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Polycomb group proteins in hematopoietic stem cell aging and malignancies

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Abstract Protection of the transcriptional "stemness" network is important to maintain a healthy hematopoietic stem cells (HSCs) compartment during the lifetime of the organism. Recent evidence shows that fundamental changes in the epigenetic status of HSCs might be one of the driving forces behind many age-related HSC changes and might pave the way for HSC malignant transformation and subsequent leukemia development, the incidence of which increases exponentially with age. Polycomb group (PcG) proteins are key epigenetic regulators of HSC cellular fate decisions and are often found to be misregulated in human hematopoietic malignancies. In this review, we speculate that PcG proteins balance HSC aging against the risk of developing cancer, since a disturbance in PcG genes and proteins affects several important cellular processes such as cell fate decisions, senescence, apoptosis, and DNA damage repair.

Keywords Hematopoietic stem cells · Aging · Leukemia · Epigenetics · Polycomb

1 Introduction

The appearance of an aged individual is often typical; skin wrinkles, hair loss, and fragility often accompany loss of visual and auditory abilities and cognitive decline. These consequences of aging are associated with an increased risk for developing age-related diseases, such as cancer, neurodegenerative diseases and diabetes. Although the phenotypic characteristics of aging are evident, their molecular causes are not well understood. In general, aging of an organism must result, to a great extent, from tissue dysfunction and degeneration as a consequence of cellular aging. Mature cells of most tissue types are short-lived and need to be continuously replenished throughout life. This process is preserved by the enduring activity of tissue-specific adult stem cells. Hematopoietic stem cells (HSCs), which reside within the bone marrow, are the best studied category of adult stem cells [1–3]. These cells are able to undergo both symmetric and asymmetric divisions, leading to their self-renewal or differentiation. A precise coordination of these cell fate decisions is essential to sustain proper blood cell production throughout the entire lifespan of the organism.

In HSCs, multiple age-dependent changes have been identified. Successively serially transplanted HSCs from several strains of mice remained able to repopulate the hematopoietic system of recipient mice [4–6]. However, these cells were less functional than their young counterparts and eventually exhausted [6–10]. Likewise, studies in which HSCs isolated from old mice were directly compared to HSCs obtained from young mice clearly showed that HSC function declines with age. Old HSCs possessed impaired homing potential and self-renewal activity and, in addition, showed a propensity toward a myeloid-lineage-skewing of differentiation potential [11, 12].

Dysfunction at the level of the stem cell compartment might underlie the decline of several age-related parameters in hematopoietic tissue function. For example, numerous impairments in the functioning of an aged immune system are found [13, 14]. These might either be attributable to an impaired activity and specificity of

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mature T cells, B cells, macrophages and NK cells, or more directly related to bone marrow failure. The changes in the functional properties of HSCs might also have more severe pathological consequences. For example, it has been shown that with advanced age the incidence of hematological malignancies increases substantially. Myelodysplastic syndromes (MDS) are the most prevalent hematological disorders in the elderly population. These syndromes are defined as clonal stem cell disorders characterized by ineffective differentiation. In patients this results into peripheral blood cytopenias in combination with a hypercellularity of the bone marrow. Ultimately, MDS often progresses into acute myelogenous leukemia (AML).

Although many theories have been put forward to explain the phenomenon of aging, studies in the mouse hematopoietic system have made it increasingly evident that there is a clear stem cell basis for age-associated changes. However, the molecular mechanisms driving functional changes in HSCs during aging remain elusive. First of all it is still a matter of debate whether aging is a programmed or stochastic process that occurs in all individual HSCs equally [11], or whether the clonal composition of the HSC compartment changes [12, 15–18]. Theoretically, the latter might ultimately result in a monoclonal dysfunctional HSC population. Second, oxidative and metabolic stress, telomere attrition and defects in DNA repair mechanisms all contribute to severe DNA damage which may result in an instable genome and the formation of toxic mutant proteins. Yet, neither of these processes in isolation can account for all age-related changes observed in HSCs. Thus, aging appears to be a multifactorial process.

There is emerging consensus that safeguarding the transcriptional "stemness" network is important to maintain a healthy HSC compartment. Gene profiling studies, for example, demonstrate that gene expression programs of aged hematopoietic stem cells are different from those in their young counterparts [12, 19–22]. Whether these changes are caused by genetic or epigenetic dysregulation of gene expression programs is still unclear. An accumulation of mutations in the DNA sequence and a dysfunctional DNA repair machinery may result in the observed changes in gene expression. However, changes in the chromatin status of the cell are also expected to have a strong causative effect in altering gene expression.

Factors that control chromatin organization, the manner in which the DNA is packed around histones, are important for safeguarding the genomic integrity as well as transcriptional activity of the cell. Several chromatin modifiers have shown to be essential in the regulation of HSC cell fate decisions [23, 24]. Dysregulation of these epigenetic factors that control the balance between self-renewal and differentiation might account for many age-related impairments in HSC function. In this review we will discuss recent evidence that supports the concept that fundamental changes in the epigenetic status of HSCs might be one of the driving forces behind many age-related HSC changes.

