

University of Groningen

The role of polycomb group proteins in hematopoietic stem cell (HSC) self-renewal and leukemogenesis

van den Boom, Vincent; Schepers, Hein; Brouwers-Vos, Annet Z.; Schuringa, Jan

Published in:

The role of polycomb group proteins in hematopoietic stem cell (HSC) self-renewal and leukemogenesis

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version

Publisher's PDF, also known as Version of record

Publication date:

2013

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

van den Boom, V., Schepers, H., Brouwers-Vos, A. Z., & Schuringa, J. (2013). The role of polycomb group proteins in hematopoietic stem cell (HSC) self-renewal and leukemogenesis. In *The role of polycomb group proteins in hematopoietic stem cell (HSC) self-renewal and leukemogenesis* (pp. 142-173). Springer.

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

Chapter 6

The Role of Polycomb Group Proteins in Hematopoietic Stem Cell (HSC) Self-Renewal and Leukemogenesis

Vincent van den Boom, Hein Schepers, Annet Z. Brouwers-Vos, and Jan Jacob Schuringa

Abstract Throughout embryonic development as well as during adult hematopoiesis Polycomb group (PcG) proteins fulfill important functions. Stem cell self-renewal but also lineage fate decisions are controlled by PcGs. Besides a role in normal hematopoiesis, PcGs are often deregulated in various types of cancer, including human leukemias. Within this chapter we will discuss the current understanding of complex composition of canonical and noncanonical Polycomb repressive complexes, how these can contribute to normal hematopoiesis, and how PcG proteins can participate in leukemic transformation.

Keywords Polycomb repressive complex • Human hematopoietic stem cells • Leukemia • Noncanonical • Stem cell self-renewal • Differentiation • AML • ALL

6.1 Introduction

Polycomb group (PcG) proteins are involved in epigenetic repression of gene transcription and generally reside in two distinct complexes: Polycomb repressive complex 1 (PRC1) and 2 (PRC2) (Fig. 6.1) (Simon and Kingston 2009). According to the classical model for PcG-mediated repression, the PRC2 complex, containing the methyltransferase EZH2, EED, and SUZ12, first trimethylates histone H3 at lysine 27 (H3K27me3) (Cao et al. 2002; Kirmizis et al. 2004; Kuzmichev et al. 2002). This epigenetic modification recruits the five-subunit PRC1 complex, most likely via the chromobox domain of the CBX subunit of the PRC1 complex (Bernstein et al. 2006; Levine et al. 2002). Subsequently, the PRC1 complex, via its RING1 subunit, can ubiquitinate histone H2A at Lysine 119 (H2AK119ub)

V. van den Boom • H. Schepers • A.Z. Brouwers-Vos • J.J. Schuringa (✉)
Department of Experimental Hematology, University Medical Center Groningen, University of Groningen, Hanzeplein 1, DA13, 9700, RB Groningen, The Netherlands
e-mail: j.j.schuringa@umcg.nl

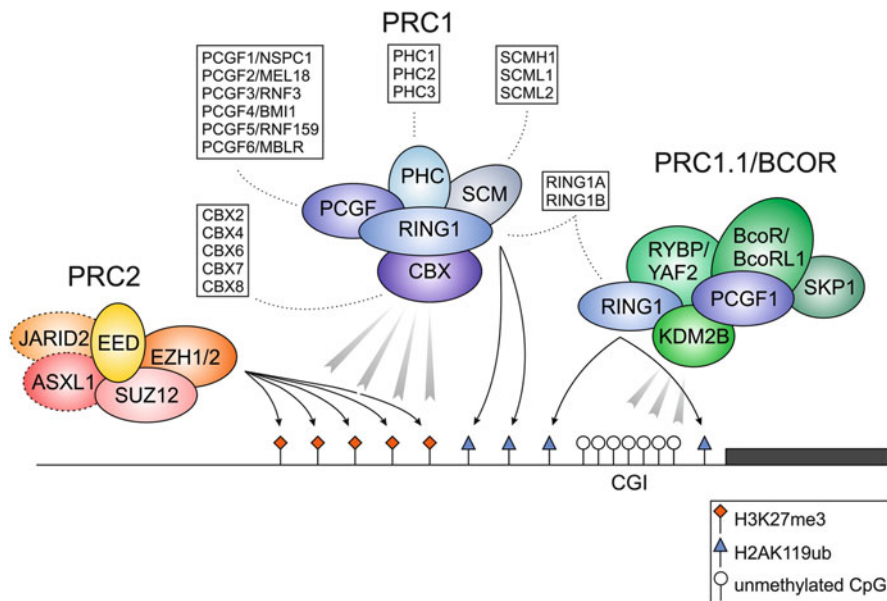


Fig. 6.1 Overview of canonical and noncanonical PcG complexes

(de Napoles et al. 2004; Wang et al. 2004). In humans, each of the PRC1 components has multiple paralog family members: six PCGF members (PCGF1/NSPC1, PCGF2/MEL18, PCGF3, PCGF4/BMI1, PCGF5, and PCGF6/MBLR), three PHC members (PHC1, PHC2, and PHC3), five CBX members (CBX2, CBX4, CBX6, CBX7, and CBX8), three Sex combs on midleg (SCM) members (SCML1, SCML2, and SMLH1), and two RING1 members (RING1A and RING1B). These paralogs allow a large diversity of distinct PRC1 complexes involved in PcG-mediated silencing (Whitcomb et al. 2007). This idea was supported by the identification of BMI1- and MEL18-containing PRC1 complexes and similarly PRC1 complexes with mutually exclusivity of CBX paralogs (Elderkin et al. 2007; Maertens et al. 2009; Vandamme et al. 2011). Indeed, PRC1 complexes were identified, which lack a CBX paralog family member but do contain RYBP or YAF2 and are targeted to PcG target genes independently of H3K27me3 (Gao et al. 2012; Tavares et al. 2012). Furthermore, some paralog family members like RING1A/B, PCGF1, and PCGF6 also reside in noncanonical PRC1 complexes such as the BCOR and E2F6 complexes (Gearhart et al. 2006; Ogawa et al. 2002; Sanchez et al. 2007; Trimarchi et al. 2001; Gao et al. 2012). Recently, the PRC1.1/BCOR complex was shown to target PcG genes by means of the KDM2B H3K36 demethylase (FBXL10), which specifically binds to unmethylated CpG islands (Fig. 6.1) (Farcas et al. 2012; He et al. 2013; Wu et al. 2013). These data show that noncanonical PRC1 complexes are involved in maintaining H2AK119ub levels as well. Also components of the core PRC2 complex display substoichiometric interactions with other (PcG) proteins. One such example is ASXL1, which is thought to mediate

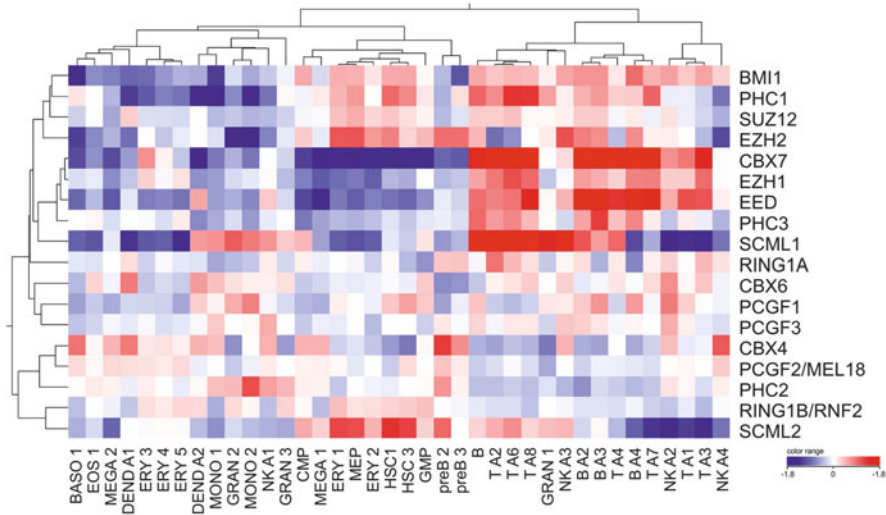


Fig. 6.2 Expression of PcG proteins in human CB cells throughout the hematopoietic compartment [based on data published by (Novershtern et al. 2011)]

recruitment and/or stabilization of the PRC2 complex to specific loci in the genome (Abdel-Wahab and Dey 2013).

The expression of PcG proteins throughout the hematopoietic compartment appears to be quite specific, with some PcGs being predominantly expressed in the immature stem/progenitor compartments, while other PcGs display a much more lineage-specific expression profile (Fig. 6.2). Taken together, these data suggest that many distinct PcG complexes exist that are likely to fulfill different functions. Yet, very little is known about possible differences in complex composition in, for instance, self-renewing hematopoietic stem cells (HSCs) versus non-self-renewing progenitors, or in normal versus leukemic stem cells (LSCs). Moreover, it is currently unclear whether specific loci would be preferentially occupied by certain PcG complexes across these different cell types. Lastly, we do not know exactly how repression of these different loci would be controlled by the various PRC complexes. Accumulating recent data indicates that molecular pathways regulating cell cycle, apoptosis, senescence, reactive oxygen species (ROS) metabolism and DNA repair are at least in part under control of PcG proteins. Whether PcG proteins fulfill similar roles in leukemia is currently unclear.

Understanding the molecular mechanisms by which PcG proteins affect stem cell fate will increase our insights into the biology of HSCs and will also aid in understanding the process of leukemic transformation, and ultimately in the identification of novel drug targets that might facilitate the eradication of LSCs. Here, we will provide an overview of the current understanding of the role of PcG proteins in HSC self-renewal and leukemogenesis.

6.2 Polycomb Function in Normal Hematopoiesis

6.2.1 Polycomb Repressive Complex 2

6.2.1.1 EZH1 and EZH2

Of all PRC2 complex subunits, the methyltransferase EZH2 has been studied in most detail. To study EZH2 function in murine hematopoiesis, conditional knockout models were required due to embryonic lethality of *Ezh2* knockout mice (O'Carroll et al. 2001). Using *Mx-Cre;Ezh2^{fl/fl}* mice it was first shown that B cell development was impaired in an *Ezh2* null background (Su et al. 2003). More specifically, pre-B cell generation was affected and accumulation of immature B cells in the bone marrow (BM) was observed. This was caused by impaired rearrangement of the immunoglobulin heavy chain gene leading to reduced μ -chain expression. Transplantation experiments emphasized that this phenotype was cell autonomous and niche independent. In contrast, pro-B cell development was unaffected indicating that EZH2 has stage-specific functions in B-lymphopoiesis. EZH2 also plays a role in T-lymphocyte generation as *Ezh2* null thymocytes fail to develop further than early CD4/CD8 double negative CD44^{int}/CD25^{hi} stage in the thymus in a transplant setting (Su et al. 2005). In contrast to adult BM cells, fetal liver cells are highly sensitive to deletion of *Ezh2* (Mochizuki-Kashio et al. 2011). Using a *Tie2-Cre;Ezh2^{fl/fl}* conditional model it was shown that levels of LSK, CMP, GMP, and MEP cells were reduced in conditional *Ezh2^{-/-}* whole fetal livers at E12.5, indicating an important role for EZH2 during fetal hematopoiesis. Strikingly, *Ezh2* null cells could efficiently reconstitute hematopoiesis in adult BM in a competitive transplant setting. In addition, H3K27me3 levels, which are very low in fetal liver *Ezh2* KO cells, were largely restored in *Ezh2^{-/-}* fetal liver cells that reconstituted the BM of recipient mice. The authors suggest a model where *Ezh1*, which is strongly induced in adult versus fetal LSK cells, may rescue *Ezh2* null cells in an adult BM environment by inducing and/or maintaining proper H3K27me3 levels in the context of an EZH1–PRC2 complex (Mochizuki-Kashio et al. 2011). Murine EZH2 overexpression studies showed enhanced long-term repopulation potential in a serial transplant setting (Kamminga et al. 2006).

It was previously shown that in *Ezh2* null embryonic stem (ES) cells H3K27me3 levels are reduced but not completely lost (Shen et al. 2008). Furthermore, EZH1 was shown to interact with PRC2 components and locates to EZH2 target genes whereby most likely (lower) levels of the H3K27me3 mark can be maintained at these loci (Margueron et al. 2008; Shen et al. 2008). Conditional deletion of both *Ezh1* and *Ezh2* in the mouse skin showed a complete loss of H3K27me3 levels further supporting a model where *Ezh1* can at least partially compensate for loss of *Ezh2* (Ezhkova et al. 2011). Recently, the specific role of *Ezh1* in hematopoiesis was addressed in a hematopoietic conditional knockout model (Hidalgo et al. 2012). Conditional deletion of *Ezh1* in the hematopoietic system using a *Vav-Cre;Ezh1^{fl/fl}* mouse model showed development of BM hypoplasia most likely

due to reduced numbers of LT-HSC, ST-HSC, MPP, and CLP cells, whereas CMP, GMP, and MEP cells were unaffected. Competitive transplantation experiments using a tamoxifen-inducible *Ezh1* conditional knockout model showed a loss of repopulating ability, which was attributed to increased levels of senescence in the primitive stem cell compartment. Increased senescence in this model is likely driven by de-repression of p16^{INK4A} since a *Vav-Cre;Ezh1^{fl/fl};Ink4a-Arf^{fl/fl}* showed restoration of the senescence phenotype. Similar to *Ezh2* knockout mice, B-cell lymphopoiesis was also affected in *Ezh1* conditional knockout mice (Su et al. 2003; Hidalgo et al. 2012). In contrast to the *Ezh2* conditional knockout model, deletion of *Ezh1* in the hematopoietic system also led to a reduction in pro-B cell numbers. Furthermore, fetal liver hematopoiesis was not affected by *Ezh1* deletion, suggesting that *Ezh2* is indeed the dominant H3K27 methyltransferase during fetal embryogenesis (Mochizuki-Kashio et al. 2011; Hidalgo et al. 2012).

