymphocytes and IL-2 also developed antimelanocyte autoimmunity [31]. Presumably, injury to differentiated cells is reparable or reversible, but injury to stem cells would cause lasting if not permanent sequelae. Effective immunotherapy may elicit an immune response to tumor antigens as well as to related stem-cell antigens, the latter of which results in an autoimmune reaction.

An association between antitumor immunity and autoimmunity was apparent in a clinical trial that directly delivered B7 costimulatory molecules encoded by a vaccinia virus into melanoma lesions. Two patients experienced objective clinical responses, but three patients developed vitiligo [32]. In another study, objective clinical responses were noted in three patients with melanoma who received a CTLA-4–blocking monoclonal antibody in combination with a peptide vaccine [33]. Among the treated

patients, 43% experienced some type of grade 3 or 4 immune-mediated toxic effects, including dermatitis, enterocolitis, hypophysitis, uveitis, and hepatitis. And in still another trial, Beck et al. [34] reported a 14% rate of response but a 21% rate of enterocolitis in patients with renal-cell carcinoma or melanoma who received a human anti–CTLA-4 monoclonal antibody (MDX-010, or ipilimumab) over a 3-year period with or without a peptide vaccine. The patients who developed enterocolitis also happened to be those who had an objective clinical response [34]. Hence, the association between autoimmunity and cancer could be explained by the theory of a stem-cell origin of cancer and cancer immunity on the basis of potential cross-reactions between antigens shared by a malignant cell and its stem cell of origin.

The theory of a stem-cell origin of cancer and cancer immunity implies that certain tumor antigens must also be stem-cell antigens, although no one has yet demonstrated that this is indeed true. I postulate that the tumor antigen Pr3, or myeloblastin, is a stem-cell antigen. Pr3 is a serine protease (i.e., a self-antigen) that is overexpressed in various myeloid leukemias and is considered to be a target of graft-versus-leukemia [35]. CTLs specific for PR1, an HLA-restricted peptide derived from Pr3, participated in the elimination of chronic myelogenous leukemia [36]. It is interesting that Pr3 is also the target of autoimmune attack in Wegener granulomatosis [37]. Because downregulation of Pr3 halts cell division and induces differentiation of a leukemia cell line [38], I reason that Pr3 is also a stem-cell antigen.

Perhaps it is not surprising that markers of stem cells and cancer cells make strange bedfellows. A case in point is the role of IL-11 in the biology of stem cells and the pathology of cancer cells. IL-11 belongs to a family of cytokines whose prototype is the leukemia inhibitory factor, a self-renewal factor for mouse embryonic stem cells. These cytokines signal through a common receptor subunit, gp130, and a ligand-specific receptor subunit. Numerous gp130 ligands are produced during early stages of embryonic development and contribute not only to the self-renewal of pluripotent cells but also to the development of numerous differentiated cell types. Binding of a ligand induces heterodimerization of its receptor with gp130 and activation of Janus kinases, which lead to the recruitment of transcription factors such as STAT3. Phosphorylation and activation of STAT3 is both necessary and sufficient for the maintenance of mouse embryonic stem cell self-renewal.

Evidence exists for the increased expression of IL-11 receptor and activation of STAT3 in prostate cancer [39]. Recombinant human IL-11 has also been shown to promote megakaryocytopoiesis in vitro [40]. Some patients with prostate cancer who experienced a gratifying and durable response to therapy also developed persistent thrombocytopenia after completion of therapy. It is difficult to attribute this unusually sustained thrombocytopenia to drug toxicity; for example, biopsies performed in these cases showed relatively intact bone marrow (unpublished data). I suspect an immune-mediated process may have occurred: Effective therapy damages prostate cancer stem cells that share certain stem-cell antigens with platelet precursor cells. Therefore, the resultant autoimmune thrombocytopenia could be a predictor of therapeutic efficacy because the treatment also happens to successfully target prostate cancer stem cells. Another implication of this observation is that IL-11 plays an important role in the

pathogenesis of a certain subtype of prostate cancer. Hence, the presence of thrombocytosis may be a clinical marker that identifies this subgroup of patients with prostate cancer.

Conclusion

My personal view of a stem-cell origin of cancer and cancer immunity is not meant to dissuade or discourage the development of new anticancer vaccines or immunotherapies. Instead, my intent is to introduce a novel perspective about the potential and limitations of such treatments according to the theory of a stem-cell origin of malignancy and its implications for cancer immunity. This theory constitutes a substantial paradigm shift that may enable us to unlock the mystery of cancer immunity and discover alternative targets for cancer therapy.

To this end, it is imperative that we understand more about the relationship between stem cells and cancer cells. Can we eradicate cancer cells more successfully by knowing more about the intricacies of stem cells? Can we enhance cancer immunogenicity by manipulating specific tumor-associated antigens that may turn out to be stem-cell antigens? At the moment, we have numerous therapeutic modalities, such as surgery, radiotherapy, and chemotherapy, that are quite effective for the treatment of some cancers in certain clinical situations. But how do we proceed beyond current norms in an effort to attain a real substantial scientific and clinical breakthrough? Perhaps a better appreciation of stem cells and the stem-cell origin of cancer and cancer immunity is another way for us to revolutionize the current understanding and treatment of cancer.

References

1. Sell S, Pierce GB (1994) Maturation arrest of stem cell differentiation is a common pathway for the cellular origin of teratocarcinomas and epithelial cancers. Lab Invest 70:6–22
2. Penn I (1994) Depressed immunity and the development of cancer [review]. Cancer Detect Prev 18:241–252
3. Pfeffer PF (1999) Cancer in organ transplanted patients [in Norwegian]. Tidsskr Nor Laegeforen 119:3792–3794
4. Schröder J, Tiilikainen A, De la Chapelle A (1974) Fetal leukocytes in the maternal circulation after delivery. I. Cytological aspects. Transplantation 17:346–354
5. Herzenberg LA, Bianchi DW, Schröder J et al (1979) Fetal cells in the blood of pregnant women: detection and enrichment by fluorescence-activated cell sorting. Proc Natl Acad Sci USA 76:1453–1455
6. Thomas MR, Williamson R, Craft I et al (1994) Y chromosome sequence DNA amplified from peripheral blood of women in early pregnancy [letter]. Lancet 343:413–414
7. Liégeois A, Escourrou J, Ouvré E et al (1977) Microchimerism: a stable state of low-ratio proliferation of allogeneic bone marrow. Transplant Proc 9:273–276
8. Bianchi DW, Zickwolf GK, Weil GJ et al (1996) Male fetal progenitor cells persist in maternal blood for as long as 27 years postpartum. Proc Natl Acad Sci USA 93:705–708

9. Starzl TE, Demetris AJ, Murase N et al (1992) Cell migration, chimerism, and graft acceptance [review]. Lancet 339:1579–1582
10. Drukker M, Katz G, Urbach A et al (2002) Characterization of the expression of MHC proteins in human embryonic stem cells. Proc Natl Acad Sci USA 99:9864–9869
11. Rouas-Freiss N, Moreau P, Ferrone S et al (2005) HLA-G proteins in cancer: do they provide tumor cells with an escape mechanism? Cancer Res 65:10139–10144
12. Paul P, Rouas-Freiss N, Khalil-Daher I et al (1998) HLA-G expression in melanoma: a way for tumor cells to escape from immunosurveillance. Proc Natl Acad Sci USA 95:4510–4515
13. Créput C, Durrbach A, Menier C et al (2003) Human leukocyte antigen-G (HLA-G) expression in biliary epithelial cells is associated with allograft acceptance in liver-kidney transplantation. J Hepatol 39:587–594
14. Lila N, Amrein C, Guillemain R et al (2002) Human leukocyte antigen-G expression after heart transplantation is associated with a reduced incidence of rejection. Circulation 105:1949–1954
15. Ugurel S, Rebmann V, Ferrone S et al (2001) Soluble human leukocyte antigen–G serum level is elevated in melanoma patients and is further increased by interferon-α immunotherapy. Cancer 92:369–376
16. Huang W-C, Wu D, Xie Z et al (2006) β2-Microglobulin is a signaling and growth-promoting factor for human prostate cancer bone metastasis. Cancer Res 66:9108–9116
17. Gough MJ, Melcher AA, Ahmed A et al (2001) Macrophages orchestrate the immune response to tumor cell death. Cancer Res 61:7240–7247
18. Peggs KS, Segal NH, Allison JP (2007) Targeting immunosupportive cancer therapies: accentuate the positive, eliminate the negative. Cancer Cell 12:192–199
19. Morley A (1998) Quantifying leukemia [editorial]. N Engl J Med 339:627–629
20. Hsueh EC, Essner R, Foshag LJ et al (2002) Prolonged survival after complete resection of disseminated melanoma and active immunotherapy with a therapeutic cancer vaccine. J Clin Oncol 20:4549–4554
21. Eggermont AM, Suciu S, Ruka W et al (2008) EORTC 18961: Post-operative adjuvant ganglioside GM2-KLH21 vaccination treatment vs observation in stage II (T3-T4 N0M0) melanoma: 2nd interim analysis led to an early disclosure of the results [abstr]. In: Proceedings of the American Society of Clinical Oncology, Chicago, IL, May 30–June 3, 2008. Abstract 9004
22. Galligioni E, Quaia M, Merlo A et al (1996) Adjuvant immunotherapy treatment of renal carcinoma patients with autologous tumor cells and bacillus Calmette-Guèrin: five-year results of a prospective randomized study. Cancer 77:2560–2566
23. Jonasch E, Wood C, Tamboli P et al (2008) Vaccination of metastatic renal cell carcinoma patients with autologous tumour-derived vitespen vaccine: clinical findings. Br J Cancer 98:1336–1341
24. Childs R, Chernoff A, Contentin N et al (2000) Regression of metastatic renal-cell carcinoma after nonmyeloablative allogeneic peripheral-blood stem-cell transplantation. N Engl J Med 343:750–758
25. The Future II Study Group (2007) Quadrivalent vaccine against human papillomavirus to prevent high-grade cervical lesions. N Engl J Med 356:1915–1927
26. Small EJ, Schellhammer PF, Higano CS et al (2006) Placebo-controlled phase III trial of immunologic therapy with sipuleucel-T (APC8015) in patients with metastatic, asymptomatic hormone refractory prostate cancer. J Clin Oncol 24:3089–3094
27. Urisman A, Molinaro RJ, Fischer N et al (2006) Identification of a novel gammaretrovirus in prostate tumors of patients homozygous for R462Q *RNASEL* variant. PLoS Pathog 2:e25
28. Nissen C, Schubert J (2002) Seeing the good and bad in aplastic anemia: is autoimmunity in AA dysregulated or antineoplastic? Hematol J 3:169–175
29. Tomer Y, Sherer Y, Shoenfeld Y (1998) Autoantibodies, autoimmunity and cancer [review]. Oncol Rep 5:753–761
30. Franzke A, Peest D, Probst-Kepper M et al (1999) Autoimmunity resulting from cytokine treatment predicts long-term survival in patients with metastatic renal cell cancer. J Clin Oncol 17:529–533, Erratum in J Clin Oncol 1999;17(4):1330

31. Dudley ME, Wunderlich JR, Robbins PF et al (2002) Cancer regression and autoimmunity in patients after clonal repopulation with antitumor lymphocytes. Science 298:850–854
32. Kaufman HL, DeRaffele G, Mitcham J et al (2005) Targeting the local tumor microenvironment with vaccinia virus expressing B7.1 for the treatment of melanoma. J Clin Invest 115:1903–1912
33. Phan GQ, Yang YC, Sherry RM et al (2003) Cancer regression and autoimmunity induced by cytotoxic T lymphocyte-associated antigen 4 blockade in patients with metastatic melanoma. Proc Natl Acad Sci USA 100:8372–8377
34. Beck KE, Blansfield JA, Tran KQ et al (2006) Enterocolitis in patients with cancer after antibody blockade of cytotoxic T-lymphocyte-associated antigen 4. J Clin Oncol 24:2283–2289
35. Molldrem JJ, Komanduri K, Wieder E (2002) Overexpressed differentiation antigens as targets of graft-versus-leukemia reactions. Curr Opin Hematol 9:503–508
36. Molldrem JJ, Lee PP, Wang C et al (2000) Evidence that specific T lymphocytes may participate in the elimination of chronic myelogenous leukemia. Nat Med 6:1018–1023
37. Franssen CF, Tervaert JW, Stegeman CA et al (1996) c-ANCA as a marker of Wegener's disease [letter]. Lancet 347:116
38. Bories D, Raynal M-C, Solomon DH et al (1989) Down-regulation of a serine protease, myeloblastin, causes growth arrest and differentiation of promyelocytic leukemia cells. Cell 59:959–968
39. Campbell CL, Jiang Z, Savarese DM et al (2001) Increased expression of the interleukin-11 receptor and evidence of STAT3 activation in prostate carcinoma. Am J Pathol 158:25–32
40. Weich NS, Wang A, Fitzgerald M et al (1997) Recombinant human interleukin-11 directly promotes megakaryocytopoiesis in vitro. Blood 90:3893–3902

Chapter 15
Drug Resistance

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There is no more dreadful punishment than futile and hopeless labor.

– Albert Camus

Précis

Elucidation of a stem-cell origin of cancers and drug resistance may steer us away from many well-intentioned but potentially futile treatments.

Introduction

Drug resistance is a prime example of the survival of the fittest: An organism equipped with some advantageous characteristics manages to outlive its competitors by chance or design. Unfortunately, our indiscriminant use of antibiotics will continue to foster drug-resistant foes. Although we continue to discover newer and better antibiotics to combat ever more potent bacterial infections, it seems as though we are always barely ahead of the drug-resistant strains. In some cases, such as malaria and tuberculosis, drug resistance is a recurring and persistent public health issue that will continue to impede progress and prosperity in the affected developing world.

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The problem with drug resistance is even more challenging for the treatment of viral infections because a virus is able to adeptly mutate and disguise its presence within host cells. This is particularly true in the case of the human immunodeficiency virus, in which drug resistance is one of the main reasons for our failed efforts to completely wipe out acquired immunodeficiency syndrome (AIDS). Similarly, we feel powerless in our encounter with drug resistance in the avian influenza virus that lies in wait to cause a catastrophic worldwide pandemic sometime in the future.

The Return of Cancer

It brings little solace to realize that drug resistance in cancer is a hundred times worse than it is with other diseases. A bacterium, plasmodium, or mycobacterium has many alien features that can be earmarked for destruction by drugs. A virus is more difficult to treat because it usurps cellular functions in the host to serve its own purposes. However, because some viral antigens are present, drugs may eventually control the virus by eliminating the affected cells or at least keeping them at bay. Unfortunately, a cancer cell is much more devious and formidable an adversary than a virus is. If a cancer cell is derived from a stem cell and the two cells are more alike than not, then it is highly unlikely that a drug will affect one cell but not the other. It will be extremely difficult if not impossible to find any drug that kills cancer cells without damaging normal ones. When it concerns the theory of a stem-cell origin of cancers and drug resistance, the question boils down to this: Is it possible to completely eliminate cancer with drugs? And if so, how?

Worst Enemies

A cruel reality all oncologists learn is that it is neither easy nor common to eradicate cancer with drugs. The vast majority of cancers afflicting humans are scarcely affected by the various cytotoxic chemotherapeutic agents available. Hence, chemotherapy alone has cured only a minority of cancers. The intractable nature of malignancy relates in part to its innate drug resistance. Recently, the biologic basis for this innate drug resistance has been traced closer and closer to a stem-cell origin of cancers [1].

Both stem cells and malignant cells are equipped with many biologic weapons that enable them to defy death in the face of noxious drug exposure: They are "armed to the teeth." It is debatable whether malignant cells acquire an increasingly drug-resistant phenotype or are selected for their drug resistance during cytotoxic therapy. However, there is consensus that *both* stem cells and malignant cells have increased expression of ATP-binding cassette (ABC) transporters and a heightened capacity for DNA repair that protect and enable them to thwart any pharmaceutical attacks. In addition, *both* stem cells and malignant cells are supplied with ample death-defying or survival-augmenting factors that render them virtually immortal. Furthermore, *both* stem cells and cancer stem cells are generally quiescent and tend

to remain in the G_0 phase of the cell cycle. This property renders them, as in the case of spores or seeds, less vulnerable to damage by external forces and allows them to regenerate when conditions are favorable. Finally, an oft-forgotten reason for enhanced drug resistance in *both* stem cells and malignant cells is that they are multipotent, with the capacity to generate heterogeneity and differentiate into a drug-resistant or "teratomatous" phenotype.

ABC Transporters

Multidrug resistance (MDR) is a phenomenon of cross-resistance to multiple cytotoxic agents gained after exposure to one of the drugs. It often develops in a patient after treatment with certain chemotherapeutic agents. A principal mechanism for the development of MDR in cancer is increased expression of ABC-transporter genes such as *mdr-1* and *brcp1*. ABC transporters are plasma membrane proteins that play an important role in diverse cellular functions, including transport of lipid and organic anions, iron metabolism, and drug metabolism. They cause MDR by virtue of their ability to extrude xenotoxic proteins from the cell.

The MDR gene product p170-glycoprotein (P-gp) is expressed by cells at the apical membranes in the liver, kidney, and intestines and at the blood–brain and blood–testis barriers. Cells with the MDR phenotype actively expel a wide variety of compounds and are characterized by lower intracellular drug accumulation and reduced sensitivity to these agents than are cells without that phenotype. Cells overexpressing P-gp display cross-resistance to several important chemotherapeutic drugs, including anthracyclines and epipodophyllotoxins.

It is interesting that P-gp is also constitutively expressed in a wide variety of stem cells. Not only does P-gp protect cells from the toxic effects of exogenous agents by pumping them out of the cell membrane, but it is also involved in the inhibition of caspase-dependent cell death pathways regardless of the presence of drugs [2, 3]. I hypothesize that cancer therapy using cytotoxic agents results in the activation of *mdr-1* and the selection of cancer stem cells in a tumor. Because the MDR phenotype is only a small piece of the big cancer stem-cell puzzle, targeting P-gp alone is unlikely to be an effective therapeutic strategy. Not surprisingly, two randomized phase III studies using valspodar, a potent inhibitor of the P-gp efflux pump, did not reveal improved response rates or survival time in poor-risk patients with acute myeloid leukemia [4, 5].

Bcrp1 mRNA is expressed at high levels in primitive murine hematopoietic stem cells and is sharply downregulated with differentiation [6]. Similarly, *mdr-1a* is highly expressed in $CD34^-$ stem cells, and this expression decreases as the stem cells differentiate. Thus, ABC transporters block differentiation and maintain cells in an undifferentiated state by expelling a differentiation-inducing factor from the interior of the stem cell [7].

A population of hematopoietic stem cells known as side population or SP cells display low Hoechst fluorescence because of an increased efflux of the dye from these cells. At least 250 bone-marrow SP cells are needed to rescue a lethally irradiated

mouse. By comparison, only a few (1–20) c-kit$^+$Sca1$^+$Lin$^-$CD34$^-$ stem cells are needed to successfully rescue such animals, whereas 500 CD34$^+$ cells are insufficient for the same task [8]. It appears that the SP phenotype is a highly conserved biologic signature of certain stem cells. So far, a stem-cell population as defined by SP cells has also been identified in other tissue types, including the skeletal muscle [9, 10] and neural tissue [11].

DNA Repair

It is anticipated that increased DNA repair contributes to enhanced drug resistance in malignant cells. But on careful examination, there appears to be a discrepancy in our understanding of DNA repair and drug resistance: How do we reconcile the finding that *impaired* DNA repair promotes cancer formation with the observation that *increased* DNA repair often occurs in cancer cells? Which comes first, and what is the relationship between these two seemingly opposing processes? It is conceivable that a stem cell or malignant cell would enhance its DNA repair capacity to minimize accumulation of DNA mutations and decrease genetic instability. Increased DNA repair could also be triggered to compensate for impaired DNA repair that might have occurred in these cells. Could this be a desperate last-ditch effort to salvage a badly damaged stem cell before it converts into a malignant cell?

I speculate that maintenance of genomic stability is a byproduct of a stem cell's ingrained ability to recognize and repair DNA damage [12, 13]. For example, the mismatch repair complexes recognize specific single mismatches or misaligned short nucleotide repeats. They recruit endonuclease and coordinate DNA resynthesis and ligation to repair damaged DNA. This increased DNA repair capability is passed on from the incipient stem cell to its derivative malignant cell. Initially, mutations within certain DNA repair pathways in a stem cell may instigate genetic instability. At some point, enhanced DNA repair may not be sufficient to fix the damage caused by the mutations in the DNA. Hence, defects in mismatch repair contribute to the development of hereditary nonpolyposis colorectal cancer, sporadic colorectal cancer, lymphoma, and leukemia. When sufficient DNA damage occurs and enhanced DNA repair, cell-cycle arrest, apoptosis, and other safety-net mechanisms can no longer save the doomed stem cell, malignancy ensues. At that point, enhanced DNA repair capability actually becomes an asset rather than a liability to the malignant stem cell, i.e., the cancer cell.

