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Review

Bmi-1, stem cells and cancer

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Bmi-1, a polycomb gene family member, plays an important role in cell cycle regulation, cell immortalization, and cell senescence. Recently, numerous studies have demonstrated that *Bmi-1* is involved in the regulation of self-renewal and differentiation of stem cells. However, the molecular mechanism underlying this biological process remains largely unclear. In the present review, we summarized the function of Bmi-1 as a transcriptional regulator of gene expression, with particular reference to stem cells.

Keywords Bmi-1; stem cell; self-renewal; cancer

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Introduction

Stem cells are characterized as unspecialized precursor cells that possess the multipotent ability to self-renew and differentiate into tissue-specialized cells. Both tissue development and homeostasis are mediated by stem cells, including embryonic stem (ES) cells and tissue stem cells (or adult stem cells) [1]. ES cells that are derived from the inner cell mass of blastocyst-stage embryos are capable of developing into the fetus. During the process, these cells generate tissue stem cells, progenitor cells, and eventually, every cell type that constitutes an organism. Tissue stem cells include somatic and germline stem cells, which develop, maintain, and repair their resident tissues in adult organisms. Self renewal is the hallmark of stem cells. Stem cells could continuously divide into two types of daughter cells. One type of daughter cell would take on the identity of the parent cell, and the other could transform into a progenitor cell that would further differentiate into specialized cell types. Both ES and tissue stem cells are capable of producing various types of differentiated cells and undergoing continuous self-replication. Stem cell research has enlightened the scientific community on the effective cell-based therapies for certain diseases such as diabetes, neurodegenerative diseases, and cancer [2]. It has been reported that the proliferation and differentiation of stem cells might be related to the regulation of *Hox* (homeobox-containing) genes, which are crucial for cell fate determination and proliferation and for the regulation of the development of an organism [3,4]. The transcriptional repression and activation of *Hox* genes could be regulated by the *polycomb group* (*PcG*) and *Trithorax-group* (*TrxG*) genes, which are essential for the maintenance of the physiological levels of the *Hox* genes during development [5,6]. PcG family proteins, which are well-known epigenetic gene silencers, have been demonstrated to be associated with the self-renewal and differentiation of stem cells [5]. Moreover, *Bmi-1*, the first identified PcG gene, has also been documented to be involved in the transcriptional repression of *Hox* genes and affect the stem cell self-renewal, embryonic development, and proliferation [7–10]. In the present review, we summarized the function of Bmi-1 as a transcriptional regulator of gene expression, with particular reference to stem cells.

Genetic Structure of *Bmi-1*

Polycomb group proteins act as epigenetic gene silencers with essential roles associated with organism development through the formation of a minimum of two multimeric complexes, i.e. the polycomb repressive complex 1 (PRC1) and the polycomb repressive complex 2 (PRC2) [5,7,11–15]. B-cell-specific Moloney murine leukemia virus integration site 1 (*Bmi-1*), which is one of the core members of the PRC1 complex, was

identified as an oncogene that cooperates with c-myc in the initiation of lymphoma [7,11]. The *Bmi-1* gene localizes on human chromosome 10p11.23 and extends over 4.9 kb, which comprises 10 exons and 9 introns. The length of the *Bmi-1* cDNA is approximately 3.2 kb (A = 959, C = 591, G = 678, and T = 975) and further, it encodes a 36.9-kDa nuclear protein consisting of 326 amino acids. The Bmi-1 protein contains a conserved RING finger domain in its N-terminal end and a central helix-turn-helix-turn-helix-turn motif (H-T-H-T), which is required for inducing telomerase activity and immortalization of human epithelial cells [12,16,17].