2 Age-associated epigenomic changes in HSCs

Epigenetic regulation of chromatin structure can be considered a gatekeeper of cellular memory, since it is crucial for establishing, maintaining and propagating transcriptional profiles of the cell [25, 26]. Epigenetic modulation involves DNA methylation of cytosine residues in CpG islands [27] and modifications of histone tails, mostly by acetylation, methylation and ubiquitination [27–29]. During symmetrical divisions, daughter cells have to inherit the same epigenetic status as the parental pluripotent cell to maintain the unique HSC cell fate. Thus, for a proper self-renewing cell division, besides the DNA sequence the chromatin structure also needs to be copied. In contrast, during asymmetrical divisions, chromatin modifications need not to be faithfully copied, leading to differential gene expression profiles and a distinct identity of both daughter cells.

Throughout life, cellular memory needs to be maintained, and proteins that control chromatin organization are therefore likely to be important for the maintenance of cell-type specific gene expression patterns during HSC aging as well [30, 31]. This interpretation is supported by a study of Chambers and coworkers [19] in which gene expression profiles of HSC isolated from young (2 months), intermediate (6 and 12 months) and old mice (21 months) were compared. Genes involved in transcriptional silencing via chromatin regulation were specifically downregulated with age. These included histone modifying genes from the SWI/SNF-complex (Smarca4 and Smarcb1), the polycomb (Ring1) and Trithorax complexes (Mll3, Mllt10), histone deacetylases (Sirt2, Sirt3, Sirt7, Hdac1, Hdac5, Hdac6) and histone acetyl transferases (Myst2). Genes involved in DNA methylation were also found to be repressed during aging. These included DNA methyltransferase (Dnmt3b) and the methyl-CpG-binding protein encoding genes (Mbd1). In a related gene expression study by Rossi and colleagues, the PcG gene Ezh2, which possesses histone-methyl-transferase activity, was found to be downregulated in old HSCs, while Smarca2 and polycomb-interacting protein Jarid1a were found to be significantly upregulated [21].

In a recent study conducted with human cells, genes involved in chromatin remodeling were also overrepresented in being differentially expressed in old human hematopoietic stem/progenitor cells (HSPCs), compared to

their young counterparts. Using micro-arrays, gene expression profiles from old HSPCs isolated from mobilized blood from individuals between 27 and 73 years were compared to gene expression profiles from young HSPCs isolated from cord blood [22]. The polycomb genes Ezh1 and Cbx7 and polycomb-associated gene Jarid2 were upregulated in old human HSPCs. Upregulation was also observed for other chromatin-modifying genes such as Rnf20, Jmjd1a, Mllt4 and Sirt2. Other chromatin regulators such as Hdac6, Ezh2, Hmgn2, Myst4, Smarca5, Cbx1 and Cbx5 were found to be significantly downregulated with age.

Together, these studies imply that genes involved in chromatin organization are frequently dysregulated upon HSC aging. In addition, both in old murine HSCs and human HSPCs, large gene clusters were identified that were coordinately up or down-regulated. This may reflect the impact of dysregulated epigenetic factors on genome-wide expression profiles [19, 22]. In agreement with this hypothesis, it has been shown that genes that were normally coordinately expressed in young to intermediate-aged tissues (from 16 month mice), failed to do so in older tissue (24 months) [32]. Moreover, aberrantly expressed genes tended to be clustered in close proximity to each other. Together, these observations support the concept of a deterioration of chromatin integrity with age (Fig. 2).

Due to the difficulty of collecting adequate cell numbers for analysis, genome-wide studies on chromatin modifications in aged HSCs are so far lacking. However, studies in other cell types have shown that several epigenomic changes indeed accumulate over time [33–35]. Although overall genomic DNA methylation has been found to decrease with age, a number of specific promoters become hypermethylated. Intriguingly, both genome-wide DNA hypomethylation and aberrant promoter hypermethylation are also epigenetic hallmarks of cancer [36, 37], which will be discussed further in this review. An age-associated decrease in histone acetylation and alterations in histone methylation patterns have also been reported [33].

In concert, chromatin dysregulation might present a plausible explanation for the numerous and broad genome-wide changes in gene expression levels observed in aged HSCs.

3 Polycomb group proteins and HSC regulation

3.1 PcG proteins

Polycomb group (PcG) proteins form an explicit class of epigenetic modulators. They assemble within multimeric protein complexes and induce transcriptional repression of target genes through catalyzation of specific histone modifications. PcGs were originally identified in *Drosophila* as repressors of homeotic (Hox) genes [38]. Together with the counteracting *Trithorax* group (TrxG) proteins, PcGs showed to be crucial for maintenance of appropriate patterns of Hox gene expression for correct axial patterning of body segments. The function of PcG proteins seemed to be highly conserved during evolution since they also showed to be important for proper development in mammals. For example, several mutant mice models that were generated displayed skeletal malformations [39–42].

Biochemical analyses have revealed that PcG proteins assemble within at least two multimeric protein complexes, termed Polycomb Repressive Complex 1 and 2 (PRC1 and PRC2) (Fig. 1) [26, 43, 44]. PRC2 contains three core subunits homologous to *Drosophila*; enhancer of zeste (E(z)), suppressor of zeste 12 (Suz12) and extra sex combs (Esc). The currently identified mammalian orthologs that have shown to function within PRC2 are Ezh1/Ezh2, Suz12 and Eed [45–48]. In addition, three different isoforms exist for Eed (Eed1, Eed2 and Eed3/4) [49, 50]. The SET-domain containing Ezh proteins catalyze trimethylation of histone 3 lysine 27(H3K27me3) and to a minor extent lysine 9 of histone 3 (H3K9me3). This modification is found to be the initiating step of gene repression.