Apoptosis

Apoptosis, or programmed cell death, is a natural, spontaneous biologic process in which defective or degenerated cells are eliminated from normal tissues. During development, it plays an important role in morphogenesis, such as in the formation

of digits and the pruning of neural connections. Apoptosis also provides a safety valve to ensure that certain undesirable and harmful mutations are not preserved in the gene pool. Elegant work by Robert Horvitz on *Caenorhabditis elegans* [14] and by Stanley Korsmeyer and Susan Cory on bcl-2 [15, 16] during the late 1980s laid the groundwork for the study of apoptosis. Since then, innumerable investigators have elucidated the many details and intricacies of apoptosis and its role in cancer formation and drug resistance.

Therefore, another way for both stem cells and malignant cells to develop drug resistance is by inhibiting apoptosis. Cells can counter apoptosis in many ways, such as by enhancing the activity of various inhibitors of apoptosis or by attenuating the effects of the nearly innumerable activators of apoptosis. Of interest is that inhibition of apoptosis is prevalent in malignant cells, just as it is universal in stem cells. One wonders whether the inherent drug resistance of a malignant cell mimics the innate imperviousness of a stem cell, providing additional support to the hypothesis that the malignant cell is a derivative of a stem cell.

"Teratomatous" Tumors

An unsolved mystery of drug resistance relates to the conflicting clinical observations that although cancer stem cells are intrinsically chemoresistant, germ-cell tumors are exquisitely chemosensitive. After all, a germ cell is a stem cell. How do we reconcile this apparent paradox?

Many oncologists consider germ-cell tumor the paradigm of a curable cancer. About 90% of germ-cell tumors are cured. But what is the basis for their curability? It would be erroneous to think that germ-cell tumor is curable because of chemotherapy. After all, only a small fraction of patients with germ-cell tumors have pure choriocarcinoma or embryonal carcinoma (about 10%), which are exquisitely sensitive to chemotherapy. The vast majority of patients have seminoma (about 50%), which can be cured by radiotherapy, or a component of teratoma (about 25%), which requires surgical resection. There are several reasons for our success in the treatment of germ-cell tumor and for its consideration as the paradigm of a curable cancer. First, we know a great deal about the biologic behaviors and clinical features of its various components. Second, we have reliable and convenient tumor markers and radiographic scans to help us monitor disease status and treatment response. And third, several therapeutic modalities can be tailored for the appropriate tumor components: chemotherapy for pure embryonal carcinoma and choriocarcinoma, radiotherapy for seminoma, and surgery for teratoma.

It is of interest that embryonal carcinoma derived from a germinal stem cell is sufficiently chemosensitive that it can be completely eradicated and readily cured by chemotherapy alone. However, germinal stem cells from the genital ridge of 21-day-old fetal mice transplanted into the testes of adult syngeneic mice actually develop into a teratocarcinoma, which is resistant to chemotherapy.

Therefore, depending on the microenvironment, a malignant germinal stem cell may manifest as an embryonal carcinoma that is sensitive to chemotherapy or as a mixed germ-cell tumor with a teratomatous component that is resistant to chemotherapy. In other words, some cancers derived from early stem cells are chemosensitive but have the potential to be chemoresistant when they develop somatic features, i.e., when they differentiate into a teratomatous phenotype. These tumors have the capacity to express diverse phenotypes and the potential to transform from one phenotype to another. In such circumstances, the tumor becomes particularly chemoresistant and cannot be cured by chemotherapy at all: Surgery may be needed to eradicate both the cancer stem cells and the teratomatous components [17].

The Enigma of Somatic Cells

An enigma about drug resistance relates to the effect of therapy on normal tissues. Conventional wisdom dictates that most chemotherapeutic agents target cells that are actively undergoing cell division, i.e., in the S phase of the cell cycle. Under the influence of cytotoxic chemotherapy, dividing cells in normal tissues such as the bone marrow, alimentary mucosa, and hair follicles are also injured and eliminated. But these normal tissues regenerate, and the lost cells are duly replaced. However, only about 5% of cells in an average breast cancer are in the S phase; no wonder it is so difficult to eradicate such tumors with chemotherapy alone. Similarly, most somatic cells in normal tissues do not divide, just like the drug-resistant cells in a teratomatous tumor, and are scarcely affected by the ravages of chemotherapy. Otherwise, one would imagine that a whole individual would be utterly wiped out by the treatment.

The Goldie–Coldman Principle

It would be improper to discuss drug resistance without mentioning the Goldie–Coldman principle [18]. This scientific treatise was of major historic importance because it provided a quantitative rationale for vanquishing drug resistance in a clinical setting. To this day, it has continued to etch a profound impression on our understanding of tumor growth, cancer mutations, and drug resistance. In fact, it still leaves an indelible mark on how we use chemotherapeutic agents and design clinical trials.

The Goldie–Coldman principle is based on a mathematic function that produces a log-kill growth curve and attempts to describe the therapeutic regression of tumors. It assumes that the doubling time of cells within a tumor is always constant. The Goldie–Coldman principle left us several enduring clinical legacies. For

example, it deduced that tumors were most curable when they were small and treated early; it suggested that log kill increased with increasing drug dose; and it proposed that extending the duration of therapy ought to enhance cure rates, especially for larger tumors. The model also predicted that drug combinations could provide multiplicative log kills and would improve therapeutic efficacy. In particular, alternating treatments ought to be more advantageous than sequential treatments because the alternating schedule might minimize drug resistance that was presumed to be emerging during treatment.

As we all know now, however, the results of many clinical trials do not generally support the Goldie–Coldman principle. To some people, it may be somewhat surprising that a seemingly solid and robust scientific model fails to produce successful clinical results. This reminds us that scientific models are only useful for the purpose of summarizing empiric observations and for designing experiments. They are valiant and valuable attempts to describe clinical phenomena, but they cannot and do not explain such phenomena if the underlying theory, principles, and assumptions for the scientific model are fundamentally flawed. Any time a discrepancy arises between a scientific model and empiric results, something may be amiss, and it warns us that we need to reassess our underlying theory, principles, and assumptions and institute an improved scientific model.

The Gompertzian Model

A most glaring misconception of the Goldie–Coldman model is the assumption that the doubling time of a tumor is always constant and its growth curve exponential. This would predicate that a tumor's growth rate always remains constant relative to its size. Clearly, this is not the typical behavior of most, if not all, human cancers. It is ironic that in 1825, more than 150 years before the Goldie–Coldman principle was published, Benjamin Gompertz reported on a superior model that described the nonexponential growth pattern of tumors [as cited in 19]. Instead of the straight line one obtains for an exponential growth curve on a semilogarithmic scale, the shape of a nonexponential Gompertzian curve deflects downward between the range of 10^2 and the clinically appreciable 10^{10} cells. In other words, the doubling time of a tumor on a Gompertzian curve increases steadily (i.e., growth slows down) as the tumor grows larger (Fig. 15.1).

The biologic basis of the Gompertzian growth curve of tumors is still unclear. Surely, interactions between cellular, stromal, and soluble elements affect the relationship between cell number and cell volume in a tumor. The fact that the fraction of dividing cells is highest when the tumor is small suggests that in an incipient tumor, the cancer-initiating cells must be undergoing unbridled symmetric cell divisions. As the tumor gets larger, though, more differentiated cancer cells are present, and the fraction of dividing cells decreases. According to the Gompertzian model, the time from the initiation of carcinogenesis to the appearance of clinical disease may be shorter than previously thought. The Gompertzian model predicts that

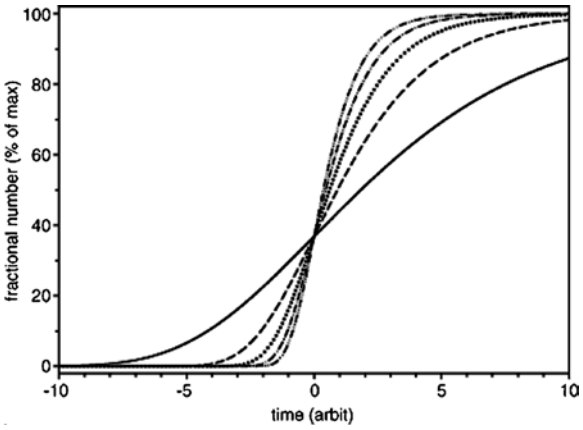

Fig. 15.1 Gompertzian curves. Instead of the straight line one obtains for an exponential growth curve on a semilogarithmic scale, the shape of a *nonexponential Gompertzian curve* deflects downward within the range of 10^2 and the clinically appreciable 10^{10} cells

greater fractional log kill in an adjuvant setting will be offset by a faster fractional tumor regrowth.

Clinical Pitfalls

Let us reexamine the assumptions underlying the Goldie–Coldman principle and illustrate why it has been modified in light of our current understanding and knowledge about drug resistance and how it may have failed according to the theory of a stem-cell origin of cancers.

The Goldie–Coldman principle predicts that tumors >1.0 cm in diameter cannot be cured with single-drug regimens. Although it is true that childhood acute lymphoblastic leukemias, other pediatric tumors, adult lymphomas, and germ-cell tumors of $>10^{10}$ cells are frequently cured with two- or three-drug regimens, both gestational choriocarcinoma and Burkitt lymphoma, two rapidly growing cancers, are curable with single drugs.

The Goldie–Coldman principle also concludes that chemotherapy needs to be initiated as soon as possible to be maximally effective. However, it is well known that most testicular cancers retain their chemosensitivities even with delayed treatment. Hence, patients with stage II testicular cancer who experienced relapse after retroperitoneal lymph node dissection and who received chemotherapy at a later date did just as well with respect to response rate and overall survival time as did the patients who received immediate adjuvant chemotherapy [20].

In addition, the Goldie–Coldman principle fails in its prediction that alternating chemotherapy sequences provides a superior clinical outcome. In the event that one cannot deliver simultaneous combination of all drugs, the model advocates alternate sequences rather than sequential blocks of treatment. However, when total doses are controlled, alternating courses of chemotherapy for the treatment of stage II breast cancer involving four or more axillary lymph nodes was found to be inferior to a crossover schedule of therapy [21].

Another shortcoming of the Goldie–Coldman principle is its assumption about absolute drug resistance. It is now well established that drug resistance tends to be relative rather than absolute. Hence, clinical data indicate that patients might still respond to chemotherapy after relapse from a complete remission induced by the same chemotherapy [22]. In another study, all parameters of disease sensitivities to treatment, i.e., response rate, response duration time, and overall survival time, were unaffected by the patients' past histories of adjuvant chemotherapy [23].

These results are perfectly in line with what is known about cancer heterogeneity: Some cells are more chemosensitive than others in a tumor (e.g., embryonal carcinoma vs. teratoma in testicular cancer). It is also consistent with what has been learned about cancer stem cells: A fraction of cells in a tumor have stem-cell features and are intrinsically refractory to chemotherapeutic agents, whereas the remainder of cells in a tumor are differentiated cancer cells, which may be more amenable to therapeutic interventions. The quality of response or remission achieved with many of our current treatments depends to a large extent on their effects on the treated tumor types.

Finally, a critical misstep in the Goldie–Coldman principle derives from an assumption that the acquisition of drug resistance occurs during treatment as opposed to an alternative possibility that drug resistance already exists before therapy. This alternative possibility explains why >30% of patients were still alive and free of disease after a radical mastectomy without any adjuvant chemotherapy [24, 25]. After 30 years of follow-up, the mortality rate of these patients reached a plateau and became indistinguishable from that of the general population [26, 27]. In another study of patients with breast cancer whose primary disease was treated by lumpectomy with or without radiation therapy, the cohort not given radiation had a significantly higher local relapse rate but similar distant metastasis rate and overall survival time [28].

The fact that the local recurrence rate without radiation was worse but had no negative effect on the distant metastasis rate or overall survival time suggested that some tumors (as in the patient population in the Fisher study) tended to be locally confined for a prolonged period (12 years). A corollary to this finding is that when metastasis develops, it is likely to occur in an entirely different group of patients with a different cancer type, in whom the odds are high that the metastasis has already occurred before the time of its initial clinical presentation. The findings that both drug resistance and metastasis occur early if not at the very beginning of carcinogenesis support the theory of a stem-cell origin of cancers, in which certain

tumors with early stem-cell origins tend to be drug resistant and metastatic from the outset, whereas other tumors with late progenitor stem-cell origins are less drug resistant and do not metastasize as much, if at all.

Clinical Gems

The clinical implications of a stem-cell origin of malignancy and drug resistance can be far-reaching. Cytotoxic agents may be more effective for the treatment of malignancies derived from late progenitor stem cells that have lost some of the power in their drug-resistance machinery. Such agents may eradicate a large proportion of the entire tumor that has lost its stem-cell characteristics and drug-resistance capability but may scarcely affect the so-called cancer stem cells, which continue to regenerate and sustain the malignancy. Hence, the tumor keeps recurring even after what seems like complete responses and durable remissions have been attained. This view explains an intriguing observation in clinical oncology, that a treatment can improve the response rate and prolong the time to progression but not provide any survival advantage to the patients treated.

According to the theory of a stem-cell origin of cancers, treatments that target the differentiated cancer cells may improve the response rate (e.g., shrinkage of the tumor volume) and even increase the progression-free survival time. However, they may not prolong overall survival time. On the other hand, treatments that target stem-cell pathways causing differentiation but not elimination of the cancer stem cells would scarcely affect the response rate but may augment the disease-free or overall survival time. We surmise that treatments that manage to target and eliminate cancer stem cells should provide improved but delayed responses as well as increased progression-free and overall survival times.

If a particular cytotoxic agent does not affect the root of cancer, namely cancer stem cells, then no matter how early, how much, or how long such treatment is used, it will not improve the ultimate clinical outcome (i.e., overall survival time) in spite of higher response rates or longer progression-free times. Consequently, early treatment using such an agent will not provide any overall clinical benefit on the basis of improved overall survival time. Hence, there was no advantage in considering early lymph node dissection for intermediate-thickness (1–4 mm) melanoma [29] or administering early external beam irradiation for low-grade gliomas (i.e., astrocytoma, oligodendroma, and mixed oligoastrocytoma) [30].

Similarly, dose-intensified therapy for some tumor types might only aggravate potential toxic effects without improving the overall survival time of patients, if it did not favorably affect the cancer stem cells. Hence, dose-escalated MAID (mesna, doxorubicin, ifosfamide, and dacarbazine) with granulocyte colony-stimulating factor (filgrastim) support did not improve the clinical outcome of patients with soft tissue sarcoma [31]. Likewise, high-dose chemotherapy and stem-cell support did not improve the overall survival time of patients with breast cancer [32]. And dose-

intensified M-VAC (methotrexate, vinblastine, doxorubicin, and cisplatin) with growth factor support increased the response rate, complete remission rate, and progression-free survival time but did not improve the overall survival time of patients with bladder cancer [33].

This notion also explains why certain maintenance therapies have not improved the overall survival time of patients with various tumors, especially if the treatment happens not to appreciably target the pertinent cancer stem cells. For example, additional chemotherapy beyond four to six cycles prolonged the time to disease progression but did not improve the overall survival time of patients with small-cell lung cancer [34]. Similarly, prolonged consolidation therapy (12 vs. 3 months of paclitaxel) after complete remission increased the progression-free survival time but not the overall survival time of patients with ovarian carcinoma [35]. Furthermore, maintenance therapy given to responding or stable breast cancer patients led to a 6-month improvement in progression-free survival time but no difference in overall survival time [36].

When the cancer stem cells in a tumor are inherently resistant to cytotoxic chemotherapy, it is expected that combination therapy using such agents would not provide results superior to those obtained with single-agent treatment. For example, combination dacarbazine therapy (i.e., the Dartmouth regimen: dacarbazine, cisplatin, carmustine, and tamoxifen) provided a higher response rate than single-agent dacarbazine did in the treatment of melanoma but did not improve overall survival time [37]. Additionally, doxorubicin plus cisplatin enhanced the response rate, complete remission rate, and disease-free survival time but not overall survival time when compared with doxorubicin alone for the treatment of uterine carcinoma [38]. In soft tissue sarcoma, Cy-VA-DIC (cyclophosphamide, vincristine, doxorubicin, and dacarbazine) did not improve the clinical outcome over that achievable with doxorubicin alone [39], and in gastric cancer, fluorouracil plus cisplatin or uracil and tegafur plus mitomycin were no better than fluorouracil alone [40]. Moreover, in extensive-staged small-cell lung cancer, adding paclitaxel to etoposide and cisplatin only caused more unacceptable toxicity without prolonging the time to disease progression or improving the overall survival time [41].

An Exercise in Futility

Elucidation of a stem-cell origin of cancers and drug resistance will, I hope, steer us away from many well-intentioned but potentially futile treatments. For instance, if cancer cells are already well endowed with potent and redundant ABC transporters (as well as other stem-cell properties they have inherited from stem cells), then treatment strategies that inhibit a particular ABC transporter seem grossly insufficient [5]. Treatments that offer nothing special and only “marinate” a potentially drug-resistant teratomatous tumor in tons of poisonous chemotherapy

(as in bone-marrow transplantation for solid tumors) seem idiotic if not insane. If malignant cells are engendered from stem cells, they are likely to be empowered with the same incredible drug-resistance capabilities. We need to be wary about promises of novel cytotoxic or noncytotoxic therapies without valid biologic principles: Any benefits derived from such treatments are likely to be modest if not superficial. To make a major breakthrough in our treatment of cancer requires that we incorporate the theory of stem-cell origin of cancers into our thinking and practice. A fundamental shift in our understanding about the origin of cancers is in order. It will permit us to better appreciate the subtleties that distinguish malignant constituents from stem-cell derivatives and allow us to design improved therapeutic strategies that target the pertinent malignant elements while sparing the equivalent stem-cell components.

An Uphill Battle

A stark reality becomes apparent. Stem cells and cancer cells have the same or similar drug-resistance machinery that renders curative treatment almost impossible for most cancers. It is as though both types of cells are capable of a two-pronged attack, with increased expression of the ABC transporters and a heightened capacity for DNA repair. Both stem and malignant cells are also fortified with abundant death-defying or survival-augmenting capabilities that virtually assure life in perpetuity. It may be a foregone conclusion that fighting drug resistance is an uphill battle. Unfortunately, the odds are against us when we have no inkling about the origin or nature of drug resistance in cancer. Worse still, we may have to repeat the curse of Sisyphus, forever pushing the boulder of cancer remission up a mountain, only to have the boulder of cancer recurrence keep rolling down the mountain again and again….

Conclusion

As long as cancer exists, drug resistance is here to stay because the very origin and nature of cancer are intimately and intrinsically linked to drug resistance. It begins to dawn on us that buried among the statistics of countless clinical trials involving hundreds if not thousands of patients lies a sad truth: Drug resistance is but one of the many manifested phenotypes that allude to cancer's infinite variability as well as its unique and distinct origins. Unfortunately, when we perceive drug resistance erroneously and tackle it superficially, our once seemingly infallible scientific models may actually lead us further astray, and our once supposedly promising treatments may in fact provide us with only marginal clinical benefits.