Role of Bmi-1 in Cancer Initiation and Progression

Bmi-1 has been demonstrated to be involved in multiple biological processes, such as embryonic development, organ formation, tumorigenesis, stem cells stabilization, and differentiation [8]. Bmi-1 is expressed ubiquitously in almost all tissues and its expression is observed to be slightly higher in the brain, spinal cord, kidney, lungs, gonads, and the placenta. However, many studies have shown that Bmi-1 expression is frequently upregulated in various types of human cancers, including lung cancer, ovarian cancer, acute myeloid leukemia, nasopharyngeal carcinoma, breast cancer, and neuroblastoma, which indicates that Bmi-1 might play important roles in cancer initiation and progression [18–23]. The oncogenic feature of *Bmi-1* has also been reported to be associated with the protection of cells from apoptosis. It has been shown that the number of lymphocytes is markedly reduced in the spleen and the thymus due to increased apoptosis in *Bmi-1*$^{-/-}$ null mice [8]. Ectopic expression of Bmi-1 protects keratinocytes from stress agent-induced apoptosis and the expression of Bmi-1 abrogates MYCN-induced sensitization of SHEP1 cells, thereby protecting cells from apoptosis [23]. In addition, numerous studies have demonstrated that expression of Bmi-1 is statistically associated with its clinical value; this suggests that Bmi-1 might be used as a diagnostic and prognostic marker of human cancer. It has been reported that the expression of Bmi-1 is upregulated in nasopharyngeal carcinoma cell lines and nasopharyngeal carcinoma tumors, and high expression level of Bmi-1 is positively correlated with poor prognosis in nasopharyngeal carcinoma patients [21]. Daniela *et al.* have reported that high expression of Bmi-1 was observed in 41 of 64 (64%) primary melanoma tissues and 117 of 165 (71%) metastatic melanoma as compared with that in the primary melanoma, indicating that Bmi-1 expression might be associated with clinical progress of malignant melanoma [24]. The clinical significance of Bmi-1 has also been demonstrated in cases of hepatocellular carcinoma, gastric carcinoma, non-small cell lung cancer (NSCLC), oligodendroglial tumor, and breast cancer [25–29].

Association between Stem Cells and Bmi-1

Increasing evidences have indicated that Bmi-1 plays an important role in the self-renewal and differentiation of human stem cells. Park *et al.* have found that *Bmi-1* is highly expressed in adult and fetal mouse and adult human hematopoietic stem cells (HSCs) using reverse transcription-polymerase chain reaction (RT-PCR) and gene expression analysis [10]. Furthermore, the number of HSCs has been shown to be markedly reduced in postnatal *Bmi-1*$^{-/-}$ mice as compared with that in the fetal liver of *Bmi-1*$^{-/-}$ mice. In addition, they have also demonstrated that the transplanted fetal liver acquired from *Bmi-1*$^{-/-}$ mice could only transiently contribute to hematopoiesis. Moreover, the expression of genes that are associated with stem cell self-renewal, cell survival, transcriptional factors, and cell proliferation, including $p16^{INK4a}$ and $p19^{ARF}$, in fetal liver cells of the *Bmi-1*$^{-/-}$ mice are observed to be completely altered. All these results indicate that Bmi-1 was necessary for the generation and differentiation of self-renewing adult HSCs. Lessard *et al.* have reported that the expression of Bmi-1 in human primitive cells was higher than that in CD34^{-} cells, which further confirmed that Bmi-1 was essential for the self-renewal, proliferation, and differentiation of HSCs, progenitor cells, and leukemia stem cells (LSCs), as well as for the implantation of stem cells *in vivo* and *in vitro* [30]. In addition, Lessard *et al.* have examined the number, migration, colony, and marrow hematopoietic microenvironment of fetal liver cells and fetal liver hematopoietic stem cells of *Bmi-1*$^{-/-}$ mice. They found that *Bmi-1*$^{-/-}$ mice with defective hematopoiesis generated much fewer fetal liver HPCs (1% when compared with 27% in the *Bmi-1*$^{+/+}$ group), which was most probably due to the inappropriate self-renewal ability of HSCs. However, these experiments excluded factors that could affect marrow hematopoiesis, such as defective HSC generation, inability to recruit the HSCs to the bone marrow, and impairment of the hematopoietic microenvironment [9,10]. Additionally, a reconstitution experiment was conducted to examine the restoration of

