Weischenfeldt et al. (2013) study extends a growing paradigm regarding the links between complex rearrangements and prostate carcinogenesis, while also considering the age dimension as a possible player in the spectrum of clinical features that contribute to disease biology.

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Growth Factor Receptors Define Cancer Hierarchies

Monica Venere,[1] Justin D. Lathia,[2,3] and Jeremy N. Rich[1,3,*]
[1]Department of Stem Cell Biology and Regenerative Medicine
[2]Department of Cellular and Molecular Medicine
Lerner Research Institute, Cleveland Clinic Foundation, Cleveland, OH 44195, USA
[3]Department of Molecular Medicine, Cleveland Clinic Lerner College of Medicine at Case Western Reserve University, Cleveland, OH 44106, USA
*Correspondence: richj@ccf.org
http://dx.doi.org/10.1016/j.ccr.2013.01.020

Normal and neoplastic tissues display cellular hierarchies that integrate extracellular cues to maintain tissue function through bidirectional signals mediated via cell surface proteins. Two papers in *Cancer Cell*, one in this issue (Day and colleagues) and one in a recent issue (Binda and colleagues), describe how Eph receptor tyrosine kinases critically define and regulate the growth of cancer stem cells.

Tumors display cellular heterogeneity through the integration of multiple supportive cell types—vasculature, stroma, and immune components—as well as diversity within the neoplastic compartment derived from genetic and epigenetic variability. Cancers co-opt transcriptional programs normally active in development and wound responses, processes in which stem and progenitor cells contribute, so it is not surprising that cancers display characteristics of stem and progenitor cells.

Recent data from human and murine models support the presence of cellular hierarchies in some advanced cancers with cancer stem cells (CSC) at the apex (Chen et al., 2012; Singh et al., 2004). The CSC field currently lacks a coherent set of criteria to define these cells. Many reports mistakenly hold that CSCs simply represent cells that form spheres in culture and tumors upon transplantation (i.e., tumor initiating cells). Rather, CSCs also mimic normal stem cells to create a dysfunctional cellular hierarchy with non-tumorigenic cells derived from the self-renewing CSC. To fulfill this feature, the CSC hypothesis needs to employ strategies to prospectively segregate tumorigenic and non-tumorigenic cells or perform functional lineage tracing studies. A critical aspect of these studies is the requirement to identify and separate discrete populations and perform functional analyses. In response, researchers have defined a number of surface molecules that are preferentially expressed by CSCs and can be interrogated in live cells. At first blush, the increasing number of these markers may engender skepticism as to the validity of the CSC hypothesis, but this viewpoint is derived from our desire to impose simplicity on an inherently complex and dynamic system. First,

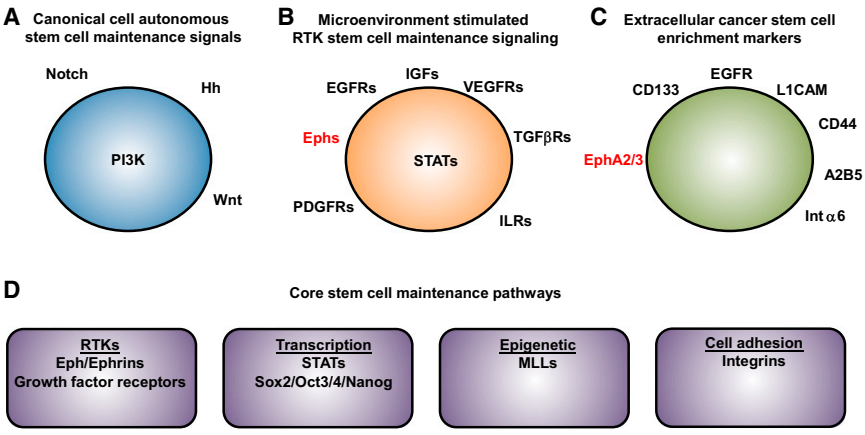

Figure 1. Intrinsic and Extrinsic Regulation of Cancer Stem Cells

(A) Schematic depicting canonical stem cell maintenance pathways shared by many normal and neoplastic stem cell systems (including Notch, Hedgehog [Hh], Wnt, and intracellular PI3 kinase signaling).

