Preface

How Could Individual Cancer Treatments Be Improved Going Forward?

This provocative and challenging question provides an ideal framework for introducing this book. “Cancer Stem Cells”. It broadly echoes the questions posed during many of my encounters with researchers, clinicians, and patients at Memorial Sloan-Kettering Cancer Center (MSKCC) in New York, as well as members of the general public not limited to the co-pedestrians in the streets and avenues of the Upper East Side of Manhattan to and fro MSKCC. After my first identification of a plant gene, which was previously unknown in any database and whose human homolog was subsequently characterized as the cancer related Jab 1, I first initiated studies to complement this plant gene in human system at the MD Anderson Cancer Center, Houston, Texas. Deeply touched by the patients pouring in from all over the globe and to help their strong will to survive against cancers, I began to shift my research focus from biotic and abiotic stress signaling in plants originally aimed at increasing global plant productivity towards targetable signaling in human cancers for benefitting the patients’ life quality worldwide. Inspired by the tremendous progress in patient focused research performed for over 100 years, and also a new and challenging opportunity to be a part of it, I started at the MSKCC as a beginner to cancer biology and clinical translational research.

My alternative perspective steered me away from conventional cancer research studies towards the poorly understood origins of cancer and the undiscovered layers of molecular control in oncogenesis. This book will introduce cancer stem cells (CSCs) to scientists unfamiliar with this area of cancer research and to clinicians interested in developing careers as physician-scientists. To provide this audience with an appropriate context for the discussions in this book, I will present my own casual appraisal of CSCs from the conclusions of prior literature, which is too extensive to list here with my sincere apologies, that laid the foundation for our current work on this topic. The goal is to synthesize a coherent set of queries so that inquisitive readers will be provoked to go online, investigate further, conceive more research themes, and even amend my thoughts here and those of the authors of the book chapters. Often, after analyzing the complexity of the questions raised by my own translationally oriented basic research and contemplating the general topic of CSCs, I find myself returning to the same fundamental issue: to improve cancer treatments we must unearth the roots of cancer and understand the soil nourishing them as much as we prune and fight against the more easily accessible, wild bushy branches of the disease. These roots include the ability to initiate, sustain and metastasize tumor growth. These are the properties of CSCs that drive tumor initiation and possibly thrive minimal residual disease in cancers, which are the focus of the chapters to follow.

Why have many human cancers remained largely incurable?

I will begin by discussing the above recurring theme: While we continue to make progress
on extending the list of curable cancers, many cancer types are still associated with extremely high mortality rates and short survival. Over the last century, we have significantly advanced our understanding of cancer, beginning with the earliest microscopic observations of transformed cancer cells and progressing to current in vitro techniques of functional interrogation and manipulation of established cancer cell lines. Through these efforts, we have learned a great deal about the cellular properties of cancer, knowledge which has undoubtedly influenced developments in clinical treatment. Extensive progress has also been made in the modeling of human cancers in animals, as in the many genetically engineered mouse (GEM) models that provide the tumors for testing drug toxicity and treatment strategies. However, studies in these animal models can only inform us to a certain degree, and the substantial gap that separates these model tumors from those of cancer patients means many of the treatment strategies fail to make the jump to real-world efficacy.

Undoubtedly, there has been significant progress in improving our understanding of oncogenesis. But until recently, most researchers in the field generally interpreted their data within a broader paradigm in which any elevated or inhibited signaling pathway intermediates were correlated to a presumed linear functional representation of the relevant genes in the bulk tumors. Oncogenes (tumor-causing) and tumor suppressor (tumor-inhibiting) genes that are represented by mutations etc., in otherwise normal developmental genes have been extensively pursued as the true targets of cancer treatment, employing several related GEM models irrespective of the fact that those models are unlikely to reflect the clinical heterogeneity of actual patient or the behavior and interaction of these tumor cells within the body. The overwhelming confidence in this paradigm continues, even though we are now realizing, based on the ongoing human cancer genome sequencing initiatives, that mutated oncogenes may be present in the mature cells of a healthy person’s body, in which malignant disease does not develop for exceptionally long times, or perhaps ever.

