Controversies in Cancer Stem Cells: Targeting Embryonic Signaling Pathways

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Abstract

Selectively targeting cancer stem cells (CSC) or tumor-initiating cells (TIC; from this point onward referred to as CSCs) with novel agents is a rapidly emerging field of oncology. Our knowledge of CSCs and their niche microenvironments remains a nascent field. CSC’s critical dependence upon self-renewal makes these regulatory signaling pathways ripe for the development of experimental therapeutic agents. Investigational agents targeting the Notch, Hedgehog, and Wnt pathways are currently in late preclinical development stages, with some early phase 1-2 testing in human subjects. This series of articles will provide an overview and summary of the current state of knowledge of CSCs, their interactive microenvironment, and how they may serve as important targets for antitumor therapies. We also examine the scope and stage of development of early experimental agents that specifically target these highly conserved embryonic signaling pathways. Clin Cancer Res; 16(12); 3106–12. ©2010 AACR.

Introduction

Although the “cancer stem cell” (CSC) concept has captured great interest recently, concerns about its scientific foundation remain because significant gaps in supporting research findings and knowledge still exist. Frequently to avoid controversy, medical researchers avoid discussing in detail the origins of CSCs, their differentiation and de-differentiation, genetic heterogeneity, symmetric and asymmetric modes of cellular division, and clonal evolution. Nevertheless, the basis for the CSC model continues to evolve, changed by new insights in a number of scientific reviews that detail innovative research in this field.

The elucidation of embryonic signaling pathways as vital processes for self-renewal was first shown in hematopoietic stem cells and later in leukemias. Despite the presence of various hypotheses to explain the origin of CSCs, use of embryonic signaling pathways are the key signatures for CSCs that allow increased self-renewal. Because both normal and CSCs share the same embryonic signaling pathways, therapeutic strategies may be unable to differentiate between the normal stem cell and CSC as targets. Efforts have been made to identify targets that may be expressed selectively in CSCs and not in normal stem cells to circumvent this outcome.

In this overview, we discuss controversies and the future perspectives in therapeutics as a bridge to a series of outstanding review articles that follow (1–5). These review articles discuss the CSC biology and development of targeted therapeutics based on embryonic signaling pathways and their association with the niche. The purpose of this series of reviews is to enable the readers to identify the current gaps in knowledge in this area and provide direction for the future development of cancer therapeutics.

Cancer Stem Cells: Controversy in Origin and Evolution

The review titled “Cancer Stem Cells and Self-renewal” by O’Brien and colleagues contains balanced perspectives on the CSC hypothesis incorporating an up-to-date summary of both hematologic malignancies and solid tumors (3). The normal tissue stem cells are capable of self-renewal by symmetric or asymmetric cell division. Progenitor cells are generated to produce more committed progenitor cells or differentiated cells to fulfill the tissue-specific functions (6). More recently, cancerous tissues have provided evidence that a small population of neoplastic cells in a tumor are capable of self-renewal and repopulation leading to naming them “tumor initiating cells.”
Increasing evidence has shown that the isolated putative CSCs overexpress the common set of stem cell genes OCT4, NOTCH1, ALDH1, FGFR1, and SOX1. The injected CSCs can initiate tumor formation and differentiate in vivo, and more importantly, limiting dilution assays show the threshold for injecting cell numbers that can cause tumor formation in vivo (7). These studies have suggested that the growth of tumors depends on a small subset of CSCs that share many features in common with the nontransformed stem cells, including self-renewal and differentiation. However, unlike normal stem cells, CSCs do not require the ability to produce multilineage differentiated cancer cells. Thus, the CSC hypothesis model proposes a hierarchical structure for cells in the tumor that results in an explanation of the functional heterogeneity often seen in solid tumors.

The counterpoint to this hypothesis is the clonal evolution model (8), which in some cases encompasses a stochastic component (6), suggesting that cancerous cells may randomly develop the capacity to proliferate extensively and regenerate tumor tissue. The clonal evolution model states that mutant clones, possessing a growth advantage, will expand to become the dominant population, thus a dominant population of proliferating cells drives tumorigenesis until new clones with additional mutations become dominant through expansion and proliferation. However, neither model alone can adequately explain the complex biology of tumor progression, resistance, and metastasis. As more data have become available, support of a combined model has been published (9). Importantly, the combined model has also been supported by Dick and colleagues who first showed the hierarchical CSC model showing that only rare cells in acute myeloid leukemia were able to re-create a serially transplantable leukemia in murine models (10). Barabe and colleagues have recently added the further insight that leukemia CSCs themselves undergo clonal evolution (9). Moreover, detailed karyotype analyses of parental and clonally derived tumor cells from human metastatic colon cancer showed the presence of chromosomal instability in the CSCs (11). On the basis of these new insights, a new CSC hypothesis model should include both the CSC hierarchical and the clonal evolution components, allowing for preexisting CSCs to be transformed into secondary CSCs. A new model containing both paradigms during tumor propagation is described in Fig. 1 and has been proposed by others (11–13).

