Cell Stem Cell
Review

Polycomb Group Proteins: Multi-Faceted Regulators of Somatic Stem Cells and Cancer

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Polycomb Group (PcG) proteins are transcriptional repressors that epigenetically modify chromatin and participate in the establishment and maintenance of cell fates. These proteins play important roles in both stem cell self-renewal and in cancer development. Our understanding of their mechanism of action has greatly advanced over the past 10 years, but many unanswered questions remain. In this review, we present the currently available experimental data that connect PcG protein function with some of the key processes which govern somatic stem cell activity. We also highlight recent studies suggesting that a delicate balance in PcG gene dosage is crucial for proper stem cell homeostasis and prevention of cancer stem cell development.

Stem Cells and Cancer

Throughout our lives, mature cells in adult tissues are continuously replenished through the activity of small populations of somatic stem cells. The defining feature of these cells is their capacity to undergo self-renewal division coupled with maintenance of multipotency. Somatic stem cells have been identified for most tissues (blood, brain, muscle, skin, gut, etc.), and harnessing their regenerative properties offers a great potential for future therapies. At the molecular level, much about self-renewal remains to be elucidated. However, we can envision this process as the fine orchestration of cell-cycle regulation with cell-fate decisions. To self-renew, stem cells must therefore enter the cell cycle and progress successfully through cell division. During this process, genome integrity has to be preserved through the coordinated regulation of cell-cycle checkpoints and DNA damage repair. While doing so, they must also ensure that at least one daughter cell restricts programs leading to differentiation, senescence, or apoptosis, thus retaining stemness.

Accumulating evidence indicates that a subpopulation of cancer cells within tumors possess stem cell-like properties. Bonnet and Dick (1997) showed that most leukemic blasts are limited in their proliferative capacity and must be constantly replenished by a rare self-renewing population of "leukemic stem cells." Similar findings have been reported for cancers of the breast, brain, colon, ovary, pancreas, and prostate (Al-Hajj et al., 2003; Li et al., 2007; O'Brien et al., 2007; Singh et al., 2003; Zhang et al., 2008b). Thus, like normal tissues, tumors appear to be organized in a hierarchy that depends on the self-renewing and ever expanding "cancer stem cell," which most likely retains remnants of the normal developmental program. In support of this model, cancer cells frequently exhibit stem cell-like gene expression and chromatin structure signatures (Ben-Porath et al., 2008; Widschwendter et al., 2007). This predicts similarities in the genes that determine self-renewal of normal and cancer stem cells and highlights the importance of identifying the key components regulating this function. As detailed below, the Polycomb Group (PcG) genes represent prime candidates for determining activity of normal and cancer stem cells. In this review, we discuss the proposed function of PcG proteins in stem cell activity with a particular focus on their role in cell-cycle regulation, differentiation, apoptosis, and senescence. We also briefly describe the growing importance of PcG genes in cancer development.

Polycomb Complexes

PcG genes were identified in *Drosophila* more than 30 years ago as regulators of anterior-posterior body patterning through the repression of *Hox* genes. They have since been recognized as global epigenetic transcriptional repressors and key regulators of cell fate (reviewed in Schwartz and Pirrotta, 2007). The Polycomb family comprises a structurally diverse set of proteins which assemble into chromatin-associated complexes. The composition of these complexes is variable and context-dependent (e.g., differentiation status). However, in mammals, two main families of PcG chromatin-modifying complexes, named Polycomb Repressive Complexes 1 and 2 (PRC1, PRC2) have been identified. The core of the PRC1 complex includes one subunit of the PCGF, CBX, PHC, SCML, and RING1 paralog groups (Figure 1, right panel) (Levine et al., 2002; Valk-Lingbeek et al., 2004). The RING1 protein has monoubiquitylation E3 ligase activity specific for the lysine 119 of H2A (H2AK119ub), a mark associated with repressive chromatin structure (de Napoles et al., 2004; Wang et al., 2004). Although less well characterized, L3MBTL and SFMBT proteins are also found in PRC1 complexes, whereas ASXL1 was recently identified in the new Polycomb Repressive H2A Deubiquitinase complex (Grimm et al., 2009; Ohtsubo et al., 2008; Peterson et al., 2004; Peterson et al., 1997; Sánchez et al., 2007; Scheuermann et al., 2010). The core of PRC2 complexes comprises SUZ12, one of the EED isoforms and the histone methyltransferase EZH1 or EZH2, which catalyze the trimethylation of lysine 27 of histone H3 (H3K27me3), another typical epigenetic silencing mark (Figure 1, left panel) (Cao et al., 2002; Cao and Zhang, 2004; Czermin

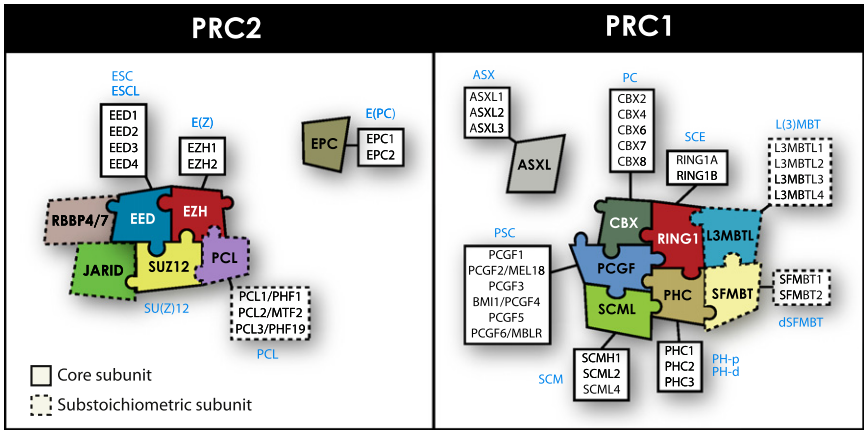

Figure 1. Diversity of PRC1 and PRC2 Complexes Formed by Vertebrate PcG Proteins

Subunits of the PRC1 (right panel) and PRC2 (left panel) complexes are indicated. The *Drosophila* homolog of each subunit is indicated in light blue. Multiple combinations of paralog subunits can generate a diversity of PRC1 and PRC2 complexes, which likely have specific and shared functions. Some subunits seem to be present in substoichiometric amounts and interact with the PcG complexes in a cell-context-dependent manner. The core subunits and the substoichiometric subunits are identified. The contacts illustrated in the diagrams are not intended to represent the actual interactions. Involvement of EPC and ASXL subunits with PRC2 or PRC1 complexes is still unclear and requires further investigations.

et al., 2002; Kirmizis et al., 2004; Kuzmichev et al., 2002). The PRC2-associated PCL proteins are not essential for complex formation and stability but are required for enhancement of its methyltransferase activity (Nekrasov et al., 2007; Sarma et al., 2008). Similarly, RBBP4/7 and JARID proteins are cofactors that help to recruit and modulate the enzymatic activity of PRC2 (Cao et al., 2002; Kuzmichev et al., 2002; Landeira et al., 2010; Pasini et al., 2008; Peng et al., 2009; Shen et al., 2009). The incompletely characterized PcG protein EPC1, which was found in a repressive E2F6-EZH2 complex, can also be considered as an additional PRC2-associated protein (Attwooll et al., 2005). When targeted to specific loci, PcG complexes repress gene expression mainly through epigenetic modification of histone tails and compaction of chromatin. The current model of gene silencing by PcG complexes is summarized in Figure 2. Detailed information about the biochemical characterization and recruitment of PcG complexes to target genes can be found in other recent reviews (Surface et al., 2010; Bracken and Helin, 2009; Schuettengruber and Cavalli, 2009; Simon and Kingston, 2009).

