**Bmi1, stem cells, and senescence regulation**

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Stem cells generate the differentiated cell types within many organs throughout the lifespan of an organism and are thus ultimately responsible for the longevity of multicellular organisms. Therefore, senescence of stem cells must be prevented. **Bmi1** is required for the maintenance of adult stem cells in some tissues partly because it represses genes that induce cellular senescence and cell death.

Many tissues are maintained throughout the lifespan of an organism by a small number of adult stem cells. These cells are unique in that they have both the ability to give rise to new stem cells via a process called self-renewal and the ability to differentiate into the mature cells of a tissue. To maintain tissue homeostasis, stem cells have developed strict regulatory mechanisms to self-renew, differentiate, and prevent premature senescence and apoptosis (see review, ref. 1). The recent observation that **Bmi1**, a Polycomb group repressor, is essential for the self-renewal of adult murine hematopoietic stem cells (HSCs) and neuronal stem cells, in part via repression of genes involved in senescence, suggests that stem cells have evolved specific mechanisms to repress senescence and to prolong their capacity to proliferate. In this Perspective, we discuss the possible role of **Bmi1** in the prevention of senescence in stem cells.

**What makes a cell a stem cell?**

HSCs are among the best-characterized stem cells. The existence of these cells was proven using clonal assays and retroviral marking (2, 3). Flow cytometry was then used to isolate HSCs based on cell-surface marker expression (4, 5). Subsequently, other types of somatic stem cells such as neuronal stem cells from the peripheral and central nervous systems have been identified (6, 7).

Stem cells possess three fundamental properties (1). First, they must self-renew, allowing the maintenance of the original stem cell population. Self-renewal is a cell division in which one or both of the daughter cells are stem cells that retain the same developmental potential as the mother cell. In contrast, proliferation is a more general term that refers to all types of mitosis, whether they yield stem cells, restricted progenitors, or terminally differentiated cells. Second, stem cells must be able to differentiate into multiple types of mature cells in order to replace the mature cells that turn over in adult tissues. Third, the total number of stem cells is strictly regulated via both extrinsic and intrinsic mechanisms, resulting in the stability of a stable stem cell pool (8–11).

**Stem cells and senescence**

Senescence is a state in which a cell no longer has the ability to proliferate. Since stem cells maintain many tissues during the lifetime of an animal, it follows that stem cell senescence must be prevented to maintain an organ throughout life. Several studies suggest that cellular senescence is accompanied by changes in gene expression, which might be regulated by epigenetic mechanisms. In support of this hypothesis, histone deacetylase inhibitors, which decondense chromatin and activate the transcription of some genes, can induce a senescence-like state in human fibroblasts (12), suggesting that conversion of some heterochromatin to euchromatin may be a feature of replicative senescence (13, 14). Other studies suggest that chromatin condensation and subsequent downregulation of certain genes might regulate senescence. Senescence accompanies changes in nuclear morphology and formation of a distinct chromatin structure, called senescence-associated heterochromatic foci (SAHF) (15). SAHF do not contain active transcription sites, and they recruit heterochromatin proteins to the genes that are to be stably repressed during senescence. It was shown that SAHF contained the retinoblastoma protein (pRB) in the EZF-responsive promoters, such as cyclin A and proliferating cell nuclear antigen pro-
Role of Bmi1 in stem cell self-renewal

Since epigenetic events such as histone modification have been implicated in senescence, it follows that genes involved in chromatin remodeling and gene expression, such as members of the Polycomb and Trithorax families, might be directly involved in decisions that affect stem cell fate, including self-renewal, senescence, and possibly aging. Polycomb and Trithorax proteins form large multimeric structures, which can lead to repression or activation of gene expression, respectively, via a concerted process of chromatin modifications (22, 23).

Both HSCs and neuronal stem cells express high levels of Bmi1 (24–26), a member of the Polycomb group of transcription repressors that was initially identified as an oncogene cooperating with c-myc in a murine model of lymphoma (27, 28). Bmi1 has a RING finger at the amino-terminus and a central helix-turn-helix domain. The RING finger domain is required for the generation of lymphoma in Eμ-Bmi1 transgenic mice (29, 30). Postnatal mice lacking Bmi1 exhibit defects in hematopoiesis, skeletal patterning, neurological functions, and development of the cerebellum (31).

It has recently been shown that Bmi1 is necessary for efficient self-renewing cell divisions of adult HSCs as well as adult peripheral and central nervous system neural stem cells, but that it is less critical for the generation of differentiated progeny (25, 26). Transplantation of Bmi1−/− fetal liver cells resulted in only transient hematopoietic cell reconstitution, suggesting that the transplanted mutant fetal liver HSCs failed to generate more HSCs but gave rise to multipotent progenitors that could sustain hematopoiesis for up to 4–8 weeks. Similarly, Bmi1 is needed for the maintenance of neural stem cells found in both the central and peripheral nervous systems. As with HSCs, the reduced self-renewal of Bmi1-deficient neural stem cells led to their postnatal depletion in vivo, but the proliferation and survival of committed progenitor cells were essentially normal (26). Given the broad ranges of phenotypic changes in Bmi1-deficient mice, including posterior transformation and neurological abnormalities (31), and its broad tissue distribution (32), it is likely that Bmi1 regulates the self-renewal of other types of somatic stem cells.