(B) Numerous microenvironmental stimulated pathways also contribute to stem cell maintenance (including Ephs, EGFRs, PDGFRs, IGFs, VEGFRs, TGFβRs, ILRs, and intracellular STAT signaling).

(C) Many of these extracellular receptors can also be used to enrich for stem cells (including Ephs, CD133, EGFR, L1CAM, CD44, A2B5, and integrin α6).

(D) Taken together, these factors contribute to the establishment of core stem cell maintenance pathways consisting of receptor tyrosine kinases (RTKs), transcription factors, epigenetic regulators, and cell adhesion.

most cancer diagnoses are based on a presumed cell of origin and pathologic features, yet no two tumors have an identical historical development, as demonstrated by the rapid definition of intrinsic molecular subtypes in many cancers. These molecular subtypes likely have differences in their CSCs, thereby limiting the universal information of a single biomarker. Second, CSCs are not a static or unitary population but rather have an evolving identity during the course of the disease driven in part by the acquisition of additional genetic lesions. The plasticity of cancer is the causative feature for why cancers of many types are so deadly.

Against this background of our inability to model the complexity of human cancers, it might seem hopeless to find utility in the study of CSCs and the cellular hierarchy. However, there are rapidly developing themes that inform possible points of fragility within cancers that may be amenable to therapeutic targeting. Whereas tissue specific stem cells have been defined as single cells with autonomous programs permitting self-renewal, sustained proliferation, and differentiation potential, this reductionist view fails to recognize that stem cells constantly sense and respond to their environment with the capacity to modify the environment either directly or through the activity of differentiated daughter cells. Therefore, it would be ideal for CSCs to activate molecular mechanisms that permit responses to stressful environments and facilitate bidirectional communication with the tumor microenvironment (Figure 1). Cells sense their microenvironment by detecting other cells through cell adhesion molecules, extracellular matrix through integins, and secreted factors through growth factor receptors. Notably, members of the receptor tyrosine kinase (RTK) family have important roles in the maintenance of the stem cell phenotype. Modulating levels of RTKs in CSCs can alter a variety of key CSC phenotypes, including the self-renewal required to maintain an undifferentiated state, tumorigenicty, and invasiveness as well as impact overall viability.

In a previous issue and in this issue of *Cancer Cell*, Binda et al. (2012) and Day et al. (2013) explore growth factor receptors that empower bidirectional communication between cells and the environment in CSC biology by defining a novel role for the Eph receptors, namely EphA2 and EphA3, respectively, in maintenance of the stem cell phenotype in glioblastoma (GBM), the most prevalent primary brain tumor. The Eph RTK subfamily has well-established roles in cancer as well as normal stem cell biology, but these studies are the first to bridge these two fields for Eph receptors. Interaction of an Eph receptor with its cell surface-associated ephrin ligand elicits bidirectional signaling between neighboring cells, although EphA2 can be activated by soluble ephrin as well (Wykosky et al., 2008). Eph signaling can influence a host of cellular processes, including migration, proliferation, and differentiation. Interestingly, depletion of EphA2 or EphA3 via ligand engagement or genetically through RNA interference drives CSCs to differentiate. Attenuation of EphA2 leads to astroglial differentiation, whereas depletion of EphA3 induces both astroglial and neuronal lineages. These findings provide support for differentiation-based therapies to target CSCs, with bone morphogenic proteins (BMP), members of the transforming growth factor-β (TGF-β) superfamily, representing the most developed driver of differentiation to date (Piccirillo et al., 2006).

