Molecular Pathways

Clinical Cancer Research

Stem Cell Quiescence

Ling Li and Ravi Bhatia

Abstract

Adult stem cells are maintained in a quiescent state but are able to exit quiescence and rapidly expand and differentiate in response to stress. The quiescent state appears to be necessary for preserving the self-renewal of stem cells and is a critical factor in the resistance of cancer stem cells (CSCs) to chemotherapy and targeted therapies. Limited knowledge about quiescence mechanisms has prevented significant advances in targeting of drug-resistant quiescent CSCs populations in the clinic. Thus, an improved understanding of the molecular mechanisms of quiescence in adult stem cells is critical for the development of molecularly targeted therapies against quiescent CSCs in different cancers. Recent studies have provided a better understanding of the intrinsic and extrinsic regulatory mechanisms that control stem cell quiescence. It is now appreciated that the p53 gene plays a critical role in regulating stem cell quiescence. Other intrinsic regulatory mechanisms include the FoxO, HIF-1α, and NFATc1 transcription factors and signaling through ATM and mTOR. Extrinsic microenvironmental regulatory mechanisms include angiopoietin-1, TGF-β, bone morphogenic protein, thrombopoietin, N-cadherin, and integrin adhesion receptors; Wnt/β-catenin signaling; and osteopontin. In this article, we review current advances in understanding normal stem cell quiescence, their significance for CSC quiescence and drug resistance, and the potential clinical applications of these findings. *Clin Cancer Res; 17(15); 4936–41. ©2011 AACR.*

Background

Adult stem cells are rare populations of cells that are able to regenerate the multiple differentiated cell types of the organ in which they reside and renew themselves (1). In contrast to germline stem cells of invertebrates, which are constantly cycling (2), mammalian adult stem cells are predominantly in a quiescent, nondividing G_0-state (3). Hematopoietic stem cells (HSCs) are among the best-studied adult stem cell populations. The quiescence of HSCs has been linked to their long-term reconstituting capacity and is critical for long-term maintenance of the stem cell compartment (1). In order to maintain a supply of mature blood cells throughout the lifetime of an individual without exhausting the HSC pool, most HSCs remain quiescent under steady state, and only a small number of them enter the cell cycle (4). However, HSCs can exit quiescence and rapidly expand and differentiate to regenerate hematopoiesis in response to stresses such as blood loss (4). Defects in the regulation of quiescence can lead to premature exhaustion of the HSC pool, causing hematological failure (3, 5). Stem cell quiescence is also closely associated with protection from myelotoxic insults (5). Similar to the role of tissue stem cells in normal tissues, several cancers are also propagated by small populations of cancer stem cells (CSCs) (6). Stem cell quiescence is highly relevant for cancer therapy because quiescent CSCs are often resistant to both conventional chemotherapy and targeted therapies, and are retained and contribute to relapse following discontinuation of therapy (6). Therefore, improved understanding of the mechanisms of stem cell quiescence is important not only to enable direct manipulation of normal stem cell function but also to develop approaches to therapeutically target quiescent CSCs.

Stem cell quiescence is controlled by both intrinsic regulatory mechanisms and extrinsic signals from the microenvironment, as shown in Fig. 1 (7). Several transcription factors play key roles in stem cell fate decisions (8). On the other hand, interactions of stem cells with the niche are critical for the long-term maintenance of HSC quiescence (9). Here, we review recent progress made in understanding the mechanisms of quiescence of stem cells, and the potential applications of these findings to cancer treatment.

Intrinsic mechanisms regulating stem cell quiescence

p53 signaling. Recent studies have shown that in addition to its important role in the cellular response to DNA damage, p53 plays a critical role in regulating HSC quiescence in steady-state conditions (8, 10). p53 is preferentially expressed in HSCs compared with more-committed progenitor cells, and promotes HSC quiescence (8). The transcription factor MEF/ELF4 modulates p53 expression