hematopoietic function in mice that were exposed to lethal radiation and transplanted with embryonic hematopoietic bone marrow cells from either *Bmi-1*$^{+/+}$ or *Bmi-1*$^{-/-}$ mice [10]. The results showed that the hematopoietic capacity was lower in *Bmi-1*$^{-/-}$ recipient mice at 4 weeks following transplantation and the donor-derived HSCs in the recipient bone marrows were undetectable at 8 weeks. No *Bmi-1*$^{-/-}$-derived HSCs were detected in the peripheral blood of the recipient mice at 16 weeks; this indicated that the hematopoietic capacity of the bone marrow cells was completely dependent on the expression of exogenous Bmi-1. Therefore, these results suggest that the decrease in the hematopoietic capacity of *Bmi-1*$^{-/-}$ mice was attributable to the defective self-renewal capability of their HSCs [10]. Further, it has been reported that Bmi-1 plays an essential role in the regulation of the self-renewal of neural stem cells (NSCs). Downregulation of the expression of Bmi-1 in NSCs could lead to lower proliferation and self-renewal ability both *in vivo* and *vitro* [31]. Upregulation of Bmi-1 expression could induce the self-renewal ability of NSCs by transcriptional repression of *INK4a* and *ARF* [32]. Heffner *et al.* have also demonstrated that Bmi-1 plays a crucial role in the process of self-renewal in CD8$^+$ T cells and promotes cellular senescence [33].

It has been reported that the epithelial components of mammary glands consist of stem cells and have the capacity to undergo self-renewal and multilineage differentiation [34]. The Hedgehog pathway has been shown to be associated with the regulation of the self-renewal and differentiation of breast stem cells; further, factors in the hedgehog pathway were found to be highly expressed in mammary stem/progenitor cells [35,36]. It has been demonstrated that *Bmi-1* is a downstream gene in the Hedgehog pathway, which implied that the modulation of the Hedgehog pathway associated with the self-renewal and differentiation of mammary stem cells might be mediated by Bmi-1. Moreover, Bmi-1 expression is upregulated up to six times when the Hedgehog pathway is activated. However, its expression is significantly downregulated when the Hedgehog pathway is blocked by small-interfering RNA (siRNA) [36].

Cancer Stem Cells

Tumor tissues are composed of heterogeneous groups of cells. Some cells are identified as cancer stem cells that are capable of causing constant expansion of existing tumors or form new tumors in the body [37]. Hewitt *et al.* have found that only 1–4% of the transplanted cells in the spleen can retain the ability of cloning after transplanting murine leukemia cells into mice with similar genetic backgrounds as donors. This observation indicated that only part of the cells from tumors could form tumors again [38]. Subsequently, Trott further demonstrated that only the cells that were isolated from a particular subgroup have high cloning ability; moreover, he proposed that $<1\%$ of tumor cells possess the quality of cancer stem cells that retain their ability to undergo self-renewal and differentiation into specialized cells [39]. In 1997, human LSCs were identified by Bonnet *et al.* [40]. It was shown that even though different types of leukemia cells could be isolated from leukemia patients, only those whose surfaces expressed markers, such as CD34$^+$CD38$^-$Thy-1$^-$, possess the ability to undergo self-renewal and form tumor *in vitro* [40].

In addition, AI-Hajj *et al.* identified and isolated cancer stem cells of CD44$^+$CD24$^{-/low}$ lineage from breast cancers tissues [41]. Furthermore, they demonstrated that these cells could be considered to be breast tumor-initiating cells since as few as 100 cells with CD44$^+$CD24$^{-/low}$ characteristic were observed to be able to form tumors in mice, whereas tens of thousands of cells without these phenotypes failed to form tumors [41]. Moreover, the expression of *Bmi-1* has been found to be upregulated up to 5-fold in CD44$^+$CD24$^{-/low}$lin$^-$ cells as compared with that in the cells isolated from the same tumor, which are the cells that are negative for cancer stem cell marker [36]. All these findings strongly suggest the existence of cancer stem cells.

