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REVIEW

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Bmi1 in development and tumorigenesis of the central nervous system

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Abstract The role of the Polycomb group gene *Bmi1* in proliferation control of lymphoid and neuronal progenitors as well as in self-renewal of haematopoietic and neural stem cells has been recently demonstrated. Here we review these recent findings with particular regard to their implications for central nervous system development and tumorigenesis.

Keywords Bmi1 · Cerebellum · Medulloblastoma · Neural stem cells

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Introduction

During development, defined genetic programmes influence tissue-specific patterning by regulating gene expression profile. This regulation involves the stable maintenance of either an active or repressed state of expression of homeotic genes by epigenetic mechanisms. Polycomb group (PcG) genes regulate gene expression by forming large multiprotein complexes at specific chromosomal sites called polycomb response elements (PREs), leading to chromatin remodelling. Among the main targets of PcG members are homeobox genes (*Hox*), which regulate several key developmental processes such as differentiation and patterning.

The first functional PcG member identified was B cell-specific Moloney murine leukaemia virus integration site 1 (*Bmi1*) gene, a homologue of the *Drosophila* PcG protein Posterior sex combs (Psc) [5]. It encodes for a 45-kDa nuclear protein that is widely expressed, e.g. in embryonic stem cells, placenta, thymus, heart, testis and brain [25].

Ablation of the *Bmi1* gene in mice leads to skeletal, neural and haematopoietic abnormalities [23], the latter characterised by severe hypoplasia of spleen and thymus and highly reduced haematopoietic cell counts in the bone marrow. Moreover, *Bmi1*-deficient bone marrow cells showed an impaired proliferative response to mitogens, especially cytokine IL-7, resulting in an abnormal expansion of committed B-cell precursors [23] and impaired self-renewing capacity of haematopoietic stem cells (HSCs) [18].

These haematopoietic abnormalities were dramatically rescued in *Bmi1*/*INK4a* double-knockout mice, providing evidence that *INK4a* locus is an in vivo downstream target of *Bmi1* [10]. Identification of the *INK4a* locus as a target of *Bmi1*, which in turn is a critical regulator of

the p53 and Rb tumor suppressor pathways, points to a role of *Bmi1* as a determining factor in cell cycle control. Because both pathways are frequently deregulated in human cancer, altered expression of *Bmi1* might be involved in tumor formation as well.

The first evidence of the critical role of *Bmi1* in tumorigenesis was provided by the development of T- and B-cell lymphomas in transgenic mice overexpressing *Bmi1* under control of the Eμ promoter [3, 8]. Conversely, the proliferative potential and self-renewing capacity of leukaemic stem cells lacking *Bmi1* is compromised [13], therefore pointing to a crucial role of *Bmi1* in controlling the proliferation of neoplastic haematopoietic cells. The onset of B-cell lymphomas in Eμ-*Bmi1* transgenic mice was accelerated by overexpression of c-Myc, thus establishing a collaboration between the *Bmi1* and c-*Myc* oncogenes, mainly due to inhibition of Myc-mediated induction of $p19^{ARF}$ and apoptosis [11].

In humans, overexpression of BMI1 was found in high-grade B-cell non-Hodgkin lymphomas (NHLs) [4], breast carcinoma [6], non-small cell lung cancer (NSCLC) [26] and, most recently, in most human colorectal cancers [12].

Various studies examining expression levels of BMI1 in relation to expression of other PcG genes in tumors have suggested the coordinated action of the different polycomb complexes in gene regulation. Deregulated expression of the PcG complex subunits results in abnormal formation of the PcG members and may contribute to tumorigenesis. For example, functional antagonism between *Bmi1* and *eed* has been shown to regulate haematopoietic cell proliferation. Similarly, in B-cell NHL, it was proposed that BMI1 expression may be regulated by a second PcG complex (ENX/EZH2/EED), which opposes the action of the BMI1-containing PcG complex, suggesting that the disturbances in the equilibrium of PcG complexes may contribute to altered cellular behaviour [24].

