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Perspective Series

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***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.

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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 E2F-responsive promoters, such as cyclin A and proliferating cell nuclear antigen pro-

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Nonstandard abbreviations used: hematopoietic stem cell (HSC); senescence-associated heterochromatic foci (SAHF); retinoblastoma protein (pRB); acute myeloid leukemia (AML); mouse embryonic fibroblast (MEF); cyclin-dependent kinase (Cdk); mouse double minute 2 (MDM2); mammary epithelial cell (MEC).

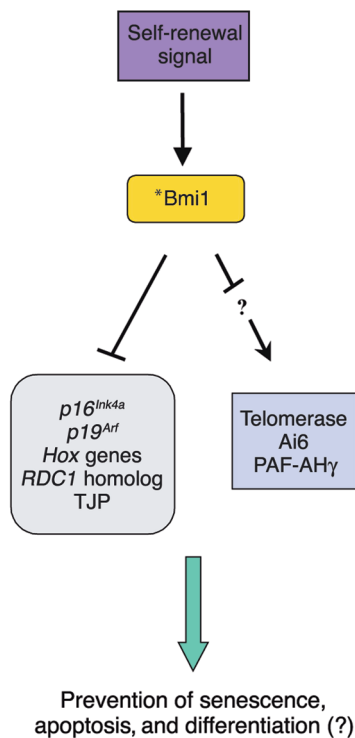


Figure 1

Postulated Bmi1 targets. Extrinsic signals for a stem cell to self-renew result in elevation of the Bmi1 level in stem cells. This allows repression of various genes including the *Ink4a* locus genes, *p16^{Ink4a}* and *p19^{Arf}*, and possibly activation, via indirect mechanisms, of some genes including telomerase, apoptosis inhibitor-6 (Ai6), and platelet-activating factor acetylhydrolase (PAF-AH γ). These genes are likely play a role in stem cell fate decisions including self-renewal and differentiation. *Sites of frequent mutations associated with cancer. TJP, tight junction protein. RDC1, chemokine orphan receptor 1.

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 *p16^{Ink4a}* 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

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).

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 E μ -*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

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 down-regulated 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 *p16^{Ink4a}* and the *p19^{Arf}* genes from the *Ink4a* locus are expressed (38). Lifespan extension by *Bmi1* is mediated in part by suppression of the *p16^{Ink4a}*-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 *p16^{Ink4a}* suppression. Furthermore, a RING finger deletion mutant acted as a dominant negative, inducing *p16^{Ink4a}* 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 *p16^{Ink4a}*. 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 *p16^{Ink4a}* and *p19^{Arf}* using different promoters (38). Enforced expression of *p16^{Ink4a}* and *p19^{Arf}* in HSCs led to senescence and apoptosis, respectively (25). In neural stem cells, *p16^{Ink4a}* 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 *p16^{Ink4a}* and *p19^{Arf}*. During the cell cycle, pRB is hyperphosphorylated by the cyclin D/cyclin-dependent kinases 4 and 6 (cyclin D/Cdk4/6) complex (39). The hyperphosphorylated pRB is unable to bind and inhibit E2F transcription factor, allowing transcription of E2F target genes that are important

for the G1/S transition, such as DNA polymerase II, cyclin E, p19, myb, and dihydrofolate reductase (40). This allows cell cycle progression. In the absence of *Bmi1*, *p16^{Ink4a}* is upregulated and prevents binding of Cdk4/6 to cyclin D, inhibiting the kinase activity. This results in hypophosphorylated pRB, which then binds E2F and inhibits E2F-mediated transcription, leading to cell cycle arrest and senescence (39). *p19^{Arf}* sequesters mouse double minute 2 (MDM2) and inhibits p53 degradation, resulting in p53-mediated cell cycle arrest and apoptosis (41, 42). Point mutations and deletion of *p16^{Ink4a}* and *p19^{Arf}* are frequently found

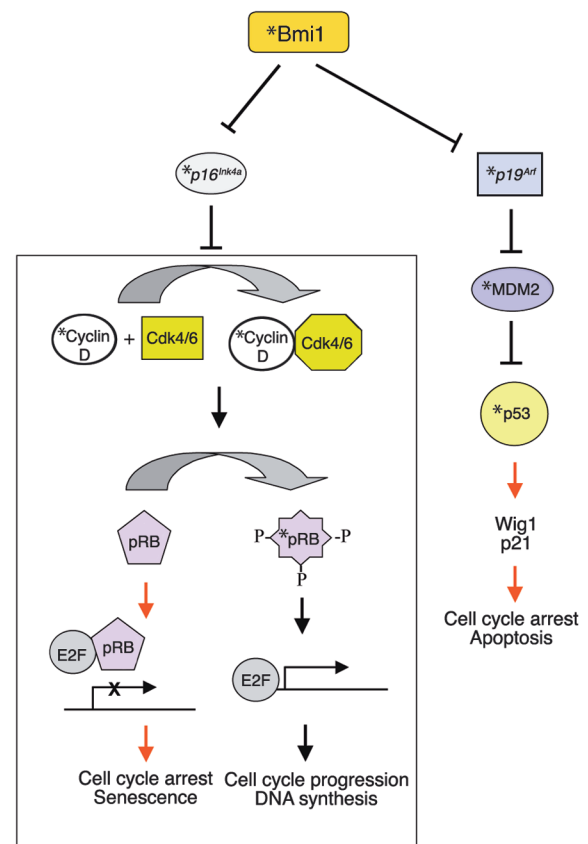


Figure 2

Regulation of cell cycle, apoptosis, and senescence by *Bmi1*. In normal stem cells, *p16^{Ink4a}* and *p19^{Arf}* genes are repressed in a *Bmi1*-dependent manner. In the absence of *p16^{Ink4a}*, 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 *p19^{Arf}*, thus preventing cell cycle arrest and apoptosis. The absence of *Bmi1* relieves the repression of the *Ink4a* locus, resulting in the expression of *p16^{Ink4a}* and *p19^{Arf}*. *p16^{Ink4a}* inhibits binding of cyclin D to Cdk4/6, resulting in inhibition of the kinase activity. This leads to a hypophosphorylated pRB, which then can bind E2F and inhibit E2F-dependent transcription, resulting in cell cycle arrest and senescence. *p19^{Arf}* 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* oncogene, which abrogates the p53/p21^{waf} 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 transplantation 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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