Regulation of Cancer Stem Cells by Cytokine Networks: Attacking Cancer's Inflammatory Roots

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Abstract
There is substantial evidence that many human cancers are driven by a subpopulation of cells that display stem cell properties. These cancer stem cells (CSC) may also contribute to metastasis and treatment resistance. Furthermore, just as normal stem cells are regulated by their microenvironment, or niche, CSCs interact with and in turn are regulated by cells in the tumor microenvironment. These interactions involve inflammatory cytokines, such as interleukin (IL)-1, IL-6, and IL-8, which in turn activate Stat3/NF-κB pathways in both tumor and stromal cells. Activation of these pathways stimulates further cytokine production, generating positive feedback loops that in turn drive CSC self-renewal. These cytokine loops and the pathways they regulate resemble those activated during chronic inflammation and wound healing, and may contribute to the known link between inflammation and cancer. Inhibitors of these cytokines and their receptors have been developed as anti-inflammatory agents. By blocking signals from the tumor microenvironment, these agents have the potential to target CSCs. Future clinical trials using these compounds will be needed to determine whether targeting the CSC population has clinical benefit.

Background
Cancer stem cells
There is increasing evidence that many human tumors display a hierarchical organization in which a subset of tumor cells with stem cell properties drives tumor growth and metastasis (1, 2). Furthermore, by virtue of their relative resistance to radiation and chemotherapy, these cells may contribute to treatment resistance and relapse after therapy. If this is the case, then more effective treatments will require effective targeting of this cell population. As is the case with their normal counterparts, cancer stem cells (CSC) are regulated by intrinsic signals as well as extrinsic signals originating in the tumor microenvironment (3–5). In the case of CSCs, epigenetic as well as genetic changes that occur during carcinogenesis result in dysregulation of self-renewal pathways. Stem cell regulatory pathways that are frequently dysregulated in tumors include the Notch, Hedgehog, Wnt, phosphoinositide 3-kinase (PI3K) NF-κB, and Jak/Stat3 pathways (6–10). These pathways may be activated via mutation of key regulatory elements. In addition, pathway dysregulation may result from altered interactions between these cells and the tumor microenvironment (11). Emerging evidence suggests that tumors and their microenvironment coevolve during tumor progression (3). Bidirectional paracrine signals coordinately regulate tumorigenic cell populations, including CSCs (7, 12–14). Tumorigenic cells in turn produce factors that attract and regulate a diverse variety of cell types that constitute the tumor microenvironment (12, 14). Inflammatory cytokines, such as interleukin (IL)-1, IL-6, and IL-8, play a pivotal role in mediating the interaction between CSCs and the microenvironment. Of interest, many of the pathways that are activated during tumor formation resemble those that mediate normal wound healing. Here, we review the links between inflammation and CSCs, with an emphasis on the cytokine networks and signaling pathways that link these processes. These pathways provide potential targets for the development of novel strategies to target CSC populations.

Inflammation and cancer stem cells
Considerable clinical evidence points to links between inflammatory states and cancer development. Epidemiologic studies have demonstrated associations between ulcerative colitis, hepatitis C, and chronic pancreatitis and the development of cancers of the colon, liver, and pancreas, respectively. Levels of chronic inflammation as assessed by serum C-reactive protein or β-amyloid are correlated with the risk of breast cancer recurrence after primary therapy (15). The development of chronic inflammation has been associated with the production of the cytokines IL-1β, IL-6, and IL-8 by a variety of inflammatory cells. Of interest, genetic
polymorphism in genes encoding these cytokines predisposes affected individuals to cancer (16). Furthermore, the Stat3/NF-κB pathway plays a critical role in inducing and maintaining a procarcinogenic inflammatory microenvironment at the initiation of malignant transformation and tumor progression (17–19). These inflammatory cytokines, including IL-1, IL-6, and IL-8, may influence tumor growth by regulating CSC populations.