Failure to down-regulate BMI1 in proliferating neoplastic cells may play a role in the pathogenesis of the tumors, but mechanisms leading to BMI1 overexpression are unclear. Gene amplification of oncogenes is a common mechanism of tumor induction, however, *BMI1* gene amplification was observed only in a minority of mantle cell lymphomas with a corresponding increase in BMI1 protein expression [4].

Apart from the role of *Bmi1* in the haematopoietic system (recently reviewed in [16, 22]), recent findings have shed new light on its involvement in self-renewal of neural stem cells (NSCs) and proliferation control of progenitor cells during central nervous system (CNS) development and possibly in formation of brain tumors.

Bmi1 and CNS development

The embryonic and postnatal development of the CNS relies on complex and tightly regulated molecular mechanisms that lead to a properly formed and functioning brain through waves of proliferation and expansion of progenitor cells and subsequent differentiation and migration of neuronal and glial cells. However, it has become increasingly evident that the adult brain is not a static structure but retains some potential in plasticity and repair, which is due not only to axonal growth and synapse remodelling but also to the existence of multipotent NSCs.

The first indication of a possible role of *Bmi1* in CNS development came from the analysis of *Bmi1*-deficient mice. In addition to haematopoietic and skeletal abnormalities, these mice develop neurological symptoms including ataxia, seizures and tremors from 3 to 4 weeks after birth [23]. Histopathological analysis revealed a normal architecture of the brain but a significant reduction of the overall size, which was particularly severe in the cerebellum wherein reduced cellularity of the granular and molecular layers were most pronounced [10].

The formation of the cerebellum is quite a well-characterised process occurring during the late embryonic and early postnatal development. Granule neurons, the most numerous cerebellar neurons, originate from a particular structure of the metencephalon, known as the rhombic lip. Granule cell progenitors migrate from the rhombic lip along the outer surface of the cerebellar anlage to form the external granular layer (EGL). Here they undergo clonal expansion and, after exiting cell cycle, start their differentiation process, which will eventually take them to the internal granular layer (IGL) upon migrating along a grid of glial cell processes. All other cerebellar neurons (Purkinje neurons and molecular layer neurons) originate at different time points from the ventricular neuroepithelium of the metencephalon.

Analysis of *Bmi1* expression during cerebellar development shows a widespread expression of the gene in cerebellar progenitor cells, which is particularly strong in the transiently amplifying granule cell progenitors located in the EGL. Newborn $Bmi1^{-/-}$ mice display a reduced number of immature EGL granule cell precursors, as identified by expression of the HLH gene *Math-1* and an increased number of postmitotic EGL granule cell precursors expressing the cyclin-dependent kinase inhibitor (CDKi) p27. Moreover, the postnatal clonal expansion of these progenitor cells was hampered and was accompanied by an increased rate of apoptosis. The identification of *Bmi1* as a downstream target of the Shh pathway, a signalling pathway essential for controlling the proliferation of EGL progenitor cells during development, provides an interpretation frame for these findings [14]. Indeed, granule cell progenitors lacking *Bmi1* are less responsive to induction of proliferation by Shh in vitro [14].

During development of the CNS, NSCs give rise to restricted progenitor cells, which differentiate into neuronal and glial cells. Some stem cells still exist in certain regions of the adult brain, namely, the subventricular zone (SZV) of the lateral ventricle in the cortex and the subgranular zone of the hippocampus.

Recently, *Bmi1* was shown to be required for the self-renewal of NSCs of the telencephalon and of the peripheral nervous system, but absence of *Bmi1* did not impair either the survival of NSCs or the proliferation of restricted neural progenitors from the gut and forebrain. Indeed, Molofsky

and colleagues [15] showed striking depletion of NSCs in adult *Bmi1*$^{-/-}$ mice, but no defects in their survival and differentiation. Moreover, *Bmi1*$^{-/-}$ glial progenitors, induced to proliferate by neuregulin, showed a similar rate of bromodeoxyuridine (BrdU) incorporation compared with wild-type cells [15].