Taken together, EZH1 and EZH2 seem to have both separate as well as overlapping functions in the hematopoietic system, whereby the loss of one methyltransferase can be partially compensated for by the other depending on the hematopoietic cell lineage and developmental stage.

6.2.1.2 EED

Apart from the methyltransferases EZH1 and EZH2, the PRC2 complex is also composed of the core subunits EED and SUZ12. The role of EED in murine hematopoiesis was first described by Lessard and colleagues using mice heterozygous for an *Eed* null allele or homozygous for an *Eed* hypomorphic allele (Lessard et al. 1999). Previously, homozygous *Eed* null embryos were shown to exert severe gastrulation defects (Schumacher et al. 1996; Faust et al. 1995). Biochemical analysis showed that EED interacts with H3K27me3 via its carboxy-terminal domain and that this interaction stimulates PRC2 methyltransferase activity suggesting a role for EED in propagation of H3K27me3 through DNA replication (Margueron et al. 2009). In murine hematopoiesis, EED likely acts as a negative regulator of cell proliferation and *Eed*^{3354/+} mice showed increased levels of myeloid progenitors and pre-B cells. In addition, at older age these mice displayed hyperproliferation of both lymphoid and myeloid cells (Lessard et al. 1999). However, classical target genes like p16^{INK4A} and p19^{ARF} and Hox genes were not upregulated in this mouse model (Lessard et al. 1999).

6.2.1.3 SUZ12

Similar to *Ezh2* null and *Eed* null mice, also homozygous deletion of the *Suz12* gene resulted in early embryonic lethality (Pasini et al. 2004). This was accompanied by a complete loss of H3K27me2/3 suggesting that PRC2 activity is completely lost in these embryos. In murine hematopoiesis, an ENU-induced loss-of-function mutation in the *Suz12* gene was identified as a suppressor of thrombocytopenia and HSC

defects in *cMpl*^{-/-} mice (Majewski et al. 2008). More recently, it was shown that crossing heterozygous *Eed* null or *Ezh2* null mutations in a *cMpl*^{-/-} background also resulted in an improvement in the thrombocytopenic phenotype, whereby increased white blood cell counts were observed (Majewski et al. 2010).

6.2.2 Polycomb Repressive Complex 1

As described above, the PRC1 complex consists of five subunits (PCGF, CBX, PHC, RING1, and SCM), and each of these subunits has a family of paralogs both in mice and humans. Below we will describe the involvement of the various paralog family members in hematopoiesis.

6.2.2.1 PCGF Paralog Family

The PCGF paralog family is composed of PCGF1 (NSPC1), PCGF2 (MEL18), PCGF3, PCGF4 (BMI1), PCGF5, and PCGF6 (MBLR). Of these, BMI1 has been studied most extensively in hematopoiesis. BMI1 was initially observed to be oncogenic in a retroviral integration site screen where it was identified as a collaborating hit in MMLV-induced B-cell lymphomas in *Eμ-myc* transgenic mice (Haupt et al. 1991; van Lohuizen et al. 1991). Homozygous deletion of *Bmi1* in mice resulted in reduced numbers of hematopoietic progenitors and more differentiated cells, eventually leading to hematopoietic failure (van der Lugt et al. 1994). More detailed analysis showed that BMI1 has a central regulatory role in self-renewal of HSCs by inducing symmetrical cell division both in mouse and human model systems (Park et al. 2003; Iwama et al. 2004; Lessard and Sauvageau 2003; Rizo et al. 2008). Accordingly, *Bmi1*^{-/-} mice displayed dramatically reduced HSC frequencies. Mechanistically, the role of BMI1 in regulating HSC self-renewal is partially explained by its ability to repress the *Ink4a-Arf* locus (Jacobs et al. 1999; Park et al. 2003). Expression of p16^{INK4A} and p19^{ARF} in HSCs induces cell cycle arrest and p53-mediated cell death. Loss of BMI1 expression most likely results in a decreased H2AK119 ubiquitinating activity of the PRC1 complex at the *Ink4a-Arf* locus, inducing expression of p16^{INK4A} and p19^{ARF}. Importantly, the hematopoietic phenotype of *Bmi1*^{-/-} mice is not only dependent on the induction of Ink4a/Arf. *Bmi1*^{-/-};*Ink4a-Arf*^{-/-} double knockout mice showed a partial recovery of hematopoietic cell counts but did not show a complete reversal of the *Bmi1* null phenotype, suggesting that other pathways are also involved (Bruggeman et al. 2005). In a separate study, competitive transplant experiments showed that *Bmi1*^{-/-};*Ink4a-Arf*^{-/-} cells showed peripheral blood chimerism levels comparable to wild-type cells, whereas *Bmi1*^{-/-} cells did not contribute at all (Oguro et al. 2006). Although HSC self-renewal clearly correlated with p16^{INK4A} and p19^{ARF} expression, the typical hypoplastic BM phenotype of *Bmi1* null mice was not completely rescued by deletion of the *Ink4a-Arf* locus.

Gene expression analysis of the HSC/MPP fraction in *Bmi1* null mice surprisingly showed premature transcriptional activation of *Ebf1* and *Pax5*, two regulators of B cell lymphopoiesis (Oguro et al. 2010). BMI1 is directly recruited to the promoters of these genes and *Bmi1*^{-/-};*Ink4a-Arf*^{-/-} cells are biased toward the B cell lineage at the expense of T cell lymphopoiesis.

A candidate *Ink4a-Arf*-independent pathway for BMI1 in controlling HSCs is regulation of ROS in the cell. *Bmi1*^{-/-} mice displayed impaired mitochondrial function due to increased expression of PcG target genes involved in ROS metabolism (Liu et al. 2009). Long-term HSCs from *Bmi1*^{-/-} mice showed increased levels of ROS and treatment of mice with the antioxidant *N*-acetylcysteine (NAC) resulted in a rescue of thymocyte cell numbers compared to non-treated *Bmi1*^{-/-} mice. Furthermore, de-regulated ROS metabolism induced activation of the DNA damage response pathway. Knockout of *Chk2*, a component of the DNA damage response pathway, in a *Bmi1*^{-/-} background resulted in partial reversal of the thymocyte phenotype observed in *Bmi1*^{-/-} mice and increased LSK cell numbers in the BM. However, *Bmi1*^{-/-};*Chk2*^{-/-} cells failed to give long-term repopulation in a competitive transplant setting similar to *Bmi1*^{-/-} mice, indicating that the HSC self-renewal phenotype of *Bmi1* null mice is not rescued by *Chk2* deletion. Interestingly, knocking down BMI1 in CD34⁺ human cord blood cells also induced increased ROS levels and apoptosis (Rizo et al. 2009). These data clearly show that apart from the role of BMI1 in regulating cell cycle and senescence through the *Ink4a-Arf* locus, BMI1 is also implicated in other pathways regulating oxygen metabolism.

PCGF1 has previously been identified as a member of the BCOR complex, containing RING1A, RING1B, BCOR, SKP1, and KDM2B (FBXL10) (Gearhart et al. 2006). Recently, the H3K36-specific demethylase KDM2B was shown to target this noncanonical PRC1 complex to unmethylated CpG islands in the promoters of lineage-specific genes in ES cells (Fig. 6.1) (Farcas et al. 2012; He et al. 2013; Wu et al. 2013). Depletion of KDM2B resulted in derepression, a loss of RING1B binding and decreased H2AK119ub levels at these target genes. In mouse hematopoietic cells, PCGF1 was picked up as a factor negative regulating self-renewal of lineage negative cells in a *Runx1* conditional knockout setting (Ross et al. 2012). PCGF1 knockdown was shown to induce expression of HOXA cluster genes and led to a loss of H2AK119ub at the promoters of these genes. Knockdown of PCGF1 in human CD34⁺ CB cells was recently shown to give a mild growth reduction in *in vitro* cultures and a loss of CFC frequency (van den Boom et al. 2013).

Mell18^{-/-} mice display severe posterior transformations of the axial skeleton in a manner similar to *Bmi1* null mice (Akasaka et al. 1996; van der Lugt et al. 1994). Similarly, analysis of the hematopoietic compartments showed a strong reduction in cellularity in the thymus and spleens in both knockout models. Both models do not completely overlap in terms of phenotype since *Mell18* null mice develop abnormalities of the lower intestine, whereas *Bmi1*-deficient mice specifically display a cerebellum defect. However, generation of *Mell18*^{-/-};*Bmi1*^{-/-} mice

showed that both proteins synergistically regulate *Hox* cluster expression (Akasaka et al. 2001). Competitive transplants using E14 fetal liver cells from *Mel18*^{-/-} mice showed a only a mild reduction in repopulating activity compared to a severe loss of repopulating activity in *Bmi1*^{-/-} cells (Iwama et al. 2004). Knockdown of MEL18 in human cord blood (CB) cells resulted in a dramatic reduction in proliferation in both BM stromal cocultures and liquid cultures and a strong reduction in CFC plating efficiency (van den Boom et al. 2013). However, while BMI1 depletion led to an upregulation of p14^{ARF} and p16^{INK4A}, MEL18 knockdown did not induce the expression of these genes. These data show that, although both involved in the regulation of hematopoiesis, BMI1 and MEL18 have distinct functions and BMI1 clearly has a much more prominent role in maintaining HSC self-renewal.

Concerning PCGF3, PCGF5, and PCGF6 no information is currently available on their potential function in murine hematopoiesis. PCGF6 knockdown in human CD34⁺ CB cells did not or only mildly affect cell growth in hematopoietic growth assays and did not alter CFC plating efficiency, suggesting that this gene is not involved in human hematopoiesis (van den Boom et al. 2013).

6.2.2.2 CBX Paralog Family

The CBX paralog family encompasses the CBX2, CBX4, CBX6, CBX7, and CBX8 proteins, which are homologues of the *Drosophila melanogaster* Polycomb protein and target the PRC1 complex to the chromatin through their interaction with H3K27me3. However, the chromodomains of the various CBX proteins show significant differences in substrate specificity in in vitro assays, since some interact with both H3K27me3 and H3K9me3, or selectively with H3K9me3 (Bernstein et al. 2006). Recently, two independent studies reported different functional modalities between CBX paralog family members in mouse ES cells (Morey et al. 2012; O’Loughlen et al. 2012). These data showed a key role for CBX7 in undifferentiated ES cells, whereas CBX2 and CBX4 were essential for lineage commitment. Gene expression data from human hematopoietic cell subsets also showed that the various CBX proteins are differentially expressed in HSCs, progenitors, and differentiated cells (Fig. 6.2) (van den Boom et al. 2013; Novershtern et al. 2011).

Two *Cbx2* (*M33*) knockout models were generated both of which showed skeletal transformations (Katoh-Fukui et al. 1998; Core et al. 1997). Interestingly, Coré and colleagues observed a reduction of cell numbers in the spleen, thymus, and BM of *Cbx2*^{-/-} mice and found both B and T cell lymphopoiesis to be affected (Core et al. 2004; Core et al. 1997). However, competitive transplant experiments using fetal liver cells from *Cbx2*^{-/-} mice did not show any change in repopulation capability compared to wild-type cells (Iwama et al. 2004). In contrast, knockdown of CBX2 in human CD34⁺ CB cells resulted in a severe phenotype in both the HSC and progenitor compartment (van den Boom et al. 2013). CBX2 knockdown cells displayed a reduced proliferation and increased apoptosis. Furthermore, the *CDKN1A* gene (*p21*) was directly targeted by CBX2 and concurrent knockdown of CBX2 and p21 partially rescued the CBX2 knockdown phenotype. It is possible

that these differences in CBX2 function between human and mouse hematopoiesis are a consequence of species-specific differences in CBX2 function. For example, where p21 is a bona fide target of CBX2/PRC1 and PRC2 complexes in human CD34⁺ CB cells, it has not been annotated as a PcG target gene in mouse cells.

CBX4 has the unique biochemical property among other CBXs that it is a SUMO E3 ligase stimulating UBC9-dependent sumoylation of the transcriptional repressor CtBP by tethering both to Polycomb bodies (Kagey et al. 2003). Interestingly, in human epidermal stem cells CBX4 preserved a slow-cycling and undifferentiated state and also prevents from senescence induction (Luis et al. 2011). Recent generation of a conditional *Cbx4* knockout mouse model showed that thymic development was impaired. However, this phenotype was caused by dysfunction of the thymic epithelial cells rather than a consequence of intrinsic defects of the developing thymocytes (Liu et al. 2013). CBX4 overexpression in murine hematopoietic cells mildly suppressed proliferation, inhibited replating potential in CFC assays, and reduced CAFC activity (Klauke et al. 2013). In contrast, knockdown of CBX4 in human CD34⁺ CB cells strongly reduced cell proliferation in BM stromal cocultures and liquid cultures and diminished CFC plating efficiency (van den Boom et al. 2013).

Cbx7 knockout mice are born in Mendelian ratios and display a slight increase in body length (Forzati et al. 2012). MEFs from these mice showed increased proliferation and decreased induction of senescence and adult *Cbx7*^{-/-} mice develop tumors in the liver and the lungs. However, effects of *Cbx7* knockout on the hematopoietic system were not studied in this report. Contrasting the tumor-suppressor role of CBX7 in other tissues, overexpression of CBX7 in murine hematopoietic cells increases proliferation in liquid cultures and enhances the in vitro proliferative capacity of LT-HSCs and ST-HSCs (Klauke et al. 2013). CBX7 overexpression in LT-HSCs and subsequent transplantation showed enhanced numbers of ST-HSCs and MPPs but not LT-HSCs at late stages after transplantation. The authors suggest a model where CBX7 preserves a HSC self-renewing state by specifically repressing genes involved in differentiation. CBX7 knockdown in human CB CD34⁺ cells led to a mild proliferative disadvantage and reduction in CFC frequencies (van den Boom et al. 2013).