During the evolution from invertebrates to vertebrates the number of PcG genes underwent significant expansion, rising from approximately 15 in *Drosophila* to 37 in Mouse and Humans. The apparent gene duplications that occurred, most notably in mammals, resulted in alternative paralogous subunits that diverged in their structural domains (Kerppola, 2009; Whitcomb et al., 2007). In some situations, this structural diversification confers to paralogs the ability to execute novel activities. For example, expression of the PRC1 protein BMI1 promotes cell proliferation and is essential for maintenance of cancer cells (Lessard and Sauvageau, 2003), while its paralog PCGF2/MEL18 (78% amino acid similarity; 97% similarity for RING domain) blocks cell proliferation and has tumor suppressive functions (Guo et al., 2007; Tetsu et al., 1998). Underlying the complexity of PcG biology, accumulating evidence indicates the existence of multiple PRC1 and PRC2 complexes whose specific function is defined by the activity of the distinct paralogs that participate in these complexes (Figure 1). A good characterization of these different PcG complexes and their specific or shared functions is greatly lacking, which impedes our ability to uncover the precise roles PcG play in stem cell activity and cancer development.

PcG and Regulation of Cell-Cycle Progression

Accumulating evidence indicate that PcG proteins play important functions in multiple cell cycle checkpoints (Figure 3).

G0/G1Transition

Integration of diverse metabolic, environmental, and stress signals dictates whether cells remain quiescent (G0) or enter the cell cycle. No role has been ascribed yet to PcGs in G0/G1 transition. However, expression levels of the PRC2 proteins EZH2 and SUZ12 are greatly reduced in serum-starved nondividing fibroblasts and rapidly increase as they enter cell cycle, whereas EZH1 levels are not affected (Bracken et al., 2003; Pasini et al., 2004). It will be important to determine whether this differential regulation of PcG protein levels also characterizes somatic stem cells and what role these changes play in their G0/G1 transition.

G1/S Checkpoint

Regulation of the G1/S transition occurs mainly through the INK4A-pRB/E2F pathway. The PcG-mediated repression of the $p16^{Ink4a}/p19^{Arf}$ locus is essential for G1/S progression (Figure 3A). The PRC1 proteins BMI1, PCGF1, PCGF2/MEL18, CBX2, CBX7, CBX8, RING1B, and the PRC2 proteins EED, SUZ12, and EZH2 have been shown in multiple cell types to bind the $p16^{Ink4a}/p19^{Arf}$ promoter directly and repress this locus (Gil and Peters, 2006; Maertens et al., 2009). EZH2 also binds to and trimethylates H3K27 in the $p15^{Ink4b}$ promoter (Kheradmand Kia et al., 2009). By repressing these CDK inhibitors, the PcG proteins enable activation of the Cyclin D-CDK4/6 complex and consequently progression through the G1 phase of the cell cycle. Stress signals appear to displace PcG complexes from the promoters of these CDK inhibitors, allowing their expression, which subsequently blocks cells in G1.

The role of PcG proteins at the G1/S checkpoint is not restricted to repressing *Ink4* CDK inhibitors. PcG complexes also require an intact pRB-E2F complex to repress a subset of cell-cycle genes (Blais et al., 2007). BMI1, RING1B, and SUZ12 bind the $p16^{Ink4a}$ locus in a pRB-dependent manner, and PRC2-dependent trimethylation of H3K27 at this locus requires an active pRB protein, indicating a regulatory feedback loop between $p16^{INK4}$ expression and pRB-E2F-PcG complexes (Kotake et al., 2007). Multiple PcG proteins directly interact with and modulate the activity of pRB-E2F. In fly and mammals, physical interaction between L3MBTL1 and pRB-E2F appears to

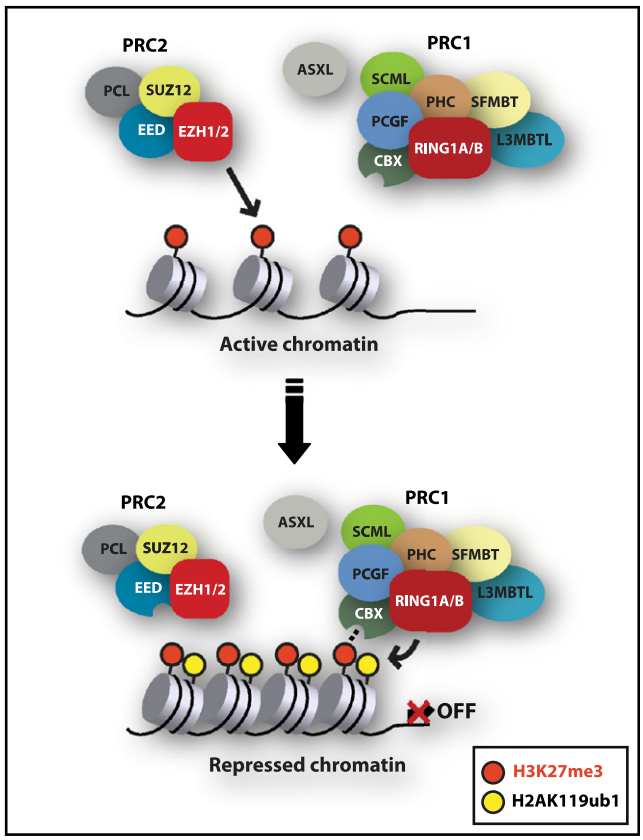

Figure 2. Coordinated Epigenetic Silencing Activity of PcG Complexes

Following recruitment of the PRC2 complex to chromatin, the histone methyltransferase EZH1/2 catalyzes the trimethylation of the lysine 27 of histone H3 (H3K27me3). Subsequent recruitment of the PRC1 complex occurs in part through affinity binding of the chromodomain of the CBX subunit to the H3K27me3 covalent mark. The PRC1 RING1 E3 ligase then monoubiquitylates the lysine 119 of histone H2A (H2AK119ub1), which was proposed to consolidate transcriptional repression by preventing access to chromatin remodelers, inhibiting RNA polymerase II-dependent transcriptional elongation and facilitating chromatin compaction (Francis et al., 2004; Zhou et al., 2008). PRC1 has also been reported to be targeted to chromatin independently of PRC2 (Boyer et al., 2006; Ku et al., 2008; Schoeftner et al., 2006). The ASXL subunit has recently been found to be involved in a H2A deubiquitinase complex required for PcG-mediated repression, but its precise role remains unclear.

be required for repression of target genes, notably *cyclin E*, an E2F2-pRB target gene crucial for S phase initiation (Lu et al., 2007; Trojer et al., 2007). Moreover, EZH2 and HDAC1 compete for interaction with pRB2/p130, indicating that PRC2 participates in the regulation of pRB/E2F activity (Tonini et al., 2004). Interestingly, E2Fs bind to and activate the *Ezh2* and *Eed* promoters, indicating the presence of a regulatory feedback loop between PcG and pRB-E2F proteins (Bracken et al., 2003). Prohibitin (PHB), an antiproliferative protein that interacts with E2F-pRB and p53, represses E2F target genes by recruiting corepressors and RING1B/PRC1 complexes. This latter interaction is essential for the transcriptional repressive function of PHB, and knockdown of PHB results in reduction of RING1B levels and increase in $p16^{Ink4a}$ expression (Choi et al., 2008). Thus, RING1B and PHB directly interact with and inhibit E2F1's transcriptional activity. Curiously, they also indirectly promote E2F1 activity by repressing $p16^{Ink4a}$ expression, which allows Cyclin D-CDK4/6-mediated phosphorylation of pRB and E2F1 release. This dual function could serve as a fine-tuning system for G1/S transition in response to mitogenic signals.