Lack of *Bmi1*, however, did affect the proliferation of cerebellar granule cell progenitors. In contrast with all other neurons of the CNS, which originate from the ventricular neuroepithelium, the granule neurons of the cerebellum arise from a second germinal zone, the EGL. The progenitor cells located in this layer undergo clonal expansion during early postnatal development and by postnatal day 20, most of the EGL cells would have differentiated and migrated inwards to their final destination, the IGL. We have recently shown that the level of *Bmi1* expression correlates with the proliferation status of granule cell precursors located in the EGL during clonal expansion: Strongest *Bmi1* expression was found between postnatal days 5 and 8, at the peak of progenitor proliferation. The absence of *Bmi1* during this period leads to a significant reduction of proliferating granule cell progenitors, which demonstrates a crucial role of *Bmi1* in the maintenance and expansion of immature granule cell precursors. However, the proliferation rate of other neuronal progenitors originating from the ventricular neuroepithelium was not affected by the lack of *Bmi1* [15].

These observations imply that *Bmi1* is involved in the control of proliferation of neural progenitor cells through different mechanisms. One possibility would be that the proliferation control varies among restricted progenitor cells from different region of the CNS. According to this hypothesis, proliferation of restricted neuronal progenitors of the forebrain and of the gut would be independent of *Bmi1*, whereas restricted granule cell precursors of the cerebellum would need *Bmi1* for efficient proliferation. However, how sure are we that all EGL cells are restricted progenitor cells, and how restricted are they really? It has recently been shown that EGL precursor cells isolated from the outermost proliferative zone of the EGL can be induced to differentiate not only into granule neurons but also into glial cells [17], therefore implying that they are not yet irreversibly committed. It is conceivable that the EGL contains not only committed progenitor cells but also uncommitted cells or even stem cells, which are still dependent on *Bmi1* for their proliferation. On the other hand, *Bmi1*-deficient cerebellar granule cell cultures lacking *Bmi1*, proliferate less efficiently and are less responsive to Shh-induced proliferation, thus suggesting that *Bmi1* is at least in part also involved in the proliferation control of more committed progenitors.

The reduced cellularity of the molecular layer due to the lack of stellate cells and of the majority of basket cells can hardly be explained as a consequence of reduced numbers of granule neurons. These neurons originate from another germinal layer, the neuroepithelium (NE), and the overall number of progenitor cells populating the NE of *Bmi1* mutant mice is clearly reduced in the newborn cerebellum [14], thus implying an impairment of self-renewal leading to postnatal depletion in keeping with the observations of Molofsky and colleagues [15]. It is intriguing that crossing *Bmi1*-deficient mice with Ink4a/ARF mutant mice leads to a significant rescue of the cerebellar phenotype observed in the granular layer but has much less effect on the reduced cellularity of the molecular layer ([10] and S. Marino, unpublished observations). These observations suggest that *Bmi1* is involved in the control of proliferation of neural progenitor cells through different mechanisms (Fig. 1). In keeping with this hypothesis, the increase in $p16^{Ink4a}$ expression contributed only in part to the reduction in self-renewal of *Bmi1*-deficient NSCs of the telencephalon [15].

Partial restoration of cerebellar and NSC defects in an Ink4a/ARF background implies that additional pathways must exist in mediating *Bmi1* functions in stem cell self-renewal and progenitor proliferation in the CNS. It is conceivable, for example, that Hox genes, which have been described to be regulated by *Bmi1*, may be involved in the process.