To study the role of CBX8 in normal hematopoiesis, both constitutive and conditional CBX8 knockout models were used (Tan et al. 2011). CBX8 deletion did not lead to changed peripheral blood cells numbers and bone marrow cellularity. Furthermore, LT-HSC functionality was not affected as shown by competitive transplant assays, suggesting that CBX8 is not involved in normal hematopoiesis. In contrast, in a separate study, overexpression of CBX8 in murine hematopoietic cells showed a phenotype opposite of CBX7, where frequencies of LT-HSCs, ST-HSCs, and MPP are all decreased (Klauke et al. 2013). Knockdown of CBX8 in human CD34⁺ CB cells resulted in a mild negative phenotype in terms of cell proliferation, similar to CBX7 knockdown (van den Boom et al. 2013). Myeloid differentiation was not affected by knockdown of either CBX7 or CBX8.

6.2.2.3 RING1 Paralog Family

The RING1 paralog family consists of RING1A and RING1B. Both proteins possess H2AK119 ubiquitinating activity (Buchwald et al. 2006; de Napoles et al. 2004; Wang et al. 2004). Deletion of both *Ring1a* and *Ring1b* was necessary to achieve genome-wide depletion of H2AK119ub (Endoh et al. 2008; Stock et al. 2007). Furthermore, H2A ubiquitination is essential for repression of developmental genes and preserving ES cell identity (Endoh et al. 2012). Although H2AK119 ubiquitination is in part BMI1 dependent, recent studies showed RING1B-dependent H2AK119 ubiquitination in the context of noncanonical PRC1 complexes (*i.e.*, PRC1-RYBP, BCOR complexes) that are targeted to the chromatin independently of H3K27me3 (Farcas et al. 2012; He et al. 2013; Wu et al. 2013; Kallin et al. 2009). *Ring1a*^{-/-} mice showed skeletal abnormalities and slight deregulation of Hox gene expression (del Mar Lorente et al. 2000). In contrast, where *Ring1a* null mice are viable, deletion of *Ring1b* led to a severe gastrulation defects and embryonic lethality (Voncken et al. 2003). The generation of a conditional *Mx-Cre;Ring1b*^{fl/fl} mouse model allowed the investigation of Ring1B function in hematopoiesis (Cales et al. 2008). This study showed that *Ring1b* depletion led to a mild increase in the primitive stem/progenitor compartment (LSK, Lin⁻ cells), whereas the total BM compartment was slightly decreased. In line with this phenotype, p16^{INK4A} was selectively activated in more differentiated cells but not in the Lin⁻ compartment, whereas the positive cell cycle regulator *CycD2* was upregulated in most compartments. Simultaneous deletion of p16^{INK4A}, p19^{ARF}, and *Ring1b* resulted in a rescue of the negative effects on proliferation of the mature compartments, although the hyperproliferative phenotype of the primitive compartment was not corrected. Knockdown of RING1B in human CB CD34⁺ cells showed severe defects in long-term expansion and progenitor frequencies (van den Boom et al. 2013).

6.2.2.4 PHC Paralog Family

Both in mice and humans the Polyhomeotic paralog family encompasses three members: PHC1, PHC2, and PHC3. *Phc1*^{-/-} (*Rae28*) mice showed skeletal abnormalities and a reduced spleen size (Takahara et al. 1997). Further studies showed involvement of *Phc1* in early B cell lymphopoiesis (Tokimasa et al. 2001). Furthermore, *Phc1* null mice displayed reduced hematopoietic progenitor activity in the fetal liver and loss of long-term repopulating activity of fetal liver cells in competitive transplant experiments (Ohta et al. 2002; Kim et al. 2004). *Phc2*^{-/-} mice also showed skeletal abnormalities and deregulated expression of Hox genes and p16^{INK4A} and p19^{ARF} (Isono et al. 2005). In contrast to *Phc1*, null mice deletion of *Phc2* did not have an apparent phenotype in hematopoiesis.

6.2.2.5 SCM Paralog Family

The sex combs on midleg (SCM) paralog family consists of SCMH1, SCML1 and SCML2. SCMH1 was first identified as a substoichiometric subunit of the PRC1 complex (Levine et al. 2002). Protein interaction studies have showed that also SCML1 and SCML2 of this paralog family can interact with other PRC1 subunits in human cells (van den Boom et al. 2013; Gao et al. 2012). *Scmh1*^{-/-} mice display characteristic features resembling other PcG knockout mouse models like skeletal abnormalities; however, studies concerning the hematopoietic system have not been reported (Takada et al. 2007).

6.3 Polycomb Function in Leukemia

6.3.1 Polycomb Repressive Complex 2

6.3.1.1 EZH1 and EZH2

Unlike its related family member EZH1, a potential role for the PRC2 member EZH2 in hematological malignancies has been heavily investigated. Both tumor suppressor as well as oncogenic functions have been described, which will be discussed here. Besides being overexpressed in a variety of malignancies (Bachmann et al. 2006), EZH2 has shown aberrant expression in mantle cell lymphoma (Visser et al. 2001), Hodgkin lymphoma (Raaphorst et al. 2000), and non-Hodgkin lymphoma (Table 6.1) (van Kemenade et al. 2001). In complex karyotype AML, overexpression of EZH2 has also been observed (Grubach et al. 2008), although in a panel of 60 AMLs separated in CD34⁺ and CD34⁻ fractions, higher expression was only observed within the CD34⁻ fraction, while the expression was significantly reduced within the CD34⁺ compartment compared to normal bone marrow CD34⁺ cells (Fig. 6.3) (Bonardi et al. 2013; de Jonge et al. 2011). In contrast, overexpression of EZH1 was observed within the AML CD34⁺ compartment compared to NBM CD34⁺ cells (Bonardi et al. 2013; de Jonge et al. 2011). More recently, mutations in the *EZH2* gene were discovered in ~22 % of follicular and diffuse large B cell lymphomas. Mutation of Tyrosine residue 641 (Y641) in the SET domain led to a severe decrease in enzymatic H3K27 trimethylation activity (Morin et al. 2010). EZH2 mutations were also reported for various myeloid malignancies like CMML, MDS, and AML (Abdel-Wahab et al. 2011; Ernst et al. 2012; Makishima et al. 2010; Cancer Genome Atlas Research Network. 2013). Although Y641 mutations were not found, these myeloid malignancies carried other *EZH2* mutations (R690, N693 and H694) that also affected the SET domain and thereby H3K27 trimethylation activity (Abdel-Wahab et al. 2011; Ernst et al. 2012; Makishima et al. 2010). In T-ALL ~18 % of the patients displayed truncating or missense mutations before the SET domain in

Table 6.1 Involvement of PcG proteins in human hematological malignancies

Canonical PRC complex	Protein	Disease	Aberrancy	ref	
PRC2	EZH1	AML	Overexpressed (AML CD34 ⁺)	(de Jonge et al. 2011)	
		AML	Overexpressed (AML CK)	(Grubach et al. 2008)	
	EZH2	AML	Reduced expression (AML CD34 ⁺)	(de Jonge et al. 2011)	
		AML	Reduced expression in CBF-mutated AML	(Grubach et al. 2008)	
	EED	AML, MDS, MPN, CMML	Loss-of-function mutations SET domain (R690, N693, H694)	(Abdel-Wahab et al. 2011; Ernst et al. 2012; Makishima et al. 2010; Ernst et al. 2010; Nikoloski et al. 2010)	
			MCL, NHL, HL	Overexpressed	(Visser et al. 2001; Raaphorst et al. 2000; van Kemnade et al. 2001)
		FL, DLBCL	Loss-of-function mutations SET domain (Y641)	(Morin et al. 2010)	
		T-ALL	Missense mutations	(Ntziachristos et al. 2012)	
		T-ALL	Reduced expression	(Simon et al. 2012)	
	SUZ12	ETP-ALL	Loss-of-function deletion/mutations SET domain (R684)	(Zhang et al. 2012)	
		MDS/MPN	Loss-of-function deletion/mutations (protein stability, EZH2 interaction, H3K27 me3 binding)	(Ueda et al. 2012)	
	PRC2 interactors	ASXL1	ETP-ALL	Loss-of-function deletion/mutations (R684)	(Zhang et al. 2012; Ueda et al. 2012)
			MDS/MPN	Loss-of-function deletion/mutations (R684)	(Ntziachristos et al. 2012; Zhang et al. 2012)
		JARID2	T-ALL	Loss-of-function deletion/mutations (S369fs, others)	(Brecqueville et al. 2012; Score et al. 2012)
	PRC1	ASXL1	MDS, MPN, AML, CML	Mutations (decrease protein stability)	(Abdel-Wahab et al. 2011; Shih et al. 2012; Abdel-Wahab et al. 2012; Schnittger et al. 2013)
JARID2			MDS/MPN, AML	Deletion	(Puda et al. 2012)
PCGF2/MEL18		AML	Overexpressed (AML CK)	(Grubach et al. 2008)	
		AML	Reduced expression in CBF-mutated AML	(Grubach et al. 2008)	
PCGF4/BMI1		AML	Overexpressed (AML CD34 ⁺)	(de Jonge et al. 2011; Chowdhury et al. 2007; van Gooliga et al. 2007)	
		AML	Overexpressed, predicts prognosis	(Chowdhury et al. 2007)	
		MDS	Overexpressed, predicts prognosis	(Mihara et al. 2006; Xu et al. 2011)	
		CML	Overexpressed, predicts prognosis	(Mohy et al. 2007)	
CBX7		HL, NHL	Overexpressed	(Raaphorst et al. 2000; van Kemnade et al. 2001; Dukers et al. 2004)	
		AML	Overexpressed	(Scott et al. 2007)	
RING1A		AML	Reduced expression (AML CD34 ⁺)	(de Jonge et al. 2011)	
		AML/MDS	Overexpressed	(de Jonge et al. 2011)	
PHC1/RAE28		B-ALL	Overexpressed	(Xu et al. 2011)	
		AML	Overexpressed (AML CD34 ⁺)	(Tokimasa et al. 2001)	
Non-canonical PRC complex		BCOR	AML	Mutated	(Grossmann et al. 2011)
	AML		Overexpressed (AML CD34 ⁺)	(Bonardi et al. 2013; de Jonge et al. 2011)	
	BCORL1	AML	Mutated	(Li et al. 2011)	
Role of PcGs proteins in hematological malignancy model systems					
gene	model	phenotype			
EZH2	Conditional Ezh2 ^{+/+} mouse model	T-ALL		(Simon et al. 2012)	
	mBM MLL-AF9 Tx model	Leukemia development in 2nd mice impaired in Ezh2 ^{-/-} cells		(Neff et al. 2012; Tanaka et al. 2012)	
	mBM Ezh2 overexpression	Myeloproliferative disease,		(Herrera-Merchan et al. 2012)	

(continued)

Table 6.1 (continued)

EED	mBM MLL-AF9/NRAS(G12D) Tx model	Reduced leukemic growth upon EED knockdown	(Shi et al. 2012)
SUZ12	mBM MLL-AF9/NRAS(G12D) Tx model	Reduced leukemic growth upon SUZ12 knockdown	(Shi et al. 2012)
PCGF4/BMI1	Primary AML	Reduced long-term growth upon BMI1 knockdown	(Rizo et al. 2009)
	huCB CD34 ⁺ BCR-ABL/BMI1	Myeloid/lymphoid transformation in vitro, B-ALL in vivo	(Rizo et al. 2010)
	mBM BCR-ABL/Bmi1 Tx model	B-ALL	(Sengupta et al. 2012; Waldron et al. 2011)
	mBM MYC/Bmi1 Tx model	B-ALL	(van Lohuizen M. et al. 1991)
	mBM HoxA9/Meis1 Tx model	Loss of serial transplantation in BMI1 ^{-/-} BM	(Lessard and Sauvageau 2003)
	mBM AML1-ETO Tx model	Loss of leukemic CFC replating in BMI1 ^{-/-} BM	(Smith et al. 2011)
	mBM PLZF-RAR α Tx model	Loss of leukemic CFC replating in BMI1 ^{-/-} BM	(Smith et al. 2011)
	mBM MLL-AF9 Tx model	Loss of transformation in HoxA9 ^{-/-} /Bmi1 ^{-/-} background	(Smith et al. 2003)
CBX8	mBM MLL-AF9/ENL Tx model	Loss of transformation in Cbx8 ^{-/-} background	(Tan et al. 2011b)
ASXL1	mBM NRAS(G12D) Tx model	loss of ASXL1 accelerated onset of leukemia	(Abdel-Wahab et al. 2012)

AML acute myeloid leukemia, *MDS* myelodysplastic syndrome, *MPN* myeloproliferative neoplasms, *CMML* chronic myelomonocytic leukemia, *MCL* mantle cell lymphoma, *NHL* non-hodgkin lymphoma, *HL* hodgkin lymphoma, *DLCLB* diffuse large B-cell lymphoma, *T-ALL* T cell acute lymphoblastic leukemia, *ETP-ALL* Early T-cell precursor acute lymphoblastic leukemia, *FL* follicular lymphoma, *CML* chronic myeloid leukemia, *mBM* mouse bone marrow; *Tx* transplantation; *CB* human cord blood; *CK* complex karyotype

EZH2 (Ntziachristos et al. 2012). Furthermore, 7 % of the T-ALLs demonstrated alterations in another PRC2 member, *SUZ12* (Ntziachristos et al. 2012). Although no deletions or mutations of *EZH2* could be found in the remaining human T-ALL cases, transcriptome analysis indicated that *EZH2* expression levels were strongly reduced (Simon et al. 2012). Conditional deletion of *Ezh2* in a mouse model led to the occurrence of T-ALL leukemias (Simon et al. 2012). H3K27 di- and trimethylation were reduced, but not absent, indicating that *EZH1* may partially compensate for the loss of *EZH2* (Simon et al. 2012). These data suggest that *EZH2* functions as a tumor suppressor in T-ALL and that loss of *EZH2* contributes to this malignancy. In a large proportion of the T-ALLs with *EZH2* or other PRC2 mutations, also oncogenic mutations in *NOTCH1* were observed (65 %) (Ntziachristos et al. 2012). This suggests that *NOTCH1* mutations and *EZH2* mutations collaborate in the induction of T-ALL.