The two models of CSC ontogeny are not mutually exclusive. In addition, CSCs are capable of dividing in a symmetric fashion, as well as asymmetrically, to sustain heterogeneity (14). Asymmetric division yields one daughter cell and one self-renewed cell, whereas symmetric division generates two self-renewed cells. This notion is often overlooked because the CSC-hypothesis model can be explained primarily by the concept of asymmetric cell division. In contrast, the clonal evolution hypothesis requires preexisting CSCs be transformed into secondary CSCs, while maintaining dormant primary CSCs.

An additional concept that often causes intense discussion concerns the origin of CSCs. CSCs may originate from either transformed normal stem cells or more differentiated progenitor cells that have acquired self-renewal capacity. Both hypotheses seem acceptable. The latter concept is shown in medulloblastoma where Hedgehog-induced medulloblastoma originates from lineage-restricted granule cell progenitors and not from neural stem cells (15). Lastly, the origin of CSCs could be derived from cancer cells that are hierarchically downstream of CSCs, but have not fully differentiated. These cancer progenitor cells may acquire self-renewal capacity and become CSCs. This hypothesis has been shown in mouse leukemia experimental models (16–19). The concept of downstream cancer progenitor cells that have acquired a stem cell-like capability for self-renewal was shown in mouse leukemia models (16–19). In human models, this concept was shown in chronic myeloid leukemia (CML) blast crisis patients. The Wnt/β-catenin pathway was found to be important in CML granulocyte-macrophage progenitor cells to maintain CSCs (20). Although this concept has not been well shown in solid tumor CSCs, it may be an important concept to consider and further research is warranted.

**Niche and the Microenvironment**

LaBarge discusses the niche as a highly specialized microenvironment, which is defined by its primary occupant the CSC and its tissue-specific tumor location (1, 21). By definition the niche plays a primary role in stem cell maintenance. Occupants of the niche include the cells, the extracellular matrix, and soluble factors released by cells or stroma. The activities in the niche are constrained in a concentration and gradient-dependent fashion thus governing polar-planar orientation (22). Within the niche, the embryonic signaling pathways for Hh, Notch, Wnt, and others direct planar-spatial aspects of cellular aggregation and orientation. The CSC is both governed by and instructs the niche, thus leading to CSC division, proliferation, differentiation, invasion, and metastasis. Disrupting the cross talk between the niche and the CSC may be one of the critical steps to circumventing resistance to therapy. Regardless of type (embryonic, progenitor, tumor initiating, etc.), the stem cell defines one to multiple overlapping, flexible microenvironments, which in turn define stem cell functional phenotypes.

LaBarge's review of the niche moves on to explore the role of the niche in invasion and metastasis. Whether CSC niches are assembled early in embryogenesis, seized from other stem cells, or occupied as an empty nest is unknown (23, 24). What is relatively clear is that the niche adapts to its occupant, which may be phenotypically different from the primary tumor. For example, primary colon tumors change from CD133hi to CD133lo as they invade and metastasize (25). Whether this is an example of phenotypic drift, clonal evolution, or different cells of origin is a subject for further study. The importance of the microenvironment was predicted by Paget's "seed and soil"
Normal Tissue

Primary Tumor

Metastatic Tumor

Cancer progenitor cell

Normal stem cell

Primary cancer stem cell (CSC)

Bulk tumor cell

Tumor formation

Secondary CSC

Metastatic CSC

Bulk tumor cell

Tumor formation

Self-renewal

DNA hits
Niche factors

Clonal evolution

DNA hits
Niche factors

DNA hits
Niche factors

DNA hits
Niche factors
theory more than 100 years ago with more recent data suggesting that the tumor vascular bed serves as a point of aggregation for clusters of bone marrow-derived cells; tumor cells “home” to these premetastatic niches as tumors lose their anchorage dependence (26). If metastatic tumor growth is the result of migratory CSCs, then are preexisting niches necessary for these cells to become established and grow? Or do CSCs create their own niche environment once they lodge in a new physical location? These are critical questions that will have an impact on design of future antitumor therapies.

Finally the review addresses the influence of the niche on its stem cell resulting in selection for a more malignant phenotype. Transcription factors such as Twist or Snail will induce EMT upon exposure to transforming growth factor β (TGF-β; ref. 27). Stromal release of TGF-β can lead normal tissues, such as mammary epithelium, to exhibit characteristics like invasion and metastasis as tumors develop. Thus the microenvironment may adaptively select the tissue stem cell and induce a malignant phenotype. The study of niche in the context of “nature versus nurture” remains an open question for intensive research.