S Phase

The PRC1 complex has been implicated in regulation of S phase initiation. Within the PRC1 complex, SCMH1 directly interacts with Geminin, an inhibitor of the DNA replication licensing factor CDT1 (Figure 3B). The PRC1 complex, comprising PHC1, SCMH1, RING1B, and BMI1, exerts E3 ubiquitin ligase activity on Geminin. PRC1-induced ubiquitination of Geminin leads to its proteasome-mediated degradation and to initiation of S phase entry and/or release of stalled DNA replication forks (Luo et al., 2004; Ohtsubo et al., 2008). Accordingly, *Phc1* deficiency impairs the ubiquitin-proteasome-mediated degradation of Geminin and blocks initiation of DNA replication. Interestingly, this regulatory role in S phase does not involve the standard chromatin-associated function of the PRC1 complex but, rather, a PRC1-mediated post-translational modification of a nonhistone protein.

As mentioned previously, L3MBTL1 represses expression of the *cyclin E* gene. In addition, L3MBTL1 physically interacts with the methyltransferase PRSET7 (Kalakonda et al., 2008). This latter protein interacts with PCNA, localizes to sites of DNA synthesis, and regulates the progression of replication forks. Knockdown of PRSET7 leads to loss of PRSET7-associated H4K20me1 marks, improper DNA replication, and massive occurrence of DNA double-strand breaks (Huen et al., 2008; Jørgensen et al., 2007; Tardat et al., 2007). Interestingly, L3MBTL1 levels increase during cell-cycle progression to peak in G1/S and S (Kalakonda et al., 2008). L3MBTL1 binds to the PRSET7-dependent H4K20me1 modification, and its accumulation mirrors the presence of this mark during S phase. Although it remains to be proven, it appears that L3MBTL1 and PRSET7 may cooperate in regulating the progression of DNA replication forks, and it would be important to find out whether, in this context, L3MBTL1 and PRESET7 act as components of the PRC1 complex.

Further evidence suggests that PRC1 and PRC2 complexes may control the timing of DNA replication. During S phase, PRC1 proteins are bound to chromatin and to newly replicated DNA. They also appear to inhibit DNA and chromatin replication in vitro (Francis et al., 2009). Interestingly, BMI1 interacts with CDC6, a protein essential for the assembly of the pre-replicative complex at origins of DNA replication. CDC6 represses $p16^{Ink4a}$/$p19^{Arf}$ in a *Bmi1*-dependent manner (Agherbi et al., 2009). In addition, the PRC2 protein EZH2 colocalizes with PCNA at foci of ongoing DNA replication, and the PRC2-mediated H3K27me3 modifications positively correlate with late replication of large DNA segments (Birney et al., 2007; Hansen et al., 2008) (Figure 3B).

G2/M and Spindle Checkpoints

Triggering of M phase is under the control of the Cyclin B/CDK1 complex while control of chromosome segregation is regulated by the APC/CDC20 complex. Following stress signals, progression into mitosis can be blocked by association of pRB with E2F, CtBP, and the PRC1 proteins CBX4 and RING1, which together bind the promoters and repress the expression of the *cyclin A* and *Cdk1* genes (Dahiya et al., 2001). Polycomb and

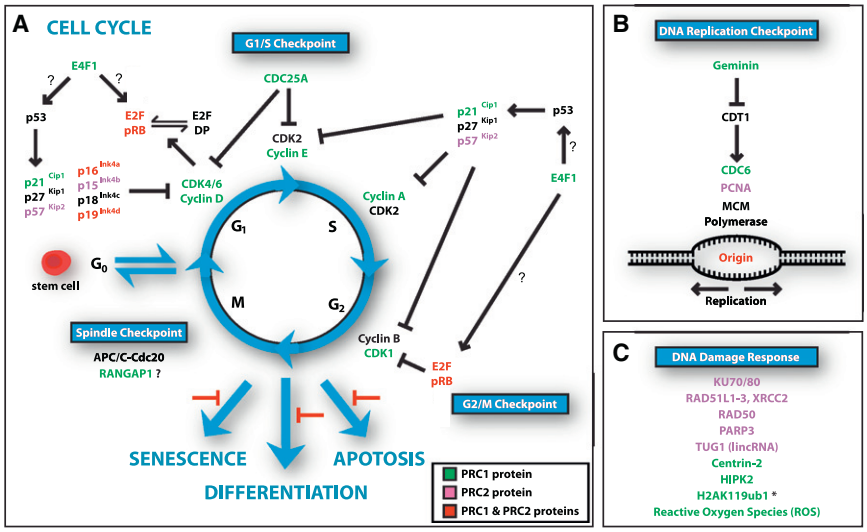

Figure 3. Regulation of Cell-Cycle Checkpoints and DNA Damage Repair Pathways by PcG

(A) The essential role of PcG proteins in somatic stem cell self-renewal is multifaceted. It involves the regulation of several cell-cycle checkpoints, DNA damage repair pathways, control of differentiation, and prevention of senescence and apoptosis programs. Cell-cycle regulators directly interacting with or repressed by PRC1 and/or PRC2 proteins are indicated in green (PRC1), purple (PRC2), and red (PRC1 and PRC2). The question mark indicates that the role of PcG proteins in the process remains to be confirmed.

(B) PRC1 proteins directly interact with Geminin and mediate its degradation, thereby stabilizing the DNA replication licensing factor CDT1. PcG proteins also interact with CDC6, colocalize with PCNA, and associate with late DNA replication origins.

(C) PcG proteins play an important role in maintaining genome integrity by regulating and interacting with multiple proteins and long noncoding RNAs (lincRNA) involved in DNA damage repair pathways. They also appear to participate in reactive oxygen species metabolism. The asterisk indicates that the involvement of the PRC1 complex in DNA damage-induced monoubiquitylation of lysine 119 of histone H2A (H2AK119ub1) remains unclear. The color code in (B) and (C) is the same as in (A).

pRB-E2F thus appear to play a dual role in cell-cycle regulation, first by enabling G1/S progression through repression of the $p16^{Ink4a}/p19^{Arf}$ locus and then by arresting cells in G2/M through repression of *cyclin A* and *Cdk1* (Figure 3A). Moreover, the p53-induced Cip/Kip CDK inhibitors, which act at both the G1/S and the G2/M transition, are also regulated by PcG proteins. EZH2 was reported to bind and repress the $p57^{Kip2}$ locus in breast cancer cells (Yang et al., 2009), whereas $p21^{Cip1}$ is repressed by BMI1 or its paralog PCFG1 (Fasano et al., 2007; Gong et al., 2006). Accordingly, knockdown of $p21^{Cip1}$ partially rescues the proliferative defects and the decreased frequency and size of *Bmi1*-deficient neurospheres.

In primitive hematopoietic cells, BMI1 interacts with the atypical ubiquitin E3 ligase E4F1 and triggers its degradation (Chagraoui et al., 2006). E4F1 is a tumor suppressor that interacts with p53 and pRB and activates both pathways (Fajas et al., 2000; Le Cam et al., 2006) (Figure 3A). *E4f1* null cells exhibit mitotic progression and chromosomal segregation defects, while ectopic expression of *E4f1* results in inhibition of the G1/S transition and cytokinesis defects. Importantly, loss of E4F1 function is sufficient to rescue the clonogenic and repopulating ability of *Bmi1* null hematopoietic stem cells (HSCs) in a $p16^{Ink4a}/p19^{Arf}$- and p53-independent manner. The mechanism of this rescue is still unknown, but it will be important to test whether this rescue is due to release of the G1/S or G2/M checkpoints or to some novel function(s) of E4F1.