In contrast, in an MLL-AF9 model of leukemia, deletion of *Ezh2* did not affect leukemic initiation, but maintenance of the leukemia was impaired. This is most likely the result of a reduction in the frequency of LSCs (Neff et al. 2012; Tanaka et al. 2012). H3K27me3 levels were strongly reduced, but not absent in these cells (Neff et al. 2012; Tanaka et al. 2012). As leukemic growth was not completely absent and H3K27 trimethylation persisted on a subset of genes, also here *EZH1* may partially compensate for the loss of *EZH2* (Neff et al. 2012; Shi et al. 2013). These results imply that *EZH2* is required for the proper maintenance of MLL-AF9-dependent LSCs, which is opposite from its tumor suppressor function in T-ALL. As also knockdown of *Eed* and *Suz12* led to a reduction in MLL-AF9/NRASG12D-induced leukemic growth, this further strengthens the dependence of these

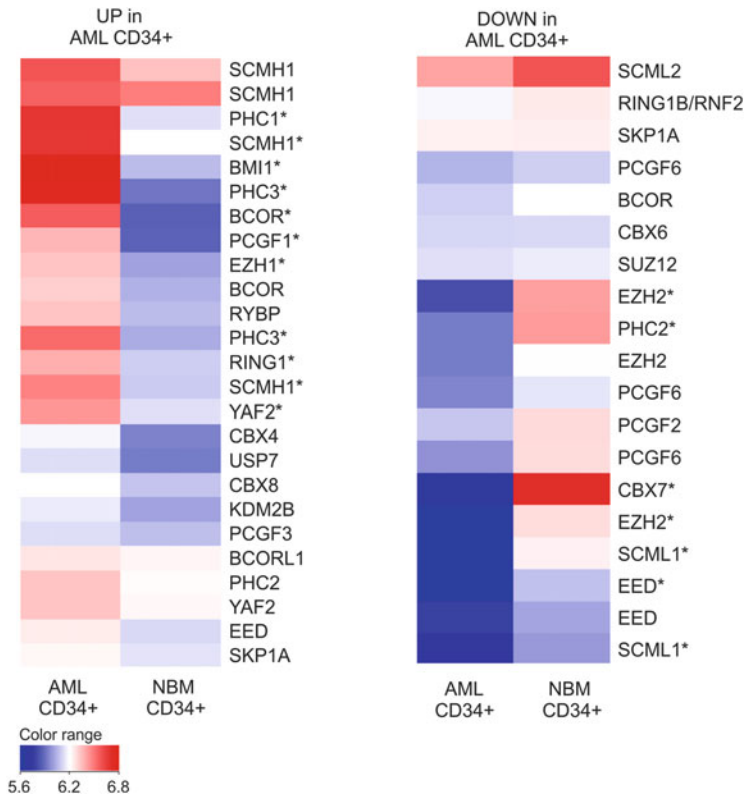


Fig. 6.3 Expression of PcG proteins in AML CD34⁺ and NBM CD34⁺ cells. Transcriptome profiling was performed on AML CD34⁺ ($n = 60$) and normal BM CD34⁺ cells ($n = 40$) (Bonardi et al. 2013; de Jonge et al. 2011). Differentially expressed genes were identified using an unpaired t-test with multiple testing correction (Benjamini-Hochberg, $p < 0.01$) and are marked with an asterisk

leukemias on PRC2 function (Shi et al. 2013). MLL-AF9-induced gene expression has been suggested to resemble an ES-like gene expression signature, which also comprises a PRC2 and MYC module (Kim et al. 2010; Somervaille et al. 2009). This MYC module is strongly reduced upon deletion of *Ezh2*, but only in secondary and not in primary leukemias. This suggests that PRC complexes play a role in the MLL-AF9-induced MYC module expression necessary for disease progression (Neff et al. 2012). In contrast, *EZH2*-deficient T-ALLs show an enhanced expression of MYC (Simon et al. 2012), suggesting that MLL-AF9 changes the gene regulatory functions of EZH2. Recent data showed that Menin, a partner of Mixed Lineage Leukemia (MLL), binds to the *Ezh2* promoter and enhances its expression (Thiel et al. 2013). Enhanced expression of *EZH2* results in a myeloproliferative disease (Herrera-Merchan et al. 2012), but together with Menin causes a block in

myeloid differentiation (Thiel et al. 2013). Since mutations that affect EZH2 methyltransferase activity have also been shown to induce MPD/MDS (Abdel-Wahab et al. 2011; Makishima et al. 2010), this suggests that this activity needs to be carefully regulated, as both hypo- and hyperactivity can result in myeloid malignancies. Therefore, the contrasting role of EZH2 as a tumor suppressor or oncogene can most likely be explained by the different genetic context in which EZH2 plays a role. The fact that PRC2 complexes are recruited by PML-RAR α and PLZF-RAR α to RAREs while PRC1 is only recruited by PLZF-RAR α further adds to this notion (Boukarabila et al. 2009; Villa et al. 2007).

6.3.1.2 EED

Unlike *Ezh2*, deletion of *Eed*, another PRC2 family member, completely abrogated MLL-AF9-mediated leukemia initiation (Neff et al. 2012). But although primary MLL-AF9 targets were not affected by *Ezh2* deletion, *Eed* deletion did affect the expression of these primary targets. These discrepancies can be reconciled with the following assumptions: 1; EZH1 partially can compensate for EZH2 activity and 2; both EZH1 and EZH2 activity is dependent upon EED. The first assumption has been confirmed in an MLL-AF9/NRASG12D leukemic model, where reduction in both EZH1 and EZH2 were necessary to reduce leukemic growth in vitro (Shi et al. 2013). Furthermore, EZH2 activity is dependent on EED (Denisenko et al. 1998) and EED mutants have been observed in patients with myelodysplastic syndrome and early T-cell precursor ALL (Ueda et al. 2012; Zhang et al. 2012). These mutations in EED will affect EZH1 and EZH2 activities and thereby contribute to aberrant myelopoiesis and lymphopoiesis. Recent evidence for this hypothesis stems from the fact that besides *EZH2* mutations in ALL, also *EED* and *SUZ12* mutations have been discovered in early T-cell precursor ALL (Zhang et al. 2012), indicating that it is the overall PRC2 activity that is inactivated. Although EED and SUZ12 knockdown reduced in vitro leukemic growth of MLL-AF9/NRASG12D cells, the reduction in in vivo leukemia development was rather modest (Shi et al. 2013). Together this suggests that PRC2 activity is required for leukemic expansion, rather than leukemic engraftment.

6.3.1.3 SUZ12

A role for SUZ12 in T-ALL development can be extrapolated from the fact that in ~7–12 % of the T-ALL cases, loss of function mutations was detected (Ntziachristos et al. 2012; Zhang et al. 2012). Downregulation of SUZ12 resulted in the expression of NOTCH1 target genes, comparable to EZH2 downregulation (Ntziachristos et al. 2012). The observation that ~33 % of early T-cell precursor ALLs has mutations in *SUZ12*, *EED*, and *EZH2* suggests that PRC2 has a tumor suppressor function in NOTCH1-mediated T-ALL.

In myeloid malignancies such as CML or other myeloproliferative neoplasms (MPNs), the role of *SUZ12* is less clear. In non-CML MPNs, like polycythemia vera (PV), essential thrombocytopenia, (ET) and myelofibrosis (MF), rare mutations in *SUZ12* have been observed (Brecqueville et al. 2012; Score et al. 2012), but a higher incidence (31 %) of *ASXL1* mutations was identified (Brecqueville et al. 2012). As *ASXL1* mutations have been shown to result in loss of PRC2 activity (Abdel-Wahab et al. 2012), it is likely that these are mutually exclusive, since they all point to a tumor suppressive mechanism of PRC2 in these non-CML MPNs. This is consistent with deletion of another substoichiometric PRC2 member *JARID2* in MPNs (Puda et al. 2012) and these data are in line with a tumor suppressive function of PRC2.

However, progression of chronic phase CML has been linked to the increased expression of *SUZ12* in a WNT-dependent manner (Pizzatti et al. 2010). Knock-down of *SUZ12* induced differentiation of these chronic phase blasts, indicating that PRC2 activity blocks differentiation. Similar, in an MLL-AF9/NRASG12D model of myeloid leukemia, reduction of *SUZ12* severely hampered leukemic expansion, as did reduction in *EED* and *EZH2* (Shi et al. 2013). These data are more in line with an oncogenic role of PRC2 activity, although it affects leukemic expansion more than leukemic engraftment/initiation. It is currently unclear if this PRC2 activity is only oncogenic in myeloid malignancies and tumor suppressive in lymphoid malignancies or whether this is imposed by the collaborating oncogenic insults like MLL-AF9 or NOTCH1.

6.3.1.4 ASXL1

ASXL1 is putative member of the PcG protein family and was recently identified as an important factor involved in regulating PRC2 activity (Abdel-Wahab et al. 2012). Mutations in *ASXL1* have been detected in a variety of hematologic malignancies, like CML, MPN, AML, and MDS (Abdel-Wahab et al. 2011; Shih et al. 2012; Cancer Genome Atlas Research Network. 2013), with a worse overall survival in MDS (Bejar et al. 2011) and AML (Metzeler et al. 2011). These mutations were shown to decrease the stability of the protein, as *ASXL1* was undetectable in cells with *ASXL1* mutations (Abdel-Wahab et al. 2012). This suggests that *ASXL1* has a tumor suppressive function in these myeloid malignancies. Downregulation of *ASXL1* led to upregulation of a gene signature that resembled the gene signature observed when MLL-AF9 is expressed in cells (Abdel-Wahab et al. 2012). The upregulated genes included genes in the posterior *HOXA* cluster (i.e., *HOXA9*) that are both classical MLL-AF9 and PcG target genes. These data suggested that *ASXL1* might modulate the activity of PcG complexes on the *HOXA* locus. Indeed, knockdown of *ASXL1* led to a decrease in H3K27me3 levels on the *HOXA* cluster, which was due to impaired recruitment of the PRC2 complex, and hence loss of *EZH2* activity at the *HOXA* cluster (Abdel-Wahab et al. 2012). *ASXL1* was found to directly interact with members of the PRC2 complex suggesting a direct role for *ASXL* in PRC2 targeting. In an *NRAS*

(G12D) model of leukemia, it was subsequently shown that loss of ASXL1 not only increased the self-renewal of leukemic cells but also accelerated the onset of leukemia (Abdel-Wahab et al. 2012). This data is consistent with leukemias induced by MLL-AF9/NRAS(G12D), where the HOX-mediated self-renewal component was supplied by the MLL-AF9 fusion (Shi et al. 2013).

ASXL1 has also been shown to form a complex with the deubiquitinase BAP1, which removes the monoubiquitin from histone H2A at Lysine 119 (Scheuermann et al. 2010). This complex (PR-DUB) which was originally identified in *Drosophila melanogaster* binds PcG target genes and is essential for repression of *Hox* genes in *Drosophila* (Scheuermann et al. 2010). This suggested a model where *ASXL1* mutations interfere with both PRC2 and PRC1 function, coordinately contributing to upregulation of the *HOX* cluster. However, both in mouse and human hematopoietic cells, knockout/knockdown of BAP1 did not induce expression of the *HOXA* genes, and loss of ASXL1 did not result in a decrease in H2AK119Ub (Abdel-Wahab et al. 2012; Abdel-Wahab and Dey 2013; Dey et al. 2012). Using a *Bap1^{fl/fl};creERT2* conditional knockout model it was shown that loss of *Bap1* induces a MDS/CMML-like disease (Dey et al. 2012). Interestingly, the authors show that apart from influencing PcG-mediated repression, the ASXL1/BAP1 complex also mediates ubiquitination, and thereby the stability of the epigenetic regulators such as OGT (Dey et al. 2012). OGT mediates O-GlcNAcylation of Ser-112 of histone H2B and can be recruited to CpG-rich transcription start sites of active genes via its interaction with TET proteins. It will be interesting to see how abrogation of these distinct functional pathways of ASXL1 contributes to leukemic transformation in ASXL1 mutant cells.

6.3.2 Polycomb Repressive Complex 1

6.3.2.1 PCGF Paralog Family

From the 6 PCGF family members, the best-described role in leukemic transformation is for PCGF4/BMI1. Although PCGF2/MEL18 expression has been shown to decrease upon differentiation of the leukemic cell line HL60, no causal role has been described yet (Jo et al. 2011). The role of BMI1 has been better documented. The first evidence for a possible involvement of BMI1 in the development of hematological malignancies came from murine models in which BMI1 was identified as a cooperating factor with MYC in the induction of B cell lymphomagenesis (van Lohuizen et al. 1991). Next, Sauvageau and colleagues demonstrated that BMI1 not only determines the proliferative capacity of normal stem cells but also of LSCs (Lessard and Sauvageau 2003). In a mouse model in which coexpression of the oncogenes HOXA9 and MEIS1 resulted in a quick onset of myeloid leukemia, no disease was observed in secondary recipients in a *Bmi1*-deficient background (Lessard and Sauvageau 2003). These data indicated that BMI1 is essential for the maintenance of HOXA9-MEIS1 LSCs in vivo. Similar, serial replating of leukemic

colony-forming units after AML-ETO or PLZF-RAR α -mediated transformation was severely hampered by deletion of *Bmi1* (Smith et al. 2011). However, lower proliferation and serial replating of leukemic stem and progenitor cells deficient for *Bmi1* were not observed in leukemias with an MLL-AF9 background (Smith et al. 2011; Tan et al. 2011). In that setting, HOXA9 was strongly upregulated by MLL-AF9 and was able to bypass oncogene-induced senescence in the absence of BMI1, as MLL-AF9 transformation was lost in the *Bmi1*^{-/-};*Hoxa9*^{-/-} background (Smith et al. 2011).

