Molecular Pathways: Stem Cell Quiescence

Ling Li and **Ravi Bhatia**

Division of Hematopoietic Stem Cell and Leukemia Research, City of Hope National Medical Center, Duarte, CA 91010

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 self-renewal of stem cells and a critical factor in resistance of cancer stem cells (CSC) to chemotherapy and targeted therapies. Limited knowledge of quiescence mechanisms has prevented significant advance in targeting of drug resistant quiescent CSC populations in the clinic. Thus improved understanding of the molecular mechanisms of quiescence in adult stem cells is critical for development of molecularly targeted therapies against quiescent CSC in different cancers. Recent studies have provided a better understanding of 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, BMP, TPO, 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.

Keywords

Adult stem cells; Quiescence; Cancer stem cells; Stem cell niche; Targeted therapy

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 self-renew themselves (1). In contrast to germline stem cell of invertebrates that are constantly cycling (2), mammalian adult stem cells are predominantly in a quiescent, non-dividing, G_0 state (3). Hematopoietic stem cells (HSC) are amongst the best studied adult stem cell populations. Quiescence of HSC has been linked to their long-term reconstituting capacity and is critical for long-term sustenance of the stem cell compartment (1). In order to both maintain a supply of mature blood cells throughout the lifetime of an individual without exhausting the HSC pool, most HSC remain quiescent under steady state and only a small number enter the cell cycle (4). However, HSC can exit quiescence and rapidly expand and differentiate to regenerate hematopoiesis in response to stresses such as blood loss (4). Defects in 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). As with normal tissues, several cancers are also propagated by small populations

Address correspondence to: Ravi Bhatia, MD, Division of Hematopoietic Stem Cell and Leukemia Research, City of Hope National Medical Center, Duarte, CA 91010, Telephone: (626) 359-8111 ext 62705, Fax: (626) 301-8973, rbhatia@coh.org.

of cancer stem cells (CSC) (6). Stem cell quiescence is highly relevant to cancer therapy since quiescent CSC 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 mechanisms of stem cell quiescence is important not only for directed manipulation of normal stem cells function, but also for development of approaches to therapeutically target quiescent CSC.

Stem cells 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 in understanding the mechanisms of quiescence of stem cells and potential applications to cancer treatment.

Intrinsic mechanisms regulating stem cell quiescence

p53 signaling—In addition to its important role in the cellular response to DNA damage, recent studies have shown a critical role for p53 in regulating HSC quiescence in steady state conditions (8, 10). p53 is preferentially expressed in HSC compared to more committed progenitor calls and promotes HSC quiescence (8). The transcription factor MEF/ELF4 modulates p53 expression and facilitates the entry of quiescent HSCs into 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 cell cycle and regulating HSC pool size under stress (11), subsequent studies have indicated that p21 plays a minimal the 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 for regulating stem cell maintenance (14, 15). The FoxO group of human forkhead proteins contains four 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). HSC 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 HSC (14). ATM is preferentially expressed in cycling HSC, and ATM-deficient mice show elevated ROS levels, lack quiescent HSC and show progressive BM failure (14). Treatment with antioxidants restores 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 HSC and regulate HSC metabolism (18). HSC 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 proven 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). Pharmacological suppression of NFATc1 or gene ablation reveals a role in regulating HSC quiescence (21). NFATc1 expression is activated by BMP and acts to repress CDK4 transcription. NFATc1 is downregulated when stem cells become activated during hair growth, relieving CDK4 repression and activating proliferation (21).

Negative regulators of mTOR: Fbw7, PTEN, PML—Several reports indicate 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 hyper-proliferation and subsequent exhaustion, and 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 promoting maintenance of quiescent HSC (27). Osteoblasts are a source of angiopoietin-1 (Ang-1), the ligand for the receptor tyrosine kinase Tie2 specifically expressed in HSCs. Tie2/Ang-1 signaling activates β1-integrin and N-Cadherin in HSC, promoting interactions with extracellular matrix and cellular components of the niche (3). Genetic mouse models indicate that Tie2 interactions maintain quiescence and enhance 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.