Both groups demonstrate an increased tumor initiating capacity for those cells with the highest level of EphA2 or EphA3 expression supporting this population as the putative CSCs within the tumor bulk. Furthermore, cells expressing EphA3 reside near tumor vasculature, a defined niche for CSCs (Calabrese et al., 2007). This finding brings to question if an Eph-ephrin gradient might exist between CSCs and the neoplastic non-CSCs akin to that described for some adult stem cell niches between the stem and differentiated cells (Genander and Frisén, 2010). In the case of GBM, the perivascular niche may support CSCs by being devoid of the ligand, whereas differentiation could be driven by increased ephrin expression on cells further from the niche. This paradigm might not hold true with EphA2, however, as ephrin-independent oncogenic activation was shown for this receptor (Miao et al., 2009). Further exploration of the full cellular hierarchy will aid in the elucidation of the role of Eph-ephrin signaling between the CSC and non-CSC populations.

The mechanism of Eph receptors in preventing differentiation of CSCs appears to lie in limiting MAPK signaling as both groups demonstrate an increase in ERK phosphorylation upon attenuation of EphA2 or EphA3. What drives higher expression of Ephs in CSCs and whether or not there is niche contribution versus a cell autonomous stem cell maintenance program is yet to be determined.

Of note, the two studies identify different Eph receptors as key in CSC maintenance, although some level of crosstalk likely exists between the Eph receptors as well as other RTKs central to maintenance of the hierarchy. It would seem this difference could not be due to differential representation within the recently identified GBM subclasses as both have highest expression in the mesenchymal and classical groups (Verhaak et al., 2010). However, Eph receptors may be informative within these subgroups, although that hypothesis would require further exploration. Importantly, both groups validate the efficacy of targeting Eph receptors in preclinical models.

In conclusion, these two reports are not simply additions of new CSC markers but rather help reinforce expanding opportunities for integrating features of normal tissue hierarchies and instructive microenvironmental cues in tumor development and maintenance that can inform advances in diagnosis and therapy.

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Breaking News on Fragile Sites in Cancer

Thomas W. Glover[1,2,*] and Thomas E. Wilson[1,2]
[1]Department of Human Genetics
[2]Department of Pathology
University of Michigan, Ann Arbor, MI 48109, USA
*Correspondence: glover@umich.edu
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Chromosome rearrangements in B lymphocytes can be initiated by AID-associated double strand breaks (DSBs), with others arising by unclear mechanisms. A recent study by Barlow and colleagues in *Cell* reports on genomic regions, termed early replicating fragile sites, that may explain many AID-independent DSBs and creates a compelling link between replication stress, transcription, and chromosome rearrangements.

Recurrent chromosomal translocations are common features of many cancers, especially lymphomas and leukemias. Most appear to be formed by the joining of two double strand breaks (DSBs). In developing B cells, DSBs are introduced into immunoglobulin loci during V(D)J recombination and class-switch recombination (CSR). Both CSR and immunoglobulin somatic hypermutation are initiated by AID, a single-strand-specific DNA cytidine deaminase targeted to DNA by transcription (Nussenzweig and Nussenzweig, 2010). AID-associated DSBs often generate one of the two breakpoints in the translocations observed in lymphoid tumors. This programmed DNA damage also puts the lymphocyte genome at risk for rearrangements with bystander loci, such as the *C-MYC* locus. Nonetheless, while many translocations are driven by off-target AID-induced DSBs, others result from poorly defined factors that might include replication errors, oxidative stress, genotoxic agents, and involvement of chromosome fragile sites.

Common fragile sites (CFSs) have been recognized for decades as hotspots for breaks occurring on metaphase chromosomes following replication stress (Durkin and Glover, 2007). Following low doses of the DNA polymerase inhibitor aphidicolin (APH), chromosome breaks can be seen at discrete genomic regions that span hundreds of kilobases, often in large genes. CFS instability is dependent on ATR signaling and associated with other DNA damage response factors (Durkin and Glover, 2007). Le Beau et al. (1998) and studies that followed showed that CFSs replicate late in S-phase and sometimes escape to metaphase with incomplete replication. For decades, two nonexclusive models have existed for CFS instability. One is that CFSs contain difficult-to-replicate sequences, leading to stalled replication forks. The second is that CFSs contain a paucity of replication origins, leading to late or incomplete replication. Support for the former came from the fact that CFSs are AT-rich and contain a high number of "flexibility peaks" (Zlotorynski et al., 2003) capable of forming secondary structures, especially when