While BMI1 is required for maintenance and self-renewal of HSCs, no data has been reported indicating that overexpression of BMI1 is sufficient to induce leukemia as a single event. However, a role for BMI1 during myeloid and lymphoid leukemic transformation has been inferred from studies indicating a correlation between high expression of BMI1 and disease progression in various leukemias and MDS/MPD (Chowdhury et al. 2007; Grubach et al. 2008; Mihara et al. 2006; Mohty et al. 2007; Xu et al. 2011; Yong et al. 2011; de Jonge et al. 2011; van Goslign et al. 2007; Raaphorst et al. 2000; van Kemenade et al. 2001; Dukers et al. 2004). In primary human acute myeloid leukemia patient samples, BMI1 was among the highest upregulated PcG genes in AML CD34⁺ cells compared to normal BM CD34⁺ cells (Fig. 6.3) and downregulation of BMI1 impaired long-term expansion and self-renewal properties of LSCs (Rizo et al. 2009). Upon aging, BMI1 expression goes down in lymphoid progenitors, resulting in an upregulation of p16^{INK4A} and p19^{ARF} (Signer et al. 2008). However, reintroduction of *Bmi1* was sufficient to render aged lymphoid progenitor cells susceptible for BCR-ABL-induced transformation (Signer et al. 2008). While one paper indicated that a C18Y polymorphism exists in BMI1 that resulted in an increase in proteasome-mediated degradation (Zhang and Sarge 2009), activating mutations in BMI1 have not been described. Although BMI1 function appears to be predominantly regulated at the expression level in tumor cells, posttranslational modifications have also been reported that alter the activity of BMI1. For instance, AKT-induced serine phosphorylation has been shown to inhibit BMI1-mediated HSC self-renewal, INK4A-ARF repression, and its ability to promote tumor growth (Liu et al. 2012). In contrast, in prostate cancer it was demonstrated that AKT-mediated phosphorylation can enhance the oncogenic potential of BMI1, independent of INK4A-ARF repression (Nacerddine et al. 2012). In AML1-ETO-positive leukemias, it was shown that aberrant signaling via mutated cKit can cause loss of Polycomb-mediated repression (Ray et al. 2013). Together, these data indicate that cytokine/growth factor signaling can directly influence PcG proteins, and it will be very interesting to further delineate the role of posttranslational modifications of PcG proteins in the future.

Over the past decades, a concept has emerged in which leukemia is regarded as a multistep process in which a number of (epi)genetic events are required in order to induce overt disease. As discussed above, to date it has not been demonstrated that expression of BMI1 alone is sufficient to induce leukemia. However, various lines of evidence suggest that BMI1 might act as an important collaborating factor in the transformation process. In a tumor model in which the oncogene TLS-ERG was introduced into human hematopoietic progenitors, in a limited number of cases the

transduced cells underwent a stepwise transformation and immortalization in which upregulation of BMI1 was identified as one of the cooperating hits (Warner et al. 2005). BMI1 can cooperate with H-RAS to induce aggressive breast cancer with brain metastases (Datta et al. 2007). Primary human epithelial cells could efficiently be immortalized by co-expressing hTERT and BMI1 (Haga et al. 2007). One report indicated that some of the X-linked SCID patients transplanted with retrovirally transduced CD34⁺ BM cells in order to re-express the IL2 γ receptor developed T cell leukemias. Integration site analysis revealed that in one patient the vectors had integrated near the *BMI1* gene (Hacein-Bey-Abina et al. 2008), leaving open the possibility that an upregulation of BMI1 might have contributed to the development of leukemia in this patient as well. Together with BCR-ABL, BMI1 is able to induce myeloid and lymphoid transformation in vitro and a serially transplantable CD19⁺ B-lymphoid leukemia in vivo (Rizo et al. 2010; Waldron et al. 2011). This collaboration between BCR-ABL and BMI1 was recently confirmed in a mouse model where B-lymphoid progenitors were transformed to B-ALL blasts upon overexpression of BMI1 (Sengupta et al. 2012). Where BMI1 induced self-renewal, the collaborating BCR-ABL oncogene prevented apoptosis and maintained proliferation (Sengupta et al. 2012).

Our understanding of the mechanisms by which BMI1 exerts its phenotypes is steadily increasing. While gain of BMI1 function might be involved in extending the lifespan of normal and LSCs by bypassing senescence, more direct control over the fate of HSC divisions appears to exist also. Although the molecular mechanisms remain to be elucidated, the symmetry of cell division of HSCs is directed toward a more symmetric mode of cell division upon overexpression of BMI1 (Iwama et al. 2004). While under normal homeostasis HSCs might divide asymmetrically, resulting in one new HSC and one daughter cell that has lost stem cell integrity and will differentiate, high BMI1 levels might dictate a more symmetric distribution of specific proteins, mRNAs, or other metabolites during mitosis whereby stem cell integrity is maintained in both daughter cells. How BMI1 would be involved in such processes remains unclear.

Protection against oxidative stress and apoptosis emerges as an important BMI1-downstream pathway as well, either by reducing p53 levels via BMI1-mediated repression of the INK4A/ARF locus or via modulation of the oxidative stress response in an INK4A/ARF-independent manner. Downmodulation of BMI1 resulted in an accumulation of ROS levels, both in knockout mouse models as well as in human CD34⁺ cells transduced with lentiviral BMI1 RNAi vectors (Liu et al. 2009; Rizo et al. 2009). In other non-hematopoietic model systems, it was also shown that downmodulation of BMI1 results in p53-mediated apoptosis, whereby ROS levels were increased (Alajez et al. 2009; Chatoo et al. 2009). The induction of ROS in the absence of BMI1 could be counteracted by treatment with antioxidants such as NAC, but appeared to be independent of INK4A/ARF in hematopoietic cells (Liu et al. 2009). However, in *Atm*-deficient astrocytes, oxidative stress resulted in an increase in ROS levels, which inhibited cell growth via a MEK-ERK1-BMI1-p16^{INK4A}-dependent pathway (Kim and Wong 2009). In *Bmi1*^{-/-} mice, the increase in ROS coincided with an increase in DNA damage

and an activation of the DNA damage repair pathways, and treatment with NAC or removal of CHK2 at least partially restored some the phenotypes (Liu et al. 2009). A number of genes that have been described to regulate intracellular redox homeostasis were found to be derepressed in *Bmi1*^{-/-} mice (Liu et al. 2009). In human CD34⁺ cells, downmodulation of BMI1 coincided with decreased expression of FOXO3 (Rizo et al. 2009). Foxo3a^{-/-} HSCs were defective in their competitive repopulation capacity, lost their quiescence, and displayed elevated ROS levels (Miyamoto et al. 2007). Thus, BMI1 might be required to protect hematopoietic stem/progenitor cells from apoptosis or loss of quiescence induced by oxidative stress conditions. In human leukemias, besides facilitating symmetric stem cell divisions, the LSC might utilize enhanced expression of BMI1 as a mode to protect itself from oxidative stress.

6.3.2.2 CBX Paralog Family

From the five CBX family members, CBX2,4,6,7, and 8, only CBX7 and CBX8 have been described to play a role in leukemia or lymphoma. CBX7 is a PcG member that has been shown to extend cellular lifespan in similar fashion as BMI1 (Gil et al. 2004). CBX7 was demonstrated to bypass senescence through repression of the *Ink4a/Arf* and the *Cdkn1a* locus (Gil et al. 2004). Enhanced expression of CBX7 was observed in human follicular lymphomas, and T cell lymphomas also appeared when CBX7 was ectopically expressed in murine lymphoid cells (Scott et al. 2007). In these lymphomas a decrease in p16^{INK4A} and p14^{ARF} expression was observed, suggesting that the CBX7-mediated bypass of cellular senescence contributes to the malignant phenotype. However, the long latency and incomplete penetrance of these lymphomas suggested that increased expression of CBX7 is not sufficient to drive lymphomagenesis on its own and that additional collaborating events are necessary (Scott et al. 2007). Overexpression of CBX7 in 5FU-treated BM cells recently confirmed the contribution of CBX7 to T-cell malignancies, although in 30 % of the malignancies also erythroid and immature leukemias were observed (Klauke et al. 2013).

The CBX family member CBX8 has been shown to interact with AF9 and ENL (Hemenway et al. 2001; Monroe et al. 2011; Garcia-Cuellar et al. 2001; Mueller et al. 2007). These proteins are common fusion partners for the *Mixed Lineage Leukemia (MLL)* gene in juvenile and adult leukemias. Although CBX family members are usually viewed as transcriptional repressors, in the context of MLL-AF9, MLL-ENL, and potentially also other MLL fusion proteins, CBX8 is actually required to induce gene expression (Tan et al. 2011). CBX8 was shown to be essential for MLL-AF9-induced HoxA9 expression (Tan et al. 2011), which had been demonstrated to be required for MLL-AF9-induced leukemic transformation (Faber et al. 2009; Zeisig et al. 2004). Indeed, deletion of *Cbx8* completely abolished MLL-AF9- and MLL-ENL-induced leukemogenesis (Tan et al. 2011). Depletion of CBX8 did not affect the binding of MLL fusion proteins to their target promoters, but rather affected the binding of RNA polymerase II and subsequent

promoter activation (Tan et al. 2011). As CBX family members were originally discovered to have repressive functions, this furthermore highlights that oncogenic MLL-AF9 and MLL-ENL fusions alter the function of CBX8.

6.3.2.3 RING1 Paralog Family

For the PRC1 complex member RING1, not much is known regarding its role in leukemia. RING1 expression is generally higher in MDS and AML as compared to normal bone marrow cells (Xu et al. 2011), and particularly within the AML CD34⁺ compartment, RING1A is significantly upregulated compared to normal BM CD34⁺ cells with no significant differences in RING1B expression (Fig. 6.3) (Bonardi et al. 2013; de Jonge et al. 2011). By means of protein pulldown assays, RING1 was found to interact with the MLL-fusion partners AF9 and ENL (Monroe et al. 2011; Mueller et al. 2007). This suggested that MLL-AF9 and MLL-ENL could potentially bind PRC1 through RING1 as well. However, for AF9 it was shown that AF9 does not directly bind RING1B, but uses CBX8 as an intermediate that binds both (Hemenway et al. 2001). As also ENL has been shown to bind to CBX8 (Mueller et al. 2007), it is therefore conceivable that CBX8 also acts as an intermediate between MLL-ENL and RING1. Very little is currently known about the different or overlapping roles of RING1A and RING1B in leukemogenesis which will need to be further investigated in detail. Intriguingly, *Ring1b* depletion had no effect on MLL-AF9-mediated transformation (Tan et al. 2011). Since depletion of *Cbx8* did severely affect MLL-AF9-induced leukemia (Tan et al. 2011), this suggests that MLL-AF9-induced leukemic transformation might be independent from canonical PRC1 signaling.

6.3.2.4 PHC Paralog Family

Expression analysis of the PHC family member PHC1 in BM mononuclear cells from patients with B-ALL indicated a complete loss of PHC1 expression (Tokimasa et al. 2001). Deletion of *Phc1* in mice leads to a complete block in B cell maturation between the pro-B and pre-B cell stages in neonatal splenocytes (Tokimasa et al. 2001), whereas T cell development appeared normal. This is consistent with its constitutive expression during B cell development and suggests that loss of PHC1 is underlying the B cell developmental arrest in ALL. How PHC1 expression is lost during B-ALL development is unclear, as Southern blot analysis has shown that both alleles were present in the B-ALL samples (Tokimasa et al. 2001). In AML CD34⁺ cells, PHC1 and PHC3 expression was found to be significantly upregulated in the AML CD34⁺ compartment (Fig. 6.3) (Bonardi et al. 2013; de Jonge et al. 2011).

6.3.2.5 SCM Paralog Family

Not much is known about a role for the SCML family members SCMH1, SCML1, and SCML2 in leukemic transformation. Grubach and colleagues have investigated the expression of various PcG genes in a panel of 126 AML patients (Grubach et al. 2008). SCML2 appeared to be significantly higher expressed in AML compared with normal bone marrow cells, especially in AML patients with an underlying t(8;21) or inv(16) translocation. We investigated gene expression in a panel of AML patients subdivided in CD34⁺ and CD34⁻ fractions and observed that SCMH1 was significantly upregulated and SCML1 was significantly downregulated in AML CD34⁺ cells compared to normal BM CD34⁺ cells (Bonardi et al. 2013; de Jonge et al. 2011).

6.4 Noncanonical PRC1 Complexes

Apart from the canonical five-subunit PRC1 complex, various other noncanonical PRC1 complexes have been described. One category of noncanonical PRC1 complexes contains RYBP or YAF2 instead of a CBX subunit and are targeted to chromatin in a manner independent of H3K27me3 (Gao et al. 2012; Tavares et al. 2012). In addition, the noncanonical BCOR and E2F6 complexes contain PCGF1/NSPC1 and PCGF6/MBLR, respectively (Gao et al. 2012; Gearhart et al. 2006; Ogawa et al. 2002; Sanchez et al. 2007; Trimarchi et al. 2001; Qin et al. 2012; Trojer et al. 2011). Recently, the E2F6 complex was shown to have H2AK119 ubiquitination activity through its RING1 subunits and to induce a repressive chromatin structure (Trojer et al. 2011; Gao et al. 2012). Whereas the complex is essential for mouse development, recent knockdown studies in human CB CD34⁺ cells did not show a dramatic phenotype upon PCGF6 shRNA expression and suggests that PCGF6 does not play an important role in normal hematopoiesis (Qin et al. 2012; van den Boom et al. 2013).