I will not even dare to delve extensively into the subject of chromothripsis, a chromosome catastrophe characterized by several gene copy numbers and cataclysmic genome disruptions, even within a single chromosome, that occurs within at least 2–3% of cancer genomes and 25% of all bone cancers, or any of the other unknown mechanisms that are challenging the thus far believed conventional model of sequential accumulation of mutations in the biogenesis of cancers. Moreover, such new observations would raise even more questions if the often overlooked layers of regulatory control and additional feedback mechanisms may impinge into other unexplored signaling cascades within cancer cells. Some of such over looked layers of control could include: (i) distinct signals in CSCs versus bulk tumor cells, (ii) an aberrant control in the early steps of polyribosome recruitment of oncogenic transcripts, (iii) altered metabolism in the cytoplasm of the CSCs involving the intracellular organelles like mitochondria, (iv) epigenetic modifiers in the functional genomic loci, etc. Nevertheless, most studies still continue to concentrate largely on tumor shrinkage rather than the biology of the disease and the impact of therapeutics on the functional cells that actually initiate tumors. It is important to note that, clinically, there is now an increasing understanding that decreasing the tumor burden is not necessarily a functional criterion for cancer cure; because of this, we must relentlessly identify and track the mechanisms of metastasis and treat the true tumor-initiating CSCs.

From Simple Concepts to Complex Signaling Networks

Unfortunately, concomitant with the persistently high proportion of incurable cancers, the list of new oncogenes and tumor suppressors continues to grow with the increasing
amounts of data provided through the efforts of the Cancer Genome Atlas (TCGA) and the International Cancer Genome Consortium (ICGC). These data suggest a complex web in which the majority of human genes and known signaling pathways are related in one way or another to cancer development in humans. Due to the dynamic nature of intra-/inter-tumor heterogeneity in human cancers, it is very important to note that the tumors utilized to generate cancer genome sequencing data or the global gene expression data can never fully represent the biologically, functionally, genetically, epigenetically and metabolically harmonized tumors in a living patient.

Therefore, the important and perhaps most relevant driver genes and their functional transcripts in the rare populations of CSCs could become nearly undetectable because of the “noise” of other gene transcripts that reflect the heterogeneity of bulk tumor tissues. Also, caution must be exercised in interpreting any single miRNA as the regulator of an entire oncogenesis program using bulk tumor tissues, especially when a small subset of distinct and detached miRNA networks are known to function in cell type-specific ways in cancers as against a large and organized tree-like network of up to hundreds of miRNAs in regulation of healthy cells. For example, the CSCs typically represent about 0.1% of the bulk tumor cell population, although that proportion may be increased under certain circumstances (e.g., hypoxia). Unfortunately, the presence of a mere 1,000 CSCs among a million bulk tumor cells, when subjected to genome sequencing, would yield data that are normally excluded as part of the accepted error rate of the instrument measurements. Thus, to pinpoint the driver gene mutations responsible for the origin of human tumors, we may perhaps need to step back and carefully look into the above lacuna in the present general race to sequence as many cancer genomes as possible. We first need cooperation and generous support from federal and state agencies, private companies, or philanthropic/nonprofit foundations for accomplishing the difficult and expensive endeavor of identification, prospective purification, functional validation, and modeling of the minor populations of CSCs from freshly resected patient tumor specimens. Then, the tumor-initiating CSC population may be subjected to comparative genome, transcriptome, proteome, and metabolome analysis. This approach is expected to identify the true driver mutations that could be functionally traced to unveil real therapeutic targets, which would nudge us much closer to durable cancer treatments.

The issues discussed above are a sample of the many reasons why the precise treatment and cure of human cancers is still not on the horizon. Another intriguing puzzle is determining why the same set of activated oncogenic growth factor receptors and their downstream signaling components play roles in other diseases, such as degenerative and metabolic diseases. Moreover, given that more than 300 different signaling proteins and about 900 signaling relationships representing feedback/feedforward cross-talks potentially operate in cancers, how can we design drugs that target these cancers without affecting the normal cells in the body and thereby causing treatment-associated morbidities? If we have not adequately resolved these bulk tumor related issues, can we effectively define personalized cancer therapies?

Thus, many targeted therapies for cancers such as melanomas and medulloblastomas have provided only transient beneficial effects and were followed by aggressive, uncontrollable relapses that resulted in faster-than-anticipated mortality. At least for now, we may have to re-evaluate the study of how a single activated growth factor receptor may influence different downstream signaling cascades in different cell types, or even within one focus of a multifocal tumor. For example, primary tumors of the prostate, kidney, etc., are known to present as multifocal cancers, even within the same organ. For example, each of the foci may be characterized by a distinct genetic defect, and yet various cell types found in the same focus might respond differently to the same gene
mutation depending on their cellular context within each focus.