Biological Functions of Bmi-1 in the Regulation of Stem Cells and Cancer Stem Cells

A number of studies have demonstrated that Bmi-1 plays an important role in the self-renewal and differentiation of human hematopoietic and LSCs [9,10,20,42,43]. Lessard *et al.* have reported that even though acute myeloid leukemia (AML) could develop in mice that were transplanted with bone marrow cells derived from either *Bmi-1*$^{+/+}$ or *Bmi-1*$^{-/-}$ recipient mouse, the stem cell number in the peripheral leukemia cells from *Bmi-1* wild-type mice was significantly higher than that in *Bmi-1*-knockout mice [30]. Furthermore, the number of leukemia cells derived from *Bmi-1*$^{-/-}$ mouse reduced by 15 ± 4 times when compared with that in the control cells from *Bmi-1* wild-type mouse following 10 days of

culture *in vitro* [30]. They further observed that the number of leukemia cells derived from *Bmi-1*$^{-/-}$ mouse in the S-phase reduced significantly and most of the cells were accumulated in the G1 phase; moreover, the number of apoptotic cells increased and their colony-forming abilities decreased. All these results strongly implied that Bmi-1 has a critical and dose-dependent role in regulating the proliferation of cancer cells and the development of leukemia. Medulloblastoma is a type of brain tumor that originates from progenitor cells from the external granular layer of the external cerebellum. It has been shown that knockdown Bmi-1 in human medulloblastoma cell lines causes inhibition of proliferation, loss of clonogenic survival, and anchorage-independent growth *in vitro*, as well as suppression of tumor formation *in vivo* [44]. Furthermore, all these phenomena have been demonstrated to be associated with increase in the expression of various important developmental regulators and differentiation factors, such as matrix metalloproteinase 3 (TIMP-3), hedgehog interacting protein (HHIP), and inhibin A (*INHBA*) genes. It is particularly noteworthy that the function of Mel-18, another Polycomb group family member, has a function that overlaps with that of Bmi-1 in the regulation of the abovementioned biological processes [44].

It has been reported that the cooperation of Bmi-1 with c-myc could induce telomerase activity and downregulate p16^{INK4a} and p19^{ARF} expression; this allows cells to bypass senescence and immortalizes them [21–23,45]. Human telomerase reverse transcriptase (hTERT) is capable of stabilizing telomeres in stem cells; this ability is important for the self-renewal and differentiation properties of the latter [46–48]. However, the molecular mechanism underlying the regulation of the differentiation of human stem cells by Bmi-1 remains largely unknown. It has been suggested that Bmi-1 might play a role in the regulation of stem cells via the stabilization of telomeres since it has been proven that Bmi-1 induces hTERT activity in normal mammary epithelial cells and nasopharyngeal epithelial cells [21,22]. Another possible mechanism of Bmi-1 on stem cell regulation is the repression of *p16*^{INK4a} *and p19*^{ARF} by Bmi-1 [17,45,49]. The proteins p16^{INK4a} and p19^{ARF}, transcribed from the same gene, namely *INK4a*, is tightly associated with the regulation of the cell cycle [50,51]. The p16^{INK4a} protein could inactivate Cdk by directly binding to Cdk4 and Cdk6, and lead to the suppression of the phosphorylation of the retinoblastoma (Rb) susceptibility protein and Cdk-dependent Rb-associated protein; as a consequence, the downstream gene of Rb is repressed and the cell cycle is arrested in the G1/S phase [52,53]. The p19^{ARF} (homolog of human p14^{ARF}) protein is capable of stabilizing p53 by antagonizing MDM2 and activating p53-dependent transcription; as a result, the cell cycle was arrested in the G1 and G2/M phases and that, in turn, lead to apoptosis [54,55]. The p16^{INK4a} and p19^{ARF} have also been demonstrated to be important targets of Bmi-1 [49,56]. Therefore, Bmi-1 could promote cell proliferation by suppressing p16/Rb (retinoblastoma protein) and/or p19^{ARF}/MDM2/p53 tumor suppressor pathways [57]. This has been supported by the observation that upregulation of Bmi-1 expression could activate the self-renewal ability of NSCs and lead to nervous system development through the inhibition of the progress of p16^{INK4a}- and p19^{ARF}- mediated senescence and apoptosis in the latter [56].