Hedgehog Signaling

The comprehensive review on “Targeting Hedgehog - a Cancer Stem Cell Pathway” by Merchant and colleagues and the review on “Targeting Notch to Target Cancer Stem Cells” by Pannuti and colleagues describe relatively novel, important targets that are associated with epithelial-to-mesenchymal transition (EMT; refs. 2, 4). The process of EMT is similar to that found in embryonic signaling pathways, and has been studied by developmental biologists for morphogenesis during embryonic development (28). The Hh signaling pathway induces expression of the gene SNAIL1, a transcription repressor of E-cadherin with a critical role in EMT. Its transcriptional upregulation is directly mediated by GLI1 expression (29). Experimentally, cyclopamine treatment of metastatic cells reduced levels of SNAIL1, -2, and GLI1, and reduced tumor regrowth in a xenotransplantation model (31). When treated with cyclopamine or small interfering (siRNA) against SMO, GLI1, and GLI2, reduced tumor regrowth in a xenograft model was observed. Additionally, shRNA directed inhibition of SMO, also inhibited tumor metastasis. Thus, these represent potential areas for future development of targeting therapeutics against CSCs to prevent or treat metastasis.

The integrated CSC hypothesis may be the most appropriate model to explain human tumor progression, particularly metastasis. EMT is associated with tumor progression in correlation with the loss of epithelial characteristics and the acquisition of a metastatic phenotype (28). Tumors cannot form metastatic colonies unless these cells contain CSC properties. To incorporate the tumor metastasis model and CSC hypothesis, Brabletz and colleagues proposed a new concept of “migrating CSCs (mCSC),” which contain both stemness and mobility characteristics (32). mCSCs that have undergone EMT can disseminate with expectations that these cells retain stem-cell function to form metastatic colonies (Fig. 1). In the colorectal cancer model, mCSCs expressed high levels of nuclear β-catenin (32). In addition to the genetic alterations, factors triggering a switch from CSCs to mCSCs may be related to secreted microenvironmental factors that induce EMT. Mani and colleagues further advanced this hypothesis and showed a direct link between the EMT and the acquisition of epithelial stem cell properties in a murine model, but definitive tests to prove the relationship between the EMT and cancer-initiating ability is required (27). It remains a challenge to design clinical trials that reflect the outcome of EMT, but these studies will be critical for the timely prevention of metastasis.

Notch Signaling

The highly conserved Notch signaling pathway in mammals consists of five membrane-bound ligands (DELTA-LIKE-1, -3, -4, and JAGGED-1, -2) and four membrane-bound receptors (NOTCH 1-4). Following ligand binding to the Notch receptor, proteolytic cleavage of the receptor results in an intracellular domain (ICN/NICD) that translocates to the nucleus. Once in the nucleus, ICN interacts with other DNA-binding proteins and activates Notch-specific gene transcription. Because both Notch ligand and receptors are located on the cell membrane, activation of the Notch receptor generally occurs through direct cell-to-cell contact. Notch signaling plays an important role in normal embryonic development and in adult tissue repair. Evidence also indicates that Notch plays a key role in normal hematopoietic stem cells (33) and gut epithelial stem cell homeostasis (34). The highly specific interactions between Notch ligands and receptors that guide short range cell-to-cell interactions is currently the subject of intense investigation (35).
Mutations within the Notch signaling pathway have been identified in many tumors (36, 37). Deregulation of the Notch pathway occurs in approximately one third of all non–small cell lung cancers and correlates with poor clinical outcome (38). Constitutive activation of Notch-1 signaling due to genetic mutations at the Notch loci is associated with T-ALL (39). The role of Notch signaling in CSCs has been best described in breast cancer, embryonic brain tumors, and gliomas (4).

Notch signaling can promote self-renewal of hematopoietic stem cells and was shown to be downregulated following cellular differentiation (40). In this model, Notch signaling was critical to the maintenance of hematopoietic stem cells in their undifferentiated state. In neural stem cells, Notch signaling has also been shown to promote proliferation, cell survival, and to inhibit stem cell differentiation (41, 42). Also, Notch blockade by a γ-secretase inhibitor slowed the growth of medulloblastoma tumor cells in vitro but also dramatically inhibited the formation of tumor xenografts in nude mice (43). Injection of large numbers of viable, rapidly proliferating medulloblastoma cells was not able to generate bulky xenografts if they had been treated previously with a γ-secretase inhibitor, whereas equal numbers of vehicle-treated tumor cells always formed large tumors.

A discussion of Notch signaling in CSCs and the role of this pathway as a potential target for antitumor therapies is discussed later in this issue of CCR Focus by Pannuti and colleagues (4). Treatment strategies combining standard therapies with Notch inhibitors is also examined. Because the best use of Notch inhibitors, like other CSC-targeted agents, will be in the context of personalized medicine, this review examines the Notch signaling pathway along with potential agents that target downstream proteins.