Similarly, breast cancers also present multiple tumor subtypes (e.g., estrogen receptor positive to negative) within the same breast, albeit to different extents. Another important example is that in case of colon cancers, the normal epithelium, with an enriched signaling pathway, proceeds through early, intermediate and late adenoma stages before culminating into a colorectal carcinoma, switching through the activation of different signaling pathways mediated by epidermal growth factor receptor, transforming growth factor receptor β, a loss of p53 function, and many more. As a result, when a patient presents to a clinician with polyps representing these varied developmental stages, it is unclear which signaling pathways should be targeted for treatment, especially since we know that there are numerous feedback controls among these signaling pathways. Thus, these intellectually challenging and not easily ignored facts, which are associated with real-world clinical situations, ultimately take us back to the important question of how to identify the cell of origin that drives tumor initiation.

From Tumor Heterogeneity to Its Cell of Origin

How many and how much of each of the above mechanisms are influenced through dynamic epigenetic changes in the affected gene loci and are relevant to the overall manifestation of the disease phenotype? Which of these are causes or consequences of changes in the tumor microenvironment of any given single tumor or single focus within a multifocal tumor? How can we attempt to solve these critical and complex puzzles in patients with the present-day basic research information? And how will personalized targeted therapies resolve these issues unless they also address tumor heterogeneity, which dynamically manifests itself three-dimensionally in a tumor, whether from center to periphery, from one side to the other, or from top to bottom? Molecular heterogeneity must also be addressed, not only among different tumors of the same type (inter-tumor heterogeneity), but also among different regions within the same tumor (intra-tumor heterogeneity).

Increasingly, there is recognition that only small populations of undifferentiated cells in bulk tumor tissue have the capacity to recreate the full tumor heterogeneity of the original tumor upon transplantation into appropriate animal models. Thus, examining the data derived from heterogeneous bulk tumor tissues is much like digging through a gold mine and expecting to find lumps of pure gold ready to be picked up. Just as raw materials from the mine must be processed to extract the refined gold, we need to purify tumor initiating CSCs from heterogeneous bulk tumor tissues.

The CSCs are largely undifferentiated cells with stem-like cell characteristics, namely self-renewal and multipotent differentiation, of adult stem cells in the same body organs (Figure 1, A–D). Adult stem cells are activated only when required, as with skin stem cell activation after skin damage to initiate the necessary tissue repair processes; upon completion, the skin stem cells return to quiescence. A widely held contention is that, analogous to adult stem cells of any given organ, the CSCs also maintain their number with self-renewal through asymmetrical cell divisions and increase their numbers as needed by proliferation through symmetrical cell divisions, thereby maintaining the stem cell number homeostasis (Figure 1A: Normal tissue). But unlike adult stem cells, the CSCs appear to exhibit a prolonged proliferation phase, undergoing more symmetrical cell divisions resulting in increased stem cell numbers and their aberrant differentiation (Figure 1A: Tumor tissue). Although it remains to be proven, the theory is plausible if, once a tumor reaches a definite size, perhaps due to the levels of tumor-secreting cytokines and growth factors, a feedback process inhibiting the proliferating CSCs sets in; this would be consistent with observations that the treatments successful in inhibiting tumor
Figure 1. Cancer Stem Cells. A. Schematic representations of adult stem cell number maintenance and their differentiation in the normal tissues versus cancer stem cell number increase and their aberrant differentiation in the tumor tissues. B. Comparative immunohistochemistry of androgen receptor (AR) and prostate specific antigen (PSA) in the human primary prostate tumor (parent tumor), the tumor-derived primary spheres (spheres), and the sphere-derived tumor (sphere tumor) respectively [see http://www.nature.com/ncomms/journal/v2/n1/full/ncomms1159.html]. C. Hypothetical representation of hierarchical arrangement of multiple mutant clones (MC1/2/3) or progenies of different CSC pools (CSC1/2/3). For color detail, please see color plate section.
burden often also increase the numbers of CSCs in many cancers.