Transforming Growth Factor-β and Bone Morphogenic Proteins—Transforming growth factor-β (TGF-β) and related molecules play important role in maintenance of quiescence. TGF-β is a potent inhibitor of stem cell growth and cycling i*n vitro* (30) - and is hypothesized to be a cardinal regulator of stem cell quiescence *in vivo*. Disruption of bone morphogenic proteins (BMP) signaling through conditional knock out 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 the TPO/MPL interactions with a neutralizing antibody reduced the number of quiescent HSCs. TPO treatment increased the expression of p57Kip2, specifically expressed in the quiescent HSC population (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 in the regulation of HSC cycling (31). N-Cadherin is present at the interface between HSC 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 HSC.

Osteopontin—Osteopontin (Opn) expressed in osteoblasts negatively regulates HSC number in the BM niche (37). Osteopontin deficient mice show an increase in HSC

numbers, suggesting that Opn inhibits HSC proliferation *in vivo* (38, 39). Normal HSC 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, polarity, and in stem cell maintenance (1). Constitutively active nuclear β-catenin signaling reduces quiescence of HSCs and blocks HSC differentiation (40). On the other hand, osteoblast-specific expression of Dickkopf1 (Dkk1), an inhibitor of canonical Wnt signaling, resulted in increased HSC cycling and reduced regenerative capacity (41). Therese 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 long-term maintenance of HSC (42). These observations suggest that fine-tuning of Wnt/β-catenin activity in the microenvironment is crucial for maintenance of stem cell quiescence).

CLINICAL TRANSLATIONAL ADVANCES

Malignant stem cells in cancer are characteristically quiescence, 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 CSC (6). As with solid tumors, several leukemias such as AML contain heterogeneous cell populations with a small percentage of quiescent leukemia stem cells (LSC) responsible for propagation of the leukemia (6). Recent studies using xenogeneic models indicate that AML LSC are localized to the BM endosteal region are non-cycling and resist elimination by chemotherapy (43). CD34+ cells from CML patients also contain quiescent cells, that are resistant to BCR-ABL tyrosine kinase inhibitors such as Imatinib mesylate (44). Primitive LSC 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 may be a critical step towards a cure for this and other CSC-driven cancers. Targeting of quiescent CSC is a difficult challenge since most conventional and targeted ant-cancer agents are ineffective in killing this population. There has been increased interest recently to develop approaches based either on activating quiescent CSC inducing their cell cycle entry and increasing their sensitivity to other treatments, or identifying agents that are capable of direct targeting quiescent CSC.

G-CSF

Granulocyte colony-stimulating factor (G-CSF) is used in the clinic to treat neutropenia and to 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 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 IM 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 enhance their elimination by cytarabine without enhancing sensitivity of normal HSCs (43). Thus these protocols may be effective with further improvement and optimization (48).

Interferon

Type I interferons (IFN-α, β) play a critical role in resistance to viral infections and innate and acquired immune responses (49). IFNs also have anti-proliferative properties on many

cell types *in vitro*. However recent observations suggest (50, 51) that IFN-α can stimulate the proliferation of HSC *in vivo*. How IFN-α signals are perceived differently in HSC compared to other cell types in which IFN-α normally suppress proliferation is unclear. The proliferative effects of IFN on HSC appear to be direct and are transient. Since increased proliferation is only seen *in vivo*, alterations in niche interactions may play a role. Two recent randomized studies showed greater reduction in BCR-ABL levels when IFN-α is combined with imatinib in the treatment of CML (52, 53). The clinical value of IFN-α in CML treatment may be related to stimulation of quiescent LSC to proliferate, increasing 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 HSC from the niche (54). AMD3100 rapidly mobilizes HSC 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 underway 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 LSC resistant to elimination with imatinib alone (57). HDACi have pleiotropic effects on cells but potential mechanisms involved in CML LSC inhibition include alteration of gene expression related to LSC self-renewal, survival and microenvironmental interactions. A clinical trial of HDACi in combination with imatinib in patients with CML in cytogenetic remission is underway (58).

Wnt inhibitors

Studies showing a role for Wnt signaling in the microenvironment in maintenance of quiescent HSC (41, 42), suggest that inhibiting Wnt may impair the dormancy of CSC. A small molecule drug ICG001 that selectively inhibits β-catenin binding to the transcriptional cofactor cyclic AMP response element binding protein (CBP) is approved for Phase 1 trial (59, 60). Several polyphenols, such as 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 direct anti-proliferative effects of Wnt inhibition on stem cells also need to be considered.