Redistribution of PHC1, RING1B, BMI1, and PCGF6 from chromatin to the cytoplasm has been detected in human cells during mitosis (Akasaka et al., 2002; Miyagishima et al., 2003). Interestingly, both BMI1 and PCGF6 are specifically phosphorylated in G2/M, suggesting that post-translational modification of these proteins might regulate their functions and their association with chromatin (Akasaka et al., 2002; Voncken et al., 1999). PCGF6 can be phosphorylated in vitro by CDK7, a kinase required during G2 for Cyclin B/CDK1 complex assembly and M phase entry (Akasaka et al., 2002). However, if and how the cell-cycle phase-specific phosphorylations affect the PRC1 complex activity remains to be elucidated. Recent studies also revealed that heterozygous mutants of *Ph-p*, a *Drosophila* homolog of PHC, display mitotic chromosome segregation defects in fly embryos and impaired chromatin condensation in imaginal discs. PH-p does not localize to chromatin during M phase but is required for mitosis as suggested by formation of chromatin bridges and chromosome breakage observed in mutant flies (Beck et al., 2010; O'Dor et al., 2006). Interestingly, heterozygous mutants of other PRC1 genes (*Pc*, *Psc*, *Asx*) also display chromatid segregation defects, whereas impaired chromatin condensation and misalignment of chromosomes at the metaphase plate have been observed in *E(z)* mutants (O'Dor et al., 2006). In contrast to other PRC1 proteins, human L3MBTL1 associates with condensed mitotic chromosomes, and its overexpression leads to chromosome segregation defects, failure in cytokinesis, and the formation of multinucleated cells (Koga et al., 1999). PRSET7 also remains associated with chromosomes during mitosis and appears to be required for chromosome condensation (Oda et al., 2009; Rice et al., 2002). As mentioned previously, these two proteins interact together, suggesting they may function cooperatively during both S phase and mitosis.

Two recent studies provided potential novel functions for PCGF2/MEL18 in regulating the G2 and M phases. PCGF2/MEL18 inhibits sumoylation of the heat shock factor HSF2 by binding to and inhibiting the UBC9 E2 conjugating enzyme. HSF2 binds the promoter of other heat shock genes during mitosis. In M phase, PCGF2/MEL18 dissociates from HSF2, which renders it susceptible to sumoylation. This enables the interaction of HSF2 with the CAP-G subunit of the condensing complex during gene bookmarking, an epigenetic memory system that allows transmission of the marks that denote active genes or genes poised for activation to daughter cells (Zhang et al., 2008a). The anti-SUMO E3 activity of PCGF2/MEL18 also affects RANGAP1, a Ran GTPase-activating protein

involved in mitotic spindle formation and postmitotic nuclear assembly. In contrast to HSF2, the PCGF2/MEL18-RANGAP1 interaction increases during mitosis, which prevents sumoylation of RANGAP1 (Zhang and Sarge, 2008). Interestingly, sumoylation of RANGAP1 is required for its association with RANBP2 and its localization to the spindle and kinetochores (Joseph et al., 2002). This pathway may thus provide a way to create a Ran-GTP gradient implicated in the assembly and orientation of the mitotic spindle (Clarke and Zhang, 2008). Disruption of the SUMO1-RANGAP1-RANBP2 complex leads to chromosome misalignments and segregation defects, a phenotype reminiscent of that observed in *Drosophila* PcG mutants (Beck et al., 2010; Joseph et al., 2002; O'Dor et al., 2006).

PcG AND DNA Damage Repair

Defects in the DNA damage response have been widely associated with aging, stem cell defects, and cancer (Ito et al., 2004; Nijnik et al., 2007; Rossi et al., 2007; Ruzankina et al., 2007). According to recent data, PcG proteins play a role in DNA damage repair (Figure 3C). The first observations were reported by Zeidler et al., who found that overexpression of EZH2 in human mammary epithelial cells leads to a drastic decrease in expression of multiple RAD51 paralogs required for homologous recombination double-strand break (DSB) repair. Overexpression of EZH2 leads to a significant decrease in the numbers of DNA repair foci, increased aneuploidy, and reduced survival rates of cells exposed to genotoxic stress (Zeidler et al., 2005). Recently, the PRC2-associated PCL1/PHF1 protein was reported to be rapidly recruited to DSBs in a KU70/KU80-dependent manner. Knockdown of PCL1/PHF1 increases the frequency of homologous recombination and sensitizes cells to irradiation. The direct physical interaction of PCL1/PHF1 with KU70/KU80 and RAD50 proteins strongly suggests that this PRC2 protein promotes the nonhomologous end joining (NHEJ) repair pathway (Hong et al., 2008). This possibility is further supported by studies showing that the PRC2 components EZH2 and SUZ12 interact with the poly (ADP-ribose) polymerase 3 (PARP3) protein, known to interact with components of the NHEJ repair pathway (Rouleau et al., 2007). In addition, 39 different long noncoding RNAs (lincRNA) were reported to be induced by DNA damage in a p53-dependent manner. Interestingly, one of them, named TUG1, recruits the PRC2 complex to repress genes that regulate mitosis, spindle formation, and cell-cycle phasing (Guttman et al., 2009; Khalil et al., 2009). Given that approximately 20% of the ~3,300 identified lincRNAs were estimated to bind to the PRC2 complex, it is likely that numerous other lincRNAs interact with PRC2 to mount an adequate DNA damage response. Interestingly, the PRC1-associated monoubiquitination of H2AK119 accumulates at DNA damage foci, but involvement of the PRC1 ubiquitin E3 ligase RING1A/B in this process remains to be clearly demonstrated (Bergink et al., 2006; Zhu et al., 2009).

The PRC1 protein CBX4, a SUMO E3 ligase, interacts with Centrin-2 and HIPK2, two proteins implicated in DNA damage repair pathways. Centrin-2 stimulates nucleotide excision repair (NER) by interacting with XPC, which recognizes DNA lesions. Sumoylation of Centrin-2 by CBX4 enhances its interaction with XPC and is essential for its nuclear localization and participation in NER (Klein and Nigg, 2009). HIPK2 is a kinase involved in transcriptional repression occurring early after the occurrence of DNA lesions and implicated in the induction of cell-cycle arrest and apoptosis. Interaction between CBX4 and the DNA damage-induced kinase HIPK2 generates an auto-regulatory loop where HIPK2 controls CBX4 intranuclear localization and phosphorylates it at threonine 495. This modification enhances the SUMO E3 ligase activity, which in turn increases sumoylation of HIPK2 and of CBX4 thereby promotes its DNA damage-induced transcriptional silencing function (Roscic et al., 2006).

Reactive oxygen species (ROS) induce double-strand breaks and base/nucleotide modifications through DNA oxidation. Accumulating evidence indicates that oxidative metabolism plays a crucial role in maintaining the self-renewal properties of somatic stem cells and that accumulation of high ROS levels leads to premature differentiation (Jang and Sharkis, 2007; Owusu-Ansah and Banerjee, 2009). Mice lacking genes involved in ROS response display genomic instability as well as defects in the maintenance of HSC quiescence and self-renewal ability (Ito et al., 2004; Zhang et al., 2007). Interestingly, antioxidant treatment of stem cells with high ROS levels restores their long term self-renewal potential (Ito et al., 2004; Jang and Sharkis, 2007; Zhang et al., 2007). To date, BMI1 is the only PcG protein that has been linked to oxidative metabolism. Chatoo et al. (2009) reported that BMI1 prevents accumulation of ROS in neurons through the repression of p53's pro-oxidant activity. *Bmi1* deficiency leads to impaired mitochondrial function and a marked increase in ROS levels, which in turn activate the ROS-mediated DNA damage response in a $p16^{Ink4a}$/$p19^{Arf}$-independent manner. Treatment with antioxidants or abrogation of the ATM-dependent DNA damage checkpoint extends the survival of $Bmi1^{-/-}$ mice and rescues the majority of their cellular defects. However, long-term repopulation potential of hematopoietic stem cells remains severely impaired (Liu et al., 2009). Although these observations argue against the possibility that defects of $Bmi1^{-/-}$ HSC result from ROS-induced activation of the DNA damage checkpoint, they do not exclude the possibility that repair and recovery of DNA lesions may be affected in the absence of BMI1. Together, these studies indicate that proteins from both PRC1 and PRC2 are involved in different DNA damage pathways and play important roles in mounting an adequate repair DNA damage response to maintain genome integrity.