Many other questions that are not yet fully answered have also arisen from the above experimental data: (1) What is the trigger for the origin of CSCs? (2) What is the cell of origin for CSCs? Is the CSC a mutated adult stem cell, progenitor cell, stem/progenitor cell with differentiation arrest, dedifferentiated adult cell, or a combination of these? (3) How do CSCs originate in the body by genetic or epigenetic mechanisms, or both? (4) How are CSCs persistently maintained as minor pools of cells, even in well differentiated tumors? (5) Are there any biological feedback controls that maintain a homeostasis among different pools of CSCs in a given bulk tumor? While it is known that genotoxic and chemotherapeutic stresses contribute to the serial development of hypoxia, aneuploidy, and cancer like genomic alterations in single-cell eukaryotes, it remains an open question whether similar cascades trigger the normal stem cells, progenitors, and the differentiated cell progeny to become tumor-initiating CSCs.

It is well known that untreated infection or long-term ulcerated wounds resulting from viral and bacterial infections can cause human cancers such as cervical and colon cancers, respectively. Infection, inflammation and the consequent reprogramming of target cells to become CSCs is one of the most plausible hypotheses, but has never been directly tested by researchers. It is also known that the terminally differentiated host cells can be reprogrammed into stem-like progenitor cells by bacterial pathogens. Because of this an altered paracrine signaling may result in the microenvironment at the infection site and this unexpected stress may destabilize the cellular homeostasis in that area resulting in possible cell-specific additional mutations and thereby the aneuploidy in adult stem cells and other competent cells in that area as they undergo reprogramming and dedifferentiation. Thus, depending on the differentiation state or cell type, and the competence of the cell, it is possible to have more than one subtype of CSCs. There is also evidence in the literature for the existence of more than one subtype of CSCs in some cancers such as brain tumors.

Importantly, it is known from animal modeling studies that when the same oncogenic mutation occurs in somatic cells as well as in stem cells, the tumor-like growths may initially result from both the cell types if the cellular proliferation was the only consequence of that mutation. But, tumor-like growth if originated from somatic cells may not be sustained for longer periods or the tumors may even regress spontaneously, as happens indeed in some cancer patients.
(sometimes viewed as ‘cancer miracles’). At the same time, the tumor growths from mutated CSCs which could result in an incurable disease as evidenced with some engineered colon cancer animal model systems by other groups. These observations reiterate the importance of the tumor microenvironment and perhaps the niche specific factors in tumor-initiation and maintenance.

Typically, once the CSCs are prospectively purified by exploiting known cell-surface markers, they are first verified for their characteristic self-renewal ability by examining in vitro sphere formation, which is a surrogate assay for their in vivo self-renewal capacity (see cover page upper strip, which shows primary spheres expressing green or red fluorescent proteins). Next, these CSCs are subjected to a gold standard experimental validation of their characteristic multipotency via transplantation of small numbers of these purified cells into animal models by exploiting limiting cell dilution approach. Most animal models of human cancer are considered to demonstrate treatment benefit if the overall tumor size decreases following a drug treatment or by target gene knockout/transcript knockdown of the predicted oncogene(s). But, the same treatments rarely cure the cancers in human patients with the same oncogene activation, raising the obvious question: WHY? Most of the above approaches are also undermined if the in vivo effects of the drug were indeed a consequence of a specific effect on target cells or a combined effect on the mobilized host cells in the animal model.

Identification of CSCs is not a simple endeavor, not only because they represent a minor population in bulk tumor tissues, but also due to dynamic changes in their markers expression profiles during tumor development. Thus, handling cell type specificity within a dynamic context of cells isolated at given stage of patient tumor development becomes the most important challenge for the basic researchers and the physician scientists. Interestingly, minor pools of undifferentiated CSCs are always maintained by still-unknown mechanisms even after the apparently fullest differentiation of CSCs into the original parent-like aberrant tumor tissues during sequential derivation and xenotransplantation of serially derived CSCs over many passages of sphere-to-tumor -to-sphere cycles in animal models. This data suggests that the CSCs are indeed tumor driving cells with tumor-initiation and maintenance functions. Thus, the CSC-sensitive tumor targeting may be an effective strategy in conferring the greatest therapeutic benefit to patients, especially when we can delineate clinically relevant novel signaling cascades that are specifically prominent in the CSCs.