PRC1 complexes consist of four core components; *Drosophila* polycomb (Pc), sex combs extra (Ring/Sce), polyhomeotic (Ph), and posterior sex combs (Psc) proteins (Fig. 1). Each of these proteins have several orthologs in mammals, classified respectively as the Cbx, Ring1, Phc, and Bmi1/Mel18 families. Cbx proteins recognize and catalyze PRC1 binding to the H3K9/27me3 mark which has been established by PRC2 [51, 52]. Subsequently, Ring1 possesses histone 2A ubiquitination activity [52–54]. Data indicate that this histone modification is the final step in gene repression since it blocks the movement of RNA polymerase along the DNA [55, 56]. However, several studies challenge this strict hierarchical recruitment model, since PRC1 and PRC2 can also function independently [57–60].

The number of genes encoding PcG family members has expanded tremendously in mammals, which resulted in a great combinatorial diversity in PRC1 and PRC2 complex compositions (Fig. 1) and molecular functions. For example, the Cbx family consists of Cbx2, Cbx4, Cbx6, Cbx7 and Cbx8. The chromodomains of different Cbx proteins were found to have different affinities toward mono-, di- and tri-methylated H3K9 and H3K27 [51]. Likewise, whereas Bmi1 can stimulate the E3 ubiquitin ligase activity of Ring1b, its homologue Mel18 does not possess this ability [53]. Distinct functional roles for Ezh1- and Ezh2-containing PRC2 complexes in gene repression were found as well [61]. Taken together, stoichiometric perturbation of

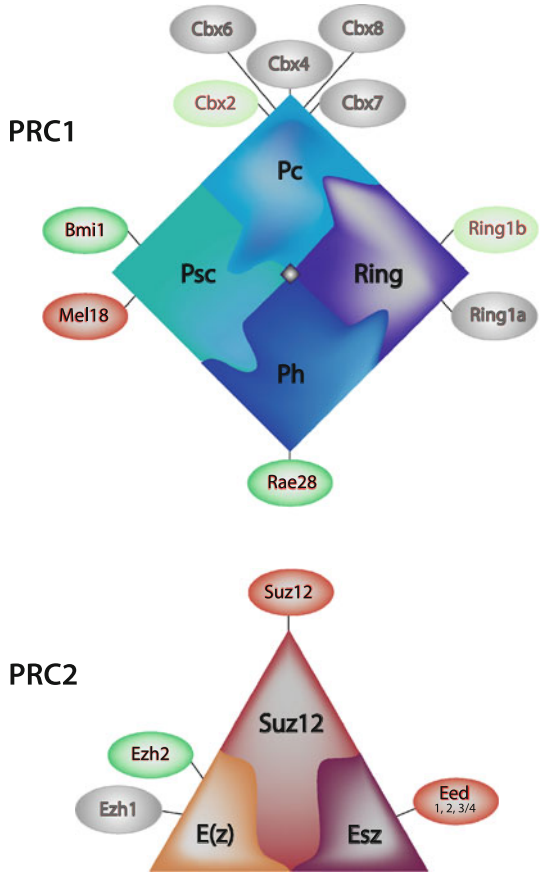

Fig. 1 Schematic representation of the two polycomb repressive complexes and their role in HSC regulation. The PRC1 complex consists of four core subunits homologous to *Drosophila* polycomb (Pc), sex combs extra (Ring), polyhomeotic (Ph), and posterior sex combs (Psc). The PRC2 complex comprises three core subunits, enhancer of zeste (E(z)), suppressor of zeste 12 (Suz12), and extra sex combs (Esc). In mammals, several different homologs exist for every distinct PRC core subunit, resulting in PRC complex composition diversity. Expression perturbation studies showed that distinct PcG genes have different functions in HSC regulation. Several PcG genes stimulate HSC or progenitor self-renewal divisions (*dark and light green balloons*, respectively), whereas others negatively affect HSC self-renewal (*red balloons*). Several PcG genes have not yet been studied for their role in HSC regulation (*grey balloons*)

one component of the PRC complex might be sufficient to modify its molecular function.

As discussed before, expression levels of several PcG genes showed to be affected during HSC aging [19, 21, 22]. One can imagine that changes in gene expression levels of PcG genes during HSC aging results in modifications of the compositions and diversity of PRC1 and PRC2 complexes. The observed age-related changes in HSCs, such as the myeloid-skewage of differentiation and reduced self-renewal abilities, might be the result of a misbalance in HSC cell fate decisions by PcG misregulation and/or altered ratios in terms of PcG homologs (Fig. 2).

3.2 PcG proteins in HSC regulation

Polycomb group proteins have shown to be key players in HSCs by determining cellular fate decisions (Fig. 1). In HSCs, they repress the transcription of lineage-specific genes. In response to extrinsic or intrinsic signals, PcG proteins can be displaced from these promoters and be recruited to stem cell-specific self-renewal genes to promote differentiation and suppress unlimited proliferation. However, during differentiation into either the myeloid or lymphoid lineage, PcGs not only repress the transcription of self-renewal genes but also of differentiation genes of the alternative lineage.