References

1. Dean M, Fojo T, Bates S (2005) Tumour stem cells and drug resistance [review]. Nat Rev Cancer 5:275–284
2. Johnstone RW, Cretney E, Smyth MJ (1999) P-glycoprotein protects leukemia cells against caspase-dependent, but not caspase-independent, cell death. Blood 93:1075–1085
3. Pallis M, Russell N (2000) P-glycoprotein plays a drug-efflux-independent role in augmenting cell survival in acute myeloblastic leukemia and is associated with modulation of a sphingomyelin-ceramide apoptotic pathway. Blood 95:2897–2904
4. Baer MR, George SL, Dodge RK et al (2002) Phase 3 study of the multidrug resistance modulator PSC-833 in previously untreated patients 60 years of age and older with acute myeloid leukemia: Cancer and Leukemia Group B Study 9720. Blood 100:1224–1232
5. Greenberg PL, Lee SJ, Advani R et al (2004) Mitoxantrone, etoposide, and cytarabine with or without valspodar in patients with relapsed or refractory acute myeloid leukemia and high-risk myelodysplastic syndrome: a phase III trial (E2995). J Clin Oncol 22:1078–1086, Erratum in J Clin Oncol 2004;22(13):2747
6. Zhou S, Schuetz JD, Bunting KD et al (2001) The ABC transporter Bcrp1/ABCG2 is expressed in a wide variety of stem cells and is a molecular determinant of the side-population phenotype. Nat Med 7:1028–1034
7. Good JR, Kuspa A (2000) Evidence that a cell-type-specific efflux pump regulates cell differentiation in *Dictyostelium*. Dev Biol 220:53–61
8. Osawa M, Hanada K, Hamada H et al (1996) Long-term lymphohematopoietic reconstitution by a single CD34-low/negative hematopoietic stem cell. Science 273:242–245
9. Gussoni E, Soneoka Y, Strickland CD et al (1999) Dystrophin expression in the mdx mouse restored by stem cell transplantation. Nature 401:390–394
10. Jackson KA, Mi T, Goodell MA (1999) Hematopoietic potential of stem cells isolated from murine skeletal muscle. Proc Natl Acad Sci USA 96:14482–14486
11. Hulspas R, Quesenberry PJ (2000) Characterization of neurosphere cell phenotypes by flow cytometry. Cytometry 40:245–250
12. Cervantes RB, Stringer JR, Shao C et al (2002) Embryonic stem cells and somatic cells differ in mutation frequency and type. Proc Natl Acad Sci USA 99:3586–3590
13. Myllyperkiö MH, Vilpo JA (1999) Increased DNA single-strand break joining activity in UV-irradiated CD34+ versus CD34− bone marrow cells. Mutat Res 425:169–176
14. Ellis HM, Horvitz HR (1986) Genetic control of programmed cell death in the nematode C. elegans. Cell 44:817–829
15. McDonnell TJ, Deane N, Platt FM et al (1989) *bcl*-2-Immunoglobulin transgenic mice demonstrate extended B cell survival and follicular lymphoproliferation. Cell 57: 79–88
16. Vaux DL, Cory S, Adams JM (1988) *Bcl*-2 gene promotes haemopoietic cell survival and cooperates with c-*myc* to immortalize pre-B cells. Nature 335:440–442
17. Tu S-M, Lopez A, Leibovici D et al (2009) Ductal adenocarcinoma of the prostate: clinical features and implications after local therapy. Cancer 115:2872–2880
18. Goldie JH, Coldman AJ (1979) A mathematic model for relating the drug sensitivity of tumors to their spontaneous mutation rate. Cancer Treat Rep 63(11–12):1727–1733
19. Laird AK (1969) Dynamics of growth in tumors and in normal organisms. Natl Cancer Inst Monogr 30:15–28
20. Williams SD, Stablein DM, Einhorn LH et al (1987) Immediate adjuvant chemotherapy versus observation with treatment at relapse in pathological stage II testicular cancer. N Engl J Med 317:1433–1438
21. Bonadonna G, Zambetti M, Valagussa P (1995) Sequential or alternating doxorubicin and CMF regimens in breast cancer with more than three positive nodes. Ten-year results. JAMA 273:542–547

22. Fisher RI, DeVita VT, Hubbard SP et al (1979) Prolonged disease-free survival in Hodgkin's disease with MOPP reinduction after first relapse. Ann Intern Med 90:761–763
23. Kardinal CG, Perry MC, Korzun AH et al (1988) Responses to chemotherapy or chemohormonal therapy in advanced breast cancer patients treated previously with adjuvant chemotherapy. A subset analysis of CALGB study 8081. Cancer 61:415–419
24. Adair F, Berg J, Joubert L et al (1974) Long-term followup of breast cancer patients: the 30-year report. Cancer 33:1145–1150
25. Ferguson DJ, Meier P, Karrison T et al (1982) Staging of breast cancer and survival rates. An assessment based on 50 years of experience with radical mastectomy. JAMA 248:1337–1341
26. Brinkley D, Haybrittle JL (1975) The curability of breast cancer. Lancet 2:95–97
27. Rutqvist LE, Wallgren A, Nilsson B (1984) Is breast cancer a curable disease? A study of 14,731 women with breast cancer from the Cancer Registry of Norway. Cancer 53:1793–1800
28. Fisher B, Redmond C, Poisson R et al (1989) Eight-year results of a randomized clinical trial comparing total mastectomy and lumpectomy with or without irradiation in the treatment of breast cancer. N Engl J Med 320:822–828, Erratum in N Engl J Med 1994; 330(20):1467
29. Balch CM, Soong S-J, Bartolucci AA et al (1996) Efficacy of an elective regional lymph node dissection of 1 to 4 mm thick melanomas for patients 60 years of age and younger. Ann Surg 224:255–266
30. Leighton C, Fisher B, Bauman G et al (1997) Supratentorial low-grade glioma in adults: an analysis of prognostic factors and timing of radiation. J Clin Oncol 15:1294–1301
31. Le Cesne A, Judson I, Crowther D et al (2000) Randomized phase III study comparing conventional-dose doxorubicin plus ifosfamide versus high-dose doxorubicin plus ifosfamide plus recombinant human granulocyte-macrophage colony-stimulating factor in advanced soft tissue sarcomas: a trial of the European Organization for Research and Treatment of Cancer/ Soft Tissue and Bone Sarcoma Group. J Clin Oncol 18:2676–2684
32. Peters WP, Rosner GL, Vredenburgh JJ et al (2005) Prospective, randomized comparison of high-dose chemotherapy with stem-cell support versus intermediate-dose chemotherapy after surgery and adjuvant chemotherapy in women with high-risk primary breast cancer: a report of CALGB 9082, SWOG 9114, and NCIC MA-13. J Clin Oncol 23:2191–2200
33. Sternberg CN, de Mulder PHM, Schornagel JH et al (2001) Randomized phase III trial of high-dose-intensity methotrexate, vinblastine, doxorubicin, and cisplatin (MVAC) chemotherapy and recombinant human granulocyte colony-stimulating factor versus classic MVAC in advanced urothelial tract tumors: European Organization for Research and Treatment of Cancer protocol No. 30924. J Clin Oncol 19:2638–2646
34. Giaccone G, Dalesio O, McVie GJ et al; European Organization for Research and Treatment of Cancer Lung Cancer Cooperative Group (1993) Maintenance chemotherapy in small-cell lung cancer: long-term results of a randomized trial. J Clin Oncol 11:1230–1240
35. Markman M, Liu PY, Wilczynski S et al (2003) Phase III randomized trial of 12 versus 3 months of maintenance paclitaxel in patients with advanced ovarian cancer after complete response to platinum and paclitaxel-based chemotherapy: a Southwest Oncology Group and Gynecologic Oncology Group trial. J Clin Oncol 21:2460–2465
36. Muss HB, Case LD, Richards F 2nd et al (1991) Interrupted versus continuous chemotherapy in patients with metastatic breast cancer. The Piedmont Oncology Association. N Engl J Med 325:1342–1348
37. Chapman PB, Einhorn LH, Meyers ML et al (1999) Phase III multicenter randomized trial of the Dartmouth regimen versus dacarbazine in patients with metastatic melanoma. J Clin Oncol 17:2745–2751
38. Thigpen JT, Brady MF, Homesley HD et al (2004) Phase III trial of doxorubicin with or without cisplatin in advanced endometrial carcinoma: a Gynecologic Oncology Group study. J Clin Oncol 22:3902–3908

39. Santoro A, Tursz T, Mouridsen H et al (1995) Doxorubicin versus CYVADIC versus doxorubicin plus ifosfamide in first-line treatment of advanced soft tissue sarcomas: a randomized study of the European Organization for Research and Treatment of Cancer Soft Tissue and Bone Sarcoma Group. J Clin Oncol 13:1537–1545
40. Ohtsu A, Shimada Y, Shirao K et al (2003) Randomized phase III trial of fluorouracil alone versus fluorouracil plus cisplatin versus uracil and tegafur plus mitomycin in patients with unresectable, advanced gastric cancer: the Japan Clinical Oncology Group Study (JCOG9205). J Clin Oncol 21:54–59
41. Niell HV, Herndon JE II, Miller AA et al (2005) Randomized phase III intergroup trial of etoposide and cisplatin with or without paclitaxel and granulocyte colony-stimulating factor in patients with extensive-stage small-cell lung cancer: Cancer and Leukemia Group B Trial 9732. J Clin Oncol 23:3752–3759

Chapter 16
Paradigm Shifts

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I must create a system, or be enslaved by another man's.

– William Blake

Précis

The idea of a cancer cell's revealing its *undifferentiated* stem-cell features instead of its becoming *dedifferentiated* from a mature differentiated cell is a major paradigm shift.

Introduction

Sometimes an idea becomes so powerful that it is almost synonymous with truth itself. We accept the idea and take it for granted in part because it makes perfect sense and seems self-evident. But we forget that at one time this same idea might have been considered ground-breaking, if not earth-shattering; once upon a time, the idea might even have been considered maverick, if not downright heretical. For example, many people thought that the earth was flat before Magellan sailed

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around it. Many more people thought that the earth was at the center of the universe before Copernicus showed otherwise. Darwin taught that all living things came into being by evolution rather than by creation, and Freud thought that psychological derailment was caused by mental illnesses rather than by demons.

This chapter illustrates how the theory of a stem-cell origin of cancers might drastically alter our current thinking about cancer, leading to important paradigm shifts.

Dedifferentiation

Dedifferentiation is anathema to the theory of a stem-cell origin of cancers. The very word implies that cancer can arise from any differentiated cells and that specialized mature cells may convert from one to another. I believe that it is unlikely for a specialized mature cell to become multipotent and undifferentiated in a natural setting and under normal circumstances. In a stem-cell hierarchy, the evolution of individual stem cells is presumed to be unidirectional and irreversible. Therefore, the phenomenon of dedifferentiation, which suggests that cellular differentiation is interchangeable and reversible, contradicts a basic tenet of the theory of a stem-cell origin of cancers. I surmise that dedifferentiation is a misconceived rather than a real phenomenon. If the theory of a stem-cell origin of cancers is correct, its first casualty would be dedifferentiation: The word dedifferentiation would be banished from the vocabulary of cancer.

Let us reexamine one particular report of dedifferentiation in some detail. Real et al. [1] performed experiments whose results suggested that differentiated cells can dedifferentiate and generate multipotent progenitor cells with self-renewing potential. It is obvious that such experiments were being performed to fulfill our overriding desire to produce sufficient numbers of stem cells for various purposes. But do the results from these experiments really reveal a sound scientific truth rather than merely demonstrating a clever research ruse? Outside of a laboratory setting, do they make any biologic sense?

It is interesting that Real's group used differentiated pigment cells obtained from quail embryos. When a cell is derived from an embryo, how differentiated can it really be? In addition, when one grows these cells in culture, which cells are actually being selected? Indeed, differentiated cells are supposed to be vulnerable and undergo apoptosis when separated from their normal microenvironment. Therefore, it is entirely plausible that undifferentiated stem cells may have been selected for the study instead. Furthermore, these so-called differentiated pigment cells can migrate and proliferate at will. Even if we give certain differentiated cells the benefit of doubt and consider that they possess stem-cell features, we become bogged down by semantics. Shall we call them differentiated stem cells? After all, certain differentiated cells do possess stem-cell features. For example, differentiated memory lymphocytes are virtually immortal and can migrate all over the body, and

differentiated hepatocytes have the capacity to differentiate into various hepatic lineages and can even regenerate a whole liver.

In the laboratory, the rules of nature can always be bent for the purposes of testing a particular hypothesis. To claim that what is observed in the laboratory is representative of even a modicum of reality is akin to violating nature's sanctity. Therefore, it should not be surprising that many experimental results do not readily translate into clinical applications. For example, it is not uncommon that many cancer treatments that successfully target certain cancer mutations or pathways in the laboratory do not work at all in the clinics. Also, a specific cancer treatment may work even though it does not target the supposedly relevant cancer mutations or pathways. It is true that one can always blame any clinical inconsistencies on individual idiosyncrasy or disease heterogeneity. One can also bury any clinical discrepancies with fancy statistics. Ultimately, we become wiser not because of our utter reliance on the scientific method but because of our humble awareness of its inherent limitation and shortcomings.

The idea of a cancer cell's revealing its *undifferentiated* stem-cell features instead of its becoming *dedifferentiated* from a mature differentiated cell is a major paradigm shift. In principle, any cell with stem-cell properties is poised to become malignant given the right conditions and circumstances. What matters is stemness in a cell – even a differentiated cell, if it possesses stem-cell properties. The stem-cell characteristic is what gives cancer its free rein. Surely, someone can always manipulate the experimental system and reprogram a mature cell to become malignant by putting stemness factors into it. But this would be a most unnatural feat whose relevance and meaning need to be questioned in the right context outside of the laboratory. Seriously, do we really expect a mature differentiated skin cell or intestinal cell to dedifferentiate into a stem cell (or cancer cell) in real life? Dedifferentiation is no more possible or real than reversing the life cycle and converting an old person into an infant, as in "The Curious Case of Benjamin Button," a short story by F. Scott Fitzgerald (1921).

Genetic Mutations

Until recently, we did not doubt that genetic mutations play a crucial role during carcinogenesis. But increasingly, we are beginning to question whether many of them are really unique, specific, or even pertinent to cancer. We have learned that many of the same mutations found in cancer can be rather innocuous and also occur in normal-appearing or nonmalignant cells (Chap. 11). Consequently, therapies that target these mutations may provide limited utility and produce only marginal benefits. It is likely that many of these mutations are either a bystander effect or background noise within the cancer cell. According to the theory of a stem-cell origin of cancers, however, only certain genetic mutations in a cancer are actually relevant. They are the cause rather than the effect of a malignant phenotype, the movers rather than the markers of a cancer cell.

I postulate that many of the same relevant factors that cause cancer or contribute to its formation also happen to be stem-cell factors. It is as though cancer comes with a stem-cell package. It has all the looks and feel of stem-cell "machinery." Perhaps the most critical components of a cancer machine, such as the engine and the brakes, are made of stem-cell parts. Cancer is like a malignant vehicle with a powerful engine but no brakes. Nobody knows how to stop its crazy driver, temper its mighty engine, or fix its defunct brakes, because nobody knows who the driver is, which the engine is, or where the brakes are. A genuine concern is that fixing just any parts of this runaway vehicle may be merely cosmetic and possibly futile.

The idea that genetic mutations relevant to cancer formation and progression tend to involve stem-cell targets is a major paradigm shift in the making. It will enable us to elucidate more comprehensively the natural history and phenotypic expression of various tumors. For example, the stem-cell theory predicts that cancer originating from an early stem cell may contain genetic defects that affect asymmetric division. Cancer arising from intermediate stem cells tends to have genetic aberrations that affect differentiation pathways. And cancer arising from late progenitor stem cells may reactivate genes that affect self-renewal. In addition, the stem-cell theory forecasts that genetic mutations affecting an early stem cell in the stem-cell hierarchy are likely to form a tumor that is more heterogeneous, metastatic, and virulent, whereas those that affect a late progenitor stem cell tend to form a tumor that is more homogeneous, localized, and indolent.

Multistep Carcinogenesis

In 1990, Fearon and Vogelstein [2] formulated a model of multistep carcinogenesis in colorectal carcinoma. Even today, this "poster child" of multistep carcinogenesis makes a lasting impression on our collective psyche about cancer.

Fearon and Vogelstein proposed a chain of events most likely to occur during the carcinogenesis of colorectal carcinoma. (1) *APC* was the gatekeeper gene, whose loss started the initial step of converting normal epithelium into early adenoma and began the whole domino effect toward malignancy. (2) The next cascade of events involved a *Ras* mutation that might be responsible for the development of an intermediate adenoma. (3) Then a deletion in *18q* caused the formation of an advanced adenoma. (4) Subsequently, a *p53* mutation transformed the adenoma into a carcinoma. (5) Finally, the development and accumulation of additional genetic changes contributed to the formation of a metastatic carcinoma. The investigators proposed that although these genetic alterations could occur in any order and were not all required to cause a malignancy, each successive step conferred a growth advantage to the nascent cancer cell. An important note about this schema is that these stepwise genetic alterations are not the only ones seen in colorectal carcinomas. But they happen to be the ones most readily identified with the specific morphologic changes involved and are being used to support the model of multistep carcinogenesis.

One should remember that this model of multistep carcinogenesis was originally proposed to explain a disturbing discrepancy about cancer: Millions of cells carrying random mutations are generated every day in every individual human; about 30,000 genes in a cell undergo mutation at a rate of about 10^{-6} times per gene per cell generation, yet why is cancer a relatively rare event? One way to resolve this paradox is to assume that multiple sequential genetic mutations need to occur. Eventually, these mutations conspire to produce a cancer. But the numbers may add up even better if we consider that not all cells are susceptible to carcinogenesis. Despite the innumerable genetic mutations possible, cancer is a relatively rare event because only certain cells (i.e., stem cells or progenitor stem cells) are actually susceptible to the effects of these mutations.

The Story of Adenoma

In 2003, Zauber et al. [3] published results that seem to contradict the classic model of multistep carcinogenesis. The centerpiece of this model is the sequence of steps in the transition from adenoma to carcinoma in colorectal cancer. Early on, adenomas acquire *APC* and *K-ras* mutations that confer growth and survival advantages on the adenomas. Accordingly, when an adenoma develops into a carcinoma, as it acquires and accumulates additional mutations (such as *p53*), the incipient genetic mutations (e.g., *APC* and *K-ras*) in the adenoma should still be present in the carcinoma. Zauber and colleagues discovered, surprisingly, that although metastases consistently showed the same specific *K-ras* mutation that their corresponding primary carcinomas had, the matched carcinoma and adenoma tended to harbor different *K-ras* mutations. Similarly, they showed that a carcinoma and its associated metastasis had identical *APC* loss of heterozygosity, but the carcinoma and adenoma did not share that *APC* loss of heterozygosity. These results exposed a gaping hole in our cherished model of multistep carcinogenesis: They suggest that some adenomas have a unique origin that is distinct from that of carcinomas and that not all colorectal adenomas progress to carcinomas.

There is another way to examine this paradox of colorectal adenoma. Why does one adenoma remain relatively benign, even at a large size, while another converts rapidly into a carcinoma? We postulate that the histogenesis of colorectal adenoma depends on its respective cell of origin. Shih et al. [4] showed that certain dysplastic cells in colonic adenomas were confined to the top or luminal regions of the crypts, whereas cells at the base of the crypts (where stem cells are located) were morphologically normal. Their "top-down" pattern of dysplastic spreading tends to be observed in larger adenomas. On the other hand, Preston et al. [5] demonstrated that dysplastic cells emanated from the base of the crypt, spread upward, and eventually involved the entire crypt. Their "bottom-up" model of the histogenesis of colorectal adenoma is compatible with rapid progression of an adenoma into a carcinoma.

I speculate that the "top-down" type of adenoma histogenesis is caused by dysplasia in a late progenitor cell, located higher at the top of a crypt. The adenoma that develops in such a cell remains relatively benign and behaves more indolently. These are the tumors that remain relatively benign even at a large size. On the other hand, the "bottom-up" type of adenoma histogenesis is caused by dysplasia in an early stem cell, located at the base of the crypt. The adenoma that develops in such a cell is potentially more aggressive and carries a more ominous prognosis. They transform easily and metastasize quickly and may even skip many stages in the multistep carcinogenesis model of colorectal cancer (Fig. 4.1). They tend to be virulent and lethal.

Thus, I submit that distinctly different types of adenomas arise from their respective stem cells or progenitor stem cells in a stem-cell hierarchy within the colonic crypts. Certain adenomas do not convert into carcinomas at all, whereas others convert into different types of carcinomas. This idea is consistent with the results of Zauber and colleagues.

It's the Stem Cell!

A major paradigm shift from our venerable model of multistep carcinogenesis is long overdue. I postulate that the type of stem cell or progenitor stem cell in which a genetic mutation occurs is possibly more important than the mutation itself during carcinogenesis. The malignant phenotype may be determined more by the expression of stem-cell features than by the accumulation of genetic mutations in a certain cell type. This principle offers a surprising prediction: Earlier stem cells require fewer mutations (i.e., they have less need for self-renewal) to become fulminantly malignant, whereas later progenitor stem cells require more mutations to become just as malignant (Fig. 2.2).

In the multistep carcinogenesis model, we envision that as a tumor evolves to become increasingly more malignant, it develops and accumulates new mutations. Hence, as a tumor advances from stage 1 to 2, and then from 2 to 3, it acquires mutations A, B, and C, as follows:

	Stage 1	Stage 2	Stage 3
Mutation	A	A+B	A+B+C

In an alternative model, I envision that the different tumor types I, II, and III have different stem-cell origins downward on the stem-cell hierarchy and accordingly display progressively fewer heterogeneous phenotypes. In this model, the cells of origin are just as important as if not more so than the type of mutations.

	Type I	Type II	Type III
Phenotype	X+Y+Z	X+Y	X

It is important to point out that in this model, a tumor does not evolve and advance from type I to II and then from type II to III. Instead, the tumor types are distinct diseases with disparate origins. Some tumors are inherently more lethal (i.e., type I), while others are intrinsically more indolent (i.e., type III). Sometimes, it may seem that a type III tumor expresses the X phenotype in the beginning only to skip the other stages and eventually transform into a type I tumor with a Z phenotype, when it is actually a type I tumor expressing one of its mixed phenotypes, X, in the beginning and the other intrinsic phenotypes, such as Z, later on under the right conditions.

This idea that patients have distinct types of cancer with unique origins rather than different mutations that occur and accumulate over time is indeed an important paradigm shift. Because they have distinct types of cancer with unique origins, their cancers respond differently to various treatments and have different clinical outcomes. An experiment of nature that can be performed to test this idea involves patients with castrate-resistant prostate cancer and bone metastasis who survived more than 20 years vs. those who survived less than 5 years after their diagnosis. I predict that the expression profile of tumors obtained from the longer-surviving patients will reveal a cancer signature compatible with a type III tumor, whereas those derived from the shorter-surviving patients will show a cancer signature consistent with a type I tumor.

Metastasis

The theory of a stem-cell origin of cancers predicts that the potential to metastasize is programmed from the start and therefore must be imprinted in primary tumors. Results from several studies lend support to this idea. A molecular profile of metastasis was found to be present in primary breast, lung, and colorectal tumors [6–8]. More importantly, these primary tumors' genetic portfolios resembled those of their metastatic counterparts more than they did those of other primary tumors or nonmetastasizing primary tumors.