Conclusion

The resistance of CSC 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 CSC 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 towards directly inhibiting survival or self-renewal of quiescent CSC through means such as epigenetic modifications. Identification of molecular mechanisms underlying enhanced survival and drug resistance of quiescent CSC will be very helpful in facilitating development of such strategies in future. (57, 60). Evaluation of treatment approaches also requires development of assays or biomarkers for quiescent CSC that can be used for patient selection and assessment (62). Realization of these objectives may indeed make it possible to target and eliminate quiescent CSC in future enhancing long-term cures for cancer patients.

Acknowledgments

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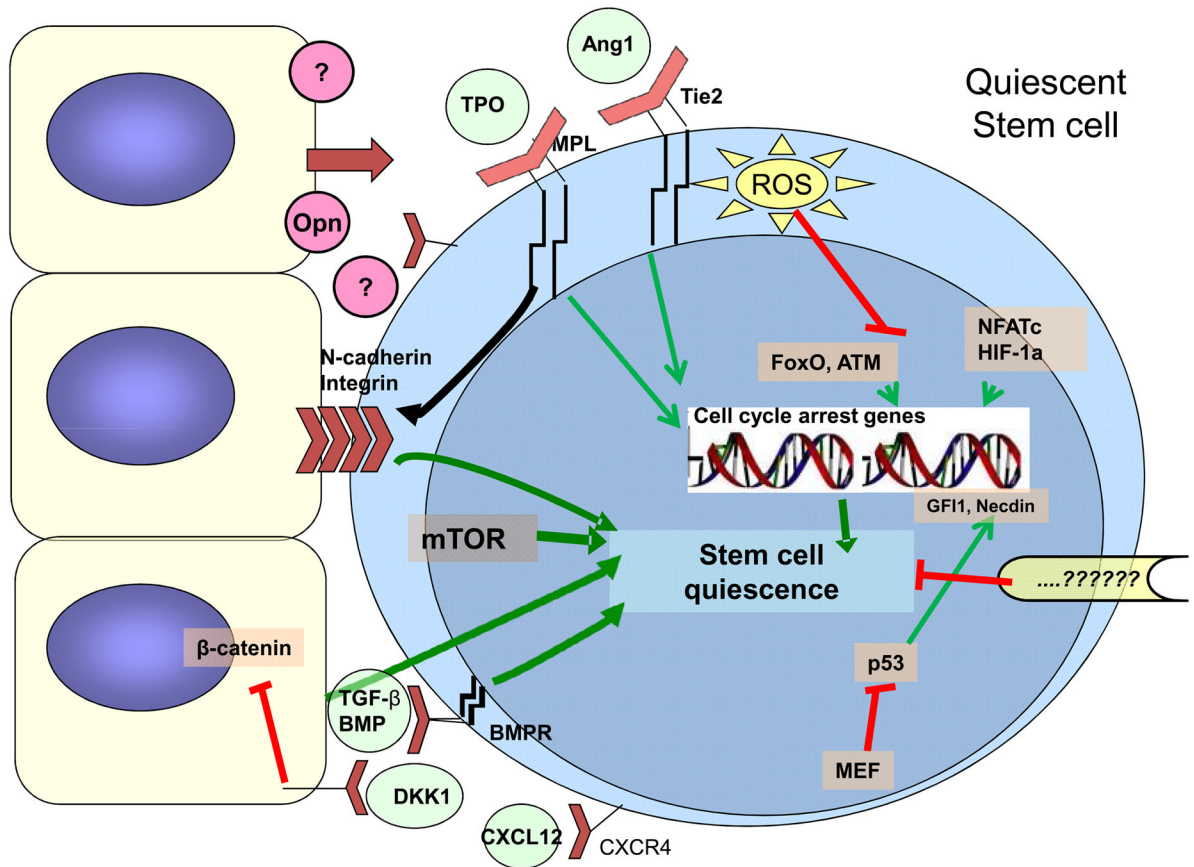## Microenvironmental cells

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. Reactive oxygen species (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 inhibiting 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 thrombopoietin (TPO) with its receptor MPL and Angiopoietin-1 (Ang-1) with its receptor Tie-2 also promote stem cell quiescence and enhance adhesion to the microenvironment through integrin and cadherin receptors. Osteopontin (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 for maintaining HSCs in a quiescent state. In future, targeting of molecular mechanisms underlying quiescence may allow sensitization of quiescent cancer stem cells to therapeutic agents.