PcG as Regulators of Cell Fate and Differentiation

The role of PcG proteins in stem cell fate is firmly established (reviewed in Surface et al., 2010; Sparmann and van Lohuizen, 2006). The genome-wide mapping of PcG target genes in mammalian and *Drosophila* cells provided a wealth of information about how PcG proteins control differentiation and cell identity. These studies revealed that PcG complexes bind to and repress numerous genes encoding key developmental regulators and signaling proteins in embryonic stem cells (ESCs). Interaction of PcG proteins with chromatin at these loci is associated with increased levels of H3K27me3 and H2AK119ub1. Importantly, these repressed PcG target genes are preferentially activated during ESC differentiation (Boyer et al., 2006; Bracken et al., 2006; Lee et al., 2006; Stock et al., 2007; Tolhuis et al., 2006). This correlates with the loss of PcG binding, loss of the H3K27me3 and H2AK119ub1 repressive marks, and increase in the active H3K4me3 modifications. Accordingly, knockout of

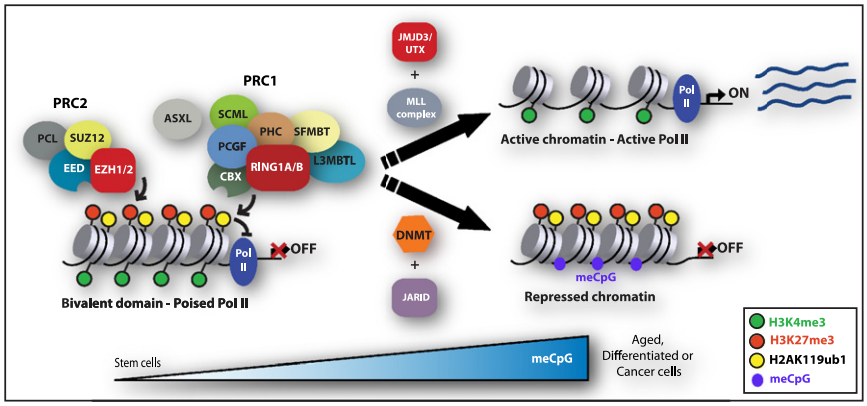

Figure 4. Resolution of Bivalent Chromatin Domains and DNA Methylation of PcG Target Genes

In stem cells, a large proportion of developmental regulators carry simultaneously both PcG-mediated H3K27me3 and H2AK119ub1 marks and the H3K4me3 modifications associated with repressive and active chromatin states, respectively. While remaining silent, these "bivalent" loci are maintained in a poised state ready for activation, given that a nonprocessive form of RNA polymerase II phosphorylated at serine 5 localizes to these loci (poised RNA Pol II). Following differentiation, these domains are resolved and retain chromatin modifications denoting either completely repressed (loss of H3K4me3) or activated (loss of H3K27me3 and H2AK119ub1) state. Resolution of the "bivalent" domains appears to require the H3K4me3 JARID demethylases (for repression) or the H3K27me3 demethylases (JMJD3/UTX) and MLL complexes (for activation). PRC1 and PRC2 proteins control DNA methylation by directly interacting with DNA methyltransferases (DNMT).

the PRC2 and PRC1 genes *Ezh2*, *Suz12*, *Eed*, or *Ring1b* leads to a loss of H3K27me3 and H2AK119ub1 marks, a spontaneous increase in developmental gene expression, and consequently, differentiation defects of embryonic stem cells (Chamberlain et al., 2008; Pasini et al., 2007; Shen et al., 2008; van der Stoop et al., 2008). Similar observations have been reported for many somatic tissues (Ezhkova et al., 2009; Caretti et al., 2004; Hirabayashi et al., 2009; Zencak et al., 2005).

Genome-wide chromatin state maps in ESCs revealed that a large proportion of developmental and tissue specific genes simultaneously carry the PcG-repressive H3K27me3 and H2aK119ub marks overlapping with the H3K4me3 modifications associated with an active chromatin state (Bernstein et al., 2006a; Ku et al., 2008; Stock et al., 2007). These loci, termed bivalent, are silent or expressed at low levels in pluripotent ESCs. In fact, these loci appear to be in a poised state ready for activation since the RNA polymerase II phosphorylated at serine 5, which is associated with paused transcription, also localizes to these loci (Stock et al., 2007). The PRC1 and PRC2 complexes are bound to these bivalent domains (Ku et al., 2008). The PRC1-mediated H2AK119ub1 modification reinforces the poised RNA polymerase II configuration and deletion of *Ring1a/b* leads to the release of the poised RNA polymerase II, and premature expression of target genes (Stock et al., 2007; Zhou et al., 2008). In differentiating ESCs, these bivalent domains are resolved and retain chromatin modifications denoting either completely repressed (loss of H3K4me3) or activated (loss of H3K27me3 and H2AK119ub1) state (Figure 4). These bivalent domains are also found in hematopoietic cells, neural progenitors, and fibroblasts, indicating they are not restricted to cultured embryonic stem cells (Mikkelsen et al., 2007; Oguro et al., 2010; Pan et al., 2007; Roh et al., 2006). Deletion of *Bmi1* in hematopoietic stem cells results in the resolution of bivalent domains at the *Ebf1* and *Pax5* B cell lineage-specific genes. Inappropriate expression of these genes in immature cells leads to premature lymphoid lineage specification associated with a loss of stem and progenitor cell populations (Oguro et al., 2010). More detailed evaluation of the mechanisms that either establish or resolve these bivalent domains in somatic stem cells will provide key information about the role PcG plays in the regulation of cell differentiation.

PcG Proteins and Prevention of Senescence

Senescence is an irreversible phenomenon where cells that should normally be able to divide (i.e., have not undergone terminal differentiation) cease to do so. It can result from normal telomere attrition following multiple rounds of divisions (replicative senescence) or from unrepaired DNA damage (stress-induced senescence). Progressive reduction of the replicative potential of stem cells is believed to represent an important factor in the normal aging of tissues. Induction of a senescence program requires an intact pRB-p16^{INK4A} pathway because deletion of *p16^{Ink4a}* bypasses this process. As mentioned previously, PcG proteins participate in the transcriptional repressive function of pRB and directly inhibit expression of the *p16^{Ink4a}* locus (Figure 3A). It is, therefore, not surprising that the majority of PcG genes prevent the onset of the senescent state. Expression of *Bmi1*, *Cbx2*, *Cbx7*, and *Ezh2* is downregulated as cells approach or enter replicative senescence. This is accompanied by decreased binding of PcG proteins to the *p16^{Ink4a}/p19^{Arf}* promoter, loss of H3K27me3, and upregulation of p16^{INK4A} and p19^{ARF} expression. Similarly, knockout or downregulation of these PcG genes by RNAi increases the expression of p16^{INK4A}/p19^{ARF} and induces premature senescence (Gil and Peters, 2006). Homozygous deletion of *Bmi1* in mice leads to a progeroid phenotype, with prematurely senescent cells and a drastic loss of hematopoietic and neural stem cells. Deletion of the *p16^{Ink4a}/p19^{Arf}* locus partially rescues the HSC and neural cell defects in these mice (Molofsky et al., 2005; Oguro et al., 2006). However, whether a combined *p15^{Ink4b}* and *p18^{Ink4c}* loss could provide an improved or even complete rescue of hematopoietic and neural defects in *Bmi1* null mutant mice has not been investigated.