Interestingly, recent whole-exome sequencing approaches in AML have identified recurrent mutations in the BCOR complex subunits *BCOR* and *BCORL1* (Tiacci et al. 2012; Grossmann et al. 2011; Li et al. 2011). *BCOR* and *BCORL1* are large nuclear proteins that act as corepressor of *BCL6* or other transcriptional regulators. Translocations have also been described like t(X;17)(p11;q12) resulting in the formation of a *BCOR-RAR α* fusion in a patient with acute promyelocytic leukemia (APL) (Yamamoto et al. 2010). Most of the identified alterations are nonsense mutations, out-of-frame insertions/deletions, or splice site mutations that most likely result in truncated proteins that lack the C-terminal nuclear receptor recruitment motif. This would suggest that *BCOR/BCORL1* would act as a tumor-suppressor gene that is inactivated by mutations in a subset of AML (Tiacci et al. 2012; Li et al. 2011). On the other hand, it has been observed that *BCOR* together with its binding partners *PCGF1* and *RING1A* in the noncanonical PRC1 complex are among the most highly upregulated PcG genes in AML CD34⁺ cells

compared to normal BM CD34⁺ cells (Fig. 6.3) (de Jonge et al. 2011), suggesting that these increased expression levels might participate in the process of leukemic transformation. In line with this notion, it was recently shown that ectopic expression of KDM2B, which targets the PRC1.1/BCOR complex to chromatin, is sufficient to transform hematopoietic progenitors (He et al. 2011). Conversely, depletion of *Kdm2b* significantly impaired Hoxa9/Meis1-induced leukemic transformation by mediating silencing of p15^{INK4B} expression through active demethylation of H3K36me₂, suggesting that KDM2B functions as an oncogene (He et al. 2011). Recently, KDM2B was shown to tether the BCOR complex to non-methylated CpG islands in developmental genes, enforcing gene repression by RING1-dependent H2AK119 ubiquitination (Farcas et al. 2012; He et al. 2013; Wu et al. 2013). Further mechanistic studies are required to gain further insight into the possible mechanisms by which the noncanonical BCOR complex might contribute to leukemia development.

6.5 Summarizing Remarks

Challenges for the future lie in the further unraveling of gene networks that are under the control of PcG proteins and how regulation of these genes affects the fate of normal hematopoietic and LSCs. It is becoming clear that multiple distinct PRC1 complexes can be composed of (most likely) specific functions, and it will be interesting to determine whether differences in complex composition exist between HSCs and progenitors, or between leukemic and normal stem cells, and ultimately how complex composition might relate to specific target gene regulation. Also, PcG proteins can act independently of the canonical PRC1 complexes, but whether and how these noncanonical PRC1 complexes may participate in leukemia development still needs to be unraveled. Posttranslational modifications of PcG proteins are beginning to be identified, although our understanding of how these affect Polycomb signaling are far from complete. Future studies will help to further delineate the role of PcG proteins in the normal hematopoietic system as well as in the process of leukemic transformation.

References

- Abdel-Wahab O, Dey A (2013) The ASXL-BAP1 axis: new factors in myelopoiesis, cancer and epigenetics. *Leukemia* 27:10–15
- Abdel-Wahab O, Pardanani A, Patel J, Wadleigh M, Lasho T, Heguy A, Beran M, Gilliland DG, Levine RL, Tefferi A (2011) Concomitant analysis of EZH2 and ASXL1 mutations in myelofibrosis, chronic myelomonocytic leukemia and blast-phase myeloproliferative neoplasms. *Leukemia* 25:1200–1202
- Abdel-Wahab O, Adli M, Lafave LM, Gao J, Hricik T, Shih AH, Pandey S, Patel JP, Chung YR, Koche R, Perna F, Zhao X, Taylor JE, Park CY, Carroll M, Melnick A, Nimer SD, Jaffe JD,

- Aifantis I, Bernstein BE, Levine RL (2012) ASXL1 mutations promote myeloid transformation through loss of PRC2-mediated gene repression. *Cancer Cell* 22:180–193
- Akasaka T, Kanno M, Balling R, Mieza MA, Taniguchi M, Koseki H (1996) A role for mel-18, a Polycomb group-related vertebrate gene, during theanteroposterior specification of the axial skeleton. *Development* 122:1513–1522
- Akasaka T, van Lohuizen M, van der Lugt N, Mizutani-Koseki Y, Kanno M, Taniguchi M, Vidal M, Alkema M, Berns A, Koseki H (2001) Mice doubly deficient for the Polycomb Group genes Mel18 and Bmi1 reveal synergy and requirement for maintenance but not initiation of Hox gene expression. *Development* 128:1587–1597
- Alajez NM, Shi W, Hui AB, Yue S, Ng R, Lo KW, Bastianutto C, O'Sullivan B, Gullane P, Liu FF (2009) Targeted depletion of BMI1 sensitizes tumor cells to P53-mediated apoptosis in response to radiation therapy. *Cell Death Differ* 16:1469–1479
- Bachmann IM, Halvorsen OJ, Collett K, Stefansson IM, Straume O, Haukaas SA, Salvesen HB, Otte AP, Akslen LA (2006) EZH2 expression is associated with high proliferation rate and aggressive tumor subgroups in cutaneous melanoma and cancers of the endometrium, prostate, and breast. *J Clin Oncol* 24:268–273
- Bejar R, Stevenson K, Abdel-Wahab O, Galili N, Nilsson B, Garcia-Manero G, Kantarjian H, Raza A, Levine RL, Neuberg D, Ebert BL (2011) Clinical effect of point mutations in myelodysplastic syndromes. *N Engl J Med* 364:2496–2506
- Bernstein E, Duncan EM, Masui O, Gil J, Heard E, Allis CD (2006) Mouse polycomb proteins bind differentially to methylated histone H3 and RNA and are enriched in facultative heterochromatin. *Mol Cell Biol* 26:2560–2569
- Bonardi F, Fusetti F, Deelen P, van Gosliga D, Vellenga E, Schuringa JJ (2013) A proteomics and transcriptomics approach to identify leukemic stem cell (LSC) markers. *Mol Cell Proteomics* 12:626–637
- Boukarabila H, Saurin AJ, Batsche E, Mossadegh N, van Lohuizen M, Otte AP, Pradel J, Muchardt C, Sieweke M, Duprez E (2009) The PRC1 Polycomb group complex interacts with PLZF/RARA to mediate leukemic transformation. *Genes Dev* 23:1195–1206
- Brecqueville M, Rey J, Bertucci F, Coppin E, Finetti P, Carbuccia N, Cervera N, Gelsi-Boyer V, Arnoulet C, Gisserot O, Verrot D, Slama B, Vey N, Mozziconacci MJ, Birnbaum D, Murati A (2012) Mutation analysis of ASXL1, CBL, DNMT3A, IDH1, IDH2, JAK2, MPL, NF1, SF3B1, SUZ12, and TET2 in myeloproliferative neoplasms. *Genes Chromosomes Cancer* 51:743–755
- Bruggeman SW, Valk-Lingbeek ME, van der Stoop PP, Jacobs JJ, Kieboom K, Tanger E, Hulsman D, Leung C, Arsenijevic Y, Marino S, van Lohuizen M (2005) Ink4a and Arf differentially affect cell proliferation and neural stem cell self-renewal in Bmi1-deficient mice. *Genes Dev* 19:1438–1443
- Buchwald G, van der Stoop P, Weichenrieder O, Perrakis A, van Lohuizen M, Sixma TK (2006) Structure and E3-ligase activity of the Ring-Ring complex of polycomb proteins Bmi1 and Ring1b. *EMBO J* 25:2465–2474
- Cales C, Roman-Trufero M, Pavon L, Serrano I, Melgar T, Endoh M, Perez C, Koseki H, Vidal M (2008) Inactivation of the polycomb group protein Ring1B unveils an antiproliferative role in hematopoietic cell expansion and cooperation with tumorigenesis associated with Ink4a deletion. *Mol Cell Biol* 28:1018–1028
- Cancer Genome Atlas Research Network (2013) Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. *N Engl J Med* 368:2059–2074
- Cao R, Wang L, Wang H, Xia L, Erdjument-Bromage H, Tempst P, Jones RS, Zhang Y (2002) Role of histone H3 lysine 27 methylation in Polycomb-group silencing. *Science* 298:1039–1043
- Chatoo W, Abdouh M, David J, Champagne MP, Ferreira J, Rodier F, Bernier G (2009) The polycomb group gene Bmi1 regulates antioxidant defenses in neurons by repressing p53 pro-oxidant activity. *J Neurosci* 29:529–542

- Chowdhury M, Mihara K, Yasunaga S, Ohtaki M, Takihara Y, Kimura A (2007) Expression of Polycomb-group (PcG) protein BMI-1 predicts prognosis in patients with acute myeloid leukemia. *Leukemia* 21:1116–1122
- Core N, Bel S, Gaunt SJ, Aurrand-Lions M, Pearce J, Fisher A, Djabali M (1997) Altered cellular proliferation and mesoderm patterning in Polycomb-M33-deficient mice. *Development* 124:721–729
- Core N, Joly F, Boned A, Djabali M (2004) Disruption of E2F signaling suppresses the INK4a-induced proliferative defect in M33-deficient mice. *Oncogene* 23:7660–7668
- Datta S, Hoenerhoff MJ, Bommi P, Sainger R, Guo WJ, Dimri M, Band H, Band V, Green JE, Dimri GP (2007) Bmi-1 cooperates with H-Ras to transform human mammary epithelial cells via dysregulation of multiple growth-regulatory pathways. *Cancer Res* 67:10286–10295
- de Jonge HJ, Woolthuis CM, Vos AZ, Mulder A, van den Berg E, Kluin PM, van der Weide K, de Bont ES, Huls G, Vellenga E, Schuringa JJ (2011) Gene expression profiling in the leukemic stem cell-enriched CD34+ fraction identifies target genes that predict prognosis in normal karyotype AML. *Leukemia* 25:1825–1833
- de Napolés M, Mermoud JE, Wakao R, Tang YA, Endoh M, Appanah R, Nesterova TB, Silva J, Otte AP, Vidal M, Koseki H, Brockdorff N (2004) Polycomb group proteins Ring1A/B link ubiquitylation of histone H2A to heritable gene silencing and X inactivation. *Dev Cell* 7:663–676
- del Mar Lorente M, Marcos-Gutierrez C, Perez C, Schoorlemmer J, Ramirez A, Magin T, Vidal M (2000) Loss- and gain-of-function mutations show a polycomb group function for Ring1A in mice. *Development* 127:5093–5100
- Denisenko O, Shnyreva M, Suzuki H, Bomszyk K (1998) Point mutations in the WD40 domain of Eed block its interaction with Ezh2. *Mol Cell Biol* 18:5634–5642
- Dey A, Seshasayee D, Noubade R, French DM, Liu J, Chaurushiya MS, Kirkpatrick DS, Pham VC, Lill JR, Bakalarski CE, Wu J, Phu L, Katavolos P, Lafave LM, Abdel-Wahab O, Modrusan Z, Seshagiri S, Dong K, Lin Z, Balazs M, Suriben R, Newton K, Hymowitz S, Garcia-Manero G, Martin F, Levine RL, Dixit VM (2012) Loss of the tumor suppressor BAP1 causes myeloid transformation. *Science* 337(6101):1541–1546
- Dukers DF, van Galen JC, Giroth C, Jansen P, Sewalt RG, Otte AP, Kluin-Nelemans HC, Meijer CJ, Raaphorst FM (2004) Unique polycomb gene expression pattern in Hodgkin's lymphoma and Hodgkin's lymphoma-derived cell lines. *Am J Pathol* 164:873–881
- Elderkin S, Maertens GN, Endoh M, Mallery DL, Morrice N, Koseki H, Peters G, Brockdorff N, Hiom K (2007) A phosphorylated form of Mel-18 targets the Ring1B histone H2A ubiquitin ligase to chromatin. *Mol Cell* 28:107–120
- Endoh M, Endo TA, Endoh T, Fujimura Y, Ohara O, Toyoda T, Otte AP, Okano M, Brockdorff N, Vidal M, Koseki H (2008) Polycomb group proteins Ring1A/B are functionally linked to the core transcriptional regulatory circuitry to maintain ES cell identity. *Development* 135:1513–1524
- Endoh M, Endo TA, Endoh T, Isono K, Sharif J, Ohara O, Toyoda T, Ito T, Eskeland R, Bickmore WA, Vidal M, Bernstein BE, Koseki H (2012) Histone H2A mono-ubiquitination is a crucial step to mediate PRC1-dependent repression of developmental genes to maintain ES cell identity. *PLoS Genet* 8:e1002774
- Ernst T, Chase AJ, Score J, Hidalgo-Curtis CE, Bryant C, Jones AV, Waghorn K, Zoi K, Ross FM, Reiter A, Hochhaus A, Drexler HG, Duncombe A, Cervantes F, Oscier D, Boulwood J, Grand FH, Cross NC (2010) Inactivating mutations of the histone methyltransferase gene EZH2 in myeloid disorders. *Nat Genet* 42:722–726
- Ernst T, Pflug A, Rinke J, Ernst J, Bierbach U, Beck JF, Hochhaus A, Gruhn B (2012) A somatic EZH2 mutation in childhood acute myeloid leukemia. *Leukemia* 26:1701–1703
- Ezhkova E, Lien WH, Stokes N, Pasolli HA, Silva JM, Fuchs E (2011) EZH1 and EZH2 cogovern histone H3K27 trimethylation and are essential for hair follicle homeostasis and wound repair. *Genes Dev* 25:485–498