It is ironic that metastatic signatures already exist in primary tumors. If a late event like metastasis had already occurred early during carcinogenesis, it would be incompatible with (and actually contradict) the model of multistep carcinogenesis. Perhaps metastasis is the wrong word to use to denote a wrong concept. Indeed, this may be the reason a putative metastatic profile has yet to be found or does not even exist. I submit that this discovery of metastatic signatures in primary tumors is yet another important move toward a major paradigm shift in cancer biology. The results support the concept of a biologic process that is altogether different from that prescribed by the model of multistep carcinogenesis. We are increasingly learning that the diverse genotypes and phenotypes of various malignancies depend on their stem cell of origin. Because various primary tumors are distinct entities with different origins, not all of them will have a so-called metastatic signature or the same metastatic signature.

Wang et al. [9] added another twist to the story when they reported that patterns of gene expression in invading or metastatic cells are transient rather than permanent. Although stable gene expression indicates an overall blueprint for invasion or metastasis, it is the transient gene expression that actually dictates the cell's immediate actions and functions.

In light of these findings, a more dynamic and interactive picture of cancer progression begins to emerge: Genetic expression and cell–niche interaction proceed spatiotemporally and affect one another reciprocally. The notion that a dynamic interplay between the tumor and the cancer niche (at both the primary and metastatic sites) plays a critical role during carcinogenesis is central to the theory of a stem-cell origin of cancers. The idea that carcinogenesis advances in waves, in layers, and in web-like networks represents a major paradigm shift. It is in stark contrast to the multistep model of carcinogenesis, which views cancer progression as a strictly linear sequence of accumulated genetic changes.

Cancer Niche

Cancer is a multicellular phenomenon: It does not occur in unicellular organisms like amebae or bacteria. In a multicellular organism, all cells are dependent on and affected by other cells. Therefore, we should not be surprised that stem-cell identity and function are greatly influenced by unique neighboring cells and the microenvironment. I anticipate that malignant cells follow the same rules and regulations as the aberrant stem cells they arise from. In other words, malignant progression parallels stem-cell function in many respects. According to the theory of a stem-cell origin of cancers, different cancer cells require certain cellular partners and specific niche conditions for their ultimate phenotypic expression, just as their stem-cell counterparts do.

The niche is an integral component of the stem-cell theory of cancer and key to answering many laboratory puzzles. One outstanding cancer enigma is that even though cancer cells are clearly demonstrated to be malignant in vitro (e.g., on the basis of clonal assays, loss of affinity), a very large number (10^5–10^7) of these cells are still required to enable "tumor take" in vivo. Although they ostensibly have stem-cell properties (e.g., immortality, multipotentiality) and malignant characteristics (e.g., invasiveness, tumorigenicity), most cancer cells do not become established very readily or easily in a natural setting or even in a laboratory setup. It is interesting that tumor take is enhanced when we use cancer stem cells (when only a few or even one cell should suffice) and especially when we place them in the right microenvironment. Without the right niche, cancer is not as automatic and autonomous as one would expect.

The theory of a stem-cell origin of cancers is likely to instigate many fundamental paradigm shifts in our current understanding of cancer. For one, our obsession with cancer genetics will shift to stem-cell targets. When we consider cancer in a cell-centric rather than a gene-centric light, we begin to appreciate

why the microenvironment around cells is of paramount importance. When we realize how a malignant cell may be influenced by its neighboring cells and its immediate niche, we begin to understand why orthotopic injection of tumor cells in experimental animals yields better tumor take than ectopic injection does. Tumor take is further enhanced when the tumor cells are co-injected with Matrigel, integrins, or adhesion molecules and when they are made to overexpress certain angiogenic factors. To better explain why certain malignant cells contain many oncogenetic changes but are not necessarily malignant and why they may ostensibly be transformed but are not tumorigenic is to introduce a major paradigm shift.

Conclusion

I believe that the stem-cell theory of cancer is concrete, resilient, and versatile. It will continue to evolve, becoming more refined as we learn more about stem cells and cancer cells. Although the theory of a stem-cell origin of cancers probably explains or predicts many aspects of carcinogenesis better than any other theories do, it is not a one-size-fits-all hypothesis. There are always exceptions to the rules. In a biologic system, it will be hard to find an idea that is 100% correct by any means.

I also believe that the theory of a stem-cell origin of cancers is destined to bring about many fundamental paradigm shifts about cancer. In the end, its place in the annals of oncology depends on whether its concepts can better accommodate the mountains of data, better explain the seemingly inexplicable biologic or clinical observations, better direct our future research strategies and therapeutic endeavors, and most importantly, facilitate or expedite more breakthrough treatments of various kinds for diverse cancers than alternative theories can. Its validation will come when it replaces our current theories about carcinogenesis and when it stands alone or beside other accepted or acceptable theories about the origin of cancers.

References

1. Real C, Glavieux-Pardanaud C, Le Douarin NM et al (2006) Clonally cultured differentiated pigment cells can dedifferentiate and generate multipotent progenitors with self-renewing potential. Dev Biol 300:656–669
2. Fearon ER, Vogelstein B (1990) A genetic model for colorectal tumorigenesis. Cell 61:759–767
3. Zauber P, Sabbath-Solitare M, Marotta SP et al (2003) Molecular changes in the Ki-ras and APC genes in primary colorectal carcinoma and synchronous metastases compared with the findings in accompanying adenomas. Mol Pathol 56:137–140
4. Shih I-M, Wang T-L, Traverso G et al (2001) Top-down morphogenesis of colorectal tumors. Proc Natl Acad Sci USA 98:2640–2645

5. Preston SL, Wong W-M, Chan AO-O et al (2003) Bottom-up histogenesis of colorectal adenomas: origin in the monocryptal adenoma and initial expansion by crypt fission. Cancer Res 63:3819–3825
6. D'Arrigo A, Belluco C, Ambrosi A et al (2005) Metastatic transcriptional pattern revealed by gene expression profiling in primary colorectal carcinoma. Int J Cancer 115:256–262
7. Ramaswamy S, Ross KN, Lander ES et al (2003) A molecular signature of metastasis in primary solid tumors. Nat Genet 33:49–54
8. Weigelt B, Glas AM, Wessels LF et al (2003) Gene expression profiles of primary breast tumors maintained in distant metastases. Proc Natl Acad Sci USA 100:15901–15905
9. Wang W, Goswami S, Sahai E et al (2005) Tumor cells caught in the act of invading: their strategy for enhanced cell motility. Trends Cell Biol 15:138–145

Chapter 17
Experimental Proof

"Holy Grail" was obtained from Google Images (www.squidoo.com/Holy-Grail)

Not everything that counts can be counted and not everything that can be counted counts.

– Albert Einstein

Précis

The results of an experiment should be used to *validate or refute a hypothesis, not to generate one*.

Introduction

When we consider what experiments need to be performed to prove the origin of cancer, many come quickly to mind. It is somewhat surprising that several pivotal experiments whose results support, confirm, or even prove the theory of a stem-cell origin of cancers have already been done. Often enough, we have been left in the dark about the real meaning or potential implications of these experiments because we do not have the benefit of a correct hypothesis. But the time has finally arrived for us to reframe the questions being asked and revisit the experiments being performed.

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Whether we like it or not, we are at the mercy of our peers, who judge our performance, and of our culture, which influences our perspectives. It takes courage to challenge our venerable traditions. In this chapter, I propose a new direction in the conduct of cancer research that is in accord with the theory of a stem-cell origin of cancers, and I discuss the experiments that have already been done or could be performed to prove the stem-cell theory of cancer.

The Culture of Cancer Research

Many people may wonder whether all the time and money we have so far committed to cancer research has been well spent. In the final analysis, are our accomplishments in cancer research commensurate with our gains in clinical benefit? For example, the death rate for cancer, adjusted for the size and age of the population, decreased by only 5% from 1950 to 2005. In contrast, the death rate for heart disease declined by 64% and, for flu and pneumonia, by 58% during that same period [1]. Is it possible that the existing avenues of cancer research may be somewhat misguided and the benefits somehow misconstrued? Perhaps the problem with translating good science into good medicine runs deeper and wider than anyone is willing to admit. The impediment to our reaping the fruits of cancer research may lie in our cultural attitude toward certain basic scientific principles.

Too often, we have assumed that laboratory results using cell lines and animal models have clinical implications. The presumed direction of translational research is from the bench to the clinics. But the importance of scientific discoveries needs to be reckoned with in the context and within the confines of the experiments being designed and performed. We should not subscribe to a culture that takes any experimental data at face value and thinks that the results are generally valid for the treatment of real cancer in real people. If we do, we are destined to suffer more disappointments and failures in our endeavor to treat cancer.

Although the use of cell lines is invaluable for elucidating the basic mechanisms of cancer, all studies that use them have some serious, insurmountable inherent limitations. The reason we use cell lines is straightforward: Cells are relatively inexpensive to keep and easy to expand within a short time. But we know only too well that many cell lines are derived from the most unusual if not extreme cases. For example, one of the most popular prostate cancer cell lines, PC-3, is derived from a tumor that caused osteolytic metastasis, which occurs in only a minority of cases. Similarly, the DU145 line originated from a metastatic tumor in the brain, which almost never occurs in conjunction with a typical prostate cancer. And LNCaP cells were obtained from a tumor in the lymph node; it will not normally form tumors, let alone metastasize, in a laboratory animal. We thus need to remember that these cell lines represent merely the fringes and not the centerpiece of cancer. They may not represent the cancers we see or treat in the clinics at all.

When we put scientific research in its rightful place, however, then this whole affair with the culture of cancer is not all that bad. There will always be special roles for both cell lines and animal models in our elucidation of the basic mechanisms of

cancer. But it is imperative for us to obtain more diverse and relevant human cell lines and collect more human tumor specimens. When it comes to finding a shortcut to understanding human cancer, we probably should move from the clinics to the bench rather than from the bench to the clinics. It makes more sense to ask the cancer questions in the clinics and test them in the laboratory than it does to ask them in the laboratory and test them in the clinics. A lasting legacy of the theory of a stem-cell origin of cancers would be the design of more pertinent laboratory experiments and the making of more insightful interpretations of the results from these experiments. I believe that adoption of this new culture will promote a revolution in cancer research and facilitate more breakthroughs in cancer therapy.

Fundamental Flaws

Perhaps there is a dark side to cancer research. However, it is important to emphasize that this perceived shortcoming of cancer research should not incriminate cancer research itself. Instead, it points out a fundamental flaw about cancer research: We pay far too much attention to the details and beauty of singular experiments but forget to adhere to the first and foremost principle of the scientific method, which is to interpret the results within the narrow scope and limited context of the experiments. Otherwise, we become myopic in our culture of cancer research. We cultivate and admire the individual trees but neglect the forest.

One shortcoming of cancer research that is often unacknowledged is that most if not all of the experimental models that recapitulate tumorigenesis do not produce metastases. Despite intensive research by many laboratories, no genetic mutations that reliably produce metastases have been identified so far. Animal models that carry various mutations do not usually develop spontaneous metastases, and the cell lines that have been selected in vivo specifically for their ability to metastasize have molecular signatures that do not differ much from those of the original cell lines. Also, there is no evidence for enhanced metastasis from solid tumors in immunodeficient animals or humans. Thus, something seems amiss about the true nature of a cancer in these experimental models when they do not reproduce metastasis at all. But one cannot deny the fact that we are just as dependent as ever on these experimental models and still vouch for them: They exert a tremendous influence on our current thinking about and understanding of cancer.

Experimental Proof Already Extant?

Sometimes when we know what we are searching for, there is a better than good chance we will find it, even if it is a needle in a haystack. However, when we do not know what we are looking for, we will not see a lath even if it is right under our nose. The stem-cell theory will tell us, I believe, exactly what we are searching for when it concerns the design of an experiment to prove both the existence and essence of a stem-cell origin of cancers.

If we know what we are looking for, evidence supporting a stem-cell theory of cancer is, quite surprisingly, prevalent and pervasive. However, such evidence is as good as none at all without the correct hypothesis, the right mind-set, and a tolerant culture. One may argue that experiments to prove the theory of a stem-cell origin of cancers have already been performed many times, except that we have not yet correctly interpreted the results in the proper light or right context. Here, I highlight a few cases of such experimental proof, several of which have also been mentioned elsewhere in the book.

Mesenchymal Origin

Once upon a time, Virchow hypothesized that cancer has a mesenchymal origin (Chap. 3). Today we are performing experiments that come close to validating his prescient ideas: Malignant cells, like stem cells and embryonic cells, express mesenchymal genes and undergo epithelial-to-mesenchymal transition (EMT).

In many ways, a mesenchymal origin is equivalent to a stem-cell origin. For instance, stem cells express transcription factors that induce the activation of mesenchymal genes. Embryonic cells express the same transcriptional factors that elaborate EMT. Conversely, the induction of EMT generates cells with properties of stem cells; they express mesenchymal markers such as N-cadherin, FoxC2, twist, and snail [2]. Recently, Yang and Weinberg [3] showed that malignant cells express the same mesenchymal genes and EMT program that mediate invasion, intravasation, transport, and extravasation during metastasis. Chang et al. [4] reinforced those results by reporting that cancer stem cells in a subgroup of breast cancers had gene signatures reminiscent of those in mesenchymal cells. These study results confirm if not prove that a multitude of stem-cell or embryonic genes, transcription factors, and pathways are at the root of many if not all malignant transformations and are seemingly resurrected during cancer progression. Results from these experiments validate the idea that malignant cells have a stem-cell or embryonic root. Malignant stem cells possess the potential to express a hybrid mesenchymal–epithelial phenotype as well as a differentiated epithelial phenotype (Fig. 17.1) and recapitulate the embryonic processes of EMT.

Transplantation Studies

What better experiments could be devised or performed to prove the theory of a stem-cell origin of cancers than those that have already been done and shown the direct transformation of stem cells into malignant cells?

Studies showing that stem cells and malignant cells were interchangeable already hinted at a close link between the two cell types (Chap. 8). A normal stem cell derived from the genital ridge [5] or an embryo [6] could be experimentally induced to form embryonal carcinoma, whereas embryonal carcinoma cells implanted into the inner cell mass of a mouse blastocyst would be incorporated into

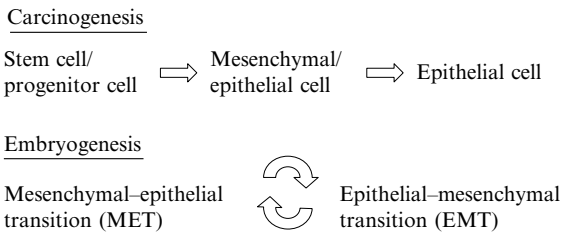

Fig. 17.1 Malignant cells have a stem-cell or embryonic root. Malignant stem cells possess the potential to express a hybrid mesenchymal and epithelial phenotype as well as a differentiated epithelial phenotype (*upper panel*) recapitulating the embryonic processes of EMT and MET (*lower panel*)

a normal developing mouse [7]. More tellingly, when human embryonic cells engineered to produce dopamine were used for the treatment of parkinsonian rats [8] or to produce insulin for the treatment of diabetic mice [9], an alarming incidence of tumors developed in the animals from these embryonic cells (Chap. 4). As predicted by the stem-cell theory, these experimental results also revealed that the more-differentiated stem cells tended to develop into a less-malignant phenotype. This same scenario, amazingly, appeared to have played out in real life when a patient with Parkinson disease who had previously received fetal allografts (gestational ages 5–6 weeks and 15–16 weeks) subsequently developed proliferation of non-neuronal tissue that obstructed his cerebral ventricles 23 months later [10]. More recently, a boy with ataxia telangiectasia who had received intracerebellar and intrathecal injections of human fetal neural stem cells developed a multifocal brain tumor of nonhost origin 4 years later [11].

Stunning experiments by Houghton et al. [12] demonstrated that bone marrow–derived cells could repopulate the stomach of C57BL/6 mice and form gastric carcinoma (Chap. 6). Oddly enough, the same experiments might also have been performed in humans: Reports of donor-cell leukemia and the development of leukemia after gene therapy using stem cells offer living proof of the theory of a stem-cell origin of cancers (Chap. 6). Similarly, stem cells derived from a grafted kidney migrated to the skin and transformed into a malignant tumor [13]. More recently, Barsky et al. [14] reported that 12% of solid cancers arising in non–sex matched transplant recipients were of donor origin.

One could hardly have devised better experiments to prove that aberrant or defective stem cells indeed directly caused the malignant tumors.

Heterogeneity Studies

Other experiments proving the theory of a stem-cell origin of cancers relate to cancer heterogeneity: The phenotypic complexity of a cancer may be related to its cell of origin in a stem-cell hierarchy. The moment of truth may have occurred with the experiments performed by Cheng et al. [15], who demonstrated that *hox* genes play

an essential role in determining the heterogeneity of ovarian cancer (Chap. 12). Similarly, Ince et al. [16] showed that the cell of origin dictates the final malignant phenotype despite the presence of the same genetic aberrations; tumor heterogeneity is decided more by its cellular origins than by its genetic makeup. In other words, the multipotentiality of stem cells is manifested as the heterogeneity of cancer cells.

Again, it is shocking that many experiments have already been done, and the results are staring right at us. Without the right hypothesis, we had no inkling what the data really meant in the real world and in the big picture of cancer. And until now, we had no idea how to fit those data into our current understanding of and prevailing views about cancer. Therefore, it is possible that many malignant phenotypes may be a "birthmark" *ingrained* in a cancer rather than a benchmark *acquired* by the cancer. I believe that these experimental results are additional evidence supporting, confirming, and even proving the theory of a stem-cell origin of cancers.

Metastasis Studies

I dare say that some of the best evidence supporting the theory of a stem-cell origin of cancers has come from the results of experiments showing that a molecular profile of metastasis is already present in certain primary tumors (Chap. 13). As expected, this metastatic signature is not present in all primary tumors, suggesting that the various primary tumors are indeed distinct entities with different cellular origins. In fact, the genetic portfolios of these primary tumors resemble those of their metastatic counterparts more than they do those of other primary tumors [17–19]. The results from these experiments expose a glaring flaw in our multistep model of carcinogenesis. They refute the conventional view that primary tumors start with the same outfit but become different when they acquire more genetic mutations. They repudiate the popular doctrine that a primary tumor undergoes metamorphosis sequentially and incrementally to become a metastatic tumor by acquiring a series of metastatic genes.

I thus argue that innumerable experiments that have already been done support, confirm, and even prove the theory of a stem-cell origin of cancers: Many metastatic phenotypes are not acquired by cancer over time but may instead be intrinsic.

Experimental Proof Still to Be Found!

A major stumbling block to performing cutting-edge research in support of the theory of a stem-cell origin of cancers is the identification and procurement of relevant stem cells. Currently, we do not even know the whole spectrum of stem

cells, let alone how to obtain them for research. How do we design or perform an experiment before its time?

Ultimately, definitive proof of the validity of a stem-cell theory of cancer will depend on finding evidence that stem cells are not created equal and on demonstrating that diverse tumors are derived from a hierarchy of stem cells in solid organs as well as in the hematopoietic system. A cellular "portfolio" or molecular signature that can be used to identify the individual stem cells needs to be established. Only then will we be able to show that aberrations in a particular stem cell lead to the formation of a unique malignant phenotype.

I propose that some experiments could be performed to definitely validate the theory of a stem-cell origin of cancers. Otherwise, we will be adding to the common sentiment that a theory without experimental proof is worthless.

Circulating Tumor Cells

I anticipate that studying circulating tumor cells will pave the way to proving the theory of a stem-cell origin of cancers. Recently, there has been substantial advancement in the quantification of circulating tumor cells. Unlike tumor cells in the primary organ or at metastatic sites, circulating tumor cells are a potentially homogeneous population of cancer stem cells that are already separated to a large extent from nonmalignant cells. This is rather like an experiment of nature in which cancer stem cells from a primary tumor are filtered and purified before they mingle again in a metastatic tumor. After all, it is the cancer stem cells that are extricated from a primary tumor and infiltrate the circulatory system. They are also equipped to endure the rigors of traveling long distances and surviving in hostile environments. The task of isolating cancer stem cells in a primary organ and at the metastatic sites is immensely more difficult than isolating those of the circulatory system because the cancer stem cells are more likely to be interspersed among differentiated cancer cells and neighboring stromal cells.

It is conceivable that cancer stem cells can differentiate in the circulation just as they do in the primary and metastatic sites. However, differentiated cancer cells are more likely to perish under duress in the circulatory system because they are generally more vulnerable. I speculate that the number and type of circulating cancer stem cells differ in different cancers according to their stem cell of origin. Hence, it is not surprising that the number of circulating tumor cells at any time during the course of disease allows the assessment of patient prognosis and is predictive of progression-free and overall survival times [20–22]. I surmise that the genetic profile of circulating tumor cells reveals their respective stem cell of origin in a stem-cell hierarchy, i.e., reflecting an intrinsic property of the tumor rather than being a simple measure of disease burden [23]. I anticipate that the molecular signature of circulating tumor cells differs in patients with clinically aggressive cancers from that in patients with indolent cancers.

Therefore, studying circulating tumor cells may enable us to elucidate the nature of unique cancer stem cells and confirm if not prove the theory of a stem-cell origin of cancers.

Hox Genes

One way to create a unique stem cell is to manipulate distinctive stem-cell genes in the same progenitor cell. An important caveat about these stem-cell genes is that they impart a unique property to the progenitor cells and produce distinctive stem cells in the spirit of a stem-cell hierarchy. The family of *hox* genes is one example of such stem-cell genes.