The Hedgehog signaling pathway has been demonstrated to be associated with the regulation of mammary stem cell self-renewal and multilineage differentiation, which are mediated by Gli transcription factors [36]. Interestingly, both Gli1- and Gli2-overexpressing mammospheres are also observed to display higher Bmi-1 expression levels. While downregulation of Bmi-1 could significantly reduce the effects of Hedgehog signaling activation on both primary and secondary mammosphere formation, which suggested that that the effects of Hedgehog signaling pathway on mammary stem cells or progenitor cells were mediated by the polycomb gene Bmi-1 [36]. Recently, Yang *et al.* reported that the ectopic expression of SALL4, which was elevated in human leukemia cell lines and primary acute myelocytic leukemia, could enhance the multipotency and self-renewal ability of HSCs. A further study demonstrated that Bmi-1 expression could be upregulated by SALL4 through the methylation of histones H3K4 and H3K9 in the Bmi-1 promoter [43]. Further, Bmi-1 has been shown to be involved in the regulation of stem cells from type-I neuroblastoma through the regulation of the self-renewal of these stem cells and controlling their specific differentiation or lineage commitment in a concentration-dependent manner [58]. The activation of the sonic hedgehog (Shh) pathway has been shown to be involved in the deregulated proliferation of progenitor cells and to lead to medulloblastoma development. All these results implied that Bmi-1 might be the downstream target of Shh signaling and that overexpression of the Shh pathway could induce rapid Bmi-1 expression [59]. Moreover, Bmi-1 has been reported to facilitate the development of Th2 cells via the stabilization of the

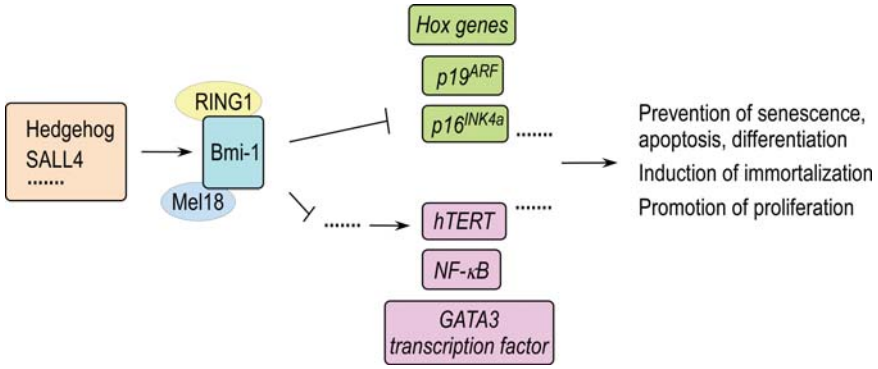

Figure 1 Bmi-1 plays important roles in the regulation of stem cells via the activation of multiple pathways Bmi-1, which could be upregulated by SALL4 and Hedgehog (Hh) signal, regulates stem cell self-renewal through repression of *Hox* genes and *INK4a* locus genes, $p16^{INK4a}$ and $p19^{ARF}$, and activation of telomerase, transcriptional factor GATA3, and NF-κB pathway. These genes and signaling are likely play a role in stem cell fate decisions including the prevention of senescence, apoptosis and differentiation, as well as the induction of immortalization and promotion of proliferation.

GATA3 transcription factor in a RING finger-dependent manner [60]. However, the underlying mechanisms are still unclear. Using Bmi-1-green fluorescent protein-knock-in mice as a model, Hosen *et al.* further confirmed that the expression of Bmi-1 is high in premature HSCs and demonstrated that Bmi-1 is downregulated once the HSCs have been differentiated into a particular lineage [61]. By employing this animal model, they could not only separate cells with differential *Bmi-1* expression into distinct subpopulations but also provide evidence that Bmi-1 is involved in stem cell differentiation [61].

