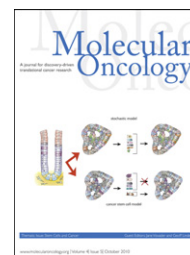


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Preface

Stem cells and cancer – The promise and puzzles

Since the declaration of war on cancer some four decades ago, there has been an exponential growth in our understanding of the genetic and cellular mechanisms that contribute to cancer. As a consequence, a number of revolutionary drugs such as imatinib and trastuzumab have emerged, representing the first wave in a new era of targeted therapy. However, the range of new weapons with a proven track record in targeting cancer is surprisingly limited, highlighting the need for new approaches to improve cancer treatment and outcomes.

The success of targeted therapies is particularly remarkable given the intratumoral heterogeneity displayed by many cancers, a feature recognized long ago by pathologists and clinicians. To date, most treatment strategies have ignored this issue of heterogeneity, and have rather utilized a uniform rather than tailored approach to therapy. This thematic issue provides a timely overview of emerging insights into understanding tumor heterogeneity as viewed through the prism of stem cell biology.

How does tumor heterogeneity arise? Tumors have long been viewed as a caricature of normal development that has gone awry. From that perspective, cellular heterogeneity represents abortive attempts by tumor cells to undergo functional differentiation. The capacity to prospectively fractionate distinct tumor subpopulations – a relatively new tool for solid tumor biologists borrowed from the hematopoietic field – has enabled researchers to dissect the issue of tumor heterogeneity for the first time. Flow cytometric analysis and cell sorting, coupled with the development of new *in vitro* and *in vivo* assays, have enabled the isolation and characterization of distinct subpopulations within tissues. This approach is proving invaluable for exploring the functional and molecular properties of discrete cellular subsets and how they contribute to cancer progression. It also has the potential to reveal important (and hitherto hidden) biomarkers relevant to tumor initiation, propagation and metastasis, thus highlighting new therapeutic targets.

Elucidating the normal cellular hierarchy within tissues is a pre-requisite to understand the ‘cell of origin’ that is targeted for neoplastic transformation and to gain insights into molecular perturbations that drive transformed cells. Stem cells, which lie at the apex of the normal tissue hierarchy, represent strong candidates as cells of origin, given that they are

long-lived and capable of self-renewal. These hallmark features are presumably necessary for the sequential acquisition of mutations or epigenetic changes that eventually culminate in cancer. Alternatively, committed progenitor cells or even mature progeny could also serve as cells of origin in cancer. Under these circumstances, cells would need to acquire stem cell-like properties, such as self-renewal. Progenitors or transit amplifying cells that are the direct progeny of stem cells represent strong candidates. Moreover, their highly proliferative nature would impart potent growth potential to cells undergoing neoplastic transformation.

The cancer stem cell (CSC) hypothesis has stimulated great interest amongst cancer biologists to explain tumor heterogeneity. This concept is quite distinct from the ‘cell of origin’ and hypothesizes that a subpopulation of cells within a tumor is uniquely capable of propagating the tumor. The stem cell-like properties of self-renewal and multipotent differentiation (at least partial) are cardinal features of CSCs. Such properties could be acquired by progenitors along the hierarchy. Alternatively, CSCs could originate in a transformed stem cell. Either way, the CSC hypothesis relies on a hierarchical model to account for tumor heterogeneity and behavior. In contrast to the CSC hypothesis, the stochastic or clonal evolution model, proposes that tumor cells are functionally equivalent in terms of their capacity to propagate tumors. However, distinct populations of cells within a tumor may respond differentially to microenvironmental factors or undergo clonal evolution, thereby accounting for tumor heterogeneity. Importantly, these two models are not mutually exclusive, since clonal evolution could equally be a property of CSCs. This hybrid concept, together with the notion that tumor cells may exhibit phenotypic ‘plasticity’, imparts an extra layer of complexity on understanding tumor heterogeneity.

There are a number of technical challenges and potential pitfalls to studying CSCs, which have helped fuel controversy in the field. Several reviews in this issue address these concerns. For example, variable markers and technical steps have often been used to isolate tumor subpopulations. In some cases, cell lines (which almost certainly have different growth properties from freshly isolated tumor cells) have been studied, while cell culture of primary cells prior to transplantation can change cell surface marker expression.

In addition, it is unclear whether *in vitro* assays such as the sphere-forming assay selectively enrich for CSCs. *In vivo* CSC assays that rely on serial tumor transplantation remain the current 'gold standard', but even here there has been a growing realization that cellular context (including the site of transplantation, the use of matrices such as Matrigel and the degree of immunosuppression for xenograft models) is a critical parameter.