I propose conducting an experiment to reproduce a series of stem cells in a prostate stem-cell hierarchy by inserting specific *hox* genes into a prostate progenitor cell. It would be very interesting to show that certain *hox* genes alone can engender diverse prostate cancer phenotypes, such as small-cell carcinoma, neuroendocrine carcinoma, ductal adenocarcinoma, and acinar carcinoma, in a particular aberrant prostate progenitor cell. I hypothesize that the presence of a certain *hox* gene confers on the cell a particular stem-cell identity that trumps other genetic mutations that may happen to be present in the same aberrant progenitor cell. The results of that experiment would potentially refute the long-standing oncologic dogma that a particular genetic mutation determines a specific malignant phenotype. More importantly, they may also prove a fundamental principle of the stem-cell theory, that the same genetic mutation occurring in different stem-cell types can lead to divergent malignant phenotypes.

The Study of Stem Cells Helps the Study of Cancer

The idea that a malignant cell resembles its respective stem cell suggests that certain stem-cell markers will be useful for diagnosing and prognosticating cancer (Chap. 10). It alludes to the possibility that studying stem cells may produce substantial dividends for the study of cancer cells. For example, certain stem-cell markers may distinguish specific stem cells in a stem-cell hierarchy as well as the unique tumor phenotypes that emanate from these cells.

Already, more and more stem-cell targets (e.g., transcription factors: Oct3/4; asymmetric division factors: PINS; self-renewal factors: wnt; morphogenesis factors: various tyrosine kinases; and differentiation inhibitors: myoblastin) are being found to have possibly pertinent or causal roles in carcinogenesis. I predict that certain stem-cell targets will be useful in distinguishing distinct groups of cancer and improving our current diagnosis and prognostication of cancer. I also anticipate that these stem-cell markers will be useful in characterizing and categorizing dif-

ferent tumor types because they portray their unique stem cell of origin within a stem-cell hierarchy. Even more importantly, and for the same reason, we may be able to purify, maintain, and study the different types of the so-called cancer stem cells. Finally, I forecast that aberrant stem-cell targets involving stemness transcription factors or asymmetric division tend to affect early stem cells, those involving morphogenesis or inhibitors of differentiation tend to affect stem cells somewhere in the middle of a stem-cell hierarchy, and those involving self-renewal tend to affect late progenitor stem cells.

The Study of Cancer Helps the Study of Stem Cells

The other side of the same coin suggests that investigation of certain malignant features will be invaluable in characterizing the elusive, enigmatic stem cells. An intriguing idea is that the study of diverse cancers on the basis of their clinical features may help us isolate and identify previously unknown stem cells. After all, the cancer cell is derived from the stem cell. With the right theory, the cancer cell may also lead us to discover the true identity of its respective stem cell. For example, an aggressive tumor with a widely metastatic phenotype will likely exhibit early stem-cell markers, whereas an indolent tumor with a limited and predictable metastatic pattern will tend to express late stem-cell targets.

Assuming that we have at last nailed the right hypothesis, the idea of an onco-niche's resembling a corresponding stem-cell niche leads to a simple but stunning implication: A particular metastatic milieu, with its unique stromal cell types and soluble factors, may provide us with the optimal medium for culturing and maintaining specific stem cells. Along with the use of selective stem-cell markers to identify specific stem cells in the stem-cell hierarchy, these special stem-cell "recipes" made from a menu of metastatic "soups" would greatly advance stem-cell research into the identification, purification, and maintenance of stem cells. I predict that early stem-cell cultures would require a more complex recipe containing various cytokines, growth factors, and cellular components, whereas late stem-cell cultures would need fewer cytokines and growth factors to keep the stem cells from differentiation.

The Ultimate Experiment

I believe that it is only a matter of time before the ultimate experiment validating the stem-cell theory of cancer is performed. Exactly when this momentous event will occur depends on when the necessary stem cells are identified and can be procured for the experiment. The results of this ultimate experiment ought to provide

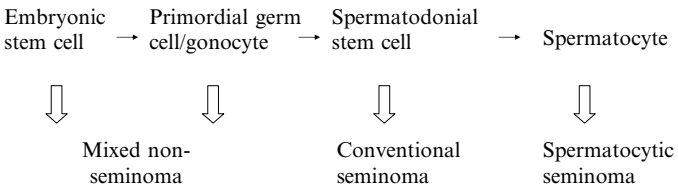

Fig. 17.2 Mixed nonseminomatous germ-cell tumor, pure seminoma, and spermatocytic seminoma are, respectively, derived from a primordial germ-cell/gonocyte, a spermatogonial stem cell, and a spermatogonia/spermatocyte

indisputable proof of cancer's origins: Specific cancer types arise from specific stem cells in a stem-cell hierarchy.

Perhaps our best hope for performing this ultimate experiment involves germ cells. After all, germ cells are the prototypical stem cells, the forebearers of all stem cells. Germ cells may be easier than any other putative stem cells to obtain for studying. I suspect that it is not by chance that germ-cell tumors are considered the paradigm of a curable cancer. Just as important is that germ-cell tumors may also offer us the best opportunity to prove the theory of a stem-cell origin of cancers.

Already, some of the most convincing experiments supporting the theory of a stem-cell origin of cancers have been performed on germ-cell tumors (testicular cancers, teratomas) (Chap. 7). According to our stem-cell theory of cancer, any germ cell that contains some degree of stemness has the potential to develop into a germ-cell tumor. I speculate that the degree of stemness in a particular germ cell, rather than the type of mutation within it, determines the type of germ-cell tumor that eventually forms. Hence, I predict that when the same mutation occurs in a primordial germ cell/gonocyte, a spermatogonial stem cell, or a spermatogonia/spermatocyte, then a mixed nonseminomatous germ cell tumor, a pure seminoma, and a spermatocytic seminoma forms, respectively (Fig. 17.2). Accordingly, epigenetic and microarray studies should demonstrate that the genetic signature or expression profile of a mixed nonseminomatous tumor, for example, resembles that of the primordial germ cell/gonocyte, whereas the same studies performed on a seminoma ought to parallel those of the spermatogonial stem cell.

A Modest Proposal

When all is said and done, the same questions remain. Why is it that many experimental treatments that seem to work amazingly well in a laboratory setting often fail to deliver in a clinical setting? How do we rectify these failures and prevent more disappointments? Here are some caveats for a modest proposal.

First, we need to start with the right hypothesis about the origin of cancer. If we start with a wrong hypothesis, then we are more likely to make the wrong assumptions, be prone to make faulty objectives and definitions, and design irrelevant experiments, ending up with bogus interpretations and conclusions.

Second, we need to know the value and limits of our experimental methods. By necessity and design, the best experiments must set conditions and limit variables. Therefore, the results of these experiments should be interpreted only within the context and confines of these restrictions. In other words, we should be conducting hypothesis-testing – not hypothesis-generating – research.

Third, we need to face the problems inherent with the use of cell lines and animal models that may represent outliers rather than the core of disease. One way to mitigate this deficiency is to both obtain and use as many pertinent human cell lines and tissue samples as possible and not omit negative data.

Fourth, we need to address the question of clinical relevance. It is important that the laboratory results are confirmed in animal models and clinical samples. I propose that it will be more fruitful and rewarding to translate research from the clinic to the bench and then back to the clinic than directly from the bench to the clinic.

Conclusion

Let us review some salient points and take-home messages about experimental proof for the theory of a stem-cell origin of cancers.

1. Adopting the correct hypothesis about the origin of cancer is imperative.
2. Full cognizance of an experiment's potential and limitations is necessary.
3. Many experiments have already provided support, confirmation, and even proof of the theory of a stem-cell origin of cancers, and new ones can be performed to validate it.

A bad hypothesis has serious consequences. The whole scientific method begins to tumble down from the top. We cannot underestimate the many ramifications and implications of a bad hypothesis. A bad hypothesis and the experiments designed to test it will continue to misinform us about the observation in question. We will continue to pose the wrong questions and design the wrong experiments. Solving cancer's origins and finding cancer's cures cannot be a more frustrating and self-defeating task when we have to work with a bad hypothesis.

The power of a hypothesis rests in its ability to explain the most common phenomena as well as the most intriguing observations. Although its strength can be determined by a paucity of exceptions, its impact may also be measured by its ability to advance an entire scientific field or discipline. Ultimately, whether a hypothesis stands or falls depends on the experiments designed to test it. Although hypothesis speaks volumes, experiment is still king. Thus, experimental proof is still considered the Holy Grail of scientific research.

References

1. Kolata G (2009) Forty years' war. Advances elusive in the drive to cure cancer. New York Times. April 24, 2009: Health
2. Mani SA, Guo W, Liao M-J et al (2008) The epithelial-mesenchymal transition generates cells with properties of stem cells. Cell 133:704–715
3. Yang J, Weinberg RA (2008) Epithelial-mesenchymal transition: at the crossroads of development and tumor metastasis. Dev Cell 14:818–829
4. Chang JC, Creighton C, Landis M et al (2008) Gene signature of cancer stem cells in an intrinsic subgroup of breast cancers with mesenchymal properties [abstr]. J Clin Oncol 26(May 20 suppl). Abstract 11009
5. Stevens LC (1964) Experimental production of testicular teratomas in mice. Proc Natl Acad Sci USA 52:654–661
6. Damjanov I, Solter D (1974) Embryo-derived teratocarcinomas elicit splenomegaly in syngeneic host. Nature 249:569–571
7. Mintz B, Illmensee K (1975) Normal genetically mosaic mice produced from malignant teratocarcinoma cells. Proc Natl Acad Sci USA 72:3585–3589
8. Roy NS, Cleren C, Singh SK et al (2006) Functional engraftment of human ES cell-derived dopaminergic neurons enriched by coculture with telomerase-immortalized midbrain astrocytes. Nat Med 12:1259–1268, Erratum in Nat Med 2007;13(3):385
9. Kroon E, Martinson LA, Kadoya K et al (2008) Pancreatic endoderm derived from human embryonic stem cells generates glucose-responsive insulin-secreting cells *in vivo*. Nat Biotechnol 26:443–452
10. Folkerth RD, Durso R (1996) Survival and proliferation of nonneural tissues, with obstruction of cerebral ventricles, in a parkinsonian patient treated with fetal allografts. Neurology 46:1219–1225
11. Amariglio N, Hirshberg A, Scheithauer BW et al (2009) Donor-derived brain tumor following neural stem cell transplantation in an ataxia telangiectasia patient. PLoS Med 6:e1000029
12. Houghton J, Stoicov C, Nomura S et al (2004) Gastric cancer originating from bone marrow-derived cells. Science 306:1568–1571
13. Aractingi S, Kanitakis J, Euvrard S et al (2005) Skin carcinoma arising from donor cells in a kidney transplant recipient. Cancer Res 65:1755–1760
14. Barsky SH, Ye Y, Xiao Y et al (2008) Insights into the stem cell origin of human cancers by studying a registry of bone marrow and other organ transplant recipients who later developed solid tumors [abstr]. J Clin Oncol 26(May 20 suppl). Abstract 110010
15. Cheng W, Liu J, Yoshida H et al (2005) Lineage infidelity of epithelial ovarian cancers is controlled by *HOX* genes that specify regional identity in the reproductive tract. Nat Med 11:531–537
16. Ince TA, Richardson AL, Bell GW et al (2007) Transformation of different human breast epithelial cell types leads to distinct tumor phenotypes. Cancer Cell 12:160–170
17. Perou CM, Sørlie T, Eisen MB et al (2000) Molecular portraits of human breast tumours. Nature 406:747–752
18. van de Vijver MJ, He YD, van't Veer LJ et al (2002) A gene-expression signature as a predictor of survival in breast cancer. N Engl J Med 347:1999–2009
19. Ye Q-H, Qin L-X, Forgues M et al (2003) Predicting hepatitis B virus-positive metastatic hepatocellular carcinomas using gene expression profiling and supervised machine learning. Nat Med 9:416–423
20. Cohen SJ, Punt CJ, Iannotti N et al (2008) Relationship of circulating tumor cells to tumor response, progression-free survival, and overall survival in patients with metastatic colorectal carcinoma. J Clin Oncol 26:3213–3221, Erratum in J Clin Oncol 2009;27(11):1923
21. Cristofanilli M, Budd GT, Ellis MJ et al (2004) Circulating tumor cells, disease progression, and survival in metastatic breast cancer. N Engl J Med 351:781–791

22. de Bono JS, Scher HI, Montgomery RB et al (2008) Circulating tumor cells predict survival benefit from treatment in metastatic castration-resistant prostate cancer. Clin Cancer Res 14:6302–6309, Erratum in Clin Cancer Res 2009;15(4):1506
23. Danila DC, Heller G, Gignac GA et al (2007) Circulating tumor cell number and prognosis in progressive castration-resistant prostate cancer. Clin Cancer Res 13:7053–7058

Chapter 18
Clinical Implications

"Thinking Outside Pandora's Box" is reprinted with permission from Tim Parish, Melbourne, Australia

Cassandra cried and cursed the unhappy hour,
Foretold our fate, but, by the god's decree,
All heard, and no one believed the prophecy.

– Aeneid 2.323, Dryden translation

Précis

Not knowing the relationship between stem cells and cancer cells, we may unwittingly expose ourselves to some lurking danger.

Introduction

There is hope that the stem-cell theory of cancer will inspire fresh ideas and new mind-sets. It will invite more debate and experiments to challenge the traditional viewpoints. Suddenly, the unconventional becomes the established, the unthinkable becomes the accepted, and vice versa. The time has finally arrived for us to formulate a new equation for solving the problems of cancer and to create a new recipe

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for improving the treatments for cancer. By the end of this chapter, one will wonder whether we have been on the wrong track the whole time about the origin of cancer and its clinical implications.

Response vs. Survival

It is well known that clinical response does not always translate to a survival advantage. Patients may experience symptomatic improvement with response to therapy but not necessarily a survival benefit with the same treatment. Almost invariably, the cancer returns again and again, until the patients no longer benefit from or can tolerate further treatments. Conventional wisdom tells us that a series of genetic mutations within cancer cells cause damage beyond repair that ultimately leads to the patients' downfall. A popular notion is that additional treatments select cancer cells that have greater drug resistance, eventually spelling the patients' demise.

This discrepancy between response and survival can be explained, however, by the presence of cancer stem cells within a tumor (Chap. 7). Treatments that eliminate differentiated cancer cells provide objective responses and symptomatic relief, but they may not affect the cancer stem cells that give rise to those differentiated cancer cells. Ultimately, it is the cancer stem cells rather than the differentiated cancer cells that drive the malignancy and determine a patient's overall survival time. For this reason, our current ways of monitoring therapeutic responses (e.g., by using the Response Evaluation Criteria in Solid Tumors (RECIST) [1]) may be grossly inadequate if not even inappropriate. Similarly, our current norms of measuring serum prostate-specific antigen (PSA) levels for prostate cancer, serum monoclonal immunoglobulin levels and the percentage of plasma cells in the bone marrow for multiple myeloma, and the BCR-ABL transcripts for chronic myelogenous leukemia (CML) consider the effects of treatment on only differentiated cancer cells and not on cancer stem cells. It is imperative that we find surrogate end points to measure the effects of treatment on cancer stem cells instead of differentiated cancer cells.

An important clinical implication of the dichotomy between cancer stem cells and differentiated cancer cells is that treatments need to affect the cancer stem cells rather than the differentiated cancer cells to achieve durable remissions and prolonged survival time. The stem-cell theory of cancer reminds us that the kinetics of therapy on the two cell types can be quite different. Hence, treatments that affect differentiated cancer cells tend to produce immediate and obvious clinical results: Patients feel better, tumors are smaller, and biomarker levels become lower. However, treatment that affects cancer stem cells may provide delayed and subtle clinical outcomes, resulting in a lag in the detection of any perceptible prolonged survival time. Therefore, we need to be both aware and wary that promising treatments may be prematurely abandoned unless and until we develop therapeutic strategies that could account for the presence of cancer

stem cells and response criteria that could be used to measure the effects of therapy on them.

Do we have treatments that affect cancer stem cells? It is possible that some chemotherapy eliminates both cancer stem cells and differentiated cancer cells in most cases of acute myelogenous leukemia, testicular cancer, Hodgkin disease, and non-Hodgkin lymphoma. These treatments also alter the onco-niche in such a manner that any residual cancer stem cells return to dormancy or homeostasis. In fact, we may already have treatments that affect cancer stem cells [2]. For example, imatinib is effective for the *treatment* of CML because it eradicates the differentiated CML cells. But imatinib does not *cure* CML because it does not affect the leukemic stem cells. In contrast, interferon-alfa may cure a fraction of patients with CML by targeting the CML stem cells. Similarly, thalidomide is an effective treatment for multiple myeloma. But this agent provides only symptomatic relief and clinical response rather than a cure because it affects only the differentiated malignant plasma cells. On the other hand, rituximab may provide a survival advantage in a fraction of patients with multiple myeloma by targeting the relevant cancer stem cells (i.e., malignant memory B cells). In addition, hormonal ablation therapy is effective for the treatment of prostate cancer because it eliminates the bulk of PSA-producing differentiated cancer cells. But to find a cure for prostate cancer, we also need to discover treatments that control the prostate cancer stem cells.

Clinical Trials

Even with meticulous selection and stratification, chances are that the cancer we treat is actually an amalgam of cancers. To make sense of the immense heterogeneity and inevitable complexity of human populations and cancers, we resort to the magic of statistics in clinical trials. Statistical analysis is a wonderful tool for us to use in seeking order out of chaos. But by itself, statistics may be insufficient for use in probing deeper into the origin, causes, and implications of cancer. Although statistical analysis does not lie, it may mislead, especially when we are not completely honest about its real potential and possible limitations.

The clinical implications of the stem-cell theory of cancer become increasingly evident when we consider that clinical trials may not be necessary and even statistics may not be needed in personalized cancer care. Personalized medicine implies that we have solved the dilemma of heterogeneity in human populations and for all diseases. In principle, personalized cancer care is possible when we know the exact origin and nature of cancer; a specific deranged stem cell results in the formation of a particular malignant phenotype according to the theory of a stem-cell origin of cancers. Hence, if we know exactly what and whom we are treating, it would be improper and unfair to subject a patient to the vagaries of clinical trials. Indeed, when we know for sure what we need to do and what we are doing, then no clinical trial or statistical analysis

is necessary to demonstrate the benefits or futility of treatment. When something is obvious, statistical analysis is superfluous. Therefore, we need to be cognizant that a greater truth may be buried beneath the statistics of clinical trials.

The Problem with Randomization

We clinicians are quite enamored of randomized studies, which seem to provide their own infallible controls. Like a stroke of magic, the power of statistics negates the curses of heterogeneity, bias, and chance in a randomized study. Somehow, the results of a randomized study seem very believable, reliable, and even definitive. It seems to be the best way to clarify any possible cause and/or effect by a specific action or event in diverse cases. Often enough, the results of randomized studies determine the standard of care or establish a gold standard for the validity of a procedure or the efficacy of a treatment.

But something is amiss with randomized studies. They tell as much as they tell not. Although a randomized study is an important and necessary device for making some sense of what is completely unclear or unknown, it cannot help us elucidate the unclear or unknown. When it creates a sense of absolute knowledge and false complacency, a randomized study may even hinder our elucidation of the unclear or unknown. For example, if a drug is effective only for the treatment of cancer subtype A, not for subtypes B or C, its benefit would still be evident in a randomized study that accepts all subtypes for therapy. If subtype A comprises only a small fraction of the entire population of treated patients, then it is just a matter of enrolling more patients into the study for us to detect a statistically significant therapeutic advantage for all subtypes. The problem with randomized studies is that when a drug is found to be effective, it is approved for all subtypes, not just the right subtype. This is one reason why not all patients benefit from some of our most promising approved drugs: They are just a number in the statistical analysis of randomized studies.

Once we solve the origin and nature of various cancers, do we still need to conduct randomized studies? In principle, when we have the capability to identify and characterize the different subtypes of a particular cancer, we should also have the know-how to design and tailor appropriate treatments for each subtype, thus reaching the very essence of personalized medicine. Using testicular cancer as an example, if we know for a fact that the best clinical treatment for teratoma is surgery, for seminoma is radiotherapy, for embryonal carcinoma is chemotherapy, and for mixed embryonal carcinoma and teratoma is chemotherapy followed by surgery, then we will be hard pressed to find a patient with any of these tumor types who wants to be arbitrarily randomized to receive any of the other prescribed treatments. Randomized study is tenable and feasible only when we are still in the dark, when we are being compelled to operate in a naive mode. It is conceivable that one day in the foreseeable future, when we have improved ways to diagnose, prognosticate, and treat the different cancer subtypes, randomized studies will become obsolete and regarded as scientific relics.

Clinical Perspectives

Many clinical aspects of cancer need to be revisited in light of the stem-cell theory of cancer. Before long, we will be abandoning some of our most traditional clinical perspectives and accepting some of the most unconventional clinical implications of cancer.

Cancer Screening

A glaring obstacle to cancer screening is heterogeneity. We are faced with the daunting task of screening for a disease that is actually many diseases with different natural histories. It seems futile to screen for a cancer that develops suddenly, progresses inexorably, and cannot be treated effectively after diagnosis anyway. It also seems superfluous to screen for a cancer that pursues an indolent clinical course and whose diagnosis or even treatment may not be necessary. The ideal cancer worth screening for has a high incidence of occurrence, evolves over a long time, adversely affects the patient's quality of life or survival, and can be successfully treated after diagnosis.