The precise mechanism whereby PcGs are recruited to their target loci remains elusive, although evidence accumulated that several transcription factors and long non-coding RNAs (lncRNA) are involved in this process [62]. Interestingly, one recent report showed that lncRNA are able to physically connect polycomb complexes with another histone modifying complex (LSD1/coREST/REST) to coordinately specify histone modifications on target genes [63]. LSD1 specifically catalyzes H3K4 demethylation. Whereas polycomb-mediated H3K27me3 is generally associated with repressive chromatin, methylated H3K4 is considered to be an active histone mark. Only recently, the existence of bivalent marks (the appearance of both "active" methylated H3K4 and "repressive" methylated H3K27 marks on the same locus) has been reported in murine CD150+ LSK HSCs and human CD34+ CD133+ HSCs [64, 65]. In embryonic stem cells, bivalent chromatin marks are thought to keep developmental genes poised for activation. Oguro et al. [66] recently discovered that Bmi1 is involved in transcriptional repression of lymphoid loci (Pax5 and Ebf1) by bivalent domains in hematopoietic stem cells. The physical interaction of polycomb complexes with an H3K4 demethylase complex mediated by lncRNA might be important for the removal of bivalent domains in HSCs upon differentiation. However, this concept needs further experimental support.

Many PcG genes show stage-specific expression patterns along hematopoietic differentiation. For example, Bmi1 is preferentially expressed in HSCs and its transcript levels decrease upon lineage specification, whereas Eed shows ubiquitous expression in all hematopoietic stages [67–69]. By analysis of the subcellular localization of PcG proteins in different hematopoietic cell populations, LT-HSCs (CD34− LSK), multipotent progenitors (CD34+ LSK) and B cell progenitors (B220+ CD43+), Kato et al. [70] showed that PRC1 complexes in primitive HSCs showed a unique composition different from progenitor cells.

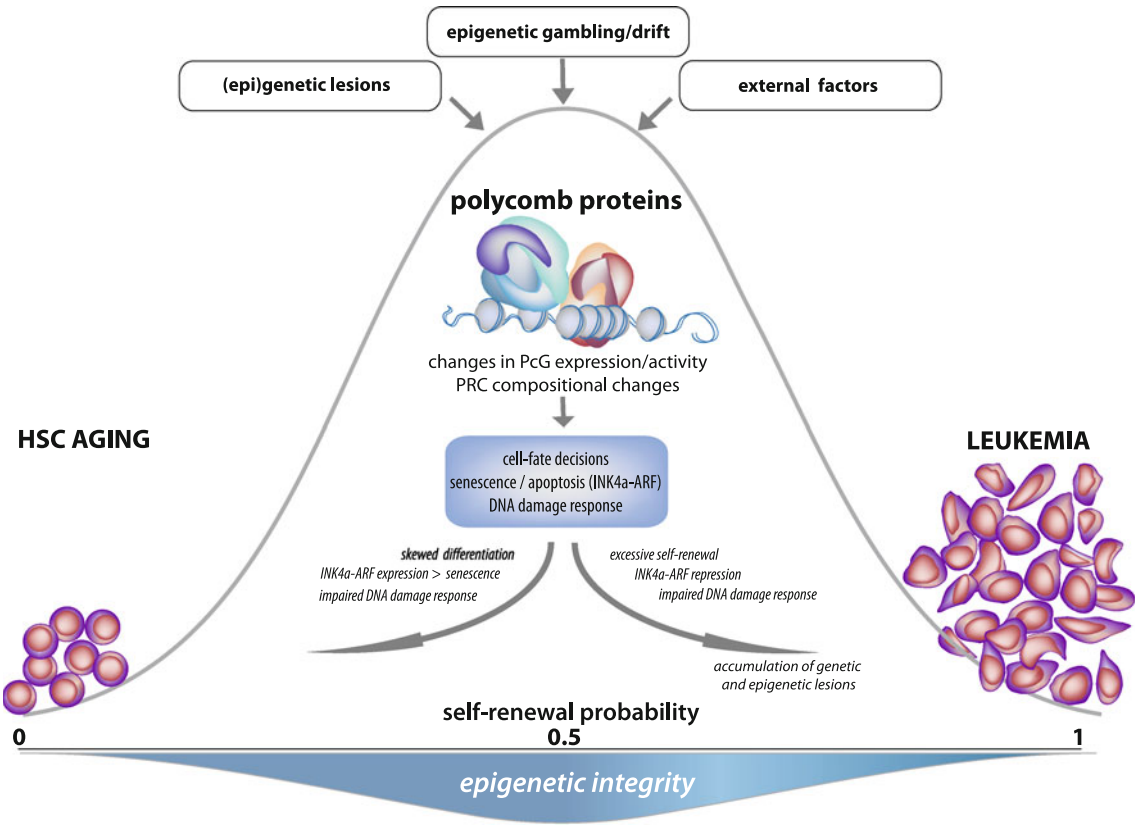

Fig. 2 Model in which polycomb group proteins balance HSC aging and cancer development. External factors, genetic or epigenetic lesions or stoichiometric changes in expression or activity can alter the function of polycomb group proteins and the composition of the polycomb repressive complexes. Polycomb group proteins are known to safeguard the epigenetic integrity of the cell. However, misregulation of polycomb group proteins can affect several important cellular processes such as cell fate decisions, the p16/p19 senescence and apoptosis pathway, and the DNA damage repair machinery. Impaired self-renewal activity, a differentiation skewage and derepression of the INK4a-ARF locus which results in HSC senescence, are common hallmarks of aging. However, malignant transformation of HSCs is generally associated with the acquisition of excessive self-renewal properties by, for example, stable repression of the INK4a-ARF locus by polycomb group proteins and improper functioning of the DNA damage response machinery. This might subsequently promote accumulation of genetic and epigenetic errors and further drive leukemic progression