BMI1 and brain tumors

The up-regulation of *Bmi1* expression during postnatal clonal expansion of EGL progenitors and the impaired proliferation of EGL cells lacking *Bmi1* in vivo and in vitro prompted us to check for overexpression of BMI1 in medulloblastomas. These neoplasms are highly aggressive embryonal tumors of childhood, developing from the uncontrolled proliferation of these very same progenitor cells. BMI1 was found to be overexpressed in the majority of human medulloblastoma analysed. Moreover, overexpression of BMI1 correlated with overexpression of PATCHED-1 (PTCH1), which is a reliable indicator of activation of the Shh pathway [14]. The involvement of the Shh pathway in the pathogenesis of medulloblastomas is well known; however, only a minority of these tumors show mutations of known members of the Shh pathway. Mutation analysis of *BMI1* at the DNA level will help to clarify if BMI1 might at least in part account for this difference.

Another aspect of the stronger growing link between developmental processes and tumor formation has been highlighted from recent data, suggesting the presence of “stem cell-like” cancerous cells in blood, breast and brain cancers (reviewed in [2, 19]). According to this theory, only a relatively small fraction of cells in a given tumor possesses the ability to proliferate and self-renew extensively. These multipotent cancer cells have been called “tumor stem cells” and have been shown to share many properties with normal stem cells in that they can self-renew and therefore be propagated for a prolonged time in culture; moreover, they can be induced to express markers of neuronal and glial lineages. So far, they have been isolated from glioblastomas, high-grade glial tumors affecting mainly middle-aged adults and from medulloblastomas by two independent groups [7, 20, 21]. Whether tumor stem cells represent a neoplastic transformation of normal stem cells or of progenitor cells or even of dif-