- Faber J, Krivtsov AV, Stubbs MC, Wright R, Davis TN, Heuvel-Eibrink M, Zwaan CM, Kung AL, Armstrong SA (2009) HOXA9 is required for survival in human MLL-rearranged acute leukemias. *Blood* 113:2375–2385
- Farcas AM, Blackledge NP, Sudbery I, Long HK, McGouran JF, Rose NR, Lee S, Sims D, Cerase A, Sheahan TW, Koseki H, Brockdorff N, Ponting CP, Kessler BM, Klose RJ (2012) KDM2B links the Polycomb Repressive Complex 1 (PRC1) to recognition of CpG islands. *Elife* 1:e00205
- Faust C, Schumacher A, Holdener B, Magnuson T (1995) The eed mutation disrupts anterior mesoderm production in mice. *Development* 121:273–285
- Forzati F, Federico A, Pallante P, Abbate A, Esposito F, Malapelle U, Sepe R, Palma G, Troncone G, Scarfo M, Arra C, Fedele M, Fusco A (2012) CBX7 is a tumor suppressor in mice and humans. *J Clin Invest* 122:612–623
- Gao Z, Zhang J, Bonasio R, Strino F, Sawai A, Parisi F, Kluger Y, Reinberg D (2012) PCGF homologs, CBX proteins, and RYBP define functionally distinct PRC1 family complexes. *Mol Cell* 45:344–356
- Garcia-Cuellar MP, Zilles O, Schreiner SA, Birke M, Winkler TH, Slany RK (2001) The ENL moiety of the childhood leukemia-associated MLL-ENL oncoprotein recruits human Polycomb 3. *Oncogene* 20:411–419
- Gearhart MD, Corcoran CM, Wamstad JA, Bardwell VJ (2006) Polycomb group and SCF ubiquitin ligases are found in a novel BCOR complex that is recruited to BCL6 targets. *Mol Cell Biol* 26:6880–6889
- Gil J, Bernard D, Martinez D, Beach D (2004) Polycomb CBX7 has a unifying role in cellular lifespan. *Nat Cell Biol* 6:67–72
- Grossmann V, Tiacci E, Holmes AB, Kohlmann A, Martelli MP, Kern W, Spanhol-Rosseto A, Klein HU, Dugas M, Schindela S, Trifonov V, Schnittger S, Haferlach C, Bassan R, Wells VA, Spinelli O, Chan J, Rossi R, Baldoni S, De CL, Goetze K, Serve H, Peceny R, Kreuzer KA, Oruzio D, Specchia G, Di RF, Fabbiano F, Sborgia M, Liso A, Farinelli L, Rambaldi A, Pasqualucci L, Rabadan R, Haferlach T, Falini B (2011) Whole-exome sequencing identifies somatic mutations of BCOR in acute myeloid leukemia with normal karyotype. *Blood* 118:6153–6163
- Grubach L, Juhl-Christensen C, Rethmeier A, Olesen LH, Aggerholm A, Hokland P, Ostergaard M (2008) Gene expression profiling of Polycomb, Hox and Meis genes in patients with acute myeloid leukaemia. *Eur J Haematol* 81:112–122
- Hacein-Bey-Abina S, Garrigue A, Wang GP, Soulier J, Lim A, Morillon E, Clappier E, Caccavelli L, Delabesse E, Beldjord K, Asnafi V, MacIntyre E, Dal CL, Radford I, Brousse N, Sigaux F, Moshous D, Hauer J, Borkhardt A, Belohradsky BH, Wintergerst U, Velez MC, Leiva L, Sorensen R, Wulffraat N, Blanche S, Bushman FD, Fischer A, Cavazzana-Calvo M (2008) Insertional oncogenesis in 4 patients after retrovirus-mediated gene therapy of SCID-X1. *J Clin Invest* 118:3132–3142
- Haga K, Ohno S, Yugawa T, Narisawa-Saito M, Fujita M, Sakamoto M, Galloway DA, Kiyono T (2007) Efficient immortalization of primary human cells by p16INK4a-specific short hairpin RNA or Bmi-1, combined with introduction of hTERT. *Cancer Sci* 98:147–154
- Haupt Y, Alexander WS, Barri G, Klinken SP, Adams JM (1991) Novel zinc finger gene implicated as myc collaborator by retrovirally accelerated lymphomagenesis in E mu-myc transgenic mice. *Cell* 65:753–763
- He J, Nguyen AT, Zhang Y (2011) KDM2b/JHDM1b, an H3K36me2-specific demethylase, is required for initiation and maintenance of acute myeloid leukemia. *Blood* 117:3869–3880
- He J, Shen L, Wan M, Taranova O, Wu H, Zhang Y (2013) Kdm2b maintains murine embryonic stem cell status by recruiting PRC1 complex to CpG islands of developmental genes. *Nat Cell Biol* 15:373–384
- Hemenway CS, de Erkenez AC, Gould GC (2001) The polycomb protein MPc3 interacts with AF9, an MLL fusion partner in t(9;11)(p22;q23) acute leukemias. *Oncogene* 20:3798–3805

- Herrera-Merchan A, Arranz L, Ligos JM, de Molina A, Dominguez O, Gonzalez S (2012) Ectopic expression of the histone methyltransferase Ezh2 in haematopoietic stem cells causes myelo-proliferative disease. *Nat Commun* 3:623
- Hidalgo I, Herrera-Merchan A, Ligos JM, Carramolino L, Nunez J, Martinez F, Dominguez O, Torres M, Gonzalez S (2012) Ezh1 Is required for hematopoietic stem cell maintenance and prevents senescence-like cell cycle arrest. *Cell Stem Cell* 11:649–662
- Isono K, Fujimura Y, Shinga J, Yamaki M, Wang J, Takihara Y, Murahashi Y, Takada Y, Mizutani-Koseki Y, Koseki H (2005) Mammalian polyhomeotic homologues Phc2 and Phc1 act in synergy to mediate polycomb repression of Hox genes. *Mol Cell Biol* 25:6694–6706
- Iwama A, Oguro H, Negishi M, Kato Y, Morita Y, Tsukui H, Ema H, Kamijo T, Katoh-Fukui Y, Koseki H, van Lohuizen M, Nakauchi H (2004) Enhanced self-renewal of hematopoietic stem cells mediated by the polycomb gene product Bmi-1. *Immunity* 21:843–851
- Jacobs JJ, Kieboom K, Marino S, DePinho RA, van Lohuizen M (1999) The oncogene and Polycomb-group gene bmi-1 regulates cell proliferation and senescence through the ink4a locus. *Nature* 397:164–168
- Jo S, Lee H, Kim S, Hwang EM, Park JY, Kang SS, Chung H (2011) Inhibition of PCGF2 enhances granulocytic differentiation of acute promyelocytic leukemia cell line HL-60 via induction of HOXA7. *Biochem Biophys Res Commun* 416:86–91
- Kagey MH, Melhuish TA, Wotton D (2003) The polycomb protein Pc2 is a SUMO E3. *Cell* 113:127–137
- Kallin EM, Cao R, Jothi R, Xia K, Cui K, Zhao K, Zhang Y (2009) Genome-wide uH2A localization analysis highlights Bmi1-dependent deposition of the mark at repressed genes. *PLoS Genet* 5:e1000506
- Kamminga LM, Bystrykh LV, de Boer A, Houwer S, Douma J, Weersing E, Dontje B, de Haan G (2006) The Polycomb group gene Ezh2 prevents hematopoietic stem cell exhaustion. *Blood* 107:2170–2179
- Katoh-Fukui Y, Tsuchiya R, Shiroishi T, Nakahara Y, Hashimoto N, Noguchi K, Higashinakagawa T (1998) Male-to-female sex reversal in M33 mutant mice. *Nature* 393:688–692
- Kim J, Wong PK (2009) Oxidative stress is linked to ERK1/2-p16 signaling-mediated growth defect in ATM-deficient astrocytes. *J Biol Chem* 284:14396–14404
- Kim JY, Sawada A, Tokimasa S, Endo H, Ozono K, Hara J, Takihara Y (2004) Defective long-term repopulating ability in hematopoietic stem cells lacking the Polycomb-group gene rae28. *Eur J Haematol* 73:75–84
- Kim J, Woo AJ, Chu J, Snow JW, Fujiwara Y, Kim CG, Cantor AB, Orkin SH (2010) A Myc network accounts for similarities between embryonic stem and cancer cell transcription programs. *Cell* 143:313–324
- Kirmizis A, Bartley SM, Kuzmichev A, Margueron R, Reinberg D, Green R, Farnham PJ (2004) Silencing of human polycomb target genes is associated with methylation of histone H3 Lys 27. *Genes Dev* 18:1592–1605
- Klauke K, Radulovic V, Broekhuis M, Weersing E, Zwart E, Olthof S, Ritsema M, Bruggeman S, Wu X, Helin K, Bystrykh L, de Haan G (2013) Polycomb Cbx family members mediate the balance between haematopoietic stem cell self-renewal and differentiation. *Nat Cell Biol* 15:353–362
- Kuzmichev A, Nishioka K, Erdjument-Bromage H, Tempst P, Reinberg D (2002) Histone methyltransferase activity associated with a human multiprotein complex containing the Enhancer of Zeste protein. *Genes Dev* 16:2893–2905
- Lessard J, Sauvageau G (2003) Bmi-1 determines the proliferative capacity of normal and leukaemic stem cells. *Nature* 423:255–260
- Lessard J, Schumacher A, Thorsteinsdottir U, van Lohuizen M, Magnuson T, Sauvageau G (1999) Functional antagonism of the Polycomb-Group genes eed and Bmi1 in hemopoietic cell proliferation. *Genes Dev* 13:2691–2703

- Levine SS, Weiss A, Erdjument-Bromage H, Shao Z, Tempst P, Kingston RE (2002) The core of the polycomb repressive complex is compositionally and functionally conserved in flies and humans. *Mol Cell Biol* 22:6070–6078
- Li M, Collins R, Jiao Y, Ouillette P, Bixby D, Erba H, Vogelstein B, Kinzler KW, Papadopoulos N, Malek SN (2011) Somatic mutations in the transcriptional corepressor gene *BCORL1* in adult acute myelogenous leukemia. *Blood* 118:5914–5917
- Liu J, Cao L, Chen J, Song S, Lee IH, Quijano C, Liu H, Keyvanfar K, Chen H, Cao LY, Ahn BH, Kumar NG, Rovira II, Xu XL, van Lohuizen M, Motoyama N, Deng CX, Finkel T (2009) *Bmi1* regulates mitochondrial function and the DNA damage response pathway. *Nature* 459:387–392
- Liu Y, Liu F, Yu H, Zhao X, Sashida G, Deblasio A, Harr M, She QB, Chen Z, Lin HK, Di GS, Elf SE, Yang Y, Miyata Y, Huang G, Menendez S, Mellinghoff IK, Rosen N, Pandolfi PP, Hedvat CV, Nimer SD (2012) Akt phosphorylates the transcriptional repressor *bmi1* to block its effects on the tumor-suppressing *ink4a-arf* locus. *Sci Signal* 5:ra77
- Liu B, Liu YF, Du YR, Mardaryev AN, Yang W, Chen H, Xu ZM, Xu CQ, Zhang XR, Botchkarev VA, Zhang Y, Xu GL (2013) *Cbx4* regulates the proliferation of thymic epithelial cells and thymus function. *Development* 140:780–788
- Luis NM, Morey L, Mejetta S, Pascual G, Janich P, Kuebler B, Cozutto L, Roma G, Nascimento E, Frye M, Di CL, Benitah SA (2011) Regulation of human epidermal stem cell proliferation and senescence requires polycomb-dependent and -independent functions of *Cbx4*. *Cell Stem Cell* 9:233–246
- Maertens GN, El Messaoudi-Aubert S, Racek T, Stock JK, Nicholls J, Rodriguez-Niedenfuhr M, Gil J, Peters G (2009) Several distinct polycomb complexes regulate and co-localize on the *INK4a* tumor suppressor locus. *PLoS One* 4:e6380
- Majewski IJ, Blewitt ME, de Graaf CA, McManus EJ, Bahlo M, Hilton AA, Hyland CD, Smyth GK, Corbin JE, Metcalf D, Alexander WS, Hilton DJ (2008) Polycomb repressive complex 2 (PRC2) restricts hematopoietic stem cell activity. *PLoS Biol* 6:e93
- Majewski IJ, Ritchie ME, Phipson B, Corbin J, Pakusch M, Ebert A, Busslinger M, Koseki H, Hu Y, Smyth GK, Alexander WS, Hilton DJ, Blewitt ME (2010) Opposing roles of polycomb repressive complexes in hematopoietic stem and progenitor cells. *Blood* 116:731–739
- Makishima H, Jankowska AM, Tiu RV, Szpurka H, Sugimoto Y, Hu Z, Saunthararajah Y, Guinta K, Keddache MA, Putnam P, Sekeres MA, Moliterno AR, List AF, McDevitt MA, Maciejewski JP (2010) Novel homo- and hemizygous mutations in *EZH2* in myeloid malignancies. *Leukemia* 24:1799–1804
- Margueron R, Li G, Sarma K, Blais A, Zavadil J, Woodcock CL, Dynlacht BD, Reinberg D (2008) *Ezh1* and *Ezh2* maintain repressive chromatin through different mechanisms. *Mol Cell* 32:503–518
- Margueron R, Justin N, Ohno K, Sharpe ML, Son J, Drury WJ III, Voigt P, Martin SR, Taylor WR, De Marco V, Pirrotta V, Reinberg D, Gamblin SJ (2009) Role of the polycomb protein EED in the propagation of repressive histone marks. *Nature* 461:762–767
- Metzeler KH, Becker H, Maharry K, Radmacher MD, Kohlschmidt J, Mrozek K, Nicolet D, Whitman SP, Wu YZ, Schwind S, Powell BL, Carter TH, Wetzler M, Moore JO, Kollitz JE, Baer MR, Carroll AJ, Larson RA, Caligiuri MA, Marcucci G, Bloomfield CD (2011) *ASXL1* mutations identify a high-risk subgroup of older patients with primary cytogenetically normal AML within the ELN favorable genetic category. *Blood* 118:6920–6929
- Mihara K, Chowdhury M, Nakaju N, Hidani S, Ihara A, Hyodo H, Yasunaga S, Takihara Y, Kimura A (2006) *Bmi-1* is useful as a novel molecular marker for predicting progression of myelodysplastic syndrome and patient prognosis. *Blood* 107:305–308
- Miyamoto K, Araki KY, Naka K, Arai F, Takubo K, Yamazaki S, Matsuoka S, Miyamoto T, Ito K, Ohmura M, Chen C, Hosokawa K, Nakauchi H, Nakayama K, Nakayama KI, Harada M, Motoyama N, Suda T, Hirao A (2007) *Foxo3a* is essential for maintenance of the hematopoietic stem cell pool. *Cell Stem Cell* 1:101–112