3.3 HSC regulation by PRC1 members

Bmi1 is the first discovered and best studied polycomb member involved in HSC regulation (Fig. 1). By a retroviral insertional mutagenesis approach in predisposed Eμ-myc transgenic mice, Bmi1 was originally discovered as a proto-oncogene that cooperates with MYC in the generation of B and T cell lymphomas [71]. The importance of Bmi1 in adult hematopoiesis became particularly apparent in Bmi1-null mutant mice [42]. Despite normal embryonic development, these mice showed hypoplastic bone marrow and reduced white blood cell counts due to impaired bone marrow hematopoiesis after birth. Park et al. showed that the number of HSCs in Bmi1−/− fetal liver was not affected but the number of HSCs in postnatal Bmi1−/− mice was severely reduced [72]. Bmi1-deficient mice died within 2 months of birth. Functional analysis of Bmi1−/− HSCs in a transplantation setting showed that these cells were incapable of self-renewal since they did not sustain long-term hematopoietic repopulating ability [67, 72]. In turn, forced expression of Bmi1 in CD34− LSK sorted HSCs promoted self-renewal [67]. This effect was restricted to HSCs, since overexpression of Bmi1 in multipotent progenitors (CD34− LSK) gave no such effects [67]. Recently, the important role of Bmi1 in human HSCs (CD34+ cord blood cells) was confirmed [67, 73, 74].

The PRC1 component Mel18, a homologue of Bmi1 with 70% identity at the amino-acid level, showed negative effects on the self-renewal activity of HSCs (Fig. 1) [75, 76]. Recently, some evidence has emerged showing that the balance between Bmi1 and Mel18 regulates HSC functioning [76]. However, this concept needs further support, for example by studying whether Bmi1 and Mel18 indeed compete for incorporation in the PRC1 complex in HSCs.

Other PcG PRC1 components are also studied for their role in adult HSC regulation, although less intensively (Fig. 1). Inactivation of Ring1a and Ring1b in mice showed distinct phenotypes. Ring1a−/− mice are fertile

but show a number of minor skeletal abnormalities along the anterior-posterior axis [41], whereas Ring1b−/− mice showed defective gastrulation and were embryonic lethal [77]. In the hematopoietic system, conditional deletion of Ring1b resulted in hypocellularity of the bone marrow. Ring1b was found to specifically restrict the proliferation of progenitors and it paradoxically promoted proliferation of their maturing progeny. Thus, Ring1b showed to have dual functions in the regulation of hematopoietic cell turnover depending on the maturational stage of cell differentiation by both targeting cell cycle activators (cyclin D2 and cdc6) and inhibitors ($p16^{Ink4a}$) [78]. PRC1 member Rae28 (a.k.a. Phc1, Edr1, Mph1) is the only member of the Phc family that has been studied for its role in hematopoietic stem cells. During embryonic development, the number of HSCs in fetal liver progressively declines in Rae28-deficient mice [79]. Rae28 showed to be essential for effective HSC self-renewal and Rae28−/− stem cells were unable to reconstitute the hematopoietic compartment upon serial transplantation experiments [79, 80].

At present, the Cbx family has been the least studied PcG PRC1 member with respect to their role in HSCs (Fig. 1). The Cbx family of proteins comprises Cbx2, Cbx4, Cbx6, Cbx7 and Cbx8, and all can bind to H3K27me3 marks through their N-terminal chromodomain. Cbx2−/− mice showed normal numbers of HSCs in fetal liver and these HSCs were fully functional even after successive serial transplantations [67]. Although no gross abnormalities on fetal liver HSC number and function was observed, 4-week-old Cbx2−/− mice showed several lymphocyte abnormalities [40]. By means of in-vitro proliferation assay using thymidine incorporation, Cbx2 appeared to be required for T cell precursor proliferation [40]. This is further supported by the observation that Cbx2 is specifically present in PRC1 complexes in lymphocytes but not in HSCs [70]. This suggests that the PRC1 complexes in HSCs have a unique composition that includes another Cbx member that can compensate for Cbx2. However, this particular Cbx protein has not yet been identified since studies on the role of Cbx4, Cbx6, Cbx7 and Cbx8 in HSC regulation are so far lacking.

3.4 HSC regulation by PRC2 members

In a 'genetical genomics' approach [81, 82] followed by functional studies, the PRC2 member Ezh2 has been identified as being important in HSC regulation and aging (Fig. 1). More than a decade ago, age-related properties of HSCs were found to be highly strain-dependent [83, 84]. For example, HSCs from of DBA/2 mice (D2) mice are more abundant in number and have a higher turnover rate than C57BL/6 (B6) HSCs. Stem cell turnover rate was shown to be correlated with mean lifespan, and has therefore been considered as being one of the most important factors that underlies the aging process [85, 86]. To uncover genes that are responsible for the differences in HSC frequency and turnover, gene expression profiling of recombinant inbred strains from DBA/2 and C57Bl/6 was conducted [87–90]. Since 30 BXD strains were fully genotyped, the genetic basis of these differences in gene expression could be determined by quantitative trait loci (QTL) analysis [91]. Two genetic regions, one on chromosome 11 and one on chromosome 18, showed to be highly deterministic for the variation in cycling activity and HSC frequency, respectively [91].

