Cancer stem cells
A nuanced perspective
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Q: What makes the cancer stem cell (CSC) paradigm a compelling focus of research?

Dr Rich: Although studying tumor cell proliferation and survival is important, what really kills our patients are tumor behaviors, like immune system evasion, therapeutic resistance, invasion into normal tissues, angiogenesis, and metastasis. All these behaviors are more likely to occur with a CSC phenotype because these cells are most likely to drive metastasis, resist therapy, and, similar to normal stem cells, modulate host-organ function. CSC functions drive important factors affecting patient outcomes.

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Dr Matsui: Tumor relapse is one reason why the eradication of the primary tumor does not necessarily translate into longer patient survival. A therapy-resistant subpopulation of tumor cells, CSCs, support tumor relapse because of their ability to regrow tumors after long periods. Yet, current drug development models focus on the short-term goal of attacking bulk tumor cells and decreasing the size of tumors. So, if a drug or a therapeutic approach does not reach that benchmark, its development may halt. By studying CSCs and their regulation, we may identify ways to attack the tumor bulk, as well as the CSCs themselves, and drive improvement in overall survival.

Dr Rich: A significant shortcoming of drug development is the overemphasis on reproducibility of consistent drug responses and the Darwinian fittest-will-survive model. Although important, this strategy neglects the fact that the complexity, adaptability, and plasticity of cancer cells are why the disease is so terrible. This may be why the endpoints we measure do not necessarily correlate with survival. So, from drug development all the way to understanding how cancer cells may function in our patients, we are not asking the right questions in the right way.

Q: What signaling pathways have emerged as central players of self-renewal, differentiation, and epithelial-mesenchymal transition (EMT) in CSCs?

Dr Matsui: There are no CSC-specific signaling pathways. CSCs “borrow” from a wide variety of known pathways. Normal stem cell pathways are used or hijacked by cancers to generate stem cell-like functions, such as pluripotency modulation to affect cell fate decisions in tumor development. CSCs also use developmental pathways because generating a tumor is similar to generating an organ. Growth control pathways known to be important in bulk tumor cell function may play a role in CSCs, as well.

Dr Rich: Important signaling pathways in CSCs include the Hedgehog, Wnt, and Notch pathways. Signaling mediators such as STAT3 and PI3K are important, also. Some have shown substantial promise in preclinical settings and even in some clinical settings. These signaling pathways may also be functional in bulk tumor cells, but we are finding certain signaling aberrations to be particularly more important to the CSC population. Preclinical studies indicate that targeting these aberrations in CSCs is more effective in controlling disease than targeting just the bulk tumor cells.

It is important to note that in most adult patients in whom cancer is most frequent, some developmental pathways are less active. So, the reality is that we are not necessarily looking for CSC-specific molecular targets, but rather the appropriate therapeutic index.

Dr Chang: Much of the current CSC research focuses on aberrations of normal stem cell signaling pathways, such as Notch, Wnt, and IGF. Many of these signaling pathways are known to function in both CSCs and their differentiated progeny, implying that certain pathways may underlie stemness plasticity and EMT plasticity. This is important because inhibiting EMT pathways and amplifying mesenchymal-epithelial transition pathways may limit dedifferentiation of nonstem cancer cells and increase the sensitivity of CSCs to conventional therapies.

Q: What insights from your work or that of others have illuminated how CSCs and their microenvironments contribute to intra- and intertumoral heterogeneity?

Dr Rich: Most cells in the body are like real estate agents—they are sensitive to location, location, and location. But one characteristic that makes CSCs so dangerous is that they actively remodel their environment to suit their needs. For example, CSCs can induce the growth of new blood vessels or modify the extracellular matrix. Also, cancer cells can be highly adaptive to their changing environments. Low oxygen, low pH, and low
nutrient levels cause necrotic “dead” zones in the tumor. These have been thought to be a consequence of cancer cells outgrowing their blood supply. However, cancer cells may drive themselves toward this environment by using CSCs as an adaptive stress response to these environmental conditions, which are similar to those of a developing fetus.

Indeed, our research indicates that necrotic regions in brain tumors are actually the best places to find CSCs. This cancer-cell-adaptive aspect may, therefore, have direct clinical relevance because development of localized therapies may not be focused on necrotic tumor regions. Rather than being a point of weakness, these extreme environmental conditions may make the tumor stronger by providing a niche that supports CSC growth. So, tumors may use the “what does not kill us will make us stronger” model of growth.