Overexpression of *Bmi1*, *Cbx7*, *Ezh2*, and *Cbx8* extends the replicative lifespan of cells by bypassing senescence in a pRB-p16^{INK4A}/p19^{ARF}-dependent manner (Gil and Peters, 2006). Importantly, overexpression of *Ezh2* prevents HSC exhaustion after replicative stress induced by serial transplantations (Kamminga et al., 2006). Prior to transformation, cells

overexpressing the *c-Myc* oncogene go through a senescent phase. Direct upregulation of *Bmi1* by c-MYC leads to repression of the $p16^{Ink4a}/p19^{Arf}$ locus and bypasses the oncogene-induced senescence program, thus promoting tumorigenesis (Jacobs et al., 1999). Again, it would be critical to determine the contribution of the other *Ink4* members to this important observation.

PcG and Apoptosis

The role of PcG proteins in the regulation of apoptosis has not been extensively examined. In *Drosophila*, overexpression of *Ph* in imaginal discs induces proliferation associated with increased JNK-dependent apoptosis (González et al., 2009). In mammalian cells, the absence of *Bmi1* leads to an increase in apoptosis, whereas its overexpression promotes survival, protects cells from stress-induced death, and is accompanied by reduced caspase activity and Poly(ADP-ribose) polymerase (PARP) cleavage (Lee et al., 2008). In lymphoid cells, BMI1 directly represses the proapoptotic *Noxa* gene. Importantly, apoptosis of $Bmi1^{-/-}$ lymphoid cells can be rescued by reducing the expression of *Noxa*, but not $p16^{Ink4a}/p19^{Arf}$ (Yamashita et al., 2008). Similarly, $Ring1b^{-/-}$ T cells, which are highly susceptible to apoptosis, express high levels of the proapoptotic gene *Bim*. The apoptosis-susceptible phenotype of these cells can be rescued by reducing the expression of *Bim*, but not other proapoptotic genes such as *Perp*, *Noxa*, or *Bax*, indicating that RING1B specifically inhibits BIM-mediated apoptosis (Suzuki et al., 2010). The fact that direct cleavage of RING1B by Caspases 3 and 9 inhibits its transcriptional repressive activity (Wong et al., 2007) suggests the presence of a positive feedback loop in apoptosis signaling pathways. E2F1 can also promote apoptosis by inducing the expression of *Bim*. Interestingly, EZH2, which interacts with E2F1, antagonizes the induction of E2F1-mediated apoptosis by directly inhibiting expression of *Bim* (Wu et al., 2010).

PcG and Cancer

The multifaceted functions of PcG proteins in the regulation of cell-cycle checkpoints, DNA damage repair, cell differentiation, senescence, and apoptosis makes them prime candidate cancer stem cell genes.

Deregulation of PcG Gene Expression in Cancer

One of the first indications that PcG proteins play a role in cancer was the identification of *Bmi1* as a *c-Myc*-collaborating oncogene (Haupt et al., 1993; Jacobs et al., 1999). Multiple other PRC1 genes are aberrantly expressed in a broad spectrum of human cancers (summarized in Table 1 and Figure 5B, reviewed in Rajasekhar and Begemann, 2007; Sparmann and van Lohuizen, 2006). *Bmi1* is overexpressed, and its locus amplified in several human leukemias and solid tumors. BMI1 activity is crucial for the maintenance of both normal and cancer stem cells, as the loss of its function leads to progressive depletion of these cell populations (Lessard and Sauvageau, 2003). Conversely, the *Bmi1* paralog *Pcgf2/Mel18* acts as a negative regulator of cell proliferation and possesses tumor suppressive functions. Overexpression of *Pcgf2/Mel18* impairs proliferation and leads to G1/S arrest, whereas deletion of this gene promotes proliferation (Tetsu et al., 1998). In line with this, loss of *Pcgf2/Mel18* expression is often found in different types of human cancers (Table 1).

The PRC2 proteins EZH2, SUZ12 and PCL3/PHF19 are also overexpressed in a variety of different human cancers (summarized in Table 1 and Figure 5B, reviewed in Rajasekhar and Begemann, 2007; Sparmann and van Lohuizen, 2006). The *Ezh2* gene is amplified in many human prostate cancer cell lines, in 27% of untreated prostate carcinomas, in 80% of xenografts, and is a good marker for aggressiveness and poor outcome in prostate and breast cancers (Kleer et al., 2003; Saramäki et al., 2006; Varambally et al., 2002). More than half of the hormone-refractory prostate cancers possess increased copies of *Ezh2*, which correlates with high expression levels of this gene. *Ezh2* overexpression and amplification is rare in early stage prostate cancers, but much more common in late stages, highlighting its potential function in tumor progression (Saramäki et al., 2006).

Mutations and Chromosome Anomalies of PcG in Cancer

Recently, a number of PcG missense mutations and chromosomal translocations have been identified in different types of human cancers (also summarized in Table 1 and Figure 5B). Somatic *Ezh2* mutations and deletions were found in 22% of germinal center diffuse large B cell lymphomas, 7% follicular lymphomas, and 12%–23% of patients with myelodysplasic and myeloproliferative disorders (Ernst et al., 2010; Morin et al., 2010; Nikoloski et al., 2010). Both monoallelic and biallelic mutations were found, and almost all are predicted to inactivate the methyltransferase activity of EZH2. This PcG enzyme, therefore, appears to have both oncogenic and tumor suppressive functions. Supporting this, heterozygous null or homozygous hypomorphic mutations of *Eed* in mice increase the incidence and reduce the latency of lymphoid tumors (Richie et al., 2002; Sauvageau et al., 2008), indicating a possible tumor suppressive role for this other PRC2 protein. Importantly, the methyltransferase activity of EZH2 depends on its interaction with EED, and mutations in EED have been reported to affect this interaction (Denisenko et al., 1998; Shumacher et al., 1996; van Lohuizen et al., 1998). Heterozygosity for a *Suz12* allele carrying an inactivating point mutation was also reported to cause an increase in numbers of platelets, megakaryocytes, and hematopoietic progenitors. Moreover, HSCs harboring a *Suz12* mutation are more competitive in repopulating the hematopoietic system than their wild-type counterparts, indicating that SUZ12 negatively regulates HSC/progenitor activity and suggesting a tumor suppressive role for SUZ12 (Majewski et al., 2008). Together, these studies indicate that a delicate balance of PRC2 gene dosage is crucial for homeostasis. Either upregulation or a decrease in PRC2 activity is sufficient to sensitize cells to transformation, whereas complete absence of PRC2 activity leads to stem cell extinction (Figure 5A).

