Cell Stem Cell
Meeting Report

Cancer and Stem Cell Biology: How Tightly Intertwined?

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Ever since the discovery of cancer stem cells in leukemia and, more recently, in solid tumors, enormous attention has been paid to the apparent stem cell nature of cancer. These concepts were the focus of the "Stem Cells and Cancer" symposium held recently at the University of California, San Francisco, and the inspiration for this overview of current research and important questions emerging in this area.

This year's annual UCSF Helen Diller Family Comprehensive Cancer Center Symposium focused on stem cells and cancer. This symposium provided a representation of all facets of stem cell biology: from the role of stem and progenitor cells in development to adult tissues, normal stem cells versus cancer stem cells, diverse model organism systems, and translational studies. In the current era, postidentification of some types of cancer stem cells, expectations surrounding the discovery of these important cancer cells are high (for example, see Al-Hajj et al., 2003; Reya et al., 2001; Wang, 2007). In particular, it is now presumed that we will be able to trace cancer's normal cell of origin, identify molecular pathways in tumorigenic subpopulations, not just the bulk tumor sample, and most importantly, develop new treatments that will lead to more durable and widespread cancer cures by targeting cancer stem cells. We focus here on the crucial questions raised during the insightful discussions that took place at the meeting.

Cancer Stem Cells and Cells of Origin

One of the areas that remains an important, yet unanswered, question is what are the normal cells of origin for cancer and their relationship to cancer stem cells. A clear distinction was made in several talks between the cells that give rise to the first form of the tumor (cells of origin), which may be stem cells, progenitor cells, or differentiated cells depending each tumor type, and the cells that propagate the tumor phenotype and exhibit self-renewal and differentiation capacity (cancer stem cells or tumor-initiating cells). For example, Irving Weissman (Stanford University, CA) showed his group's viewpoint, illustrating how different cell lineages in the hematopoietic system fit into the traditional multistep cancer progression model often referred to by cancer biologists as the Vogelgram (Fearon and Vogelstein, 1990). According to the minimum seven-step "Weissmangram," (Rossi et al., 2008) the earliest step on the path to hematopoietic malignancies must take place in the hematopoietic stem cell (HSC) population that exhibits the self-renewal properties and longevity required for an accumulation and maintenance of mutational events. The limited life span of progenitor cells combined with their inability to self-renew precludes eventual cancer progression unless additional mutations or epigenetic events that confer these properties accumulate. When these additional events occur in progenitor cell populations derived from mutant HSC clones, cancer progression occurs. Thus, the phenotype of the cancer stem cell may be more similar to the normal progenitor population than to the HSC itself. There is evidence that this concept holds true in mouse models of solid cancer (Joseph et al., 2008; Zheng et al., 2008). The mutations or epigenetic events needed for this process include those that confer avoidance of the immune response and apoptotic evasion, among others. This model highlights the principle that the phenotype of the cell of origin need not match that of the cancer stem cell.

These ideas are also in line with recent data from Michael Clarke (Stanford University, CA), who showed that in mice triply deficient in *Trp53, Ink4a*, and *Arf* expression, long-term hematopoietic engraftment is served by cells that phenotypically resemble multipotent progenitors, cells that are not normally capable of self-renewal (Akala et al., 2008). These data suggest that progenitor cells can acquire self-renewal ability if they receive the correct combination of genetic alterations. Normally, with progressive differentiation, there is restriction of self-renewal potential, but the combined loss of cell-cycle regulators allowed self-renewal to be reinstated in more mature cells, such as multipotent progenitors, but not in cells further down the lineage hierarchy, such as common myeloid progenitors. Also fitting with this concept, data and questions from several talks, including those from Sean Morrison (University of Michigan, Ann Arbor, MI) and Meenhard Herlyn (Wistar Institute, Philadelphia, PA), raised the possibility that the phenotype of the cancer stem cell from early-stage lesions may be distinct from the phenotype of cancer stem cells from advanced tumors.

The logical extension of this model, in that it combines cell and molecular biology of cancer cells, was also one of the first discussion points raised at the Symposium: the hard question that arises is whether cancer stem cells, assuming they exist, will be too difficult to target. More precisely, if the cancer stem cell phenotype can change as tumors evolve, how can we expect to successfully target this population to achieve more effective therapy? Could the non-stem component of the cancer cell population evolve to acquire stem cell properties? The question was posed with the assumption that during the initial stages of

tumorigenesis, the lesion is relatively homogenous, but that heterogeneity can arise during tumor progression. Sean Morrison pointed out that we do not yet know whether the events that accumulate in the cancer stem cell subset, or the non-stem cell component of the tumor population, arise due to epigenetic or genetic mechanisms. Certainly, determining the relative degree of reversible versus irreversible alterations will be important to address this question. Gerard Evan (University of California, San Francisco, CA) posed a related question, in that he questioned why only some cancer cells are self-renewing, and asked what might distinguish the non-stem-like cells in a tumor that might evolve to exhibit cancer stem cell activity from other subsets that cannot? Finding a mechanistic basis for cancer stem cell functions will be key advances required to answer these important questions.