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**Dr Rich:** Our work, along with that of others, has shown that hypoxia is one of the key drivers of tumor heterogeneity. As CSCs have been shown to thrive in hypoxic microenvironments, heterogeneous oxygenation areas within a tumor may allow for variations in the levels and types of microenvironmental cytokines and inflammatory cellular infiltrates, leading to tumor heterogeneity and treatment resistance.

**Dr Matsui:** It is important to note that CSCs may work in concert with nonstem cancer cells to mold the microenvironment. Our laboratory has shown that nonstem multiple myeloma plasma cells induce bone marrow stromal cells to secrete a cytokine that increases CSC self-renewal and clonogenic potential. Our model proposes that CSCs go on to differentiate into nonstem multiple myeloma cells, thereby completing a cytokine-mediated, feed-forward loop, which results in the expansion of CSCs and differentiated cancer cells, along with greater alteration of the microenvironment. Such complex interactions afford good opportunities to consider new strategies to simultaneously target CSCs, tumor bulk, and microenvironment.

**Q:** How do CSC properties differ between the primary and secondary tumors? How do these differences inform knowledge about disease progression including recurrence, resistance, and metastasis?

**Dr Chang:** This is a difficult question. Clinically, we know that treating metastatic disease is very different from treating the primary tumor. We know the outcome of metastatic cancer is very different from that of early-stage primary cancer. For example, when confined to the breast, breast cancer is curable. But, if you find even one cancer cell in the bone or liver, that cancer no longer becomes curable, at least in the traditional sense. We have some data that lead us to believe that CSCs are more upregulated in metastatic tumors. But, until we have more data from paired primary and metastatic biopsy samples from the same patient, we would not have definitive answers.

**Dr Matsui:** Microenvironmental differences may partially account for differences in CSCs in primary tumors versus those in metastatic tumors. Understanding how changes in the microenvironment of the primary tumor may trigger initial metastasis is important to understanding how cancer progresses. We need to consider, too, that CSCs in transit may be even more different than those at the metastatic site.

**Dr Rich:** Studies have shown that for some types of cancer, the genetic profile of the metastatic tumor is completely different from that of the primary tumor. CSCs may allow for this diversity because of genetic changes conferred by exposure to therapy and inherent intratumoral heterogeneity. Importantly, studies indicate high levels of stennis among clusters of metastasis-inducing circulating tumor cells. Collectively, this implies that CSCs may drive metastasis as their genetic profile changes. This may alter therapeutic approaches to account for CSCs as genetically “moving targets.”

**Dr Chang:** I agree. Circulating tumor cells, either single cells or clones—are they different from the primary tumor and metastatic tumors? That is an important hypothesis and probably true.

**Q:** What are the challenges of translating CSC research from the laboratory to the clinical setting?

**Dr Rich:** Interlaboratory variability in how studies are conducted and the techniques used are an ongoing limitation. This may explain some inconsistencies we have seen in CSC studies. A more fundamental challenge is, however, how to replicate accurately in the laboratory a tumor’s biology and microenvironment as it exists in a patient. To that end, the CSC research field has seen some improvement of the in vivo models.

For example, our laboratory has shown recently that organoid cultures of patient-derived glioblastoma CSCs more accurately recapitulate parental tumor heterogeneity compared with that of patient-derived glioblastoma CSC sphere cultures. So, there is growing enthusiasm for model development. But it will be challenging to develop models that truly allow for personalized medicine because of factors such as heterogeneous CSC populations within and between tumors and a lack of uniform CSC biomarkers. As a result, therapy development may need to focus on pan-CSC treatment strategies or combination treatment strategies, in which several distinct CSC populations are targeted.

**Dr Chang:** Although it is difficult to replicate in the laboratory what happens to CSCs in patients with many other types of tumors, studying breast cancer is less difficult. For example, getting repeat biopsies in metastatic lung cancer is almost impossible. But, we are seeing a very close correlation between in vivo models in our patient-derived xenografts and what happens clinically to CSCs.

**Dr Matsui:** Another major challenge in transitioning CSC-directed agents and strategies into the clinic is that we do not really know what the best clinical endpoints to study are. A common criticism of the CSC paradigm is that if these cells truly are the drivers of cancer development, then overall survival is an appropriate endpoint. This is the “cleanest” and most important clinical endpoint. But, this is more appropriate in the late stages of drug development used in large randomized trials. Although bulk tumor reduction is commonly used as a shorter-term endpoint, CSCs are rare and slow-growing. Overall survival is, therefore, not the easiest metric to confirm that a CSC therapy is active. More clinical development of CSC biomarkers is needed to survey quantify changes in CSC populations to support drug development in shorter, early-phase trials of CSC therapies.