**Invoking PDX<sup>CSC</sup> Cancer Models**

Moreover, compared to cancer cell lines or bulk tumor cells that are normally transplanted in multiples of millions for inducing tumors in immunocompromised animals, CSCs are required only on the order of hundreds, or sometimes less. This confirms that bulk tumors contain only a minor population of CSCs and, therefore, that bulk tumor analysis may largely identify tumor-associated or differentiated cell targets rather than targets specific to the tumor-driving function of CSCs. Because the primary tumor tissue volumes obtained from surgical resections are highly heterogeneous, with tissue composition that is variable depending on the tumor stage, size, necrosis, etc., the ability to isolate sufficient numbers of CSCs may also be inconsistent and challenging. Although it is by no means an alternative to conventional banking of flash frozen tissues used to identify the physiological status of disease at the time of biopsy or surgery, the live biobanking approach offers a renewable resource of functional tumor tissue for real-time basic and preclinical studies. The well-characterized patient-derived xenograft (PDX) cancer model systems may therefore be far superior to any other contextual models and form a harmonized, reproducible, and renewable platform for addressing original patient tumor heterogeneity. However, it must be kept in mind that most human tumor xenografts, including the PDX tumors, consist of about 40–50% mobilized host animal cells. Because of
differences in human versus mouse cytoki-
nins as well as their signaling cross talks, it
will be important to investigate the human
CSC-derived PDX (PDX^{CSC}) cancer models.
These models will be useful in developing
individually tailored patient CSC-specific
therapeutics for successfully treating patient
specific cancers. Importantly, it is necessary
to frequently validate PDX^{CSC} cancer models
with comparisons to the parental PDX can-
cer models by testing for the expression of
known functional genes, and by short
tandem repeat analysis for confirming the
maintenance of gross genomic integrity over
many passages during the renewal of the
tumor tissues. Nevertheless, the invoking
PDX^{CSC} cancer models offer an excellent
opportunity to obtain sufficient numbers of
functional CSCs in a faithfully renewable
fashion for biochemical and molecular anal-
ysis and for the discovery of novel and use-
ful biomarkers.

For example, by exploiting an established
subcutaneous xenograft model of primary
human prostate carcinoma [PDX prostate
cancer model], it was found that all the dif-
ferentiated cells in the primary human
prostate tumor xenografts have expressed
androgen receptor (AR) and its downstream
target, prostate specific antigen (PSA), which
have been the most widely employed clinical
markers for prostate cancer (Figure 1B).
However, the sphere-forming prostate CSCs
(spheres) isolated from the patient tumor or
the primary PDX (patient tumor) tumor
were largely undifferentiated, expressing low
levels or none of these markers. Upon ortho-
topic transplantation of these CSCs into
mouse prostate, the resulting PDX^{prostate CSC}
cancer model represents a well-differentiated
parent-like tumor tissue re-expressing the
markers AR and PSA, as can be seen in the
sphere-derived tumor (sphere tumor).

Through the limiting cell dilution experi-
ments, the prospectively purified tumor-
initiating CSCs were specifically found to be
enriched with clinically relevant and also
self-perpetuating NF-kB – IL-6 signaling.
Please refer to the cover page, lower strip
for an example of immunohistochemical
staining of nuclear NF-kB shown in bright
field followed by green immunofluorescence
in the blue background of nuclear staining
with DAPI. A subset of human prostate can-
cer patients that had undergone the radical
prostatectomy and thus had negative mar-
gins for expression of AR and PSA, su-
cumbed to death more quickly than others.
Interestingly, the above negative margins of
this moribund subset of patients have been
found to contain an increased levels and
activation of NF-kB.

Thus, we propose that the enriched
NF-kB signaling is evolved as a possible
anti-apoptotic mechanism in the CSCs, con-
ferring resistance to antiandrogen or AR
antagonist treatments. Increased NF-kB sig-
aling is being found in the CSCs isolated
from a variety of other organ tumors, and
more recently, an involvement of activated
NF-kB has also been suggested in the
expression of characteristic markers of
CSCs such as Sox9. Our and others’ hypo-
thesis that the NF-kB signaling, which
occurs at heightened levels in CSCs and
which plays an important role in tumor initia-
tion, could persist in CSCs and regenerate
tumors over many generations, may be veri-
ified through serial transplantation assays by
employing different PDX^{CSC} cancer models.