Ezh2 was found to be transcriptionally controlled by the chromosome 18 locus [7] and HSCs of BXD mice that had inherited the B6 allele at the chromosome 18 locus showed significant higher expression levels of Ezh2 than mice that carried the D2 allele. Along hematopoietic differentiation, Ezh2 expression levels significantly decreased [7, 68] and enforced expression of Ezh2 prevented HSC exhaustion, as shown by repeated serial transplantations [7]. Whereas control HSCs cease to show long-term repopulating ability after three serial transplantations, the function of Ezh2 overexpressing stem cells was not impaired. Ezh2-deficient mice have no clear HSC phenotype, although B and T cell development and VDJ rearrangement showed to be affected [92, 93]. Likewise, inactivation of its homolog Ezh1 did not affect HSC function [92, 93]. In a recent study [94], the function of Ezh1 and Ezh2 was shown to be partially redundant with respect to self-renewal in embryonic stem cells. Upon loss of Ezh2, Ezh1 incorporated into PRC2 and compensated for its loss. This might also hold true for HSCs and might explain the lack of HSC phenotypes in knockout mice. However, several differences between Ezh1 and Ezh2 molecular functions have also been reported and these do not fully comply with functional redundancy [61].

In contrast to the effect of Ezh2 on HSCs, its binding partners Eed and Suz12 were shown to have negative effects on HSCs and progenitors (Fig. 1) [69, 95, 96]. Eed loss-of-function using hypomorphic mice resulted in hyperproliferation of both primitive and more mature lymphoid and myeloid progenitors [69]. With age, the defects in proliferation capacity of progenitor cells in Eed mutant mice deteriorated and ultimately developed into leukemia [69]. Mutations in Suz12, which resulted in a truncated inactive form, enhanced HSC activity [95]. In another study, a sensitized Mpl−/− background was used to detect subtle changes in HSC function in mice heterozygous for individual PRC2 components. Ezh2, Eed and Suz12 were all found to restrict HSC activity [96]. The results for Ezh2 obtained in this study were not consistent with reports by Kamminga et al. [7], that were discussed above. Since both studies were performed in C57Bl/6 mice, the genetic background cannot account for this discrepancy. Yet, Ezh2

gene dosage and/or the balance between PRC2 complexes including either Ezh2 or its homolog Ezh1 might be important. Since we hypothesize that a strict balance in PRC complex compositions is central for HSC fate, we speculate that downregulation or overexpression of a PcG member does not necessarily have to result in strict opposite phenotypes. For example, upon overexpression of one particular PcG member (Cbx2), its family members (Cbx4, Cbx6, Cbx7 and Cbx8) will most likely be outcompeted from the complex. In this case, the function of PRC complexes exclusively containing the overexpressed PcG member (Cbx2) will be studied. However, upon downregulation, the stoichiometry of PcG family members becomes disturbed and it will be difficult to foresee which homologs will take its position in the PRC complex and thus what exactly will be studied. Unfortunately, proteomic analysis regarding PRC complex compositions after PcG gene expression modifications is generally lacking.

3.5 PcG target genes in HSC

In HSCs, the most important target identified for PcG genes thus far has been the tumor suppressor locus INK4a-ARF. This locus encodes two proteins, $p16^{Ink4a}$ and $p19^{Arf}$. p16 is a key regulator of cellular senescence since it functions as a cyclin-dependent-kinase-inhibitor and hampers cell cycle progression by activating the retinoblastoma (RB) pathway. p19 is important for p21/p53-mediated cell cycle arrest and apoptosis [97, 98]. In Bmi1−/− mice, the INK4a-ARF locus showed to be responsible for the lymphoid and neurological defects, as a double deletion of Bmi1 and INK4a-ARF partially rescued the phenotype [99]. In mouse and human Bmi1-deficient HSCs, ineffective self-renewal has also been attributed to derepression of the INK4a-ARF locus and subsequent premature senescence [67, 72, 74]. p16, but not p19, was found to be a key-target of Ring1b in hematopoietic cells as well [78]. p16 was found to be upregulated in response to Ring1b deletion and INK4a-deficiency rescued the expansion defects of Ring1b-deficient lymphoid and myeloid precursor cells.

Chromatin immunoprecipitation experiments in mouse embryonic fibroblasts showed that INK4a-ARF is a critical target of many other PcG proteins as well [57, 100–102]. They repress its transcriptional activity by direct binding and catalyzation of H3K27 trimethylation. Ectopic expression of different PcG members in primary fibroblasts was shown to result in bypass of senescence and cellular immortalization [7, 57, 100–102].

Interestingly, several studies showed that p16 expression markedly increased with age in the hematopoietic and neuronal system and in the pancreas [103–105]. In the hematopoietic system, this increase was found to be restricted to the stem cell compartment (Lin^-, $Sca1^+$, $cKit^+$, $CD34^{low}$, $Flk2^{low}$), because other bone marrow cells did not express p16 in an age-dependent fashion [103]. Using both p16-deficient and overexpression mice models, p16 was shown to promote aging by restricting stem cell self-renewal potential [103–105]. For instance, whereas normally the reconstitution ability of HSC declines with age, Janzen et al. [103] showed that this impairment could partially be mitigated by repressing p16. Thus, deficiency of a critical polycomb target gene, p16, showed to delay the age-associated decline of several adult stem cell types and therefore strongly argues for the involvement of PcG proteins in the aging process (Fig. 2).