**Dr Chang:** Endpoints more specific to CSC biology, such as time-to-second—or subsequent metastasis, may be more appropriate than endpoints used to measure the efficacy of conventional therapies. Measuring onset of new metastases may be more important than measuring tumor shrinkage. In addition,
complementary assays that, for example, determine the number of functional CSCs in biopsy samples are needed to gauge the robustness of clinical endpoints of CSC therapies.

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Q: Do you see the targeting of CSCs becoming part of standard multimodality therapies?
Dr Chang: Since CSCs play a role in promoting treatment resistance, CSC-directed therapies will be useful as combinatorial anticancer agents with conventional therapy to limit resistance.

Designing appropriate CSC-directed therapies that sensitize the cells to conventional therapy will help to make combination therapies more effective. For example, it is known that autophagy supports CSC maintenance and that the epigenetic profile of CSCs is different from that of nonstem cancer cells. Our laboratory has shown that breast cancer CSCs can be sensitized to conventional therapy in vivo through autophagy inhibition and epigenetic reprogramming. If similar sensitivities are functional in the CSCs of patient tumors, the combination of CSC-sensitizing or -differentiating drugs with conventional therapies may be a promising treatment strategy.

Dr Matsui: Developing CSC-specific treatments is becoming a necessity. Therapeutic strategies that more specifically target CSCs may, for example, exploit immunogenic differences between CSCs and tumor bulk cells, or may be more effective in extremely slow-growing tumors. Of course, targeting the tumor bulk is also important because the tumor bulk is likely what makes cancer patients sick. Therefore, it may be important to treat the tumor bulk first to provide enough time to lessen disease severity.

Multimodality therapies will require using agents that can target multiple compartments within a tumor in appropriate sequential or concurrent regimens. I believe that CSC-directed therapy and conventional therapy combinations will likely be part of the entire anticancer armament we use to treat or cure our patients’ cancers.

Q: What are some other outstanding questions about CSCs that need to be answered?
Dr Rich: Those of us working in the CSC field must hold ourselves to the highest standards of research because our patients deserve it. The complexity of CSCs actually represents the complexity of cancer itself. Clearly, we need better models. It requires using patient-derived tissues. We need a systems approach. We tend to take an analytic approach. We need to realize that the biology of cancer—biology of everything—is a system. Everything interacts. If we ignore the interactions, we cannot truly understand how the entire system works.

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Dr Matsui: I agree. We must accept that there are differences from CSC to CSC. There are differences in the environments, even within a tumor, as well as between patients. You need to understand how adaptability is driven and works—by treating patients, doing surgery, or by cells outgrowing their micro-environments. If you understand how cells will behave in a dynamic way to different stimuli, I think it will provide the potential to corral them and push them into a direction where you can get them. Studying only 1 or 2 aspects of the dynamic biology of CSC, I believe, will probably not give us the clinical advancement we desire.

Q: What other aspects of the CSC field are important to communicate to oncologists?
Dr Matsui: Remember, we fear tumor cells because of their functional capabilities. Most cancer research has focused on proliferation and cell survival as ways of trying to destroy tumor cells. But, we know that many patients with advanced disease do not do well, even when traditional therapies have provided good responses. I believe CSCs explain many events, such as metastasis, relapse after complete remission, and drug resistance, that oncologists face in clinical practice.

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Dr Rich: Oncology requires partnerships between clinicians, scientists, and the pharmaceutical and biotech industries. We all have a great deal of work to do. Certainly, CSCs show the complexity of our challenges. But, I think they offer opportunities to create paradigms in which everyone is working together. Practicing oncologists, who at this point might consider themselves a bit separate from these concepts, are making real-life observations that are moving the field forward.

Dr Matsui: Although treatment is our highest priority, I think that there are other clinical aspects to the science behind CSCs that can be useful now. I think understanding a tumor’s CSC profile, and how it drives disease, may become a useful prognostic indicator.

Dr Chang: I believe that the relationship between immunoncology and CSCs is the next frontier in cancer research. We now understand that the host immune system, including tumor-infiltrating lymphocytes, macrophages, and neutrophils, is of critical importance. Understanding that in relation with CSCs and heterogeneity will be critical in understanding and treating solid and, probably, liquid tumors.