Three reviews in this issue principally focus on the 'cell of origin' question. In their review on the cell of origin in lung cancer, Sutherland and Berns (2010) outline current evidence suggesting that different histological subtypes arise from different cancer-initiating cells throughout the lung. Evidence to date suggests a bronchioalveolar stem cell (BASC) as a potential early target for K-ras activation and tumorigenesis in non-small cell lung cancer (NSCLC). However it remains to be established whether this cell (marked by CC10, SPC) is a true stem cell or an early progenitor. Other subtypes, such as small cell and squamous cell lung cancer may arise from different stem and progenitor cells. Interestingly, although small cell lung cancer appears to have a neuroendocrine origin, a common cell of origin with NSCLC cannot be ruled out, given that some tumors exhibit non-small cell features. The absence of a robust *in vivo* assay has been an impediment to delineating lung cancer stem cells and the normal lung epithelial hierarchy. Nevertheless, recent progress has been made in understanding cell types prone to malignant transformation.

In their review, Vries et al. (2010) describe how insights gained from studying Wnt targets have been exploited to identify stem cells in the intestinal and gastric antrum. Notably, the Wnt pathway appears to be one of the main pathways regulating epithelial self-renewal in the intestine. The Wnt target Lgr5 appears to mark both intestinal and gastric stem cells. Its location at the base of the intestinal crypts and gastric glands helps address a long debated question regarding the precise location of stem cells in these organs. However, as with many discoveries, a new level of complexity is now apparent. In stomach, the existence of two stem cells now seems likely – a self-renewing Lgr5-positive stem cell that contributes to the daily renewal of gastric epithelium, as well as a Lgr5-negative quiescent stem cell that appears to be activated during emergency or inflammatory injury. In the intestine, the stem cell has been shown to act as the cell of origin for tumor development, while lineage-tracing experiments in the stomach suggest that the Lgr5-positive stem cell may also be the target for oncogenesis.

Goldstein et al. (2010) also discuss the cell of origin issue for prostate cancer, the majority of which have luminal cell features. The review outlines current knowledge of the epithelial cell hierarchy in normal prostate and the possible role that precursor cells play in tumor initiation. There are now different studies to support either a luminal or basal cell of origin for this disease. The review summarizes the link between prostatic stem cells and prostate cancer, highlighting three critical functional properties of prostatic stem cells: castration-resistance (i.e. cells that survive androgen ablation but may be indirectly androgen responsive), the capacity to self-renew and to regenerate prostatic tissue. Their work has revealed basal cells with stem cell features that are Sca-1⁺,

CD49^{hi} and express high levels of Trop2. Much less is known about the presence of cancer stem cells in primary prostate cancer, although it seems clear that properties shared by primitive prostate cells and castration-resistant prostate cancer cells, including self-renewal pathways could elucidate novel prostate cancer targets.

There is considerable evidence for CSCs in a variety of tumor types. Breast cancer was the first solid tumor in which CSCs were identified. McDermott and Wicha (2010) outline evidence to support the hierarchical organization of normal human breast tissue, as well as evidence to support breast stem or luminal progenitor cells as targets for transformation. CSCs appear to exhibit chemo- and radio-resistance, possibly mediated by Wnt and Notch signaling. Indeed, a significant number of key signaling pathways relevant to stem cell biology appear to be implicated in CSC function. The concept of 'migratory cancer stem cells' is discussed, where it is speculated that CSC seeding may require an epithelial–mesenchymal transition. The review provides a comprehensive overview of pathways utilized by CSCs, noting current strategies (such as targeting Hedgehog, NOTCH, AKT and CXCR1) that are under investigation in pre-clinical and clinical trials to exploit potential vulnerabilities of CSCs. There may be inherent difficulties in evaluating CSC targets in clinical trials, given that typical trial endpoints usually rely on bulk tumor shrinkage defined by RECIST criteria. Surrogate endpoints evaluating cells expressing CSC markers may therefore prove important. MicroRNAs likely play an important role in breast CSC regulation, as reported in recent studies on the Let-7 and mir200 families. Key transcription factors, such as BMI1 and ZEB1, that have important roles in stem or progenitor cell function appear to be linked to complex miRNA networks. The combination of high throughput RNAi screens and small molecule screens is also discussed as a potential means of identifying novel CSC inhibitors.