Nonetheless, critical INK4A-ARF-independent PcG targets in HSC also must exist, as the effect of both Eed and Suz12 downregulation showed to be INK4a-ARF independent [69, 95]. Hox genes such as HoxB4 and HoxA9, cell cycle-related genes such as p21, p27, cyclin D2, Geminin, and cdc6, C/EBPα target genes, and lineage developmental regulator genes (Pax5 and Ebf1) have all been suggested as being direct PcG targets in HSCs [66, 75, 78, 95, 96].

4 Polycomb group proteins and the DNA damage response

During each division of HSCs, either symmetrical or asymmetrical, replication of the genome results in copy errors. During evolution, several different DNA damage response mechanisms have developed that function as an initial step to repair these errors. However, during aging, deficiencies in the DNA damage repair system have shown to limit the function of HSCs [106, 107]. Appropriate regulation of DNA repair mechanisms is therefore crucial to maintain a proper HSC pool size with age, since extensive DNA damage is likely to trigger cellular senescence and apoptosis.

Very recently, several PcG proteins were identified to be involved in DNA damage responses in multiple cell types, including neural stem cells [108–111]. In an attempt to identify factors that are specifically enriched in the chromatin-associated proteome after DNA damage, Chou et al. combined stable isotope labeling of cells in culture (SILAC), chromatin fractionation, and quantitative mass spectrometry and compared protein abundance of normal cells with cells damaged by UV-irradiation. Three polycomb group proteins, Suz12, Cbx8 and Ezh2, were found to be recruited to sites of damaged DNA. Depletion of these proteins caused cells to become more sensitive to ionizing radiation [108]. In a different study, Bmi1 and Ring1b were found to be recruited to sites with double stranded breaks (DSB) [111] and contributed to the ubiquitinilation of γ-H2AX, an initial step in DSB repair.

Bmi1 knockdown resulted in increased sensitivity to ionizing radiation and simultaneous depletion of Bmi1 and Ring1b resulted in an even severe accumulation of DNA damage after insult [111]. Bmi1 also has shown to be involved in DSB repair in neuronal stem cells (NSC) [109]. Overexpression of Bmi1 in these cells resulted in enhanced recruitment of ataxia-telangiectasia-mutated (ATM) kinase (ATM) to DSBs. ATM constitutes a crucial component of the DNA double-strand break (DSB) response machinery. Overexpression of Bmi1 subsequently resulted in resistance of NSC to radiation. Together, these results show a clear link between polycomb group proteins and the DNA damage response, and suggest yet another mechanism whereby PcG might safeguard genomic stability and cellular integrity during aging (Fig. 2).

5 Polycomb group proteins, aging, and hematological malignancies

The incidence of developing cancer increases exponentially with age [112]. The median age of diagnosis of acute myelogenous leukemia (AML) is 65 years [113] and fewer than 10% of the patients are children [114]. The observation of the age-distribution of cancer has been fundamental to the origin of the multi-stage theory of cancer [115]. This theory implies that most cancers arise from the accumulation of several mutations, and the probability of acquiring a sufficient number of detrimental mutations increases with time [112]. Because the lifespan of HSCs can surpass that of the organism [4, 116] and because of their potentially extensive replicative history, primitive hematopoietic cells are thought to be particularly subject to the accumulation of errors. However, according to the clonal succession theory, most cells in the HSC compartment are quiescent and only a few are activated at any given time [117]. In theory, dormancy lowers the risk of replication errors. This has recently been supported by advanced label-retaining studies [118].

Nevertheless, substantial evidence suggests that aging HSCs are likely targets for leukemic transformation. For example, it has been found that only a subfraction of cells, termed leukemic stem cells (LSC), possess the ability to initiate and sustain leukemia. Intriguingly, these LSCs express the same immunophenotypical markers (CD34+, CD38−) as normal human HSCs [119–121], which suggests that these rare LSCs originally derived from healthy HSCs. Second, the unique intrinsic property of stem cells to self-renew indefinitely is the foremost important criteria for malignant transformation, besides growth factor independency, differentiation blockage and escape from apoptosis. However, it has also been suggested that leukemias can arise from more committed progenitors that have acquired ‘stemness’ properties that allows them to self-renew infinitely. Yet, theoretically, malignant transformation of an HSC that already possesses self-renewal capacities requires fewer reprogramming events than transformation of a more differentiated cell without such self-renewal ability, and this is therefore more likely to occur in HSCs.