The ASXL proteins have been implicated in cancer development by recent studies reporting *Asxl1* mutations in 17%–25% of acute myeloid leukemias, 33%–43% of myelomonocytic leukemias, and 10%–18% of myelodysplasic syndromes and hyperproliferative disorders (Table 1). The majority of *Asxl1* alleles are heterozygous frameshift mutations caused by duplications, insertions, substitutions, or deletions of nucleotides and are predicted to encode an ASXL1 protein lacking the C-terminal region comprising the nuclear receptor box and

Table 1. Expression, Mutations, and Chromosomal Translocations of PcG Genes in Human Cancers

	Aberrant Expression	
Subunit	Expression	Cancer Type
PRC2 Components		
Ezh2	overexpression and/or amplification	B cell non-Hodgkin lymphomas,[35] bladder cancers,[1,5,29,41] breast cancers,[2,5,8,15,27] colon cancers,[5,22] glioblastoma,[5] Hodgkin lymphomas,[28] oral cancers,[5] liver cancers,[33] lung cancers,[5] lymphoma,[5] mantle cell lymphomas,[37] melanomas,[2] prostate cancers,[2,36] testicular cancers[5]
Suz12	overexpression and/or amplification	breast cancers,[14] colon cancers,[14,42] germinal cell-derived tumors,[20] liver cancers,[14] lung cancers,[20] mantle cell lymphomas,[20] melanomas,[20] parathyroid and pituitary adenomas[20]
Pcl3/Phf19	overexpression	colon cancers,[39] skin cancers,[39] lung cancers,[39] rectal cancers,[39] cervical cancers,[39] uterus cancers,[39] liver cancers[39]
PRC1 components		
Cbx7	overexpression	follicular lymphoma[32]
	loss	pancreatic cancers[13]
Phc1	loss of heterozygosity	acute lymphoblastic leukemias[34]
Phc3	loss of heterozygosity	Osteosarcomas[12,17]
Bmi1	overexpression and/or amplification	acute myeloid leukemias,[31] breast cancers,[30] gastrointestinal tumors,[30] lymphomas,[3,30,35] medulloblastomas,[18] neuroblastomas,[26] lung cancers,[38,30] parathyroid and pituitary adenomas[30]
Pcgf2/Mel18	loss	cutaneous squamous-cell carcinomas,[30] gynecological tumors,[30] prostate cancers[30,40]
Ring1a	loss	clear-cell renal-cell carcinomas,[30] testicular germ-cell tumors[30]
Ring1b	overexpression	bladder cancers,[30] breast cancers,[30] colon tumors,[30] gynecological tumors,[30] kidney cancers,[30] larynx cancers,[30] livers cancers,[30] lung cancers,[30] lymphomas,[30] melanomas,[30] pancreas cancers,[30] parathyroid and thyroid cancers,[30] prostate cancers,[30] skin cancers,[30] thymus cancers,[30] urinary tract cancers[30]
	Mutations	
Subunit	Type of Mutation	Cancer Type
PRC2 Components		
Ezh2	missense	diffuse large B cell lymphomas,[23] myelodysplasic/myeloproliferative syndromes[10,25]
Epc1	truncation	acute lymphoblastic leukemias[24]
PRC1 Components		
Phc3	missense	osteosarcomas[9,12]
Asxl1	frameshift, truncation	acute myeloid leukemias,[4,7] myelodysplasic/myeloproliferative syndromes[4,6,11]
	Chromosomal Translocations	
Subunit	Fusion Gene Partner	Cancer Type
PRC2 Components		
Suz12	Jazf1	endometrial stromal sarcomas[16,19]
Pcl1/Phf1	Jazf1, Epc1	endometrial stromal sarcomas[21]
Epc1	Asxl2	acute lymphoblastic leukemias[24]
PRC1 Components		
Asxl2	Epc1	acute lymphoblastic leukemias[24]

See Supplemental Information for detailed numerical references.

PHD domain (Boultwood et al., 2010; Carbuccia et al., 2009; Gelsi-Boyer et al., 2009). Interestingly, in acute myeloid leukemias, ASXL1 and NPM1 mutations appear to be mutually exclusive, ASXL1 mutations being present in 34% of non-NPM1-mutated cases (Carbuccia et al., 2010). Moreover, *Asxl*$^{-/-}$ mice have defects in lymphoid and myeloid progenitor cell frequency and differentiation, whereas multipotent progenitor and HSC populations appear to behave normally (Fisher et al., 2010). Missense mutations are also found in PHC3 in osteosarcomas (Deshpande et al., 2007; Iwata et al., 2010). More extensive analysis of PcG mutations in human cancers will likely be revealing.

Some PcG genes are involved in recurrent chromosomal rearrangements detected in human cancers (see Table 1). In addition, PcG proteins have been shown to be recruited to target genes by physically interacting with a number of chimeric fusion proteins involved in leukemia, such as PLZF-RARA and TMPRSS2-ERG (Figure 5D) (Boccuni et al., 2003; Boukarabila et al., 2009; García-Cuéllar et al., 2001; Hemenway et al., 2001; Villa et al., 2007; Yu et al., 2010).

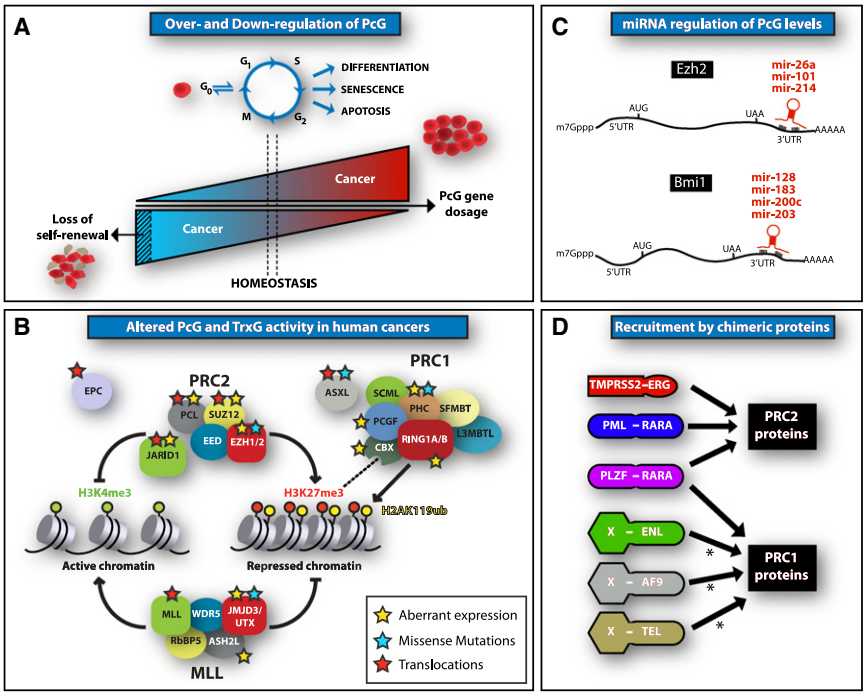

Figure 5. Deregulation of PcG Activity in Cancer

(A) Proposed model for PcG gene dosage and its effect on stem cells and cancer development. Briefly, adequate PcG gene expression levels seem to be essential to maintain homeostasis and normal stem cell function. Gain or loss of PcG functions, such as deregulated expression or mutations, lead to increased tumor development, possibly by modifying the composition or perturbing the stoichiometry of the complexes. In turn, complete ablation of PcG activity appears to be detrimental and leads to impaired self-renewal and loss of stem cells.

(B) Schematic representation of the balance between PcG-mediated H3K27me3 and MLL complex-mediated H3K4me3 modifications in the maintenance of a transcriptional program. The function of many proteins from these complexes is altered by aberrant expression (yellow star), by missense mutations (blue star), or by chromosomal translocations (red star) in human cancers (Agger et al., 2009; Dalgliesh et al., 2010; Krivtsov and Armstrong, 2007; van Haaften et al., 2009; Wang et al., 2009; Xiang et al., 2007), likely leading to changes in transcriptional programs and cell states.

(C) Expression of *Ezh2* and *Bmi1* is regulated at the post-transcriptional level by miRNAs, targeting their 3′UTR, leading to transcript degradation.

(D) Multiple chimeric proteins resulting from chromosomal translocations found in human cancers interact with PRC1 and PRC2 complexes and appear to recruit them to target genes. AF9. ENL and TEL are found in multiple chromosomal translocation-derived fusion proteins in human cancers. The asterisk indicates that, although actual recruitment of PRC1 by the fusion proteins remains to be proven, the AF9, ENL, and TEL domains interacting with PRC1 proteins are present in the fusion proteins and are essential for their oncogenic activity (Boccuni et al., 2003; García-Cuéllar et al., 2001; Hemenway et al., 2001).