Although cancer screening has been shown to be beneficial in the case of cervical and colorectal cancers, it has not worked in the case of lung cancer. Why is this so? Bach et al. [3] demonstrated that screening for lung cancer with a computed tomographic scan of the chest resulted in an increased number of lung cancers being diagnosed and surgeries being performed. Surprisingly, they did not detect any declines in the diagnosis of advanced lung cancer or in death from lung cancer.

The theory of a stem-cell origin of cancers accounts for this phenomenon of time bias by predicating that both indolent and virulent tumors have diverse stem-cell origins. It contradicts current cancer models that assume that indolent tumors evolve and become virulent tumors. Cancer screening is more effective for indolent tumors because they are present for a prolonged period and are thus more amenable to earlier diagnosis. However, earlier diagnosis and treatment of these tumors may exert only marginal or modest effects on their clinical course. In contrast, virulent tumors develop quickly, become lethal rapidly, and cannot be detected easily on screening. Unless they can be treated successfully, the diagnosis and treatment of these tumors are likely to provide only minimal or minor effects on the clinical course of disease.

Therefore, some tumors may not need to be screened for, and other tumors cannot be screened for, according to the stem-cell theory of cancer. Similarly, some tumors do not need to be cured, and others cannot be cured. Knowing this difference means that we can cure some cancers but only conquer others. Conquest of cancer implies that we know our capabilities as well as our limitations. It implies that we treat different cancers accordingly so that patients have the opportunity to experience the best clinical outcome with available therapeutic modalities. In essence, this is what personalized cancer care is really about. Surely,

cancer conquest is not as glamorous as cancer cure. But it is a more realistic and reasonable goal when we consider the theory of a stem-cell origin of cancers and understand its clinical implications.

Cancer Targets

Perhaps our technologic advances have raced far ahead of our theoretical stance. Cutting-edge technologies such as genomic sequencing, microarrays, and proteomics have produced far more data than we can possibly assimilate. But more knowledge does not necessarily mean more enlightenment. We need to improve our current theories, which may propel us into a higher intellectual and conceptual sphere. Otherwise, the burden of information may paradoxically hinder our reaching loftier heights. I believe that the theory of a stem-cell origin of cancers will empower us to elucidate basic cancer biology, design pertinent cancer experiments, understand available cancer information, and discover effective cancer therapies.

Although we have learned many valuable lessons, we are still at a loss to explain the biologic and clinical relevance of various cancer targets (Chap. 11). It is unclear whether many of these cancer targets are an impetus or an impediment in our quest to understand and treat cancer. Surely, we need to go beyond the genetic mutations, signaling pathways, and molecular signatures to fully grasp their clinical implications. The theory of a stem-cell origin of cancers offers us a unique opportunity to delve into the real meanings behind the cancer targets for the diagnosis, prognosis, and therapy of cancer.

The stem-cell theory of cancer predicts that many pertinent tumor antigens are also stem-cell antigens, and vice versa. In addition, many common tumor markers have embryonic origins. For example, various tyrosine kinases are important morphogens that are normally expressed during embryogenesis but reemerge and resurge during carcinogenesis. Other well-known stem-cell antigens strongly expressed in a variety of cancers include c-kit in serous ovarian carcinoma, testicular carcinoma, malignant melanoma, and small-cell lung carcinoma [4, 5] and CD34 in dermatofibrosarcoma, epithelioid sarcoma, and solitary fibrous tumors [6]. I anticipate that many as-yet-unknown tumor antigens will be found to play essential roles in the basic functions of a stem cell and may help us identify unknown stem-cell markers. Conversely, stem-cell markers will be invaluable in our discovery of germane tumor antigens.

Diagnosis and Prognosis

The theory of a stem-cell origin of cancers introduces a major paradigm shift concerning cancer heterogeneity. It suggests that cancer would be better classified according to its origin from a hierarchy of stem cells with unique stem-cell antigens.

This is a giant step forward from our traditional ways of diagnosing and prognosticating cancer using histologic criteria, immunohistochemical methods, and/or molecular markers. In many respects, this theory may drastically change the way we understand and think about cancer in the future.

Despite a decrease in the incidence of some cancers (e.g., prostate and cervical cancers), the overall mortality rate associated with these cancers has scarcely changed at all [7]. How do we reconcile the observation that we have improved screening, diagnosis, and treatment of these cancers with the fact that a fraction of them remain inherently lethal? I postulate that a proportion of all cancers originate from an earlier stem cell and remain relatively constant for a particular type of cancer. Our current diagnostic and prognostic capabilities cannot identify or distinguish such tumors very well. Furthermore, our treatments hardly affect their inexorable clinical course and do not improve the dismal outlook for patients who harbor such cancers.

An unexpected reward of stem-cell research is that certain stem-cell antigens may turn out to be invaluable diagnostic and prognostic markers. I propose that malignant cells derived from early stem cells contain early stem-cell antigens. They are the ones that cause the notorious cancers that pursue an aggressive and lethal clinical course. In contrast, malignant cells derived from late stem cells contain late stem-cell antigens; they cause the incidental cancers that follow a more benign clinical course and are more amenable to therapy. Therefore, I predict that early stem-cell markers can be used to diagnose and prognosticate the more virulent cancer subtypes and late stem-cell markers can be used to diagnose and prognosticate the more indolent subtypes. Thus, the clinical implications of the stem-cell theory of cancer cannot be denied or ignored; one day it may completely overhaul our current standards of diagnosing and prognosticating cancer.

Therapeutic Implications

The stem-cell theory of cancer has sweeping implications for how we define our mission and deploy our resources to treat cancer. Some examples follow.

Chemoprevention

The clinical implications of the stem-cell theory of cancer have no boundaries. Consider its relevance in chemoprevention. Currently, we believe that premalignant tumors and metastatic cancer have a common origin. Because cancer advances in a stepwise fashion from a premalignant lesion to a metastatic cancer, there are ample opportunities for us to intervene in some of those steps and arrest if not altogether prevent cancer progression.

But the clinical implications of chemoprevention would be vastly different if certain premalignant tumors were actually diseases that are different from meta-

static cancers, according to the theory of a stem-cell origin of cancers. If certain cancers are indeed different because of their distinct stem-cell origins, then effective treatment for one cancer might be completely inappropriate for another. Consequently, it is not surprising that several chemopreventive agents (e.g., retinoids, celecoxib, finasteride) proven to be beneficial for the treatment of certain premalignant lesions (e.g., oral leukoplakia, colonic polyps, and prostate tumors, respectively) [8–10] happen to be completely ineffective for the treatment of metastatic cancers (e.g., head/neck, colorectal, and prostate cancers, respectively). The official explanation for this observation is that advanced cancers acquire additional mutations that render them more recalcitrant to the same therapy. But how do we explain a corollary observation that many therapeutic regimens proven to be beneficial for the treatment of metastatic cancers happen to be ineffective for the chemoprevention of premalignant lesions? It is intriguing that cytotoxic drugs effective for the treatment of metastatic breast, prostate, and other cancers are in fact inactive and poor chemopreventive agents in premalignant tumors of the same cancers. I propose an alternative explanation for these observations, according to the stem-cell theory of cancer: Metastatic cancers do not necessarily arise from any premalignant tumors; some metastatic cancers are an altogether different disease with a different cellular origin from that of certain premalignant tumors (Chap. 16).

When it concerns chemoprevention, modulation of the onco-niche becomes even more relevant. But before we manipulate any onco-niche, we need to know when and how a normal stem-cell niche becomes an abnormal onco-niche. What cascades of events precipitate the development of an onco-niche when the normal stem-cell niche factors no longer exert a homeostatic effect on aberrant stem cells? I speculate that an onco-niche fosters rogue stem cells, awakening them from dormancy and releasing them from asymmetric division and promoting rampant self-renewal, invasion, and migration. Not surprisingly, there are increased inflammatory activity and abundant angiogenesis factors in an onco-niche. Perhaps chemoprevention works by mitigating the effects of inflammation, oxidation, angiogenesis, and/or hypoxia in an onco-niche. Thus, by decreasing the level of these factors in an onco-niche, we may be able to attenuate the malignant potential of certain cancers.

Surgery

An unspoken, perhaps forgotten enigma of cancer is its relative drug sensitivity. After all, when treated with cytotoxic agents, a tumor may regress while most normal tissues and organs are either left virtually intact or eventually heal. Only because a tumor rarely goes into complete remission and instead keeps recurring despite treatment do we consider that cancer is generally drug resistant. Does this dichotomy of drug sensitivity vs. resistance allude to a secret of cancer's complex nature and mysterious origin?

Awareness is growing that it is the cancer stem cells that provide a tumor with its drug-resistant phenotype. Whether this drug-resistant phenotype is born or bred may be moot. Like a spore, a cancer stem cell is dormant and virtually impervious

to harm or insult. It has a built-in survival kit that enables it to expel toxins and avoid programmed cell death. But one should also remember that these drug-resistant mechanisms are intrinsic in the makeup of normal stem cells and are normally designed to eliminate differentiated cells that have become damaged, deformed, or otherwise liable to affect the whole organism. In a strange quirk of nature, these same mechanisms for ensuring the survival of normal stem cells are being used to guarantee the success of cancer stem cells.

An important clinical implication of the stem-cell theory of cancer relates to the role of surgery in overcoming drug resistance. Surgery is particularly efficacious for the removal of cancer stem cells that remain locally confined for a prolonged period. Whether surgery succeeds in eliminating drug resistance and eradicating cancer stem cells depends on the selection of appropriate patients with the right tumor types. An important note is that the theory of a stem-cell origin of cancers provides us with the rationale for and some clues to who may be the ideal candidates and what may be the right tumors for primary surgery, metastatectomy, and neoadjuvant or adjuvant treatment strategies.

Indeed, this is yet another facet of the drug-resistance story. So far, we have focused on the more obvious aspects of drug resistance: dormancy, multidrug resistance, DNA repair, and apoptosis. Often enough, we have neglected to mention another key aspect of drug resistance: the indolent but intractable differentiated tumor, which behaves just like normal tissue that is left virtually intact or that eventually heals in the face of cytotoxic therapies. Like their respective cancer stem cells, certain differentiated cancer cells are inherently dormant and multidrug resistant; they are more than adept at undergoing DNA repair and avoiding apoptosis. Like a malignant "teratoma," such tumors are invulnerable to the effects of various therapies and need to be surgically extirpated (Chap. 15).

Vaccine Therapy

The theory of a stem-cell origin of cancers also has important clinical implications concerning the basic nature of cancer immunity and our design of cancer vaccines. If a cancer cell and its stem-cell counterpart were more alike than not, then any distinction between them would be minimal and quite subtle. If cancers were derived from stem cells, then cancer vaccines might not work in most cancers. I predict that cancer vaccines will be more efficacious for the treatment of well-differentiated or virally induced tumors. Because malignancies derived from early stem cells are likely to be less immunogenic, vaccines or immunotherapies designed for such cancers may not be as effective as they are for those malignancies derived from late stem cells that may display altered "self" or "foreign" antigens and tend to be more immunogenic. An important clinical implication of the stem-cell theory of cancer and cancer immunity, therefore, is that vaccines should not be indiscriminately designed for all malignancies. Our limited resources are best served when we establish priorities for the use of cancer vaccines in the right tumor types (Chap. 14).

Stem-Cell Therapy

This is the dawn of stem-cell research. This is also high time for stem-cell therapy. There is great hope and much promise in the air that one day stem cells may fulfill their potential to cure innumerable debilitating and devastating diseases. Already, Tabar et al. [11] have managed to differentiate embryonic stem cells into neurons that could be used to heal Parkinson disease. Transplantation of these cells into rats improved the animals' mobility. Similar experiments will be performed in monkeys before the treatment will be tried in humans. Soon enough, clinical trials using stem cells will be conducted to treat humans with spinal cord injury, motor degenerative diseases [12], macular degeneration, impaired heart muscle, and damaged skin.

But if cancer is derived from rogue stem cells, then we have a problem. According to the stem-cell theory of cancer, the very real potential risks of stem-cell therapy cannot be more harrowing. Are we opening a Pandora's Box with stem-cell therapy? The indiscriminant use of stem cells for therapeutic purposes could unleash grave consequences. Not knowing the relationship between stem cells and cancer cells, we may unwittingly expose ourselves to some lurking danger. Already, leukemia has developed in several patients enrolled in a clinical trial that used stem cells as a vehicle for replacing specific genes ([13]; Chap. 6). If cancers are indeed derived from rogue stem cells, and an aberrant stem-cell niche can promote the development of malignant cells from normal stem cells, then the use of stem cells for any clinical purpose has the potential to be hazardous under certain conditions. For these reasons, extra precautions need to be taken in any such endeavor. Stem cells are rather like fire in that they can both serve us and burn us.

A real and present danger awaits cancer therapy that targets stem-cell components. Because such components (e.g., Hh, wnt) may constitute the most crucial aspects of a cancer cell, treatments targeting them could be particularly efficacious but also potentially toxic. Let us consider a worst-case scenario, in which targeting Hh and wnt in the cancer cell causes cognitive or affective impairments and neurodegenerative disorders because Hh and wnt play a role in normal brain functions as well as in cancer. The same treatment might also perturb wound healing and bone remodeling because Hh and wnt also play roles in normal connective tissue and bone functions.

We simply cannot afford to deny or ignore the theory of a stem-cell origin of cancers, especially when it forecasts that an effective cancer therapy affecting cancer stem cells may also cause serious, severe toxic effects as well as irreversible and permanent complications by injuring normal stem cells.

Future Research Directions

A major shift in our understanding and thinking about the origin of cancer has important implications for the direction and nature of cancer research in the future. Some examples follow.

Cancer Stem Cells

When it concerns the origin of cancer stem cells, there is a big difference between cancer that arises from a malignant stem cell and cancer that arises from a cell with stem-cell features. When cancer arises from a malignant stem cell, it is the affected *cell type*, namely, stem cell or progenitor stem cell, that determines the formation and behavior of the cancer. However, when cancer arises from a cell with stem-cell features, it is the *cell content*, such as genetic mutations, that dictates the formation and behavior of the cancer. In the former case, unique cancers derived from distinct stem cells or progenitor stem cells will be diagnosed even though they may contain the *same* genetic mutations, whereas in the latter scenario, unique cancers containing *different* genetic mutations will be diagnosed because these mutations cause the specific stem-cell aberrations.

The prognostication of cancer also needs a substantial makeover if cancer arises from malignant stem cells rather than from cells with stem-cell features. In the former case, the prognosis of cancer depends more on its malignant stem cell of origin rather than on any genetic mutations. The same genetic mutations tend to be more harmful when they occur in an earlier stem cell than in a later progenitor stem cell. They may even be harmless when they occur in a somatic or differentiated cell. I predict that tumors arising from stem cells are completely different from and more dangerous than those arising from progenitor stem cells or from cells with stem-cell features.

Thus, cancer, being derived from rogue stem cells, inherits a complete malignancy package that includes immortality, self-renewal, immunity, heterogeneity, metastasis, drug resistance, and so on (see Chap. 6, Fig. 6.1). It will not be easy to eradicate such resilient and versatile cells with our current arsenal of radiotherapy, surgery, cytotoxic drugs, novel targeted agents, and immunotherapy. In contrast, a cancer cell with a specific genetic mutation that involves a certain stem-cell function (e.g., Hh, wnt) may be more amenable to treatments that selectively target and rectify the particular genetic aberration in question. So far, most cancer treatments have either worked or not worked at all according to the former premise; only rarely have they provided the reliable clinical efficacy and benefit that are predicted by the latter premise.

Stem-Cell Research

An important clinical implication of the stem-cell theory of cancer is that stem-cell research may drastically transform our understanding of cancer and, conversely, that cancer research will inevitably revolutionize our perspectives about stem cells. It is entirely plausible that cancer research overlaps with stem-cell research because the two disciplines have common ground.

Assuming that there is a real and close relationship between cancer and stem cells, I believe that research on one could enhance the research on the other. Thus, progress in various aspects of cancer research could influence stem-cell research, and vice versa. For example, hypoxia appears to play an important role in the pathogenesis of cancer because cancer stem cells self-renew and invade more easily in a hypoxic microenvironment. Interestingly enough, hypoxia is also an integral component of the stem-cell niche; under hypoxic conditions, stem cells mobilize well and do not differentiate. Because acidosis is closely linked to hypoxia, I suspect that acidosis is also an important characteristic of the stem-cell niche. Malignant cells activate certain enzymes, such as Na^+/H^+ exchangers and carbonic anhydrase XI or XII, which produce acidosis. Considering that malignant cells are derived from stem cells, as in the theory of a stem-cell origin of cancers, I predict that stem cells also prefer an acidic microenvironment and that Na^+/H^+ exchangers and carbonic anhydrase XI or XII will be found to play an integral role in stem-cell biology.

Cancer Epigenetics

At first, it seems perfectly logical to think that hypermethylation plays an important role in carcinogenesis. After all, a plethora of data indicates that hypermethylation of certain promoters in a multitude of genes occurs in various cancers. Naturally, we have concluded that hypermethylation of these promoters plays a causal role in carcinogenesis. It is just a matter of time before someone begins to design some ingenious demethylation drugs for the treatment of cancer on the basis of this idea. Before long, we will have found sufficient financial incentives and channeled our finite human resources into what may seem like a sound and promising biologic and clinical investment.

But unfortunately, someone needs to tell the Emperor that he is naked! No one complains when demethylation therapy by itself works in only a minority of patients for some very obscure cancers. We have become all too familiar and accustomed to the raised and dashed hopes of yet another supposed blockbuster treatment. Although the science of epigenetics is impeccable, its price may be too high in the economy of cancer. If only we would realize that the epigenetics of cancer is no more than an endorsement of its stem-cell signature and origin, then we may be spared the disappointment about its clinical implications. We cannot afford more outrage and the repeated disillusionment that results when the promises provided by some fantastic experiments performed on the basis of a wrong theory about the origin or nature of cancer turn out to be empty.

The idea that cancer cells have an innate hypermethylated stem-cell signature cannot be more eye-opening. The consequence of our thinking that hypermethylation is a reflection of a cancer's stem-cell origin and features rather than a marker of the cancer itself may fundamentally change our current view of cancer epigenetics. Just imagine the clinical implications of this complete turnaround. Thus, cancer epigenetics provides us with yet another proof that cancer has intrinsic stem-cell

features and is under the influence of an onco-niche that may be very similar to a stem-cell niche.

Angiogenesis

When it concerns cancer, there is still much to learn about angiogenesis. In principle, when we have the wrong hypothesis about the origin of cancer and the role of angiogenesis in carcinogenesis, we are prone to misinterpret our clinical observations, design the wrong experiments, misunderstand their results, and misdirect our cancer therapies and strategies. Consider the following viewpoints.

1. Does antiangiogenesis actually shut off blood supply to a tumor like turning off a vascular spigot? If so, we may actually be diminishing the delivery to a tumor of other drugs given in conjunction with the antiangiogenesis agent.
2. Or, does antiangiogenesis in effect cut off blood supply to a tumor like pruning the vascular branches? If so, we may paradoxically be enhancing angiogenesis, increasing hypoxia, and causing other negative-feedback effects, thereby actually exacerbating the malignant process.
3. Or, does antiangiogenesis possibly manipulate the onco-niche in such a way that cancer stem cells become more indolent or even dormant? This notion suggests that antiangiogenesis therapy should be given in conjunction with cytotoxic treatments, which reduce the overall tumor burden, thus providing optimal conditions for adjunct or maintenance therapies to work.

These divergent viewpoints about antiangiogenesis and cancer therapy illustrate the plight of our not having the right hypothesis about the origin of cancer and the nature of angiogenesis at our disposal. Without the correct hypothesis, we will continue to encounter obstacles in designing experiments, assimilating data, and developing therapies.

Conclusion

One cannot help but be perplexed when faced with recurrent extraordinary coincidences. It seems inescapable that the many hallmarks of cancer – heterogeneity, metastasis, immortality, immunity, and so forth – considered to be the touchstone of a cancer cell also happen to be the cornerstone of a stem cell. When we connect more dots, the big pictures of normal stem cells and rogue cancer stem cells become increasingly more superimposable. As we learn more about the biologic intricacies of cancer cells and stem cells, the clinical implications of their putative relationship become more strikingly obvious. The take-home message is straightforward and simple: We should neither ignore nor deny the sheer power of the normal stem cell and the clinical implications of its malignant counterpart, the cancer stem cell.

Otherwise, even the best of our premonitions according to the stem-cell theory of cancer may go unheeded, like Cassandra's prophesies.