Cancer Signaling Pathways

Several key concepts that emerged during the meeting were identified as crucial for our understanding of how molecular pathways regulate normal tissue stem cells, cells of origin, and cancer stem cells. One theme that reappeared in several presentations is the importance of understanding the coordination of different pathways in a particular stem cell type. While many individual pathways have already been linked to stem cell regulation, such as the Wnt, Hedgehog, Notch, Hippo, etc. cascades, as demonstrated by Yuh Nung Jan (University of California, San Francisco, CA), Thomas Rando (Stanford University, CA), Judith Kimble (University of Wisconsin, Madison, WI), and many others at the meeting and in published literature (reviewed in Blanpain et al., 2007), it is clear that simply considering each pathway in isolation will result in missing the big picture. Instead, we must now consider that most of these pathways have coordinated efforts and crosstalk. As Elaine Fuchs (Rockefeller University, New York, NY) articulated, a number of these pathways may function to reciprocally control stem cell activation versus stem cell quiescence and tipping this balance may result in inappropriate stem cell activation. As further discussed by Thomas Look (Harvard Medical School and Dana-Farber Cancer Institute, Boston, MA), the overlap of key molecular pathways and their relationship to mutational patterns found in cancer cells has important implications for therapy. A detailed analysis of collateral pathway defects in cancer is required to understand how stem cells are regulated and to effectively treat cancer. For example, use of gamma secretase inhibitors to inhibit activated Notch signaling is unlikely have a therapeutic effect on tumors that have deleted *PTEN*, given that in such cells, the Akt pathway dominates control over the proliferative state. It likely will be useful to combine multiple drugs that antagonize individual pathways in order to target cancer stem cells. This goal is complicated by the need to simultaneously ensure that the various signaling cascades required for normal tissue homeostasis are maintained intact and highlights a specific challenge facing the field.

A second emerging concept in attempts to elucidate stem cell molecular control is that regulatory pathways and signaling events are not simply on or off, but rather are fine-tuned with thresholds to achieve a desired set point. Abrogation of stem cell maintenance or function can occur both in settings of excessive pathway stimulation and also in response to the complete elimination of a pathway; the correct concentration and localization of regulatory molecules is critical for proper stem cell activity. This concept has been beautifully demonstrated in numerous studies of morphogen gradients during development, and now is relevant at minimum for the Wnt, Shh, and Bmp cascades, as described by Thomas Rando, Arturo Alvarez-Buylla (University of California, San Francisco, CA), Elaine Fuchs, and Ron DePinho (Harvard Medical School and Dana-Farber Cancer Institute, Boston, MA). Alvarez-Buylla's work reveals that disruption of a structural signaling hub, the primary cilium, through *Kif3a* mutation blocks Shh signaling and results in defects in the proliferative zones of the mouse brain late in development (Spassky et al., 2008). It will be interesting to determine if other stem cells use similar specialized cell structures to translate changes in the extracellular milieu into modified intracellular signaling responses. The idea of exquisite regulation of levels of signaling events has been even more directly shown to be related to cancer biology, as low levels of Ras and downstream receptor tyrosine kinase signaling stimulates proliferation while higher levels initiate cell-cycle arrest and, in some cases, senescence (Dankort et al., 2007). As mentioned below, the manipulation of reversible changes in stem cells will depend on the development of experimental systems capable of "dialing" the levels of gene products in vivo and in culture settings. Regulation of gene expression by endogenous microRNAs is hypothesized to operate based on such a rheostatic mechanism, and the mounting evidence that microRNAs regulate differentiation may be one major mechanism that links these concepts (Bartel and Chen, 2004; Wang et al., 2007).