PcG and Cancer Epigenetics

Aging is the strongest demographic risk factor for cancer, and several lines of evidence point to an association between aging and changes in DNA methylation at specific loci. Epigenetic silencing by DNA hypermethylation is prevalent in cancers and represents an efficient mechanism for inactivation of tumor suppressor genes. Interestingly, PcG target genes identified in ESCs are more likely to be hypermethlyated in aged somatic cells (Teschendorff et al., 2010). A large proportion of the PcG target methylation signature is present in ovarian cancer, follicular lymphoma, and glioblastoma multiforme (Martinez et al., 2009; O'Riain et al., 2009; Teschendorff et al., 2010). In fact, the PcG target genes identified in ESCs are 12 times more likely to be hypermethylated in cancers than non-PcG targets (Ohm et al., 2007; Widschwendter et al., 2007). A recent study showed that 50% of genes frequently hypermethylated in colon or prostate cancers are premarked by PcG proteins and by the PRC2-mediated H3K27me3 mark for de novo methylation in cancer (Schlesinger et al., 2007). The link between DNA methylation and polycomb target genes in cancer is further supported by the fact that PRC2 directly controls DNA methylation through interaction of EZH2 with the DNA methyltransferases DNMT1, DNMT3A, and DNMT3B. This interaction allows the recruitment of DNMTs to PRC2-repressed genes and their subsequent methylation (Viré et al., 2006). The PRC1 proteins CBX4 and CBX7 also interact with DNMT3B, whereas BMI1 forms a ternary complex with DNMT1 through direct interaction with the DNMT-associated protein DMAP1 (Kim et al., 2008; Mohammad et al., 2009; Negishi et al., 2007). It was estimated that approximately 47% of DNMT3B target genes are bound by PRC1 and PRC2 and that DNMT3B-PcG-bound genes in normal cells are good predictors of epigenetically silenced loci in colon cancer (Jin et al., 2009). Since most of the PcG targets are lineage and differentiation determinants, these studies suggests that gradual de novo DNA methylation of PcG targets locks cells in an undifferentiated state and predisposes them to malignant transformation (Figure 4).

Recently, many large intervening noncoding RNAs (lincRNA) in the *Hox* loci have been found to be deregulated during progression of breast cancer. The lincRNA HOTAIR, which recruits the PRC2 complex to silence the *HoxD* cluster, is overexpressed in primary breast tumors and metastases. In fact, high HOTAIR levels appear to be a good indicator of metastatic progression, and its knockdown decreases cancer invasiveness, especially in cells expressing high levels of PRC2 proteins (Gupta et al., 2010; Rinn et al., 2007). Conversely, ectopic expression of HOTAIR increases invasiveness and metastasic potential of epithelial cancer cells and induces relocalization of the PRC2 complex to bind target genes in a pattern similar to that observed in embryonic fibroblasts. The resulting differential gene expression pattern is likely responsible for the invasive phenotype because reduction of PRC2 activity in HOTAIR-overexpressing cells inhibits metastasis (Gupta et al., 2010). It is likely that numerous other lincRNAs are also deregulated in cancers, thus generating a new mechanism to redirect PRC2 complex activity and "reprogram" cells to an undifferentiated and/or malignant state. Interestingly, interaction between the lincRNA ANRIL and the PRC1 protein CBX7 in prostate cancer cells has recently

been found to be crucial for repression of the $p15^{Ink4b}/p16^{Ink4a}/p19^{Arf}$ locus (Yap et al., 2010). Since all CBX proteins have sequence-specific RNA binding activity, they also likely interact with multiple lincRNAs and thereby target the PRC1 complex to specific loci (Bernstein et al., 2006b).

In recent years, small noncoding RNAs have been recognized as major players in regulatory pathways, and many of them are deregulated in cancers. Multiple miRNAs are repressed in ESCs and marked by H3K27me3 modification, indicating that PRC2 complexes regulate their expression (Marson et al., 2008). In addition, several miRNAs were found to bind PcG transcripts and inhibit their expression (e.g., mir-26a and mir-214 target *Ezh2* transcript [Juan et al., 2009; Sander et al., 2008]) (Figure 5C). Interestingly, MYC decreases the expression of mir-26a and, thereby, contributes to tumorigenesis by allowing expression of *Ezh2* (Sander et al., 2008). mir-101 targets the 3′UTR of *Ezh2* mRNA and promotes its degradation. Expression of this miRNA is downregulated in bladder transitional cell carcinoma, hepatocellular carcinoma, and prostate tumors, which correlates with high *Ezh2* levels (Friedman et al., 2009; Su et al., 2009; Varambally et al., 2008). Almost 40% of prostate cancers and 67% of metastatic stage tumors harbor loss of one or both mir-101 alleles. Overexpression of mir-101 inhibits proliferation and reduces the invasiveness potential and colony formation activity of cancer cells, a phenotype similar to that found with *Ezh2* knockdown (Cao et al., 2010; Friedman et al., 2009; Varambally et al., 2008).

The PRC1 gene *Bmi1* is also targeted by multiple miRNAs. Expression of *Bmi1* is post-transcriptionally inhibited by mir-128, mir-183, mir-200c, and mir-203, which target the 3′UTR of the transcript. The mir-203 locus is silenced by DNA methylation in hepatocellular carcinoma and squamous cell carcinoma of the esophagus (Furuta et al., 2010; Kozaki et al., 2008). Similarly, mir-200 and mir-183 are often suppressed by DNA methylation in invasive cancers, providing a link between miRNAs and the deregulation of PcG in cancers. Human glioblastomas display reduced levels of mir-128, whereas ectopic expression of mir-128 inhibits glioma cell proliferation and xenograft growth. *Bmi1* downregulation by mir-128 in glioma cells leads to a decrease in H3K27me3 levels, upregulation of $p21^{Cip1}$, and a block in self-renewal (Godlewski et al., 2008). Normal mammary and breast cancer stem cell populations show reduced expression of mir-183 and mir-200 family members, resulting in increased *Bmi1* levels. In addition to inhibiting the ability of normal mammary stem cells to form mammary ducts, expression of mir-200c suppresses breast cancer stem cell proliferation, clonogenicity, and tumorigenic potential (Shimono et al., 2009). Recently, a link between miRNA, *Bmi1*, and epithelial-mesenchymal transition (EMT) was also established. Carcinoma invasiveness and metastasic spread is promoted by the establishment of an embryonic EMT program. Expression of the ZEB1 protein activates epithelial-mesenchymal transition (EMT) and contributes to the maintenance of stem cell self-renewal. This protein also suppresses expression of mir-200c, mir-203, and mir-183, which results in high levels of BMI1. Highly invasive tumors express high levels of ZEB1, which appears to be essential for the expansion of primary and metastic pancreatic and colorectal tumors (Wellner et al., 2009).

Concluding Remarks

Through chromatin silencing and other non-histone functions, PcG complexes are involved in the regulation of multiple molecular programs essential for stem cell activity. Such programs involve cell cycle checkpoints and progression, DNA damage repair, differentiation, apoptosis and senescence. Aberrant expression, inactivating mutations, chromosomal translocations and recruitment of PcG proteins and complexes by chimeric proteins or lincRNAs are observed in human tumors. The frequency of these newly reported mutations/anomalies now suggests that deregulation of PcG activity is central to cancer development and cancer stem cell biology. A clear picture of the exact regulatory events involving PcG complexes in stem cell self-renewal and cancer is hindered by the multiplicity of PcG complexes and by the distinct functions of the different PcG proteins within these complexes. Future studies will have to address this issue.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental References and can be found with this article online at doi:10.1016/j.stem.2010.08.002.

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