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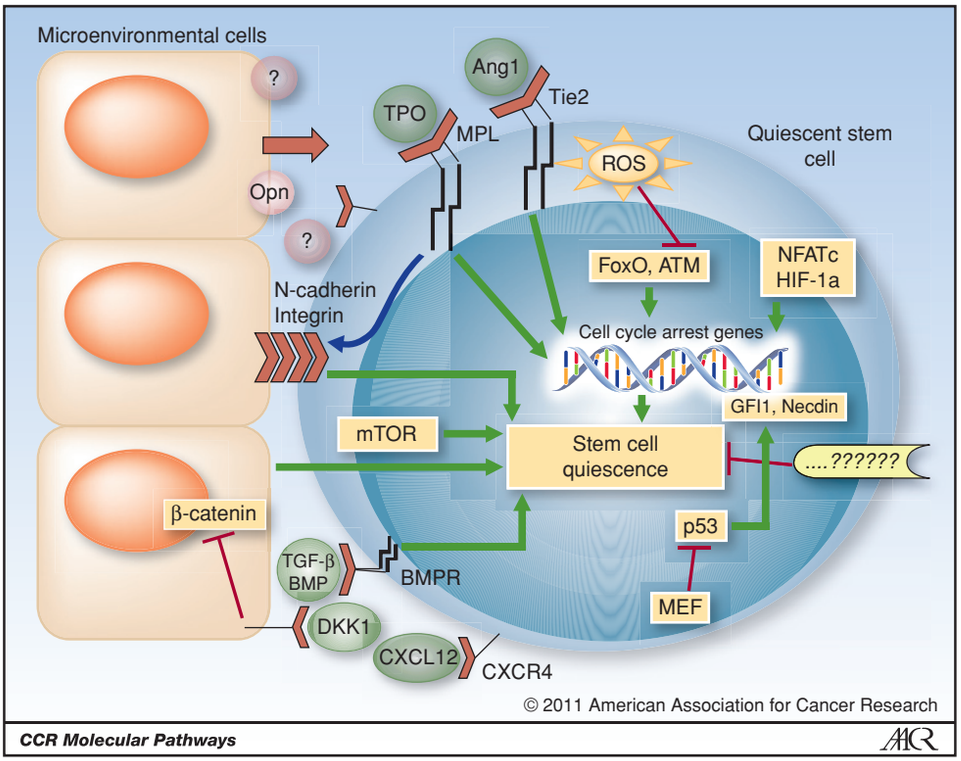

Figure 1. Pathways to stem cell quiescence. Stem cell quiescence is controlled by both intrinsic regulatory mechanisms and extrinsic signals from the microenvironment. Several transcription factors play key roles in promoting stem cell quiescence. The transcription factor p53 is a critical regulator of quiescence in the steady state. ROS in stem cells regulate expression of the transcription factors FoxO and ATM, which in turn act to regulate ROS levels in stem cells and maintain stem cell quiescence. A role for HIF-1α and NFATc in regulating stem cell quiescence has also been shown. Factors that inhibit mTOR also contribute to stem cell quiescence. Interactions of stem cells with microenvironmental supportive cells are critical for the long-term maintenance of HSC quiescence. TGF-β and BMPs produced by microenvironmental cells are important regulators of stem cell quiescence. Interactions of TPO with its receptor MPL and Ang-1 with its receptor Tie-2 also promote stem cell quiescence and enhance adhesion to the microenvironment through integrin and cadherin receptors. Opn from the microenvironment is also reported to mediate stem cell quiescence. Wnt signaling has complex effects on stem cells, but there is evidence that Wnt signaling in the microenvironment plays an essential role in maintaining HSCs in a quiescent state. In the future, targeting of the molecular mechanisms that underlie quiescence may allow the sensitization of quiescent CSCs to therapeutic agents.

and facilitates the entry of quiescent HSCs into the cell cycle (10). MEF^{null} HSCs display increased quiescence that is p53 dependent, and are resistant to the myelosuppressive effects of chemotherapy and radiation. Although earlier studies suggested a role for the p53 target gene *p21* in restricting HSC entry into the cell cycle and regulating the HSC pool size under stress (11), subsequent studies indicated that p21 plays a minimal role in regulating HSC quiescence under steady-state conditions (12). Two other p53 target genes, *Gfi-1* and *Necdin*, have been identified as important regulators of quiescence (8, 13).