Recently, a number of studies have documented that the activation of nuclear factor kappa B (NF-κB), which is a transcription regulator, is associated with the regulation of stem cells. Aberrantly active forms of NF-κB have been observed in different types of cancer, including breast cancer, colon cancer, non-small cell lung cancer, squamous head and neck cancer, and gastric cancer [62–66]. It has been reported that the NF-κB pathway is activated in LSC population but not in normal hematopoietic stem cells [67]. Inhibition of NF-κB with the proteasome inhibitor MG-132, which is a well-known inhibitor of NF-κB, could induce leukemia-specific apoptosis [68]. NF-κB pathway inhibitors preferentially inhibit breast cancer stem-like cells [69]. Tumor necrosis factor (TNF-α) could promote the proliferation of adult NSCs via the IKK/NF-κB signaling pathway [70]. Li *et al.* found that abnormal activation of NF-κB at an early stage of mesenchymal stem cell not only inhibits the differentiation of mesenchymal stem cells but also enhances the proliferation and invasion abilities of fibroblast-like synoviocytes (FLSs) [71]. However, the biological significance of NF-κB pathway on stem cells remains largely unknown. In 2001, Cheng *et al.* reported that the Notch-1 signal transduction pathway, which is an important regulatory mechanism of stem cells, is associated with the induction of *NF-κB* expression [72]. They found that the DNA binding and transcriptional activities of NF-κB were dramatically decreased in HPCs that were derived from Notch-AS-Tg (Notch-1 antisense transgenic) mice; further, it was found that the decrease in NF-κB activity in HPC was due to transactional repression of NF-κB subunits by Notch-1 factor. It has been reported that Bmi-1 is upregulated by NF-κB in Epstein–Barr Virus-negative Hodgkin lymphoma (HL) cells [73]. In brief, Bmi-1 plays important roles in the regulation of stem cells via the activation of multiple pathways (**Fig. 1**).

However, the precise mechanism of Bmi-1 on the regulation of chromatin remodeling still remains largely unclear. Cao *et al.* demonstrated that Bmi-1 and other components of the PcG complexes bind to the promoter of *HoxC13* and lead to the H2A ubiquitylation and *Hox* gene silencing, which might be implicated in the changes observed in cancer and stem cell self-renewal abilities that occur as a result of Bmi-1 function [74]. In addition, Posterior Sex Combs, a subunit of the *Drosophila* PRC core complex, has been shown to inhibit chromatin remodeling and transcription efficiently by forming an assembly with RING1 to recruit chromatin [75].

Recently, an 11-gene signature was described as a conserved Bmi-1-driven pathway, which defines stem cell-ness of highly invasive tumors of multiple tissue origin and correlates with therapy failure [21].

Furthermore, the expression level of Bmi-1 has been shown to be negatively correlated with the therapy of NSCLC patients. Disease-free survival for stage I and II patients who had received adjuvant therapy was better in the case of Bmi-1 negative patients when compared with their Bmi-1 positive counterparts [27]. Moreover, Guo *et al.* indicated that ablation of Bmi-1 expression in tumors by various therapeutic approaches might help in cancer treatment [76]. All these studies suggested that the PcG protein Bmi-1 could also be a valid target for cancer therapy.

Conclusions

Bmi-1, a member of the PcG family, has been reported to be associated with the initiation and progression of various types of tumor-initiating cells, which might originate from cancer stem cells. Further, numerous studies have demonstrated that Bmi-1 plays vital roles on the self-renewal and differentiation of stem cells through multiple pathways *in vitro* and *vivo*. Hence, it is of great clinical value to further understand the molecular mechanism underlying the regulation of Bmi-1 on stem cells, which not only provide a better understanding of the roles of Bmi-1 in the growth and differentiation of stem cells, particularly cancer stem cells, but also provide insights for the establishment of new strategies and effective clinical therapies for the treatment of tumors.

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