With respect to preclinical and transla-
tional studies, as outlined in Figure 2, it will
also be very informative to generate the
PDX and PDX^{CSC} cancer models in a
longitudinal fashion, beginning at a
patient’s primary diagnosis, through therapy
resistance, metastasis, and all the way until
a patient’s death, followed by warm autopsy.
Such strategies will significantly augment
our understanding of the evolution of can-
cer development in the clinical setting of
therapy resistance. In collaboration with
and under the leadership of Dr. John
Healey, Chief of the Orthopaedic Surgical
Service at MSKCC, as well as with many
intellectual consultations with Dr. Irving
Weissman of Stanford University, we have
been developing live biobanking of rare
bone primary tumors and all other meta-
static tumor specimens, as the majority of
mortality associated with the most common cancers result from tumor metastasis to distant organs. Out of an expected 700,000 new invasive cancers per year, about 300,000 cases were associated with bone cancers, which are expected to double by the year 2020 in the United States. Moreover, 7–15% of the total metastatic bone cancers were derived from unknown and uncharacterized rare primary cancers. Normally, the metastasized tumor cells and CSCs will be in limited numbers during their initial dormancy period, which could last for decades in a given patient before emerging as a detectable metastatic tumor. Thus, the patient-specific development of PDX and PDX\textsuperscript{CSC} cancer models is expected to facilitate the discovery of novel CSC markers that could aid in the early clinical detection of cancer metastasis in patients who have presented with primary tumors.

The overall tumor growth in the animal models incorporates a cooperative paracrine signaling between the host animal cells and the transplanted guest human tumor cells, including the CSCs. It is also unknown if the crosstalk between cells of mouse stroma and the human tumor cells in the PDX cancer models are comparable to that seen in real patient tumors with only human stroma in reference to the patient-specific CSCs. Moreover, since murine and human cytokines are not the same, any observed results related to signaling in the animal models must be carefully verified with the tumor-derived cells from an actual patient as soon as they are available. Thus, it may be important to employ animal models that are humanized to express a relevant set of human cytokines which are known to participate in the dysregulated proliferation and aberrant differentiation of CSCs. Nevertheless, the live biobanking of tumor tissues through the proposed PDX models unveils a paradigm shift in the development of patient-specific individual cancer treatment strategies, as well as in the field of next-generation tissue biobanking, which is Live Tumor Tissue Banking. Recently emerging next-generation targeted genome editing approaches involving Talens, CRISPR/Cas, etc., may also be extended to the above-mentioned PDX\textsuperscript{CSC} cancer models for validating the targets and perhaps for correcting the cancers, at least in the in vivo model systems. But the challenge still remains as to how to target the CSCs specifically in solid tumors, which brings us back to the important question of what are the CSC-specific cell surface markers that may be exploited in this important goal.

Finally, the live biobanking approach described above for generating viable tumor tissues also has the potential for a broader

Figure 2. A schematic for strategic development of live biobanking of patient-specific and disease-stage specific tissue, including therapy-resistant PDX and PDX\textsuperscript{CSC} cancer models, starting from patient diagnosis and proceeding through warm autopsy. For color detail, please see color plate section.
applicability well beyond the realm of its suitability to cancers, and it may eventually open up “cure models” of diseases in general.

**Therapy Resistance—How to Understand the Problem?**

Currently, the major challenge in treating cancers hinges upon whether the minimal residual disease (residual cancer) and the development of therapy resistant disease were the consequence of context-dependent emergence of therapy resistant clones by acquiring fresh mutations or were due to therapy-dependent activation of definite subsets of cancer stem cell pools that were otherwise dormant and now resulting in generation of parent-like differentiated heterogeneity. Thus, it is expected from the current treatment strategies that we could encounter a sort of heterogeneity even within an observed therapy resistance (resistance heterogeneity). Targeted therapies developed to date against activation of a given signaling pathway intermediate (e.g., mutant clone 1; MC1) are of only transient benefit to patients, because they invariably develop resistance followed by an emergence of new mutant clones (mutant clones: MC2 and MC3). It is unknown how exactly such therapy-resistant clones emerge, bypassing the same pathway intermediates during tumorigenesis. Most importantly, it is unknown why the new clones (MC2 and MC3) are prevented from emerging before targeted therapy against MC1. It also remains unknown whether the new MCs emerge as a result of newly acquired mutations or if they proliferate afresh from the pre-existing mutant clone. In the former scenario, any other non-tumor stromal cells could also acquire such new mutations, whereas in the latter scenario, the newly emerged clones MC2 and/or MC3, etc., may have been inhibited by the functional MC1 as modeled in Figure 1C.