Cancer, including leukemias and lymphomas, has long been viewed purely as a genetic disease. Many patients with leukemia carry an abnormal karyotype as a consequence of chromosomal translocations. These translocations cause in-frame fusion of two otherwise separated genes. The resulting fusion product often disturbs proper regulation of proliferation, differentiation, growth factor signaling, senescence or apoptosis. Traditionally, these genetic abnormalities were considered to be the sole cause of malignant transformation of the cells. Typical examples of translocations frequently observed in AML patients are t(15;17) PML-RARa, t(8;21) AML1-ETO, Inv(16), CBFb-MYH11, t(7;11) NUP98-Hoxa9, and several varieties of 11q23 MLL rearrangements [122]. The ‘Philadelphia Chromosome’, the result of t(9;22) BCR-ABL fusion, is found in 95% of the patients with chronic myelogenous leukemia (CML) which often progresses into AML, [123] but also in a subset of patients with acute lymphoblastic leukemia (ALL) [124]. However, it has been shown that some chromosomal translocations associated with hematopoietic malignancies can be present in healthy (aged) individuals, and do not seem to cause leukemia per se [125–128], arguing for cooperating events. An additional argument for the requirement of cooperating mutations for full malignant transformation is the observation that some oncogenic fusion proteins do not cause leukemia with 100% penetrance after cloning and expressing in murine hematopoietic cells, and leukemia develops only after many months [129, 130]. Together, these observations resulted in the (at least) two-hit model of leukemogenesis, stating that malignant transformation depends on both the activation of proto-oncogenes and deactivation of tumor-suppressor genes by genetic alterations [131]. Hence, cancer has been viewed as a disease driven by progressive genetic abnormalities, including chromosomal translocations and mutations in tumor suppressor genes (e.g. INK4a-ARF) and oncogenes.

However, about a decade ago it became apparent that cancer is also associated with profound epigenetic changes [132–134]. Tumor cells are associated with genome-wide DNA hypomethylation, gene specific hypermethylation, as well as genome-wide histone modifications [37, 135–138]. Whereas DNA hypomethylation results in chromosomal instability, both promoter hypermethylation and specific histone modifications represents an efficient mechanism for inactivation of tumor suppressor genes such as INK4a-

ARF. Although it is now widely accepted that epigenetic alterations are common hallmarks of human cancer, epigenetic alterations are still viewed largely as a consequence of genetic lesions.

Yet, recently a critical role for polycomb (dys)regulation in the initial stages of tumorigenesis became apparent. First, it has been demonstrated that genes that are normally suppressed by polycomb complexes in stem cells are far more likely to undergo cancer-specific promoter DNA-hypermethylation than non-PcG target genes [139–141]. Half of the genes frequently hypermethylated were found to be premarked with Ezh2-containing PRC1 complexes that catalyzed the recruitment of DNA-methyl transferases (DNMT) in prostate and colon cancer, but not in healthy cells [140]. In a different study, Cbx7-containing PRC1 complexes were shown to be able to recruit DNMT to genes frequently found to be hypermethylated in cancer [142]. Next, in a study by Teschendorff et al., it was shown that aging contributes to this process. In aged blood cells and bone marrow-derived mesenchymal stem cells, silencing of PcG targets by promoter hypermethylation predisposed cells to become malignant [143].

Polycomb group genes are often deregulated in various types of cancer, including those of the hematopoietic system [144, 145]. For example, Bmi1 overexpression is associated with both myeloid and lymphoid malignancies [146–149] and Cbx7 was expressed at elevated levels in human follicular lymphomas [150]. In turn, Ezh2 is overexpressed in several different types of lymphomas [149, 151–153]. Very recently, a number Ezh2 mutations that inactivate its methyltransferase SET domain have been identified in different types of hematological malignancies, including in follicular and diffuse large B cell lymphomas [154] and in myeloproliferative syndromes [155, 156].

Taken together, we hypothesize that epigenetic dysregulation, for example by PcG misexpression, may precede genetic changes in premalignant progenitor or stem cells and set the stage for accumulation of additional genetic and epigenetic errors such as mutations, promoter hypermethylation and chromosomal instability that, together, may further drive tumor progression (Fig. 2).

6 Additional remarks and future prospects

Polycomb group proteins are key factors in HSCs by regulating the transcriptional 'stemness' profile of the cell. Epigenetic changes imposed by PcG proteins can result in extensive effects on cell fate as they orchestrate DNA compaction and thereby affect expression of numerous genes. Traditionally, cellular aging and cancer, of which the incidence increases with age, have been viewed as genetic syndromes driven by the sequential accumulation of mutations. However, it is now clear that both hematopoietic stem cell aging and hematopoietic malignancies are accompanied by many epigenetic changes, including changes in PcG expression and function. This dysregulation can be held accountable for the disturbance of a variety of cellular processes, including senescence, DNA repair and cell fate decisions (self-renewal vs. differentiation), and it may contribute to aging as well as facilitating malignant transformation of hematopoietic cells. We therefore speculate that PcG proteins regulate the balance between aging (by limiting stem cell self-renewal) and the risk of developing cancer (excessive self-renewal) (Fig. 2).

Whether dysregulation of PcG proteins in aged and malignant hematopoietic cells truly comprise the initial step of cellular aging and/or malignancy remains largely unknown, since at present there is no mechanistic evidence of a direct causal relationship. However, an intrinsic property in biology constitutes "epigenetic gambling" or "epigenetic drift". Subtle changes, that alter the epigenetic landscape of cells, constitute a mechanism to provide random changes and sufficient variation in cellular gene expression programs to ensure survival of the population (of cells or species) (Fig. 2) [157]. Changes in PcG expression and its activity that occur during normal aging, are unlikely to uniformly affect all individuals. In addition, epigenetic modifications are thought to mediate, at least in part, the relationship between the environment and the genome. They could therefore provide a direct link between various external factors, such as radiation, exercise, and nutrition, which all have been associated with healthy aging, and the risk of developing cancer (Fig. 2). Further elucidation of the epigenetic background of hematopoietic stem cell aging and aging of other adult stem cell types could lead to strategies that stimulate healthy aging and prevent cancer development.

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