**Interleukin-1**

The IL-1 family consists of IL-1α, IL-1β, and the antagonist IL-1Ra. These receptors bind IL-1, which is produced in response to infection or tissue injury, resulting in activation of NF-κB and downstream targets IL-6 and IL-8. Local production of IL-1 by tumor-associated macrophages promotes angiogenesis, tumor growth, and metastasis, whereas blocking the IL-1 receptor inhibits these processes in mouse models (20, 21). Elevated levels of IL-1 expression in cancer patients are correlated with advanced metastatic disease (22, 23). Furthermore, gene expression profiling shows higher IL-1 expression in breast CSCs compared with their more differentiated counterparts (7).

**Interleukin-6**

IL-6 is a pleiotropic cytokine that is secreted by a wide range of cells and plays a crucial role in immunoregulation. Elevated levels of IL-6 have been associated with chronic inflammatory states, sepsis, hypertension, obesity, insulin resistance, and poor survival in cancer patients, increasing their risks for developing malignancies (24, 25). In cancer patients, high levels of IL-6 are associated with poor patient outcome, and in preclinical models, IL-6 has been shown
to promote tumorigenesis, angiogenesis, and metastasis (26, 27). IL-6 has been shown to be a direct regulator of breast CSC self-renewal (13), a process that is mediated by the IL-6 receptor/GP130 complex through activation of Stat3 (Fig. 1). In inflammatory cells, IL-6–mediated Stat3 signaling selectively induces a proangiogenic, tumorigenic microenvironment (28). Stat3 activation in turn leads to transcriptional activation of NF-κB in inflammatory cells that secretes additional IL-6 and IL-8 acting on tumor cells. Thus, these cytokines generate a positive feedback loop between immune cells and tumor cells that further stimulates the tumor stem cell components, accelerating metastasis and therapeutic resistance (Fig. 1). Using mouse xenografts, we recently demonstrated that bone marrow mesenchymal stem cells are recruited to sites of growing breast cancers by gradients of IL-6 (12). Furthermore, IL-6 is a key component of a positive feedback loop involving these bone marrow mesenchymal stem cells and breast CSCs (12). Sethi and colleagues (29) recently demonstrated that IL-6–mediated Jagged1-Notch1 promotes breast cancer metastasis to bone. Because Notch is also a stem cell regulator, this suggests that IL-6 may regulate stem cells through multiple pathways. These studies identify IL-6 and its receptor as attractive therapeutic targets.

**Interleukin-8**

IL-8 is a proinflammatory cytokine that functions in different biologic processes, such as neutrophil chemotaxis and angiogenesis. It activates multiple intracellular signaling pathways by binding its receptors, CXCR1 and CXCR2. Within the tumor microenvironment, a diverse variety of cells, including mesenchymal cells, macrophages, and immune cells, secrete IL-8 (30). Serum IL-8 levels in patients with cancer have been associated with aggressive cancer behavior and poor prognosis (31, 32). Using gene expression profiling, we previously identified the IL-8 receptor CXCR1 as being highly expressed on breast CSCs (33). Recombinant IL-8 increased breast CSC self-renewal and tumor growth. In contrast, blocking this receptor in mouse xenografts with repertaxin, a small-molecule inhibitor, significantly reduced the breast CSC population, leading to decreased tumorigenicity and metastasis.

**NF-κB pathway**

The NF-κB family is composed of 5 related transcription factors: p50, p52, RelA (p65), c-Rel, and RelB (34, 35). In resting cells, NF-κB proteins are predominantly found in the cytoplasm, where they are associated with the IκB family of proteins (Fig. 1). Activation of NF-κB by diverse signals results in ubiquitin ligase-dependent degradation of IκB and nuclear translocation of NF-κB protein complexes. A number of cytokines, including IL-6 and IL-8, are regulated by NF-κB. In addition, a positive feedback loop was recently shown to maintain a chronic inflammatory state in tumor cells. Of interest, this loop involves the microRNA let7, as well as Lin28, a factor involved in embryonic stem cell self-renewal (7). This feedback loop is maintained by IL-6–mediated Stat3 activation, which in turn activates NF-κB, affecting Lin28 and let7 (Fig. 1). The specific role of IL-6 in maintaining this inflammatory loop in breast CSCs was recently demonstrated (7, 10). NF-κB may play an important role in normal breast physiology, as well as in carcinogenesis. In an HER2-neu model of mammary carcinogenesis, suppression of NF-κB in mammary epithelium reduced the mammary stem cell compartment, resulting in delayed onset of HER2-neu–induced tumors (36). NF-κB has also been implicated in the regulation of mouse mammary stem cells during pregnancy. Elevated levels of progesterone during pregnancy induce RANK ligand (RANKL) in differentiated breast epithelial cells. In turn, RANKL stimulates breast stem cell self-renewal via activation of NF-κB in these cells (37, 38). The increased incidence of aggressive breast cancers associated with pregnancy (39) may result from activation of similar pathways in breast CSCs (37, 38).