References

1. Eisenhauer EA, Therasse P, Bogaerts J et al (2009) New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer 45:228–247
2. Huff CA, Matsui W, Smith BD et al (2006) The paradox of response and survival in cancer therapeutics. Blood 107:431–434
3. Bach PB, Jett JR, Pastorino U et al (2007) Computed tomography screening and lung cancer outcomes. JAMA 297:953–961, Erratum in JAMA 2007;298(5):518
4. Arber DA, Tamayo R, Weiss LM (1998) Paraffin section detection of the c-kit gene product (CD117) in human tissues: value in the diagnosis of mast cell disorders. Hum Pathol 29:498–504
5. Strohmeyer T, Reese D, Press M et al (1995) Expression of the c-kit proto-oncogene and its ligand stem cell factor (SCF) in normal and malignant human testicular tissue. J Urol 153:511–515
6. Natkunam Y, Rouse RV, Zhu S et al (2000) Immunoblot analysis of CD34 expression in histologically diverse neoplasms. Am J Pathol 156:21–27
7. Leaf C (2004) Why we're losing the war on cancer (and how to win it) (Avastin, Erbitux, Gleevec...the new wonder drugs might make you think we're finally beating this dreaded scourge. We're not. Here's how to turn the fight around.) Fortune Magazine, March 22, 2004, pp 77–92
8. Hong WK, Endicott J, Itri LM et al (1986) 13-cis-retinoic acid in the treatment of oral leukoplakia. N Engl J Med 315:1501–1505
9. Steinbach G, Lynch PM, Phillips RK et al (2000) The effect of celecoxib, a cyclooxygenase-2 inhibitor, in familial adenomatous polyposis. N Engl J Med 342:1946–1952
10. Thompson IM, Goodman PJ, Tangen CM et al (2003) The influence of finasteride on the development of prostate cancer. N Engl J Med 349:215–224
11. Tabar V, Tomishima M, Panagiotakos G et al (2008) Therapeutic cloning in individual parkinsonian mice. Nat Med 14:379–381
12. Pucéat M, Ballis A (2007) Embryonic stem cells: from bench to bedside. Clin Pharmacol Ther 82:337–339
13. Hacein-Bey-Abina S, von Kalle C, Schmidt M et al (2003) A serious adverse event after successful gene therapy for X-linked severe combined immunodeficiency. N Engl J Med 348:255–256

Chapter 19
Curing Cancer

Modified from the original image, "The Angry Crab," obtained from Google Images (www.clipartpal.com/clipart/animal/crab1.html)

Cure sometimes, treat often, comfort always.

– Hippocrates

Précis

The panacea everyone is searching for *may actually be a conquest of rather than a cure* for cancer.

Introduction

Is curing cancer actually or even possible? Many of us still feel a preternatural fear about the "C" word. Even for those of us who are healthy and well, cancer is anathema: A diagnosis of cancer brings grim tidings. However, there is growing optimism that we will cure cancer some day in the not-too-distant future. I predict that the theory of a stem-cell origin of cancers will be a major force driving us toward this goal in both the research and clinical arenas.

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A rude awakening awaits us, though, if we expect an eventual cure of all cancers. Indeed, many of us are taken aback when we learn that the survival rate of patients with metastatic cancer has scarcely changed over the last three decades [1]! Despite the exponential explosion in our knowledge about cancer during that same period, only a modest incremental improvement in cancer cure has been gained. Until now, each victory in our war against cancer has been a Pyrrhic one. Likely, we will be unable to make the leap into the brave new world of curing cancer when our views about the origin of cancer remain erroneous and obsolete. Unless we take a giant step forward in understanding the origins of cancer, we will have very few breakthroughs but many heartbreaks in our treatment of cancer.

Conquest vs. Cure

Around 2003, the rate of cancer death started to decline for the first time in recorded history [2]. Is this a sign of things to come? Are we on the verge of curing cancer? Have we figured out how to penetrate rather than only make a dent in the armor of cancer? Have we finally acquired the necessary ammunition to wipe cancer off the face of this world? When it comes to curing cancer, we have come a long way, but we have farther still to go. I believe that one of our best weapons in the battle to beat cancer is to unlock the secrets of its origins.

As they say, the devil lies in the details. One suspects that the truth about curing cancer is buried somewhere in the statistics. Something about the statistics is dubious, though. The numbers may not lie, but they do not tell everything. What we thought to be a significant decrease in the rate of cancer death in the United States was an improvement of 3,014 cancer deaths per year, but the total cancer death toll was still about 540,000 persons per year [2]. Thus, even though the downward trend in cancer death is promising and encouraging, the fact remains that any improvement in cancer survival is modest at best. One cannot help but notice that many people still die and will die of cancer. Despite our best efforts to prevent, detect, and treat them, many cancers still maim and kill. But why is that so? Again, I suspect that the truth about cancer death that many of us know but dare not ask lies in the origin of cancers.

Waging a war against cancer means that we need to learn and know more about how the enemy works. According to the theory of a stem-cell origin of cancers, diverse cancers arise from distinct stem cells and stem-cell derivatives. This is the source of cancer's tremendous heterogeneity and enormous complexity. Soon enough, we will learn which cancers need to be resected and which can only be controlled. We will realize that some cancers are potentially curable whereas others are likely to be lethal despite our best efforts. *Winning* the war against cancer means that we need to realize that even though they may be treatable, not all cancers are curable. In the end, we will find that there is NO one-treatment-fits-all, NO magic elixir for curing cancer. We will come to accept that the panacea everyone is searching

for is not a cure but rather a conquest of cancer. This is a bittersweet pill, but we all must eventually swallow it.

Personalized Therapy

Often enough, our choice of therapy for a particular cancer is based on empiric results rather than scientific evidence. Many of us will admit that we do not have the slightest idea (let alone the full details) of a treatment's mechanism of action. I hope that with improved knowledge about the origin and nature of cancer, we will be designing personalized therapies that are more effective and less toxic in the future. We will be making more "smart bombs" and fewer "dirty bombs" against the relevant cancer cells on the basis of their exact origins and precise nature.

Assuming that cancer stem cells inherit stem-cell properties because they arise from normal stem cells, one may question whether it is at all possible to find treatments that eliminate the cancer stem cells without affecting normal stem cells since the two cell types are more alike than not. This concern is legitimate because indiscriminant treatments may not only damage cancer stem cells but also harm normal stem cells, thereby causing serious, severe side effects as well as persistent and permanent complications. Despite our best intentions, a promising treatment based on a flawed hypothesis may cause unexpected results and even effects that are the opposite of what we intend: We may use treatments that actually aid rather than aim for the all-important cancer stem cells because we do not know what is going on or what we are doing. For example, single-agent angiogenesis inhibitors may produce a paradoxically unfavorable effect and cause more angiogenesis, with enhanced hypoxia and possibly worsening tumor invasion and increased metastasis in the treated tumors [3, 4].

Alternatively, if cancer stem cells acquire stem-cell properties from or mimic normal stem cells, then there is a chance that cancer stem cells are inherently different from normal stem cells. Hence, we may be able to distinguish between the two cell types and design different treatments accordingly. Tantalizing evidence suggests that it is possible to target unique stem-cell pathways that have become deranged in malignant stem cells but not in normal stem cells. For example, *Pten* dependence distinguishes leukemia-initiating cells from hematopoietic stem cells [5]. Consequently, agents like rapamycin and temsirolimus, which correct *Pten* deficiencies and directly target the leukemic stem cells, may be efficacious for treating certain leukemias. Differences between normal and cancer stem cells may also reside in posttranscriptional and posttranslational modifications. Perhaps this is the reason why proteosome inhibitors such as bortezomib may eliminate cancer stem cells but spare normal stem cells. Cyclopamine is another promising agent for the treatment of several cancers because it inhibits a stem-cell signaling pathway, hedgehog. Whether cyclopamine could distinguish cancer stem-cell from normal stem-cell pathways and selectively affect pertinent malignant stem-cell targets remains to be seen.

It should not be surprising that our best cancer treatments are successful because they happen to modulate pertinent stem-cell targets. Whether by chance or design, they eliminate or induce differentiation of the relevant cancer stem cells. Because cancer (and normal) stem cells rarely undergo cell cycling, express more drug-resistant transporter proteins, and have increased DNA-repair capabilities and enhanced antiapoptotic or survival mechanisms, they are generally less susceptible to treatment than differentiated cancer cells are. However, I believe that personalized therapy will be realized once we elucidate the exact origins and precise natures of various cancers. According to the theory of a stem-cell origin of cancers, cancer stem cells may be amenable to specific therapeutic modalities, including chemoprevention, differentiation therapy, surgery, maintenance therapy, and targeted therapy. I will discuss each of these modalities in turn.

Chemoprevention

Undoubtedly, chemoprevention will play a critical role in our battle against cancer. If possible, it is better to prevent a problem than it is to fix it. Protection from the sun, exogenous hormones, noxious chemicals, and unhealthy diet, particularly during the most formative time of our lives, is crucial for the prevention of many cancers. Already, our campaign against smoking has provided better results in decreasing the incidence and mortality of many cancers than any available treatments have.

According to the theory of a stem-cell origin of cancers, a stem cell is relatively quiescent. As in the case of a spore, this inactivity makes the stem cell less vulnerable to injury by external forces. If a stem cell happens to sustain some damage, it has a superior ability to repair the insult and heal itself. Under normal circumstances, this capability of stem cells to minimize damage and maximize repair makes the development of cancer a relatively uncommon and late event in a person's life span. When a stem cell loses its quiescence, it becomes cancerous more readily. It becomes increasingly exposed and vulnerable to the various dangers that happen to come its way. A universal theme about the effects of prolonged smoking, sun exposure, exogenous hormones, noxious chemicals, and unhealthy diet is that all of these induce incessant cellular proliferation and inflammation, which promote tissue damage. The stem cell is required to leave its safe haven and become chronically active with tissue repair, asymmetric division, and self-renewal. Under such conditions, the chance for error and for the development of cancer is increased.

Therefore, chemoprevention has a new meaning and implication according to the theory of a stem-cell origin of cancers. For example, when stem cells already contain germ-line mutations that predispose them to further mistakes, cancer development is likely to accelerate. Under such circumstances, a stem cell possesses intrinsic defects that impair its ability to repair the damage. Because these genetic deficiencies are inherited, there is less room for error and less time for the error to manifest as cancer. And because the onset of cancer shifts to fast forward in these

high-risk cases, chemoprevention may be able to delay but not altogether prevent the development of cancer.

Differentiation Therapy

If we cannot prevent cancer, perhaps we can modulate it to become less malignant. Central to the theory of a stem-cell origin of cancers is the notion that a cancer cell is trapped in the body of a stem cell. It can no longer differentiate into a more governable entity that obeys various homeostatic or regulatory rules. In this, we are reminded of an oncologic aphorism that control of differentiation is equivalent to suppression of carcinogenesis. Accordingly, one key to cancer therapy is to induce differentiation of a poorly differentiated tumor.

It is amazing how certain factors have distinct, even diametrically opposed functions in different cell types. For example, TGF-β inhibits the differentiation of specific stem cells but stimulates the growth of their progeny cells in their respective niches. BMP-6 may restrict stem-cell renewal by suppressing Wnt signaling and may induce osteoblasts to undergo differentiation. We postulate that certain bone factors (e.g., soluble ErbB3) like BMP-6 attenuate the progression of prostate cancer by both limiting malignant stem-cell renewal and producing an osteoblastic response to bone metastasis [6]. Therefore, differentiation therapy should be included as an important therapeutic option in our armamentarium against cancer by targeting both cancer stem cells and their accomplices, accessory cells.

Unfortunately, many challenges and obstacles in the use of differentiation agents in cancer therapy remain. It is true that transretinoic acid and arsenic trioxide for treating acute promyelocytic leukemia and imanitib for treating chronic myelocytic leukemia are successful in part because they induce differentiation of the malignant clones. Demethylation or deacetylation agents, such as azacytidine and decitabine, may be effective for the treatment of myelodysplastic syndrome because they induce differentiation of aberrant stem cells in the bone marrow into erythrocytes, leukocytes, and platelets. But differentiation therapy is not yet feasible for use in most cancer types, and the question is how to increase its reliability and utility in the foreseeable future. I surmise that differentiation therapy is plausible for only certain targets or pathways in certain tumor types. Like other therapeutic modalities, it is one of many options and part of a general scheme of personalized therapy for cancer.

One way to improve differentiation therapy is to alternate its use with that of cytotoxic agents. Thus, we could first use a differentiation agent to induce differentiation of malignant stem cells into a more malleable phenotype, which can then be resected by surgery, or into a more vulnerable phenotype, which can then be eliminated with cytotoxic agents. This logic suggests that the opposite sequence – using cytotoxic chemotherapeutic agents before differentiation agents – may not be as effective because the cytotoxic agents are less likely to affect malignant stem

cells. Hence, acute promyelocytic leukemia is a curable disease when treated with transretinoic acid–based induction therapy followed by chemotherapy. Currently, about 90% of patients with newly diagnosed acute promyelocytic leukemia experience complete remission, and more than 70% of these patients are cured. However, this therapeutic regimen works only for acute promyelocytic leukemia, not for any other types of leukemia or for any solid tumors, as would be expected of a personalized therapy.

Surgery: To Operate or Not to Operate?

Another way to improve current cancer therapy is to consolidate its benefit with that of surgery. But to operate or not to operate? That is the question. Until recently, surgery was generally not recommended when a cancer was already widely metastatic. The rationale for this principle seems plain and simple enough: If the systemic cancer is ultimately fatal, why bother with the local tumor, which may not even be symptomatic? In other words, surgery to remove the tumor in such cases may cause more morbidity than the tumor itself does. However, accumulating data suggest that extirpation of the primary tumor in the face of metastatic disease provides a superior clinical outcome in special situations.

This has been shown to be true in the case of renal-cell carcinoma (RCC) and perhaps breast cancer. A study of the European Organisation for Research and Treatment of Cancer (EORTC) demonstrated that the median survival time of patients with metastatic RCC who underwent radical nephrectomy and treatment with interferon-alfa (IFN-α) was longer than that of patients who received IFN-α alone: 17 vs. 7 months [7]. Another randomized study, performed by the Southwest Oncology Group (SWOG), yielded similar results: The median survival time of patients with metastatic RCC who underwent radical nephrectomy and treatment with IFN-α-2b was longer than that of patients who received IFN-α-2b alone: 11.1 vs. 8.1 months [8]. Recently, a retrospective study by Rapiti et al. [9] suggested that complete resection of primary tumors in patients with metastatic breast cancer also increases survival time.

I postulate that the drug-resistant phenotypes (as expressed by the ATP-binding cassette transporter, antiapoptotic, and DNA repair proteins) in cancers are traceable to normal stem cells that passed on these properties to the cancer stem cells. Certain subtypes of cancer stem cells reside at and tend to be confined to the primary site for a prolonged time during carcinogenesis. Therefore, one of the best-kept secrets about drug resistance may turn out to be a perpetual well of certain cancer stem cells that are locally confined and should be resected along with the primary tumor. Another often overlooked aspect of drug resistance, which may be just the other side of the same coin, is the fact that these cancer stem cells are predisposed to differentiation and formation of heterogeneous mature progeny. The formation of a teratomatous component of the tumor by the cancer stem cells in the

primary site is yet another cause for drug resistance in a tumor that needs to be surgically extirpated.

It makes sense that local therapy should work best on tumors that tend to be confined and exhibit delayed metastasis. By nature, these tumors cannot be completely eradicated by any systemic therapies. However, it is evident that not all tumor subtypes will have these unique biologic or clinical characteristics. Therefore, I suspect that local therapy is appropriate only for certain subtypes of cancer. Selection of local surgery for the right subtypes of cancer will be critical in our efforts to improve overall clinical outcome [10]. For example, surgery for teratomas and yolk sac tumors, subtypes of germ-cell tumors, is both necessary and beneficial. However, other subtypes of germ-cell tumors that have a systemic nature, such as choriocarcinoma and embryonal carcinoma, are better treated with cytotoxic chemotherapy and are minimally affected by local therapy. Therefore, another way to eliminate drug resistance is to directly remove the cancer stem cells and the differentiated "teratomatous" components of the tumor at the primary site.

Maintenance Therapy

Even if a tumor cannot be completely eliminated because of its advanced stage, we may still want to render the remaining disease less threatening by using some form of maintenance therapy to decrease the chances for its recurrence. Again, the idea behind maintenance treatment is to keep the cancer stem cells from continuous self-renewal. Maintenance treatments attempt to establish a quasi–stem cell niche in which the microenvironment maintains homeostasis, keeping stem cells quiescent and malignant cells dormant.

Some fascinating experiments have shown that malignant cells do not behave malignantly when they are placed in an embryonic microenvironment (Chap. 8). These intriguing experimental results support the theory of a stem-cell origin of cancers: Cancer cells are like stem cells gone berserk, but a proper embryonic or stem-cell microenvironment may be able to tame those rogue stem cells. Obviously, it would be impossible to reverse a grown person into embryonic form in real life. The closest thing to achieving reconstitution of an embryonic or stem-cell state in adult humans may be the administration of a concoction of certain fruits and nuts. After all, fruits and nuts are embryos of potential plants. I wonder whether such an elixir would provide the "magical" powers of an embryonic or stem-cell niche for controlling malignant cells. Indeed, it would be a miracle to learn that the theory of a stem-cell origin of cancers holds the secret to the making of a panacea and the finding of our long-sought Holy Grail of a "cure" for cancer.

If we consider cancer to be like a weed, then cutting it at its stem will bring only temporary relief because it will grow back in no time. After all, we are only removing the bulk of differentiated cells that are readily and easily replaced in a tumor. However, demolishing a weed at its root is likely to be more effective because we

are going after the so-called cancer stem cells that drive and replenish the whole tumor. I propose that an alternative way of controlling the weed problem is by manipulating the soil. After all, the onco-niche is the cradle of cancer, and a tumor is less likely to run amok if the niche is right. Again, it is premature to devise an effective maintenance therapy without sufficient knowledge about the nature of a stem-cell niche or onco-niche. I postulate that maintenance therapy, unlike differentiation therapy, should be administered after cytotoxic therapies. An example of maintenance therapy's providing possible clinical benefit is the use of ketoconazole (and possibly, abiraterone) for castrate-resistant prostate cancer [11].

Targeted Therapy

You need not worry that you are alone in having doubts about the promises of targeted therapy. The failure of most targeted therapies to completely eradicate advanced cancers attests to this. It is not easy to devise treatments that target malignant cells while sparing normal stem cells when the former cells are derived from the latter and the two cell types are more alike than not. According to the theory of a stem-cell origin of cancers, targeted therapy that happens to be more efficacious may also affect normal stem-cell functions and cause serious toxic effects and chronic complications because of collateral damage to normal stem cells.

But not all is gloom and doom. An abnormal stem cell giving rise to a cancer cell may contain unique features besides its stem-cell properties. I anticipate that the best way to detect and identify these unique features is not to compare cancer cells with normal cells (as we have done so far) but instead to compare cancer cells with their normal stem-cell counterparts (which we have not been able to do thus far). I predict that these unique features are more likely to be found in tumors arising from late progenitor stem cells than in those from early stem cells; therefore, I believe that targeted therapies are more likely to work in tumors arising from late progenitor stem cells.

Our experience with antiangiogenesis therapy so far has been somewhat illuminating. It illustrates our desire to design a cancer therapy that targets cancer cells but not normal tissues. Unbeknownst to many of us, most angiogenesis inhibitors may actually target stem-cell features in a tumor but less so in normal tissue. After all, both tumor and normal tissues need a healthy vascular system to provide them with an abundant supply of oxygen and other nutrients. But somehow, the endothelial "address" in a tumor may be different and distinguishable from that in a normal tissue. This is in line with the idea that although angiogenesis may be an important component of the general tissue microenvironment, it is distinct or unique for a particular stem-cell niche or onco-niche. Consequently, many angiogenesis inhibitors may affect only malignant cells, sparing normal tissues.

Another promising strategy relates to the development of therapy that targets cancer networks rather than pathways. We have already learned the hard way that biologic systems are riddled with redundancies. Networks of redundant pathways

are both complex and simple. Nature uses networking to ensure that a particular activity is both potent and resilient. A web of networks permits changes or adjustments to occur rapidly and smoothly. At the same time, the changes are controlled by abundant checks and balances. When we focus treatment on a particular cancer target in a specific pathway, the effect of therapy may become obscured or diluted in this complex web of networks. I propose that it would be more efficacious to administer treatments that affect an entire web of networks at a particular stage of the cancer. For example, an inflammatory network may be at play during the early stages of carcinogenesis. Then various inflammatory factors in the network start to overwhelm potential negative checkpoints and inhibitory safeguards. The inflammatory network thus wreaks havoc on the stem-cell niche and induces irreparable damage to the stem cells themselves. An ideal therapeutic strategy might involve agents that close this inflammatory floodgate. In the same vein, a lifestyle that attenuates this inflammatory overdrive (e.g., healthy diet, no smoking) might prevent or delay the progression of cancer.

Basic Rules of Science

I do not pretend to be Nostradamus. I am not Pollyanna, either. Nobody wants to be the boy who cries wolf about the coming of cancer. But when it concerns designing cancer cures according to the theory of a stem-cell origin of cancer, we need to remember some basic rules of science.

First of all, experiments need to reflect, not violate, the basic laws of life. It is impossible to "un-ring" the bell of life. As the wheel of life turns, time proceeds along one irreversible path. Tadpoles grow to be frogs, mature female frogs lay eggs, which in time become tadpoles, and the whole life cycle begins anew. The basic laws of life tell us that life cycles do not reverse in time to go the other way around. However, we do know how to take a cell from the body of a frog and make it become a tadpole. Our experimental ingenuity knows no bounds. We certainly know how to perturb the balance of nature and accomplish many incredible or seemingly impossible feats in the laboratory. We applaud and congratulate each other for being so daring and clever. But to infer any special implications from such scientific accomplishments would be a grave mistake. We are only fooling ourselves if we believe that these experimental oddities have the slightest resemblance to reality. We have reason for concern when we fail to put these experimental results into their rightful place and fail to obey the basic laws of life. In so failing, we will encounter even more delays in our quest to find a cure for cancer.