A third aspect of the molecular regulation of stem cells that received emphasis at this meeting was the importance of the pathways that regulate the stem cell niche (Sneddon and Werb, 2007). The niche can be defined as the microenvironment in which the stem cell resides that specifies lineage potency and self renewal. The niche is defined both by surface molecules expressed on adjacent cells and by soluble and adherent components in the extracellular surroundings. Therefore, the niche model defines methods of extrinsic regulation of stem cell behavior and position. This concept clearly spans stem cells in distinct organisms and diverse environments during development, after injury, with age, in cancer stem cells and also raises therapeutic implications. The niche can actually specify the numbers of stem cells, as was demonstrated in work from Judith Kimble. She described how a single cell in the distal tip of the *C. elegans* germ cell organ controls the number and location of germ line stem cells and presented evidence that Wnt signals participate in this regulation. Using another model, Thomas Rando indicated that Wnt can be involved in the extrinsic suppression of muscle stem cell function with age and also showed that the dominant pathway controlling this population switches from Notch to Wnt during the aging process (Brack et al., 2007, 2008). An obvious implication of niche-mediated control of stem cells is that non-stem cells may acquire stem cell properties if they happen to fall into the correct niche. Therefore, it seems conceivable that cancer stem cells could emerge if non-stem cells encounter an optimal microenvironment within the tumor mass or in surrounding normal tissues. In other words, an extrinsic "reprogramming" of more committed bulk cells back into stem cells may be possible. Given that cancer stem cells often exhibit blocked differentiation, this reversion process

may occur more easily in non-stem cancer cells. Stuart Orkin (Harvard Medical School, Dana-Farber Cancer Institute, and Children's Hospital, Boston, MA) stressed that reprogramming of normal cells into ES-like cells may require both driving the pluripotent state and simultaneously preventing differentiation. Coordination of these two processes may be achieved in response to altered expression of epigenetic regulators, some examples of which have been shown to be elevated in cancer. Reprogramming requires a precise orchestration to balance pluripotency with differentiation, and perturbation of this balance could lead to the acquisition of aberrant stem cell programs in non-stem cells. Fred de Sauvage (Genentech, Inc.) presented some of his company's recent progress in developing cancer therapeutics based on Hedgehog pathway functions in cancer cells and in niche cells. His comments underscored that future therapeutic manipulations will need to consider the combined molecular aspects of niche and stem cell regulation.

Technologies Needed

Beyond all the insight gained from the studies described above and elsewhere, it is clear from the panel discussion that new technologies are needed to elaborate on the intersections of stem cell biology and cancer. First, as discussed by Sean Morrison, Zena Werb (University of California, San Francisco, CA), Shahin Rafii (Weill-Cornell Medical College, New York, NY), and others, there are considerable technical limitations inherent in the assay(s) used to detect cancer stem cells. The typical method involves cell sorting based on surface molecule expression and subsequent transplantation of the isolated population(s) into immunocompromised mice (Purton and Scadden, 2007). However, this paradigm cannot fully predict the potential of a given population, or subset therein, in human subjects or in the absence of manipulation and, thus, may not reflect the true activity of the putative cancer stem cell under examination. For example, improved animal models and culture conditions will depend on new developments in order to mimic the stromal components that accompany cancer cells in their normal environment. One solution discussed by Max Wicha (University of Michigan, Ann Arbor, MI), particularly for breast cancer stem cell work, was his laboratory's use of the protocol established by Kupperwasser et al. to "humanize" the mouse mammary gland. In this model, human mammary gland stromal cells are introduced in the graft to encourage the growth of both normal human mammary gland stem cells, as well as breast cancer samples from patients (Kuperwasser et al., 2004). Possible technical advances that may contribute to the design of improved animal models include the development of polymers, growth factors, or other reagents. When combined with the identification of appropriate tissue-specific stromal components and the establishment of methods for their purification, this collection of materials will likely be useful for studies using human samples. Clearly, advancing the ability to mimic the stroma of stem cells will require a more thorough characterization of the tumor microenvironment and the niche for normal stem cells. Until improved, humanized models are available, the study of endogenous murine tumors, which can more easily be manipulated in syngeneic and/or immune compromised murine transplant recipients, will be critical to evaluate in parallel with patient tumor samples, as demonstrated by Jeff Rosen (Baylor College of Medicine, Houston, TX). As well, development of better culture systems that more faithfully read out stem cell behavior in precisely defined conditions is urgently needed for biologic and therapeutic studies.

In addition, discussions at the symposium often underscored that our current understanding of the nature of stem cells in multiple tissues and situations and how those populations relate to a given type or subtype of cancer is still rudimentary. Therefore, while not a specific technological development, more studies to understand the basic biology of these cells, as well as defining the hierarchy in different normal tissues, was identified as another absolutely necessary advance. As pointed out by Irving Weissman, we have yet to apply a cellular version of Koch's postulates to establish a causal relationship between a specific cell population and a specific form of cancer; we still do not yet know the precise identity of, or how to prospectively isolate, the cells that give rise to most types of tumors. It is also unknown whether the observed parallels between hematopoietic malignancies and HSCs will be relevant for solid malignancies of epithelial origin. For example, as noted by Ron DePinho, hematopoietic cells may be wired differently than other cells for apoptosis. Similarly, Zena Werb and Elaine Fuchs noted the crucial role of adhesion regulation, organization on the basement membrane, and spindle polarity in epithelial cells (for example, see Ewald et al., 2008). One particular interesting question that no attendee could answer definitively was why certain tissues seem to be resistant to cancer, despite rapid proliferation, as in the small intestine. Could there be a stem cell explanation? Although one can postulate that the colon may be particularly bombarded by environmental and bacterial toxins that are known to initiate tumors, it may also be that the small intestine has better intrinsic tumor suppressor mechanisms in its stem cells. One explanation may lie in differences in the body's intrinsic protection for individual tissues so that they cannot all be affected by the same mechanistic defect. In other words, perhaps multiple quality control mechanisms are in place in the body's stem cells. It will therefore be critical to determine the unique and important mechanisms that constrain self renewal for each type of stem cell and cancer cell in distinct tissues.