**Clinical–Translational Advances**

Solid tumors are composed of heterogeneous cell populations that interact in complex networks. As is the case in developing organs, tumor cells interact with and in turn are regulated by these components in the microenvironment. Metastatic tumor cells also recreate complex cellular micro-environments at metastatic sites. More than 120 years ago, Paget proposed the "seed and soil" hypothesis of tumor metastasis (40). Reframed in a modern context, the "seeds" are the CSCs and the "soil" is the rich microenvironment, which is composed of diverse cell types that interact with tumor cells via cytokine networks. These networks regulate CSCs and their progeny, which form the tumor bulk. Elucidation of these pathways may provide new targets for therapeutic development. Examples of such pathways include the cytokines IL-6 and IL-8 and their receptors IL-6R and CXCR1. Blockade of these cytokine pathways reduced breast CSCs in preclinical models (33). Clinical trials using IL-6–blocking antibodies have been initiated for the treatment of multiple myeloma, and early results are encouraging (41). Furthermore, an anti–IL-6R antibody, tocilizumab, has been approved for the treatment of arthritis (42) and has little clinical toxicity. A small-molecule CXCR1 inhibitor, repertaxin, was developed to block rejection in renal transplant patients, and early results from clinical trials suggest that it is well tolerated. Phase I clinical trials combining this cytokine receptor/inhibitor with chemotherapy are being planned. NF-κB also represents an attractive therapeutic target. Preclinical studies suggest that the NF-κB inhibitor parthenolide is able to target leukemic stem cells, and early-stage clinical trials for the treatment of leukemia with this agent are in progress. Together, these trials will determine the feasibility of targeting CSCs by blocking interaction of these cells with the tumor microenvironment.

The CSC model has important implications for clinical trial design. Currently, tumor response rate is determined by tumor size as described by Response Evaluation Criteria
in Solid Tumors (RECIST). For many tumors, regression does not correlate with increased patient survival (43–45). Because CSCs may constitute only a minor fraction of a tumor, agents that target this population may not produce tumor regression. In fact, stem cell–targeting agents would be expected to have more dramatic effects in the adjuvant setting than in advanced-tumor settings (46). This suggests that in advanced disease, it will be necessary to combine CSC-targeting agents with debulking approaches such as chemotherapy or radiation therapy.

The time-to-tumor progression may prove a more useful clinical endpoint than tumor regression in such studies. However, because the non–stem-cell fraction of tumors may still retain a proliferative capacity, it is important to use accurate criteria to define tumor progression to ensure that patients are not removed from treatment prematurely. The evaluation of CSC biomarkers, such as CD44, CD133, and aldehyde dehydrogenase-1, in serial biopsies may provide a tool to assess the efficacy of CSC-targeting agents (47–49). Circulating tumor cells may also provide a valuable source of CSC populations for biomarker analysis. These assays will need to be able to capture circulating CSCs, which may not express antigens that are currently used, such as EpCAM. A neoadjuvant trial design may prove to be particularly useful for assessing the effects of CSC-targeting agents, because acquiring tissue before and after treatment enables one to assess the efficacy of CSC targeting.

In addition, the effects of these agents on increasing the complete pathologic response rate, an accepted clinical endpoint, can be readily assessed. Ultimately, randomized trials will be required to determine whether successful targeting of CSCs improves patient outcome.

Disclosure of Potential Conflicts of Interest

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