Reactive oxygen species: FoxOs and ATM. Reactive oxygen species (ROS) play an important role in regulating stem cell maintenance (14, 15). The FoxO group of human forkhead proteins includes 4 members (FoxO1, FoxO3a, FoxO4, and FoxO6) with both distinct and overlapping functions. FoxO proteins are activated in response to oxidative stress (16, 17), and up-regulate genes involved in ROS detoxification and cell-cycle arrest (16). HSCs from FoxO1, FoxO3, and FoxO4 triple knockout mice exhibit increased levels of ROS, increased cycling, apoptosis, and defective long-term repopulating activity (15). The HSC defect resulting from loss of FoxOs can be rescued by antioxidant administration. ATM, a cell-cycle checkpoint regulator activated after DNA damage, also regulates ROS levels in HSCs (14). ATM is preferentially expressed in cycling HSCs, and ATM-deficient mice show elevated ROS levels, lack quiescent HSCs, and show progressive bone marrow (BM) failure (14). Treatment with antioxidants restores the quiescence and BM reconstitutive capacity of $ATM^{-/-}$ HSC.

Hypoxia inducible factor-1α. The transcription factor hypoxia inducible factor-1α (HIF-1α) is stabilized under low-oxygen conditions, such as are present in the BM environment (18, 19). HIF-1α levels are elevated in HSCs and regulate HSC metabolism (18). HSCs from HIF-1α–deficient mice show reduced quiescence and decreased numbers following transplantation, myelosuppression, or aging (19). Overall, these data indicate that precise regulation of HIF-1α levels is required for maintenance of HSC quiescence.

Nuclear factor of activated T cells c1. The hair follicle has also proved to be a good model system for studying

stem cell quiescence. Stem cells are localized in the bulge region of the follicle (20, 21). The transcription factor nuclear factor of activated T cells c1 (NFATc1) is preferentially expressed in the bulge relative to proliferative basal cells in the epidermis (20). Both pharmacological suppression of NFATc1 and gene ablation have revealed that it plays a role in regulating HSC quiescence (21). NFATc1 expression is activated by bone morphogenic protein (BMP) and acts to repress CDK4 transcription. NFATc1 is down-regulated when stem cells become activated during hair growth, relieving CDK4 repression and activating proliferation (21).

Negative regulators of mTOR: Fbw7, PTEN, and PML. Several reports have indicated that negative regulators of mTOR, including Fbxw7 (22), PTEN (23, 24), and PML (25), can maintain stem cell quiescence. Deletion of these genes in mice leads to strikingly similar phenotypes of stem cell hyperproliferation and subsequent exhaustion, and a defective repopulating potential.

Extrinsic mechanisms regulating stem cell quiescence

Stem cells are localized to niches formed by cells that provide a microenvironment that supports their growth and regulates their fate (9, 26). Interaction of stem cells with the niche is crucial for the long-term maintenance of quiescence.

Tie2/angiopoietin-1. Osteoblasts within the BM provide a niche that promotes the maintenance of quiescent HSCs (27). Osteoblasts are a source of angiopoietin-1 (Ang-1), the ligand for the receptor tyrosine kinase Tie2, which is specifically expressed in HSCs. Tie2/Ang-1 signaling activates β1-integrin and N-cadherin in HSCs, promoting interactions with the extracellular matrix and cellular components of the niche (3). Genetic mouse models indicate that Tie2 interactions maintain quiescence and enhance the survival of HSCs by preventing cell division (28). In humans, Ang-1 is expressed in mesenchymal stem cells (29), suggesting that these cells may provide a niche for quiescent human HSCs.

TGF-β and bone morphogenic proteins. TGF-β and related molecules play an important role in maintaining quiescence. TGF-β is a potent inhibitor of stem cell growth and cycling *in vitro* (30), and is hypothesized to be a cardinal regulator of stem cell quiescence *in vivo*. Disruption of BMP signaling through conditional knockout of Bmpr1a in osteoblasts resulted in increased HSC numbers (31). Conditional ablation of Bmpr1a also activated quiescent hair follicle stem cells to proliferate (32). Quiescent $SOX2^+$ neural stem cells in the subgranular zone are depleted by genetic deletion of Bmpr1a or infusion of the BMP antagonist Noggin (33).

Thrombopoietin. Mice deficient in the MPL receptor or its ligand, thrombopoietin (TPO), have fewer HSCs in the BM (34). MPL-positive HSCs in close contact with TPO-producing osteoblastic cells at the endosteal surface are quiescent (35, 36). Inhibition of TPO-MPL interactions with a neutralizing antibody reduced the number of quiescent HSCs, and TPO treatment increased the expression of p57Kip2, which is specifically expressed in quiescent HSC populations (35).