Second, a key component missing in the current cancer stem cell "tool box" is the ability to visualize both the tumor stem cells and the compartments in which they reside. Thus, new methods for imaging tumors and transplanted cells are required, along with the development of reporter genes or other biosensors that will allow for monitoring the activity of normal stem cells and cancer cells. Owen Witte (University of California, Los Angeles, CA) outlined the importance of the concept of multiscale imaging and monitoring of tumors. This model emphasizes the need to image the tumor, assess its biological activity, and to put these in perspective with whole-body, physiological readouts that can be monitored in patients to assess drug response or disease progression. One currently used imaging technology that can be included in multiscale imaging paradigms is Positron Emission Tomography (PET) (Radu et al., 2008), which allows tumor imaging based on the metabolic activity of cancer cells. PET and other imaging systems have made it evident that some tumors are rapidly growing while others are not. Ironically, the basis of this technology highlights yet another limitation in the field that was raised during the panel discussion: our limited understanding of tumor metabolism. Distinctions between

metabolic control in stem cells versus progenitor cells, cancer stem cells, or even the bulk cancer population itself have not been made. Thus, an expansion of the repertoire of established signaling pathways (as discussed above) and their impact on cell-type-specific metabolism will likely be important.

A third dire need identified for all of stem cell biology, both in normal tissues and cancerous tissues, is the ability to characterize the molecular and biochemical properties of cell populations at the single-cell level. The field also requires improved methods to isolate pure populations of defined cells. While the ability to analyze some changes in single cells may prove challenging in the short term, some existing methods might now be used to analyze small numbers of relatively homogeneous cells. For example, the ability to characterize gene expression, genetic alterations, epigenetic modifications, and posttranslational events in specific cellular subpopulations would go a long way toward answering questions related to the evolution of cancer cells and cancer stem cells. The development of genetic tools to induce reversible changes in stem cell populations and within the niche will also aid in testing the importance of specific altered events that are detected in tumor samples.

Therapeutics

One of the most beneficial aspects of applying stem cell biology to cancer research is the resulting conceptual advance with respect to designing therapeutic mechanisms to specifically reach the tumorigenic cells themselves. That is, instead of focusing predominantly on signals that regulate cell proliferation, thinking about cancer as a stem cell disease opens new pathways as potential clinical targets (Wang, 2007). Examples include self-renewal (although this process remains somewhat ill-defined at the molecular level), promoting differentiation, blocking the niche, targeting key cell surface markers, and enhancing immune surveillance. Proof of principle studies in a variety of different tumor models indicate that induction of differentiation, blocking the niche, and attacking cells expressing key surface markers are all promising anticancer stem cell strategies that can now be considered. In addition, Jeremy Rich (Duke University, Durham, NC) reviewed his recent, exciting studies that exploit distinct properties of cancer stem cells versus bulk cancer cells in their ability to promote angiogenesis and to restore radiation sensitivity. Jeff Rosen presented compelling evidence that cancer stem cells from genetically distinct patient tumors have different responses to chemotherapy. Irving Weissman also described exciting work to show that leukemic stem cells exhibit a special ability to escape immune surveillance mechanisms; this finding will be of tremendous importance if solid cancer stem cells also share the same capacity.

Conclusion

Cancer biology and stem cell biology have certainly become, and will forever be, closely entwined. A vast number of questions have arisen following the initial reports that identified cancer stem cells. These remain early days in the field, and many details and variances have yet to be elucidated. The cancer stem cell model may not apply to all human cancers, and some mouse tumors may also not fit the paradigm. What is clear, however, is that these two fields intersect and, in doing so, have engaged an increasing number of outstanding investigators eager to test the relationship using their own experimental arsenal. Discoveries made in cancer stem cell biology will certainly feed back into an improved understanding of normal stem cells and tissue homeostasis. A fresh look at cancer from a stem cell perspective promises to bestow upon the field of oncology an even deeper understanding of this terrible disease. Without a doubt, studies of cancer stem cells fall within the lines that define stem cell biology, and the discipline will not be complete without them.

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