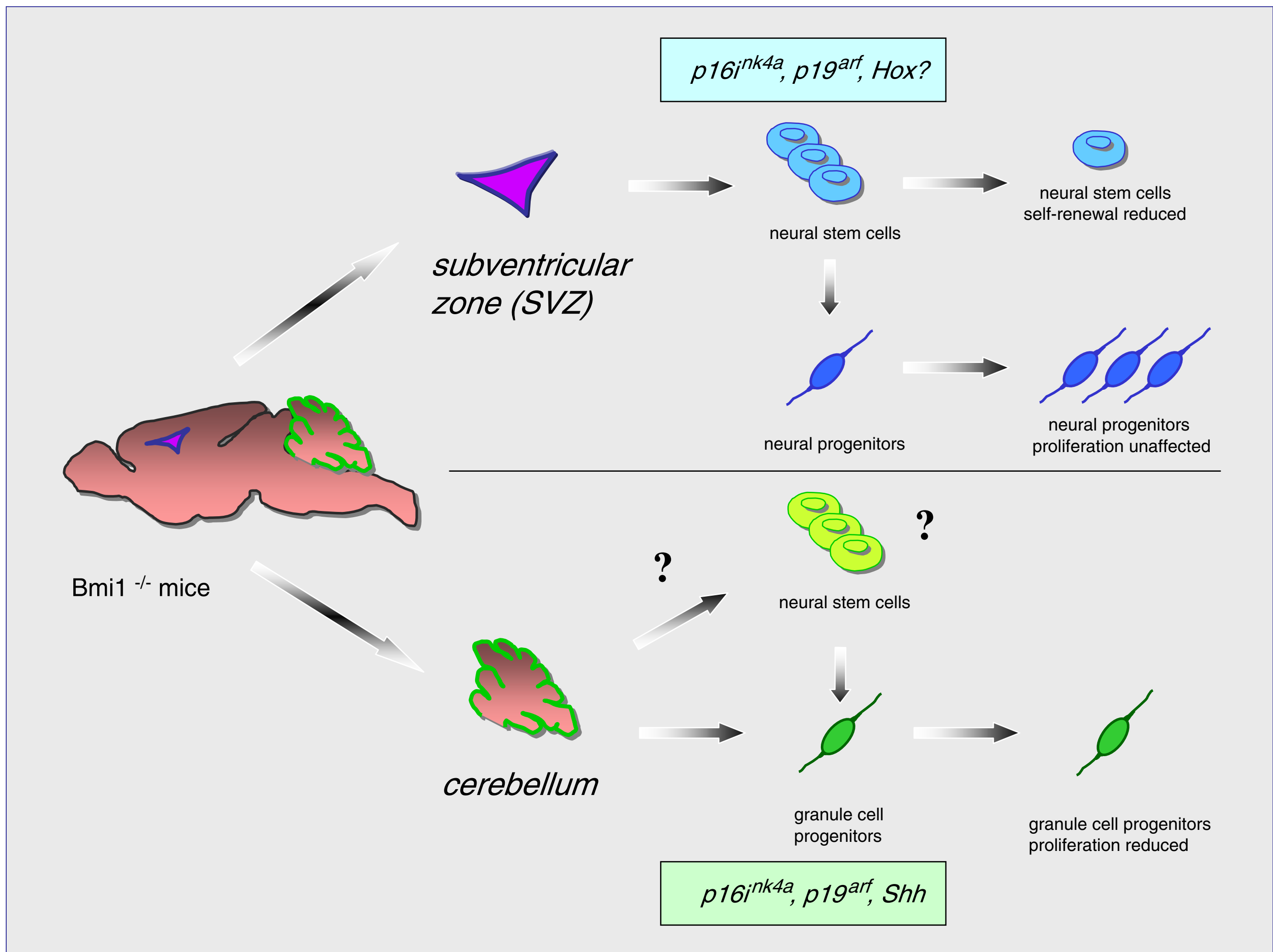

Fig. 1 Role of *Bmi1* in stem cell self-renewal and progenitor proliferation in the CNS. *Upper panel*: *Bmi1* deficiency leads to reduced self-renewal capacity of neural stem cells of the telencephalon and consequently to their postnatal depletion. Restricted neural progenitors from the forebrain proliferate normally in the absence of *Bmi1*. $p16^{Ink4a}$ and $p19^{arf}$ are downstream effectors of *Bmi1* repression. *Lower panel*: In the cerebellum, *Bmi1* plays an essential role in the transient expansion of immature granule cell precursors as a downstream player of the Shh pathway. Whether *Bmi1* is crucial for the proliferation of all granule cell precursors or only of a subset of them (neural stem cell?) is unclear

ferentiated cells that have regained some stem cell properties remains to be clarified. Recently, BMI1 expression has been detected in cancerous stem cells, and withdrawal of mitogen did not correlate with down-regulation of BMI1 expression in neurospheres derived thereof, as is the case with neurospheres derived from non-neoplastic NSCs [9], in agreement with a mitogen-independent proliferation of neoplastic cells. The authors suggest that persistent high level of BMI1 expression correlates with greater capacity of self-renewal and might represent a general mechanism contributing to tumorigenesis in the CNS. However, additional studies are needed to demonstrate that also brain tumors consist of cells organized in a hierarchy based on their proliferation and differentiation capacity, as has been shown for acute myeloid leukaemia and breast cancer. Moreover, we did not observe significant overexpression of BMI1 protein in the glioblastomas analysed, in keeping with a more specific role of BMI1 in neoplastic transformation of EGL progenitor cells giving rise to medulloblastoma. However, it is also conceivable that lack of significant overexpression of BMI1 in the glioblastomas analysed simply reflects a more pronounced differentiation of most cells constituting these tumors and does not exclude the possibility that the cancerous stem cells indeed express BMI1 in these tumors.

Overexpression of BMI1 in medulloblastoma and in cancerous stem cells isolated from these and other brain tumors is intriguing and might open new interesting therapeutic options (reviewed in [1]). It should be stressed, however, that additional studies such as selective overexpression of *Bmi1* in mice are needed to prove the pathogenetic role of *Bmi1* in the development of these neoplasms.

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