Second, the time has arrived for us to revise the popular tenet about moving cancer therapy from the bench to the bedside. I propose that it is more productive to move cancer therapy from the clinic to the laboratory and then back to the clinic again. Nowadays, we place emphasis and set priorities on the basis of translational research, i.e., going from bench to bedside. But we need to remember that although experiments are essential and powerful tools for testing and confirming reality, they

do not by themselves represent or even reflect reality. We need to be reminded that although experiments are vital for science, they offer only a limited view of reality. Only in the clinics do we experience reality. In the laboratory, we examine and elucidate those experiences, and the whole cycle perpetuates itself. Although research may merge with reality at some point, we should not blindly mix research with reality. If we lose this perspective and accept research to be reality itself, then we should brace ourselves for many more shocking disappointments about curing cancer. Again and again we have learned that what cures cancer in a contrived cell culture or a manipulated mouse model does not constitute a cure for cancer in a human patient. We need to realize that experimental evidence is not the whole truth or even reality. In the end, we must always return to the clinic and put everything we have learned from the laboratory into its right perspective and proper context.

Conclusion

This is a historic time as far as curing cancer is concerned. The theory of a stem-cell origin of cancers tells us that we may be either close to or right on the threshold of "curing" cancer. I maintain that the theory of a stem-cell origin of cancers is not a simple facelift of an old idea about cancer. At the very least, it will be an extreme makeover of our current views of cancer. For the first time, we have an opportunity to adopt an entirely novel mind-set, not only about the origin and nature of cancer, but also about the way we diagnose cancer, perform cancer research, and design cancer therapy for the foreseeable future. Looming over the horizon, or maybe even just around the corner, is the dream – long believed to be impossible – of "curing" cancer. Now, we must seize and savor the opportunity to conquer cancer once and for all.

References

1. Leaf C (2004) Why we're losing the war on cancer (and how to win it) (Avastin, Erbitux, Gleevec...the new wonder drugs might make you think we're finally beating this dreaded scourge. We're not. Here's how to turn the fight around.) Fortune Magazine March 22, 2004, pp 77–92
2. Jemal A, Siegel R, Ward E et al (2007) Cancer statistics, 2007. CA Cancer J Clin 57:43–66
3. Ebos JML, Lee CR, Cruz-Munoz W et al (2009) Accelerated metastasis after short-term treatment with a potent inhibitor of tumor angiogenesis. Cancer Cell 15:232–239
4. Pàez-Ribes M, Allen E, Hudock J et al (2009) Antiangiogenic therapy elicits malignant progression of tumors to increased local invasion and distant metastasis. Cancer Cell 15:220–231
5. Yilmaz ÖH, Valdez R, Theisen BK et al (2006) *Pten* dependence distinguishes haematopoietic stem cells from leukaemia-initiating cells. Nature 441:475–482
6. Lin S-H, Lee Y-C, Choueiri MB et al (2008) Soluble ErbB3 levels in bone marrow and plasma of men with prostate cancer. Clin Cancer Res 14:3729–3736

7. Mickisch GH, Garin A, van Poppel H et al (2001) Radical nephrectomy plus interferon-alfa-based immunotherapy compared with interferon alfa alone in metastatic renal-cell carcinoma: a randomised trial. Lancet 358:966–970
8. Flanigan RC, Salmon SE, Blumenstein BA et al (2001) Nephrectomy followed by interferon alfa-2b compared with interferon alfa-2b alone for metastatic renal-cell cancer. N Engl J Med 345:1655–1659
9. Rapiti EJ, Verkooijen HM, Vlastos G et al (2006) Complete excision of primary breast tumor improves survival of patients with metastatic breast cancer at diagnosis. J Clin Oncol 24:2743–2749
10. Tu S-M, Lopez A, Leibovici D et al (2009) Ductal adenocarcinoma of the prostate: clinical features and implications after local therapy. Cancer 115:2872–2880
11. Tu S-M, Millikan RE, Mengistu B et al (2001) Bone-targeted therapy for advanced androgen-independent carcinoma of the prostate: a randomised phase II trial. Lancet 357:336–341, Erratum in: Lancet 2001;357(9263):1210

Chapter 20
Epilogue

In the Japanese Zen tradition, this brushed circle, "enso," is used as a symbol of the "circle of enlightenment." Others call it the "infinity circle." Enso could also be interpreted as "mutual circle" or "circle of togetherness": the oneness of life, the beginning and end of all things, and the connectedness of existence

An expert is someone who knows more and more about less and less until eventually he knows everything about nothing.

– Anonymous

Précis

Without the right road map at a major crossroad, we risk making the wrong turn or taking a more circuitous route.

Introduction

Recently, the role of stem cells in health and disease has garnered increasing attention. Stem cells offer a potentially unlimited source of replacement cells for the treatment of various diseases and disabilities, including Alzheimer disease, Parkinson disease, heart diseases, spinal cord injuries, strokes, diabetes mellitus, and burns. Stem-cell therapy is like a fountain of youth in which young, healthy

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cells are generated to replace old, defective ones. Almost no realm in medicine is untouched by the innate power of stem cells. But there is still much that needs to be learned about stem cells. We need to be cognizant of both the assets and potential hazards of stem-cell therapy. We should not ignore the fact that stem cells also have a dark side.

The time has arrived for us to write a new book on the origin of cancer. I submit that *a unified theory* (Preface) about the stem-cell origin of cancer will radically transform our current view of cancer. It will expand cancer research and elevate cancer therapy into the stratosphere. By repudiating the *cancer myths* (Chap. 2) and causing a major *paradigm shift* (Chap. 16), a unified theory of cancer will enable us to make sense of an ever-growing, never-ending information base and make a major overhaul in our conduct of cancer research and our design of cancer therapy. Henceforth, a unified theory of cancer will help us refocus our attention, redirect our resources, and reinvest our time to improve the diagnosis and treatment of cancer. I believe that a correct unified theory of cancer is a prerequisite in our war against cancer and our goal of conquering it.

Cancer's Boogeyman

In many quarters, cancer is still a six-letter word nobody wants to utter, a curse one chooses to hush, and an embarrassment one prefers to hide. Often enough, cancer appears clinically untouchable and scientifically incorrigible. The scale and scope of this silent killer's menace have no bounds.

Not knowing cancer's boogeyman has its consequences. Sometimes, it takes a cancer crisis to provide us with the best motivation and ideal opportunity to explore the origin of cancer and to prove the theory of a stem-cell origin of cancers.

In many respects, cancer is a disease of stem cells. For a corrupted stem cell, it is not a matter of *if* but rather of *when* a cancer will become manifest. The theory of a stem-cell origin of cancers is still a work in progress. We witness its common tracks without realizing that one day it may completely revamp our current schemes of everything and anything related to cancer.

Of course, many investigators still have their reservations about *how cancer relates to stem cells* (Chap. 6). It is hard to subscribe to the stem-cell theory of cancer when there are so many *"unknown unknowns" about stem cells* (Chap. 5). They object to any discourse about an entity that is still poorly defined and not easily amenable to study. It is true that we have no idea about the total number or the absolute types of extant stem cells. We also have no inkling whether these stem cells are exquisitely transitory or entirely dependent on their microenvironment or *niche* (Chap. 8). These deficiencies confound our understanding and complicate any studies on the stem-cell origin of cancers. Perhaps "*stem cell*" or "*cancer stem cell*" is actually the wrong term for describing a cancer-initiating cell (Chap. 7). Nevertheless, it will be a shame if we allow semantics to confuse the issues, impede any progress, or block the truth.

Legend of the Fall

At the core of the theory of a stem-cell origin of cancers is the idea of a stem cell that would be a cancer cell: A stem cell–like cell or a cell with stemness features that would be the mother and father of all cancers. Could this be a theory for the ages, our long-sought unified theory of cancer? The clinical fallout from this idea cannot be overstated. There is bound to be a major overhaul in just about everything and anything that deals with cancer diagnosis, prognosis, and treatment. There will be paradigm shifts and culture shocks, and our overall view of cancer will likely be changed forever.

It is important to emphasize again that many of the comments and opinions expressed in this book are strictly my own. I am hopeful that a collision of ideas about the origin of cancer will make us bolder and wiser. I want to show that a simple idea has immense implications. The theory of a stem-cell origin of cancers is a story about the legend of the fall, how a stem cell falls from grace and becomes a cancer cell. It explains why a cancer cell mimics a stem cell in many ways and how it makes a mockery of our many efforts to cure cancer. I believe that a correct theory about the origin of cancer bodes well for the future of oncology. It will force us to ask the right questions, envision the right research, and dispense the right treatments. It will enable us to use our time, energy, and resources more efficiently and effectively in our perennial war against cancer.

The Curse of Job

This book is all about the origin of cancer. In it, I have tried to convince readers that the origin of cancer has a great deal to do with its *cells of origin* (Chap. 4). In all likelihood, these putative cancer-initiating cells are some sort of stem cells or progenitor stem cells in a stem-cell hierarchy. I speculate that a unique stem cell or progenitor stem cell gives rise to a unique cancer type. In other words, distinct cancer types have distinct stem cells or progenitor stem cells of origin. Just as the essence of stemness already exists within certain stem-cell types, the essence of malignancy may already exist within the very stem cells or progenitor stem cells that engender the cancer.

The dichotomy between stem cells and malignant cells reminds me of many stories. As in the allegory of the curse of Job, it seems as though the good things given to us by stem cells can be stripped away from us suddenly and unreasonably by cancer cells. What is the purpose and meaning of all these misfortunes and ills amidst the omnipresent goodness and health? How can we let cancer have such free rein? This cannot be a simple matter of apples turning rotten. It is more bewildering than the sudden appearance of a violent storm. Just like Job, we would very much like to complain more and accept less the inevitability of cancer. Perhaps when it concerns cancer, we should be rewarded more for vision and perseverance and less for faith and patience.

The Big Fix

This book is unique because it focuses on the clinical aspects of the stem-cell theory of cancer. When a theory is alive and well in the clinics – i.e., it relates to metastasis, heterogeneity, immunity, drug resistance, etc. – it is likely to be both real and pertinent. After all, experiments can always be done to test all kinds of theory, although of course, the best experiments are those performed to test the correct theory. But how do we know which theory is the right one? I believe that a correct theory should be able to explain clinical observations and natural phenomena, not just interpret laboratory results and experimental data. Therefore, I propose that *the ultimate experiment* to test or prove a theory needs to be performed in the clinics (if at all possible) rather than in the laboratory (Chap. 17).

I believe that this book is a testament that the stem-cell theory of cancer is just as alive and well in the clinics as it is in the laboratory. I want to convince readers that the *clinical implications* of the stem-cell theory of cancer are what make it so pertinent and powerful (Chap. 18). I have tried to show that even though many of the ideas expressed here are quite abstract and may be particularly difficult to prove in the laboratory, their importance could not be more relevant to clinical practice.

Therefore, I did not intend this book to "fix" the stem-cell theory of cancer or fit it into a grander scheme of things. I just want to remind readers that we are not on a fact-finding mission about the origin of cancer. Rather, many facts may already be here, and we just need to gather them and put them into their rightful places.

A Tale of Two Cells

Already, many of the most enduring hallmarks of cancer, such as its heterogeneity, can be linked to its stem-cell origin. Cancer heterogeneity is often shrouded in vague words such as "dedifferentiation" to a different phenotype or "transformation" to an alternate phenotype. Perhaps inherent *heterogeneity of cancer* is not so farfetched after all when we consider that it is ultimately derived from a pluripotent stem cell that is also quite capable of expressing diverse phenotypes (Chap. 12). Therefore, I believe that a malignant cell actually evolves from a more premature phenotype rather than converts into one: *Cancer is a revelation of undifferentiated stem-cell features rather than a manifestation of the dedifferentiated cellular processes.*

In addition, it seems more than mere coincidence that many unique properties of a cancer cell also happen to be the basic characteristics of a stem cell. Consequently, it will be difficult to separate many fundamental mechanisms of a cancer cell from those of its corresponding stem cell of origin. For example, cancer and stem cells harness the same *immunotolerance* machinery (Chap. 14) and contain the same *drug-resistance* capabilities (Chap. 15). The list seems to go on and on. A unified theory of cancer accounts for the recurring and persistent phenomenon of *oncology's recapitulating ontogeny* (Chap. 9): Many carcinogenic processes recapture embryonic events, and many embryonic molecules resurface during carcinogenesis. In other

words, malignant roots have stemness seeds. Hence, malignant (e.g., metastatic) potential may be present from the start, and the relevant cancer antigens may also be stem-cell antigens. More than ever, the distinction between a stem cell and a cancer cell becomes blurred and dubious.

Old Dogma and New Doctrine

To some people, the theory of a stem-cell origin of cancers is obvious. One may even venture to declare that this idea is downright self-evident. Yet, why is it so difficult for us to assimilate this theory into our conscience and our culture? It is a shame that many of our most erudite discourses about cancer (e.g., dedifferentiation, multistep progression) actually contradict the basic tenets of this theory. Most troubling of all is that many of our current therapeutic strategies (e.g., chemoprevention, targeted therapy, cancer vaccines) also run counter to the basic principles of this theory.

If we accept this theory of a stem-cell origin of cancers, then its antithesis, dedifferentiation of mature committed cells to cancer, becomes the grandest fallacy in modern oncology. Perhaps we can manipulate cells and control experiments in the laboratory. We demonstrate that differentiated cells can become malignant by acquiring certain mutations that give them stem cell–like properties and render them dedifferentiated. However, we need to be aware that when mature committed cells acquire countless other mutations that may or may not mediate stem cell–like properties, they do not always become malignant. We need to be wary about translating and extrapolating what is observed in the laboratory to what actually happens in the clinic. To put it bluntly, I contend that the idea of dedifferentiation is patently bogus and misguided, according to the theory of a stem-cell origin of cancers.

Recent research results also threaten to topple another dogma of our time, the model of multistep progression of cancer, which holds that premalignant mutations occur in early tumors, whereas metastatic mutations develop in late tumors. Instead, we have learned that a *metastatic signature* may already be embedded in many primary tumors (Chap. 13). The discovery that primary tumors have a molecular profile resembling that of their corresponding metastatic tumors (more so than that of another primary tumor) has immense clinical implications. Instead of a low-grade tumor's evolving into a high-grade tumor or an early-stage tumor's progressing to a late-stage tumor, we may be dealing with distinct cancers arising from unique stem cells or progenitor stem cells. The consequence of multiple and cumulative genetic or epigenetic changes is different when they involve different cell types of different cellular origins. It means that we need to think twice about the rationale for screening for the early detection of certain cancers. We need to be prepared for some unconventional and unexpected insights when it concerns treatment of certain cancers with chemoprevention, selected targeted agents, or cancer vaccines. I believe that a new theory is in order to better explain some of our most intriguing observations and to help us design novel therapies for some of the most deadly cancers.

Trade-offs

Despite our best efforts and intentions, cancer research becomes misguided without a compass to inform us about the origin of cancer. Surely, our reverence of and deference to the stem cell cannot be more justified. However, as we pay homage to the power and potential of the stem cell, we must not lose sight of its malfeasance in the form of a cancer cell, in which that power and potential are misused. Although it is important for us to instill hope in our research on the origin of cancer and to inspire enthusiasm in our search for a cancer cure, we need to be ever aware that in our quest to find a panacea for cancer, we may encounter many sinister, menacing monsters along the way.

It is human nature to assume that to be seen is to be believed or, even better, to be proven. Research is already under way to uncover the identity of stem cells. It is only a matter of time before we have to face the ultimate dilemma from this endeavor: Is our quest to solve the mystery of stem cells and discover their magic? Or is this, perhaps, risky business? In pursuit of our blind ambition and lofty goals, are we playing Russian roulette? Nobody wants to cry wolf. However, one cannot help but ponder whether we have unwittingly made a deal with the devil concerning stem-cell research and perhaps stem-cell treatment, in light of the theory of a stem-cell origin of cancers.

We need to know in no uncertain terms that life is full of trade-offs. One purpose of this book is to help us reckon with the potential promises and drawbacks of stem-cell research and stem-cell therapy in the context of a unified theory of the origin of cancer. Alas, it seems as though what stem cells give, they may also take back. When it concerns cancer, does the stem cell of origin mean that we are damned if we do and damned if we don't? When it concerns cancer therapy, does the origin of cancer necessarily imply that there will be more upsides than downsides? To receive a diagnosis of cancer is like Persephone's being snatched from earthly bliss and condemned to eternal hell. Although cancer treatments may palliate symptoms, prolong life and, occasionally, cure the disease, they all entail some degree of sacrifice and suffering for all patients and their loved ones; even when Persephone tasted the life-sustaining pomegranate, she was constrained to periodically return to the underworld. The theory of the stem-cell origin of cancers does not guarantee a happy ending. Although stem-cell research offers us hope for a miracle to cure many devastating diseases, we must also be prepared for some inevitable trade-offs when it comes to reaping the fruits of such endeavors.

Quo Vadimus?

In many respects, the theory of a stem-cell origin of cancers is actually a gentle awakening, not a rude wake-up call. It is not the theory to end all theories, but a beginning. It is supposed to empower us with all of its unique clinical implications and perspectives so that we can take a broader and deeper view of cancer and appre-

ciate the mysteries of this dreaded and dreadful disease. The theory provides us with a novel frame of reference for better understanding the intricacies of cancer, its heterogeneity, metastasis, immunity, drug resistance, and so forth. It provides us with an alternative avenue for studying the relevance of the various *cancer targets* and pathways…perhaps in a more appropriate context (Chap. 11). Most importantly, it is destined to affect how we practice clinical oncology, our *diagnosis, prognosis*, and treatment of cancer (Chap. 10).

This discourse about the origin of cancer suggests that the truth may lie just below the surface and within reach. But without the right road map at a major crossroad, we risk making the wrong turn or taking a more circuitous route. Without a solid game plan, we may drop the ball rather than take the winning shot. Whatever the eventual outcome may be, the day of reckoning will come sooner or later. Whatever the ultimate truth may be, I hope that our endeavors will allow us to reach tangible clinical benefits as soon as possible. Sometimes, the truth can be unsettling, but at other times, it may embolden us so we dare to hope again for an incredible miracle.

In *The Brothers Karamazov* by Fyodor Dostoevsky, Ivan hallucinated that a gentleman-devil was complaining to him that "many doctors diagnose beautifully, but have no idea how to cure you." For a majority of cancers, this statement still holds true. We have come a long way in diagnosing and treating all sorts of cancers, but often enough, even when a particular cancer is cured, we have no idea why or how it happened. Perhaps a once-in-a-lifetime change in our theory about the origin of cancer will enable us to understand why and how some *cancers are cured* and why and how some others can be conquered, but not cured (Chap. 19).

Final Thoughts

To find a unifying theory for the origin of cancer cannot be a more daunting but urgent task. Our desire to understand cancer and our ability to conquer it depend on the success of this mission. Although it is unlikely that cancer will wipe out an entire population or our whole species anytime soon, its toll on our being and psyches cannot be easily ignored or denied.

We face an inconvenient truth about cancer. In terms of human condition and cost, we are dealing with something big and getting bigger. It is only a matter of time before someone formulates a unified theory for the origin of cancer. Many people are knowledgeable and qualified enough to undertake this job. But someone needs to take the helm, now if not sooner. Unfortunately, we all have other obligations to worry about and be distracted by: career, grant funding, marriage, family, etc. More than ever before, to be competitive and successful we need to be very specialized and focused. Sometimes it takes a maverick with prescient drive and a messianic urge just to initiate such an exhausting but exhilarating task. It might even take an amateur with broad vision and wide interests to stay engaged and complete this momentous and monumental mission.

Everyone loves a great story. Cancer can be either a mystery or horror show. It is often a tragedy and occasionally a comedy. It can be a drama or even a thriller with more than its share of heroes and villains. As we revisit the vast unknowns of cancer, we can easily be held hostage by dogmas or myths and be bombarded by hype or hoax. A benchmark for a successful story is that it touches our hearts and souls and is worthy of retelling. In the long run, the story of a stem-cell origin of cancer will be found to be beneficial and cost effective for all humankind.

Glossary

Cancer cells A generic term for malignant cells that includes both cancer stem cells and differentiated cancer cells.

Cancer immunity An antitumor immune response, either positive or negative.

Cancer targets Targets for cancer biomarkers or anticancer therapy; used as an umbrella term to describe molecular and/or genetic alterations in cells, some of which may be good targets for cancer biomarkers or anticancer therapy and others, not.

Onco-niche A specialized microenvironment that "houses" cancer cells just as a stem-cell niche houses stem cells.

Progenitor stem cells Relatively more mature stem cells or progenitor cells with stem-cell properties that might be the actual targets of tumorigenic transformation; also defined in this book as cancer-initiating cells.

Stem cells Undifferentiated cells with self-renewal and differentiating capacities.

Stemness Properties or potential that make a stem cell, including effects from the stem-cell niche.

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