Thus, it will be interesting to discover if one mutant clone affects the proliferation of other mutant clones and thereby impact overall tumor development in a dynamic fashion. This is likely to contribute to a possible altered tumor heterogeneity and thereby a sort of resistance heterogeneity. For example, if the MC1 is exerting a hierarchical control on the expression of MC2 and then MC3, targeting the MC1 will only increase the levels of MC2, but not MC3 as depicted in the microenvironment A of Figure 1C. On the other hand, if the MC1 is exerting a parallel control on the expression of MC2 and MC3, targeting MC1 will increase both the levels of MC2 and MC3, as depicted in the microenvironment B of Figure 1C. Alternatively, it also may be possible that newly emerged targeted therapy-resistant clones (MC2 and MC3) are derivatives of different subsets of CSC pools (CSC2 and CSC3) that were otherwise dormant until the CSC1 progeny were functionally inactivated or eliminated during primary targeted therapy. Because the targeted therapy-resistant clones eventually re-create the tumor heterogeneity comparable to the original parent tumor, which is also the characteristic of stem cell multipotency, the therapy resistance may be of the stem cell origin. Thus, it becomes important to delineate the hierarchy of CSC subsets and their combined complex interactions associated with the functional development of tumor within the context of its microenvironment (Figure 1C; Compare Microenvironment A versus Microenvironment B). One can execute more intelligent experiments if one subset indeed exerts control over the other subsets of CSCs.

Finally, it will also be important to understand the effects of targeting individual mutant clones on the overall metabolism of the CSCs in their respective microenvironments. For example, it is unknown if targeting CSCs in their original tumor microenvironment will require different ammunition from what would be required after targeting the predominant MC1-3/CSC1-3 in the bulk tumors. The reason I highlight this point is to emphasize that we should not exclude any potential direct cross talks between CSCs and their differentiated progeny, irrespective of the tumor stroma.
Thus, many targeted therapies developed to date, based solely on bulk tumor analysis, may have to be re-examined if we hope to develop durable therapies against the cells of cancer origin in the actual clinical settings of the patients.

I have also developed a simple small-scale protocol to screen for small molecule inhibitors against CSCs. The assay uses the stem cell self-renewing characteristics such as sphere formation in vitro to identify and functionally test the self-renewing properties of single cells harvested from bulk tumor samples (Figure 1D). This assay can be used to screen various candidate molecule inhibitors in search of compounds that specifically disrupt the sphere-forming capacity. We have previously verified this principle in human CSCs isolated from primary spheres to secondary sphere formation assays and/or to direct functional tumor-initiating transplantation studies. Thus, the lack of sphere-formation and/or tumor-initiation in xenografts will be the preliminary test of efficacy for any compounds that specifically target CSCs. Moreover, the drugs that affect CSCs can be distinguished from those that act on bulk tumor without interfering with tumor relapse. The targets of such CSC specific drugs would likely have long-term clinical benefit in patients, and we have demonstrated their effect directly on tumor initiation in the human patient prostate CSC-derived xenograft (PDX prostate CSC) cancer model system (http://www.nature.com/ncomms/journal/v2/n1/full/ncomms1159.html), along with our ongoing studies in human patient breast CSC-derived xenograft (PDX breast CSC) cancer model system.

Nevertheless, it may not be purely hypothetical to consider that comparable to bacterial quorum sensing, whereby the bacteria use concentration of signaling molecules released in their environment to sense the number of bacteria and function, the unaffected CSCs or MCs may orchestrate their function depending on their altered microenvironment in the tumors undergoing targeted therapies (Figure 1C). Importantly, clinical data exist suggesting that, in addition to the mutational status of a tumor cell, the age of the patient also plays a role in manifesting the cancer development. Thus, identifying the role for age-dependent changes in the tumor microenvironment and its consequences on the function of CSCs will be other important avenues to pursue further investigations for developing successful and durable therapies to cancer patients.