Bmi1 may also play a key role in some types of cancer (33–35). In approximately 11% of cases of mantle cell lymphoma, the malignant cells have a three- to seven-fold amplification of Bmi1 DNA and express high levels of the protein, implicating this gene in this invariably lethal form of lymphoma. In a mouse model of senescence of mouse HSCs during serial transplantation (19). On the other hand, telomerase-deficient HSCs can be serially transplanted only twice, accompanied by an increased rate of telomere shortening, indicating that telomerase is nonetheless needed to prevent premature loss of telomere function during serial transplantation (20, 21).

motors, and silenced the expression of E2F-responsive genes during senescence but not during quiescence (15). Formation of SAHF and silencing require an intact pRB pathway, since inhibition of p16Ink4a prevents SAHF formation and leads to DNA replication. These results provide a molecular mechanism for the maintenance of the senescent state and demonstrate the importance of pRB as a tumor suppressor.

HSCs have an impressive regenerative potential, as demonstrated by transplantation experiments using limited numbers of cells. In mice, serial transplantation is possible for four to six passages, suggesting that individual HSCs are capable of extensive self-renewal but may not be immortal. Even though HSCs express telomerase (16, 17), it is not sufficient to completely prevent telomere erosion during aging (18). Overexpression of the catalytic subunit of the telomerase enzyme in hematopoietic cells prevents telomeres from shortening during serial transplantation of bone marrow. However, even HSCs overexpressing telomerase could be serially transplanted no more than four times, as is the case with wild-type HSCs; this suggests that a telomere-independent mechanism regulates replicative
leukemia, Bmi1 was essential for the maintenance of leukemic cells (36). Enforced expression of Hoxa9/Meis-1 in both normal and Bmi1-deficient mouse fetal liver cells, followed by transplantation, initially resulted in infiltration of the bone marrow by cells that looked like acute myeloid leukemia (AML) blasts, and mice developed a bone marrow infiltrate that resembled AML. However, only Bmi1 wild-type AML could be serially transplanted. Taken together with the detection of high levels of Bmi1 in human AML stem cells (25), these results suggest that Bmi1 is also required for the self-renewal of leukemic stem cells.

Bmi1 and senescence
In WI-38 human fetal lung fibroblasts, Bmi1 is downregulated when the cells undergo replicative senescence, but not when they are quiescent. Additionally, Bmi1 extends replicative lifespan but does not induce immortalization when overexpressed (37). In the absence of Bmi1, both the p16Ink4a and the p19Arf genes from the Ink4a locus are expressed (38). Lifespan extension by Bmi1 is mediated in part by suppression of the p16Ink4a, dependent senescence pathway and requires an intact pRB pathway, but not the p53 tumor-suppressor protein. The RING finger and helix-turn-helix domains of Bmi1 were required for lifespan extension and p16Ink4a suppression. Furthermore, a RING finger deletion mutant acted as a dominant negative, inducing p16Ink4a and premature senescence (37).

Normal mouse embryonic fibroblasts (MEFs) reach replicative senescence after seven passages in culture, whereas MEFs from Bmi1−/− mice show a premature-senescence phenotype at the third passage. This was correlated with increased expression of p16Ink4a. Re-expression of Bmi1 in Bmi1−/− MEFs prevented premature senescence (28). Overexpression of Bmi1 gave a proliferative advantage and extended MEF lifespan. Furthermore, unlike human fibroblasts, Bmi1 could immortalize MEFs.

Downstream targets of Bmi1
Gene-profiling studies suggest that Bmi1 modulates HSC self-renewal through the regulation of genes important for stem cell fate decisions, as well as survival genes, antiproliferative genes, and stem cell–associated genes (Figure 1) (25). The previously mentioned Bmi1 target, the Ink4a locus (28), encodes p16Ink4a and p19Arf using different promoters (38). Enforced expression of p16Ink4a and p19Arf in HSCs led to senescence and apoptosis, respectively (25). In neural stem cells, p16Ink4a deficiency partially restored the ability of Bmi1-deficient stem cells to self-renew (26).

Figure 2 illustrates regulation of the cell cycle and senescence by Bmi1. In normal stem cells, p16Ink4a and p19Arf genes are repressed in a Bmi1-dependent manner. In the absence of p16Ink4a, the cyclin D/Cdk4/6 complex can phosphorylate pRB, allowing the E2F-dependent transcription that leads to cell cycle progression and DNA synthesis. In addition, MDM2-mediated p53 degradation causes low p53 levels in the absence of p19Arf, thus preventing cell cycle arrest and apoptosis. The absence of Bmi1 relieves the repression of the Ink4a locus, resulting in the expression of p16Ink4a and p19Arf. p16Ink4a inhibits binding of cyclin D to Cdk4/6, resulting in inhibition of the kinase activity. This leads to a hypophosphorylated pRB, which then binds E2F and inhibits E2F-dependent transcription, resulting in cell cycle arrest and senescence. p19Arf inhibits MDM2, which mediates ubiquitin-dependent degradation of p53, thus leading to accumulation of p53 protein in the cell. This leads to induction of various p53 target genes involved in cell cycle arrest and apoptosis. Proteins affected by high and low levels of Bmi1 are shown by black and red arrows, respectively. *Sites of frequent mutations associated with cancer.
in many types of human cancers, which implicates them as key regulators of immortalization and/or senescence checkpoints.