Adult glioblastomas are generally clinically aggressive neoplasms with abysmal outcomes. In a review on brain tumor cells, Dirks (2010) notes that the recent discovery of cycling precursor cells in the postnatal mammalian brain, coupled with the ability to prospectively isolate and study these cells using specialised culture techniques has provided an important paradigm for the study of an analogous brain tumor hierarchy. Controversies surrounding CSC markers such as CD133 (Prominin 1) are highlighted, together with an overview of other potential markers such as SSEA-1/CD15 and $\alpha 6$ integrin. A note of caution is raised about culture conditions potentially altering markers, and thus the ability to define populations with different tumorigenic abilities. It now seems unlikely that a single marker will define all brain tumor stem cells. The discovery of proliferative activity in the postnatal brain, particularly in the subventricular zone (where the majority of tumors appear to arise), has revolutionized efforts to identify the cell of origin and to dissect the role of specific oncogenic lesions in target precursor cells. A key point to emerge from this review is the importance of stem cell pathways in fuelling tumor cell growth and therapeutic resistance. Evidence to date would suggest that brain CSCs are proliferative, and this could provide a therapeutic window to spare normal neural stem cells. Several approaches currently being pursued

by the field to exploit potential targets are outlined, including BMPs, which promote differentiation and attenuate the tumorigenic phenotype.

Pancreatic tumors represent one of the most aggressive solid tumors and are a frequent cause of cancer related deaths. [Lonardo et al. \(2010\)](#) note that whilst gemcitabine has modestly improved median survival in patients with advanced disease, new approaches are required to improve on generally dismal outcomes. They review the apparent conflicting literature on CD133 expression and pancreatic CSCs. Interestingly, CD133⁺ cells appear to be resistant to gemcitabine cytotoxicity. The identification of key stem cell-associated pathways involved in pancreatic cancer, including sonic hedgehog (Shh) and mTOR, which have high activity in CD133⁺ cells has raised the possibility that 'triple therapy' with gemcitabine and Shh and mTOR inhibitors represents a promising approach to reduce tumorigenic burden and CSC activity. As with other tumor types, the potential overlap between normal stem cell and CSC markers represents a challenge to optimizing targeted therapy and the need to find exclusive CSC markers.

The existence of CSCs also represents an attractive mechanism to explain tumor dormancy. A number of malignancies are characterised by late recurrence – indeed in breast cancer metastatic relapse may occur even up to 20 years following diagnosis. The review by [Essers and Trumpp \(2010\)](#) explores long-term quiescence that has been best characterised in dormant hematopoietic stem cells (dHSCs). This rare cell is almost permanently in a G₀ quiescent state and may divide only about 5 times per lifetime in its specialized niche. However extrinsic hematopoietic insults (such as bleeding or cytotoxic therapy) can reactivate dHSCs, which can then be rendered susceptible to anti-proliferative therapy. This notion appears to apply to counterpart leukemic stem cells, which can be activated through priming with G-CSF, arsenic trioxide or IFN α . A two-step model whereby transient activation and proliferation of dormant cancer stem cells followed by efficient targeting with cytotoxics of targeted therapy (such as imatinib for CML) represents a compelling – and testable – new approach to tumor dormancy.

Not all tumor subtypes conform to the CSC hypothesis. In their review, [Shackleton and Quintana \(2010\)](#) provide an overview of tumor-propagating studies in malignant melanoma. Their own work demonstrates that melanoma propagating cells are not rare and highlights the importance that methodology plays in defining CSCs. In the context of their assay, the presence of extracellular matrix and NOD-SCID-IL2R $\gamma^{-/-}$ immunocompromised mice are critical to revealing tumorigenic capacity. The authors also comment on *in vitro* clonogenicity not being a valuable surrogate for melanoma cell tumorigenicity. The review discusses phenotypic plasticity (or interconversion) and clonal evolution as potential mechanisms that drive melanoma propagation. For example, tumors derived from either CD133⁻ or CD133⁺ cells re-establish heterogeneous expression of this marker, suggesting phenotypic switching. Genetic instability is also likely to be a major driving force behind melanoma progression. Although the CSC model may not apply to certain melanomas, a recent report provides evidence for enrichment of CSCs from primary melanomas using a single marker,

perhaps indicating that early lesions could initially be sustained by a CSC.

Collectively, these reviews provide a timely overview of many key discoveries and controversies that are informing debate and future research. Regardless of one's view on the frequently debated issues surrounding cancer stem cells, the importance of stem cell biology to cancer biology cannot be disputed. Elucidating the role of stem and progenitor cells in normal cell fate specification will almost certainly lead to the identification of new therapeutic targets. Similarly, exploring cancer stem cell behaviour and signaling pathways will almost certainly lead to the development of new therapeutic armaments in the war on cancer.

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