N-cadherin and integrins. The adhesion molecules N-cadherin and β1-integrin are required not only for HSC anchoring to the niche but also for regulation of HSC cycling (31). N-cadherin is present at the interface between HSCs and osteoblastic cells (31). Tie2/Ang-1 signaling induces β1-integrin and N-cadherin–dependent HSC adhesion (3). MPL/TPO signaling also up-regulates β1-integrin in HSCs (36). Therefore, β1-integrin and N-cadherin may be key downstream targets of Tie2/Ang-1 and MPL/TPO signaling in HSCs.

Osteopontin. Osteopontin (Opn) expressed in osteoblasts negatively regulates HSC number in the BM niche (37). Opn-deficient mice show an increase in HSC number, suggesting that Opn inhibits HSC proliferation *in vivo* (38, 39). Normal HSCs demonstrate a long-term engraftment defect in an $Opn^{-/-}$ microenvironment (39).

Wnt/β-catenin signaling. Wnt signaling plays a vital role in cellular proliferation, movement, and polarity, and in stem cell maintenance (1). Constitutively active nuclear β-catenin signaling reduces HSC quiescence and blocks HSC differentiation (40). On the other hand, osteoblast-specific expression of Dickkopf1 (Dkk1), an inhibitor of canonical Wnt signaling, results in increased HSC cycling and reduced regenerative capacity (41). These findings suggest that Wnt pathway activation in the niche limits HSC proliferation and preserves self-renewal (41). Other studies have shown that microenvironmental β-catenin plays an important role in the long-term maintenance of HSC (42). These observations suggest that fine-tuning of Wnt/β-catenin activity in the microenvironment is crucial for maintaining stem cell quiescence.

Clinical-Translational Advances

Malignant stem cells in cancer are characteristically quiescent, and the dormancy of these small populations protects them from elimination following cancer treatment, contributing to cancer relapse (1). In several malignancies, including breast and colon cancer, relapse can occur more than a decade after the initial treatment. These late relapses can be explained by the survival and long-term persistence of dormant CSCs (6). As with solid tumors, several leukemias, such as acute myelogenous leukemia (AML), contain heterogeneous cell populations with a small percentage of quiescent leukemia stem cells (LSCs) responsible for propagation of the leukemia (6). Recent studies using xenogeneic models indicate that AML LSCs are localized to the BM endosteal region, are noncycling, and resist elimination by chemotherapy (43). $CD34^+$ cells from patients with chronic myelogenous leukemia (CML) also contain quiescent cells that are resistant to BCR-ABL tyrosine kinase inhibitors such as imatinib mesylate (44). Primitive LSCs persist in the BM of CML patients in cytogenetic remission on imatinib treatment (44), and stopping treatment frequently leads to disease relapse even in patients in whom BCR-ABL transcripts are no longer

detectable by PCR (45). It is likely that overcoming LSC dormancy will be a critical step toward attaining a cure for this and other CSC-driven cancers. Targeting of quiescent CSCs is a difficult challenge because most conventional and targeted anti-cancer agents are ineffective at killing this population. Recently, there has been increased interest in developing approaches based either on activating quiescent CSCs, inducing their cell-cycle entry and increasing their sensitivity to other treatments, or identifying agents that are capable of directly targeting quiescent CSCs.

Granulocyte colony-stimulating factor. Granulocyte colony-stimulating factor (G-CSF) is used in the clinic to treat neutropenia and mobilize HSCs to the circulation (46). G-CSF has also been used to enhance the sensitivity of leukemia cells to cytotoxic agents (47). G-CSF treatment induces proteolytic enzyme release in the BM, leading to degradation of the adhesion molecules CXCL12 and CXCR4. G-CSF treatment significantly enhanced inhibition of CML LSC by imatinib *in vitro*. However, a clinical pilot study failed to confirm that combined G-CSF and imatinib treatment can eliminate LSC in CML patients (48). Recently, G-CSF treatment was shown to efficiently activate dormant human AML stem cells in the endosteal niches, and to enhance their elimination by cytarabine without enhancing the sensitivity of normal HSCs (43). Thus, these protocols may prove to be effective after further improvement and optimization (48).