Wnt/β-catenin Signaling Pathway

Wnt signaling plays a vital role in cellular proliferation, cellular movement, establishment of cell polarity, as well as in stem cell maintenance (44). Wnt signaling has been defined as occurring either through the canonical or non-canonical pathways. Canonical Wnt signaling is characterized by the stabilization and cytoplasmic accumulation of β-catenin, which then translocates to the nucleus to facilitate the activation of a variety of WNT target genes. In humans, 19 members of the WNT family and 10 members of its receptor family (FRIZZLED) have been described (45). The large variety of potential ligand-receptor combinations...
hints at the multitude of complex cellular downstream effects that are possible following Wnt activation.

Mutations within this highly conserved signaling pathway occur frequently in human cancers. Activating mutations of β-catenin have been reported in a variety of tumors including melanomas, ovarian, medulloblastoma, and endometrial (46–48). In addition, activation of the Wnt/β-catenin pathway has been shown to play an important role in non–small cell lung cancer (49). Many reports have indicated that dysregulation of Wnt signaling occurs in breast cancer, with up to 80% of malignant breast carcinomas having repressed or absent Frp1 expression (50). Additionally, up to 50% of breast tumors have been shown to have hypermethylation of the adenomatous polyposis coli (APC) gene (51).

Because Wnt signaling plays such a critical role in the regulation of stem cell populations, targeting this pathway may yield important clinical benefits for cancer patients. For example, Wnt signaling is essential for maintaining colonic crypts and for the regulation of cellular differentiation (52). Studies suggest that Wnt signaling is also involved in the regeneration of epidermal and gastrointestinal stem cells (53, 54). The formation of epithelial tumors in mice has been linked with the activation of Wnt signaling in epidermal stem cells (55). Cutaneous squamous cell carcinoma stem cells are dependent upon β-catenin signaling to maintain their stem cell properties (49, 56). Thus, targeting the Wnt/β-catenin pathway may yield clinical benefits by inhibiting the proliferative capacity of CSCs or possibly force terminal differentiation.

The role that Wnt signaling plays in the expansion of stem cell colonies or conversely the induction of cellular differentiation is controversial. These dramatically different outcomes may depend on specific ligand-receptor pairings or on yet unknown factors. Interestingly, a new CBP/β-catenin antagonist ICG-001 may specifically drive stem cells toward differentiation.

The development of antitumor agents that target the Wnt signaling pathway represents a novel and potentially useful way of limiting tumor growth. Because this pathway is used primarily during embryogenesis and tissue repair in the adult, significant levels of toxicities are not expected. A detailed examination of Wnt/β-catenin inhibitors with potential clinical utility is provided by Takahashi-Yanaga and Kahn in this issue of CCR Focus (5).

Conclusion

The CSC hypothesis, which once seemed to be accepted by only a small number of investigators, has undergone evolitional changes in its concept and has become more widely accepted. Merging the clonal evolution concept with the CSC hypothesis seems to provide more flexibility for the CSC hypothesis. This merging of ideas has led to more evidence for genetic instability in CSCs and more support for the explanation of how CSCs metastasize. The emerging de-differentiation concept holds that a few progenitor tumor cells may develop into CSCs. The de-differentiation concept has been tested in induced nuclear reprogramming experimental models (57). Thus, CSCs seem to be distinct among tumor types due to their microenvironment and cell origin.

The most important concept to emerge from the CSC controversy is that a number of signaling pathways unique to normal stem cells may be operating in CSCs, and that these offer new targets for cancer therapy. Embryonic signaling pathways are the first and most obvious places to target with therapeutics for CSC. The CSC niche, and possibly EMT, have the potential to be drug targets for CSCs. Additional target evaluation and drug screening may be done using nuclear reprogramming technology, which includes a powerful tool to dedifferentiate somatic cells into embryonic stem cell-like stem cells. This technology may be applied to generate CSCs for the purpose of drug screening. Further research should evaluate potential cross-talk between the three embryonic signaling pathways and other pathways, including ErbB family and PI3K/AKT (see Fig. 2). Proof of principle trials examining drug combinations that target these pathways are currently underway at the National Cancer Institute.

Efforts, other than in vivo xenografts, directed toward improving CSC readout are critically important to advance CSC-targeting therapy. Although we did not debate the different types of in vivo CSC xenograft models, the relative frequency of CSCs is extremely dependent on the specific experimental conditions. Thus, comparisons between CSC models may be difficult to interpret. Clinical trials designed to evaluate anti-CSC investigational agents will need to critically evaluate CSC biomarkers and may require novel designs and endpoints for validation.

Disclosure of Potential Conflicts of Interest

The authors have no potential conflicts of interest.

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