**Integrating CSCs with the Mouse Hospital Concept and the Prospects**

The above critical appraisal is aimed at targeting CSCs in vivo, which relies on precise identification of unique cell surface markers associated with the CSCs (http://www.genomeweb.com/proteomics/sloan-kettering-team-ids-non-psa-producing-cells-potentially-linked-prostate-can); this, in turn, facilitates their prospective purification, which eventually paves the way for discovery of novel biomarkers and/or also functional therapeutics. Furthermore, once the unique cell surface markers in the functional CSCs are identified, the patient’s own T cells can also be reprogrammed to target the CSCs. This recently developed approach may be another worthy avenue to expedite, especially in the context of the dynamic nature of cell surface markers expression in CSCs. Thus, debulking of the bulk tumors (largely by surgery) followed by the therapeutic targeting of CSCs by one or more methods, like those suggested above, would be a productive approach to the prospective and successful treatment of cancer patients. Single cell genome sequencing, and analyses of the epigenome, kinome, proteome, and metabolome in CSCs and in PDX CSC cancer models are expected to provide a hitherto unrecognized landscape of tumor initiation mechanisms and lead to treatment strategies that can successfully and predictably eliminate patient tumors.

All the above discussion calls for the functionally focused specialization in the existing bulk tumor based-mouse hospital concept, which may be referred to as ‘PDX CSC specialized -mouse hospital’. Here, each type of PDX CSC cancer models could
be a representative of the given type of cancer patient and the overall infrastructure being live tumor tissue resource of human cancers and their CSCs for translational studies. In the bulk tumor tissue based mouse hospital approach, the same structure and procedures as in phase 1 and phase 2 human clinical trials are performed simultaneously in mouse models and humans. As the clinical trials in mice are expected to move faster, integrating such data as a predictive of human response needs many other careful considerations. For example, a group of independently developed PDX and PDXCSC models of a given cancer type may apparently reflect the comparable intertumor heterogeneity as expected among the real life patients of given cancer types; but, the mouse tissue heterogeneity is also added up into the same tumor models. Thus, the signaling interplays between the mouse and the human cells, their influence on the overall PDX development, and their therapy response may have to be carefully considered before exploiting the mouse clinic conclusions for clinical trials in human patients. Otherwise, we may continue to cure cancers in mice without similar impact in human patients especially when we are convinced of patient specific individualized therapies for cancers that did not have contribution from animal signaling cross talks. A simple take-home lesson would be to employ functionally validated CSCs to generate harmonized PDXCSC cancer models, engineered to express relevant human cytokines and make them available to all researchers globally as a common platform for verifying the consensus and for making new discoveries. Any preclinical treatment data derived from these humanized mouse models of PDXCSC may guide expectations for treatment outcomes in patients and, thus, the models serve as extremely valuable tools for achieving successful clinical outcome. We have just began state of the art preliminary studies standardizing the PDX and PDXCSC cancer models for noninvasively determining the growth, permeability, and metabolic status of the tumors growing in orthotopic sites and their metastatic spreads as means to gauge therapy responses. The therapy response characteristics of the PDXCSC models of a particular cancer type to a particular patient CSC-specific therapy protocol in the mouse hospital would enhance the chances for developing precise, successful, and timely treatment options to the respective patients in the companion human clinics. Thus, the above proposed studies and ideas on the structurally integrated and yet parallel settings of human patient clinic and PDXCSC-mouse hospital could bring our clinical decisions much closer to accomplishing a cure for all cancer patients.

Classification of the Chapters

The following chapters have been written by an excellent group of researchers and scholars who discussed many of the queries and topics mentioned above. I hope that many readers will be also inspired by the provocative hypotheses and novel ideas in the following chapters. For the convenience of readers, I have classified the 35 chapters in this edition into following 6 sections:

1. Essentials of Cancer Stem Cells and Conceptual Modeling (Chapters 1–10),
2. Stem Cells in Liquid Tumors (Chapters 11 and 12),
3. Stem Cells in Solid Tumors (Chapters 13–18),
4. Cancer Stem Cells in Tumor Metastasis Perspective (Chapters 19–22),
5. Novel and Potential Targets in Cancer Stem Cells (Chapters 23–31), and

Thank you for your interest in the book and your appraisals are welcome. Vinagolu K. Rajasekhar Email: Dr.VKRajasekhar@gmail.com or Vinagolr@mskcc.org