Interferon. Type I interferons (IFN-α and -β) play a critical role in resistance to viral infections and innate and acquired immune responses (49). IFNs also have antiproliferative properties in many cell types *in vitro*. However, recent observations suggest that IFN-α can stimulate the proliferation of HSCs *in vivo* (50, 51). How IFN-α signals are perceived differently in HSCs compared with other cell types in which IFN-α normally suppresses proliferation is unclear. The proliferative effects of IFN on HSCs appear to be direct and are transient. Because increased proliferation is only seen *in vivo*, alterations in niche interactions may play a role. Two recent randomized studies showed a greater reduction in BCR-ABL levels when IFN-α was combined with imatinib for treatment of CML (52, 53). The clinical value of IFN-α in CML treatment may be related to stimulation of quiescent LSCs to proliferate, increasing the sensitivity to imatinib.

CXC motif receptor-4 antagonists. CXCL12 [stromal cell-derived factor-1 (SDF-1)] binding to receptor CXC motif receptor-4 (CXCR4) plays an important role in HSC localization to the niche (54). AMD3100 is a bicyclam molecule that selectively and reversibly antagonizes CXCL12-CXCR4 interactions, with subsequent dislodgement of HSCs from the niche (54). AMD3100 rapidly mobilizes HSCs and is approved for stem cell mobilization in combination with G-CSF. AMD3100 also disrupts interaction between AML blasts and the BM stroma, mobilizing blasts to the peripheral blood and sensitizing them to chemotherapy (55). Clinical trials testing AMD3100 as a strategy to sensitize leukemic cells to chemotherapy are under way in patients with AML (54).

Histone deacetylase inhibitors. Histone deacetylase inhibitors (HDACi) have shown promise as a therapy for several cancers (56). In contrast to most other proapoptotic agents that preferentially target dividing cells, HDACi can induce apoptosis in nonproliferating cancer cell lines (56). The combination of HDACi and imatinib induced apoptosis in quiescent CML LSCs that were resistant to elimination with imatinib alone (57). HDACi have pleiotropic effects on cells; however, potential mechanisms involved in CML LSC inhibition include alteration of gene expression related to LSC self-renewal and survival, and microenvironmental interactions. A clinical trial of HDACi in combination with imatinib in patients with CML in cytogenetic remission is under way (58).

Wnt inhibitors. Studies have shown that Wnt signaling in the microenvironment has a role in maintaining quiescent HSCs (41, 42), suggesting that inhibition of Wnt may impair the dormancy of CSCs. The small-molecule drug ICG001, which selectively inhibits β-catenin binding to the transcriptional cofactor cyclic AMP response element binding protein (CREB), has been approved for phase 1 trial (59, 60). Several polyphenols, including quercetin, epigallocatechin-3-gallate (EGCG), curcumin, and resveratrol, have been implicated as inhibitors of Wnt/β-catenin signaling and are being considered for clinical trials, although the specificity of these agents is unclear (60). Therapeutic monoclonal antibodies against Wnt-1 and Wnt-2 have been shown to inhibit Wnt signaling and suppress tumor growth *in vivo* (60). However, when using this approach, the potential directly antiproliferative effects of Wnt inhibition on stem cells also need to be considered.

Conclusions

The resistance of CSCs to chemotherapy may be explained by their state of dormancy. Recent studies have improved our understanding of the mechanisms that maintain stem cells in a quiescent state and suggested possible strategies to target these difficult-to-eliminate populations. Dormant CSCs may be activated by targeting the extrinsic or intrinsic mechanisms that maintain them in a quiescent state, potentially rendering them susceptible to targeted or conventional chemotherapy (61). The kinetics of CSC activation and sensitization need to be better understood for optimal design of such combinatorial approaches. Other strategies are aimed toward directly inhibiting survival or self-renewal of quiescent CSCs through means such as epigenetic modifications. The identification of molecular mechanisms that underlie the enhanced survival and drug resistance of quiescent CSCs will be very helpful in facilitating the development of such strategies in the future (57, 60). Evaluation of treatment approaches also requires the development of assays or biomarkers for quiescent CSCs that can be used for patient selection and assessment (62). The realization of these objectives may indeed make

it possible to target and eliminate quiescent CSCs in the future, and enhance long-term cures for cancer patients.

Disclosure of Potential Conflicts of Interest

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