Mice lacking Bmi1 showed induction of both p16\(^{ink4a}\) and p19\(^{arf}\) in various hematopoietic and neuronal tissues (25). Overexpression of p16\(^{ink4a}\) and p19\(^{arf}\) in adult HSCs induced cell cycle arrest and apoptosis via the pRB and the p53-dependent pathway, respectively. Double deletion of the Bmi1 and p16\(^{ink4a}\)/p19\(^{arf}\) genes partially rescued the phenotypes observed in Bmi1-deficient mice (28), suggesting that p16\(^{ink4a}\), p19\(^{arf}\), and p53 are downstream effectors of Bmi1 that are involved in the control of the proliferation and survival of HSCs during self-renewing cell divisions (Figure 2). Therefore, Bmi1 maintains the HSC pool in part by repressing genes involved in cellular senescence. Increased expression of the p53 target gene Wig1 in Bmi1\(^{-/-}\) bone marrow suggests that the p19\(^{arf}\) pathway may have been activated in Bmi1\(^{-/-}\) hematopoietic cells. Wig1 is a double-stranded RNA-binding protein and inhibits tumor growth in vitro, suggesting that it may function in stress-induced p53 responses (43). The observation that p53-deficient mice have increased numbers of stem cells is consistent with the notion that p53 might be a downstream effector of Bmi1 (44). In addition, some of the Hox9 family of genes are also affected in Bmi1-deficient hematopoietic tissues and neurospheres (25, 26). Determination of the relative contribution of each of these pathways to the regulation of HSC self-renewal will require careful analysis of the HSCs from double- or triple-knockout mice.

There is evidence that Bmi1 might regulate telomerase expression in human mammary epithelial cells (MECs) and might play a role in the development of human breast cancer. Bmi1 is overexpressed in several breast cancer cell lines and postselection human MECs immortalized with human papilloma virus E6 oncoprotein, which abrogates the p53/p21\(^{wd}\) pathway (45), suggesting that Bmi1 might be involved in immortalization. Postselection MECs can be obtained by regular feeding of a heterogeneous population of MECs from primary mammary tissue. During this process, the p16\(^{ink4a}\) gene is progressively silenced and not expressed in postselection MECs (46, 47). Overexpression of Bmi1 in postselection MECs bypasses senescence, extending replicative lifespan and immortalizing MECs. This is associated with human telomerase reverse transcriptase (hTERT) expression, which leads to induction of telomerase activity. Although hTERT is a direct target of c-Myc-induced transcription in MECs (48, 49), Bmi1 appeared to act independently of c-Myc. Since Bmi1 is a transcription repressor, induction of telomerase is probably mediated by an indirect mechanism. Deletion analysis of the Bmi1 protein suggested that the RING finger, as well as the conserved helix-turn-helix domain, was required for its ability to induce telomerase and immortalization. These data suggest that Bmi1 directly or indirectly regulates telomerase expression in MECs and might play a role in the development of human breast cancer. However, Bmi1 induction of telomerase is cell type specific; Bmi1 fails to induce telomerase in fibroblasts (45). This is consistent with the observation that Bmi1 overexpression did not immortalize human fibroblasts (37). It is not known whether Bmi1 is involved in telomere function in normal breast stem cells.

**Future directions**

Bmi1 maintains the stem cell pool by preventing premature senescence, either through repression of genes involved in senescence or perhaps through induction of telomerase to prevent telomere shortening. It is very likely that Bmi1 is important for maintenance of multiple types of somatic stem cells, since it is widely expressed and Bmi1-deficient mice have developmental defects in other organs. Bmi1 is also important for maintenance of leukemic stem cells and perhaps other tumorigenic stem cells; therefore, Bmi1 could be used as a molecular target to induce senescence in cancer stem cells (50).

Since Bmi1 maintains the HSC pool size and regulates key genes implicated in senescence and aging, it is of interest to determine whether expression of Bmi1 and its target genes changes during stem cell transplanation and/or aging. Whether stem cells undergo senescence during aging is controversial (51–53). In C57BL mice, in which most HSC studies have been performed, HSC numbers increase with age without losing overall function (54–56). However, HSC senescence might occur during aging in certain other strains of mice (57, 58). The number of times that HSCs can reconstitute the bone marrow of lethally irradiated mice is limited in serial-transplantation experiments. This observation might be either a result of an intrinsic stem cell aging program that occurs only when stem cell proliferation far exceeds that seen during normal aging, or a result of damage to the stem cells that is secondary to the stress of the transplant. In either model, it is possible that the loss of stem cell activity is mediated by Bmi1 or its downstream targets.

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