- Mochizuki-Kashio M, Mishima Y, Miyagi S, Negishi M, Saraya A, Konuma T, Shinga J, Koseki H, Iwama A (2011) Dependency on the polycomb gene *Ezh2* distinguishes fetal from adult hematopoietic stem cells. *Blood* 118:6553–6561
- Mohty M, Yong AS, Szydlo RM, Apperley JF, Melo JV (2007) The polycomb group BMI1 gene is a molecular marker for predicting prognosis of chronic myeloid leukemia. *Blood* 110:380–383
- Monroe SC, Jo SY, Sanders DS, Basrur V, Elenitoba-Johnson KS, Slany RK, Hess JL (2011) MLL-AF9 and MLL-ENL alter the dynamic association of transcriptional regulators with genes critical for leukemia. *Exp Hematol* 39(1):77–86
- Morey L, Pascual G, Cozzuto L, Roma G, Wutz A, Benitah SA, Di CL (2012) Nonoverlapping functions of the Polycomb group Cbx family of proteins in embryonic stem cells. *Cell Stem Cell* 10:47–62
- Morin RD, Johnson NA, Severson TM, Mungall AJ, An J, Goya R, Paul JE, Boyle M, Woolcock BW, Kuchenbauer F, Yap D, Humphries RK, Griffith OL, Shah S, Zhu H, Kimbara M, Shashkin P, Charlot JF, Tcherpakov M, Corbett R, Tam A, Varhol R, Smailus D, Moksa M, Zhao Y, Delaney A, Qian H, Birol I, Schein J, Moore R, Holt R, Horsman DE, Connors JM, Jones S, Aparicio S, Hirst M, Gascoyne RD, Marra MA (2010) Somatic mutations altering EZH2 (Tyr641) in follicular and diffuse large B-cell lymphomas of germinal-center origin. *Nat Genet* 42:181–185
- Mueller D, Bach C, Zeisig D, Garcia-Cuellar MP, Monroe S, Sreekumar A, Zhou R, Nesvizhskii A, Chinnaiyan A, Hess JL, Slany RK (2007) A role for the MLL fusion partner ENL in transcriptional elongation and chromatin modification. *Blood* 110:4445–4454
- Nacerddine K, Beaudry JB, Ginjala V, Westerman B, Mattioli F, Song JY, van der Poel H, Ponz OB, Pritchard C, Cornelissen-Steijger P, Zevenhoven J, Tanger E, Sixma TK, Ganesan S, van Lohuizen M (2012) Akt-mediated phosphorylation of Bmi1 modulates its oncogenic potential, E3 ligase activity, and DNA damage repair activity in mouse prostate cancer. *J Clin Invest* 122:1920–1932
- Neff T, Sinha AU, Kluk MJ, Zhu N, Khattab MH, Stein L, Xie H, Orkin SH, Armstrong SA (2012) Polycomb repressive complex 2 is required for MLL-AF9 leukemia. *Proc Natl Acad Sci USA* 109:5028–5033
- Nikoloski G, Langemeijer SM, Kuiper RP, Knops R, Massop M, Tonnissen ER, van der Heijden A, Scheele TN, Vandenberghe P, de Witte T, van der Reijden BA, Jansen JH (2010) Somatic mutations of the histone methyltransferase gene EZH2 in myelodysplastic syndromes. *Nat Genet* 42:665–667
- Novershtern N, Subramanian A, Lawton LN, Mak RH, Haining WN, McConkey ME, Habib N, Yosef N, Chang CY, Shay T, Frampton GM, Drake AC, Leskov I, Nilsson B, Preffer F, Dombkowski D, Evans JW, Liefeld T, Smutko JS, Chen J, Friedman N, Young RA, Golub TR, Regev A, Ebert BL (2011) Densely interconnected transcriptional circuits control cell states in human hematopoiesis. *Cell* 144:296–309
- Ntziachristos P, Tsigros A, Vlierberghe PV, Nedjic J, Trimarchi T, Flaherty MS, Ferres-Marco D, da Ros V, Tang Z, Siegle J, Asp P, Hadler M, Rigo I, Keersmaecker KD, Patel J, Huynh T, Utro F, Poglio S, Samon JB, Paietta E, Racevskis J, Rowe JM, Rabadan R, Levine RL, Brown S, Pflumio F, Dominguez M, Ferrando A, Aifantis I (2012) Genetic inactivation of the polycomb repressive complex 2 in T cell acute lymphoblastic leukemia. *Nat Med* 18:298–303
- O’Carroll D, Erhardt S, Pagani M, Barton SC, Surani MA, Jenuwein T (2001) The polycomb-group gene *Ezh2* is required for early mouse development. *Mol Cell Biol* 21:4330–4336
- Ogawa H, Ishiguro K, Gaubatz S, Livingston DM, Nakatani Y (2002) A complex with chromatin modifiers that occupies E2F- and Myc-responsive genes in G0 cells. *Science* 296:1132–1136
- Oguro H, Iwama A, Morita Y, Kamijo T, Kamijo T, van Lohuizen M, van Nakauchi H (2006) Differential impact of Ink4a and Arf on hematopoietic stem cells and their bone marrow microenvironment in Bmi1-deficient mice. *J Exp Med* 203:2247–2253

- Oguro H, Yuan J, Ichikawa H, Ikawa T, Yamazaki S, Kawamoto H, Nakauchi H, Iwama A (2010) Poised lineage specification in multipotential hematopoietic stem and progenitor cells by the polycomb protein Bmi1. *Cell Stem Cell* 6:279–286
- Ohta H, Sawada A, Kim JY, Tokimasa S, Nishiguchi S, Humphries RK, Hara J, Takihara Y (2002) Polycomb group gene rae28 is required for sustaining activity of hematopoietic stem cells. *J Exp Med* 195:759–770
- O’Loughlen A, Munoz-Cabello AM, Gaspar-Maia A, Wu HA, Banito A, Kunowska N, Racek T, Pemberton HN, Beolchi P, Laval F, Masui O, Vermeulen M, Carroll T, Graumann J, Heard E, Dillon N, Azuara V, Snijders AP, Peters G, Bernstein E, Gil J (2012) MicroRNA regulation of Cbx7 mediates a switch of Polycomb orthologs during ESC differentiation. *Cell Stem Cell* 10:33–46
- Park IK, Qian D, Kiel M, Becker MW, Pihalja M, Weissman IL, Morrison SJ, Clarke MF (2003) Bmi-1 is required for maintenance of adult self-renewing haematopoietic stem cells. *Nature* 423:302–305
- Pasini D, Bracken AP, Jensen MR, Lazzerini DE, Helin K (2004) Suz12 is essential for mouse development and for EZH2 histone methyltransferase activity. *EMBO J* 23:4061–4071
- Pizzatti L, Binato R, Cofre J, Gomes BE, Dobbin J, Haussmann ME, D’Azambuja D, Bouzas LF, Abdelhay E (2010) SUZ12 is a candidate target of the non-canonical WNT pathway in the progression of chronic myeloid leukemia. *Genes Chromosomes Cancer* 49:107–118
- Puda A, Milosevic JD, Berg T, Klampfl T, Harutyunyan AS, Gisslinger B, Rumi E, Pietra D, Malcovati L, Elena C, Doubek M, Steurer M, Tosic N, Pavlovic S, Guglielmelli P, Pieri L, Vannucchi AM, Gisslinger H, Cazzola M, Kralovics R (2012) Frequent deletions of JARID2 in leukemic transformation of chronic myeloid malignancies. *Am J Hematol* 87:245–250
- Qin J, Whyte WA, Anderssen E, Apostolou E, Chen HH, Akbarian S, Bronson RT, Hochedlinger K, Ramaswamy S, Young RA, Hock H (2012) The polycomb group protein L3mbtl2 assembles an atypical PRC1-family complex that is essential in pluripotent stem cells and early development. *Cell Stem Cell* 11:319–332
- Raaphorst FM, van Kemenade FJ, Blokzijl T, Fieret E, Hamer KM, Satijn DP, Otte AP, Meijer CJ (2000) Coexpression of BMI-1 and EZH2 polycomb group genes in Reed-Sternberg cells of Hodgkin’s disease. *Am J Pathol* 157:709–715
- Ray D, Kwon SY, Tagoh H, Heidenreich O, Ptasinska A, Bonifer C (2013) Lineage-inappropriate PAX5 expression in t(8;21) acute myeloid leukemia requires signaling-mediated abrogation of polycomb repression. *Blood* 122:759–769
- Rizo A, Dontje B, Vellenga E, de Haan G, Schuringa JJ (2008) Long-term maintenance of human hematopoietic stem/progenitor cells by expression of BMI1. *Blood* 111:2621–2630
- Rizo A, Olthof S, Han L, Vellenga E, de Haan G, Schuringa JJ (2009) Repression of BMI1 in normal and leukemic human CD34(+) cells impairs self-renewal and induces apoptosis. *Blood* 114:1498–1505
- Rizo A, Horton SJ, Olthof S, Dontje B, Ausema A, van Os R, van den Boom V, Vellenga E, de Haan G, Schuringa JJ (2010) BMI1 collaborates with BCR-ABL in leukemic transformation of human CD34+ cells. *Blood* 116:4621–4630
- Ross K, Sedello AK, Todd GP, Paszkowski-Rogacz M, Bird AW, Ding L, Grinenko T, Behrens K, Hubner N, Mann M, Waskow C, Stocking C, Buchholz F (2012) Polycomb group ring finger 1 cooperates with Runx1 in regulating differentiation and self-renewal of hematopoietic cells. *Blood* 119:4152–4161
- Sanchez C, Sanchez I, Demmers JA, Rodriguez P, Strouboulis J, Vidal M (2007) Proteomics analysis of Ring1B/Rnf2 interactors identifies a novel complex with the Fbx110/JhdmlB histone demethylase and the Bcl6 interacting corepressor. *Mol Cell Proteomics* 6:820–834
- Scheuermann JC, de Ayala Alonso AG, Oktaba K, Ly-Hartig N, McGinty RK, Fraterman S, Wilm M, Muir TW, Muller J (2010) Histone H2A deubiquitinase activity of the Polycomb repressive complex PR-DUB. *Nature* 465:243–247
- Schnitger S, Eder C, Jeromin S, Alpermann T, Fasan A, Grossmann V, Kohlmann A, Illig T, Klopp N, Wichmann HE, Kreuzer KA, Schmid C, Staib P, Peceny R, Schmitz N, Kern W,

- Haferlach C, Haferlach T (2013) ASXL1 exon 12 mutations are frequent in AML with intermediate risk karyotype and are independently associated with an adverse outcome. *Leukemia* 27:82–91
- Schumacher A, Faust C, Magnuson T (1996) Positional cloning of a global regulator of anterior-posterior patterning in mice. *Nature* 384:648
- Score J, Hidalgo-Curtis C, Jones AV, Winkelmann N, Skinner A, Ward D, Zoi K, Ernst T, Stegelmann F, Dohner K, Chase A, Cross NC (2012) Inactivation of polycomb repressive complex 2 components in myeloproliferative and myelodysplastic/myeloproliferative neoplasms. *Blood* 119:1208–1213
- Scott CL, Gil J, Hernando E, Teruya-Feldstein J, Narita M, Martinez D, Visakorpi T, Mu D, Cordon-Cardo C, Peters G, Beach D, Lowe SW (2007) Role of the chromobox protein CBX7 in lymphomagenesis. *Proc Natl Acad Sci USA* 104:5389–5394
- Sengupta A, Ficker AM, Dunn SK, Madhu M, Cancelas JA (2012) Bmi1 reprograms CML B-lymphoid progenitors to become B-ALL-initiating cells. *Blood* 119:494–502
- Shen X, Liu Y, Hsu YJ, Fujiwara Y, Kim J, Mao X, Yuan GC, Orkin SH (2008) EZH1 mediates methylation on histone H3 lysine 27 and complements EZH2 in maintaining stem cell identity and executing pluripotency. *Mol Cell* 32:491–502
- Shi J, Wang E, Zuber J, Rappaport A, Taylor M, Johns C, Lowe SW, Vakoc CR (2013) The Polycomb complex PRC2 supports aberrant self-renewal in a mouse model of MLL-AF9;Nras (G12D) acute myeloid leukemia. *Oncogene* 32(7):930–938
- Shih AH, Abdel-Wahab O, Patel JP, Levine RL (2012) The role of mutations in epigenetic regulators in myeloid malignancies. *Nat Rev Cancer* 12:599–612
- Signer R, Montecino-Rodriguez E, Witte O, Dorshkind K (2008) Aging and cancer resistance in lymphoid progenitors are linked processes conferred by p16Ink4a and Arf. *Genes Dev* 22:3115–3120
- Simon JA, Kingston RE (2009) Mechanisms of polycomb gene silencing: knowns and unknowns. *Nat Rev Mol Cell Biol* 10:697–708
- Simon C, Chagraoui J, Krosil J, Gendron P, Wilhelm B, Lemieux S, Boucher G, Chagnon P, Drouin S, Lambert R, Rondeau C, Bilodeau A, Lavallee S, Sauvageau M, Hebert J, Sauvageau G (2012) A key role for EZH2 and associated genes in mouse and human adult T-cell acute leukemia. *Genes Dev* 26:651–656
- Smith KS, Chanda SK, Lingbeek M, Ross DT, Botstein D, van Lohuizen M, Cleary ML (2003) Bmi-1 regulation of INK4A-ARF is a downstream requirement for transformation of hematopoietic progenitors by E2a-Pbx1. *Mol Cell* 12:393–400
- Smith LL, Yeung J, Zeisig BB, Popov N, Huijbers I, Barnes J, Wilson AJ, Taskesen E, Delwel R, Gil J, Van Lohuizen M, So CW (2011) Functional crosstalk between Bmi1 and MLL/Hoxa9 axis in establishment of normal hematopoietic and leukemic stem cells. *Cell Stem Cell* 8:649–662
- Somervaille TC, Matheny CJ, Spencer GJ, Iwasaki M, Rinn JL, Witten DM, Chang HY, Shurtleff SA, Downing JR, Cleary ML (2009) Hierarchical maintenance of MLL myeloid leukemia stem cells employs a transcriptional program shared with embryonic rather than adult stem cells. *Cell Stem Cell* 4:129–140
- Stock JK, Giadrossi S, Casanova M, Brookes E, Vidal M, Koseki H, Brockdorff N, Fisher AG, Pombal A (2007) Ring1-mediated ubiquitination of H2A restrains poised RNA polymerase II at bivalent genes in mouse ES cells. *Nat Cell Biol* 9:1428–1435
- Su IH, Basavaraj A, Krutchinsky AN, Hobert O, Ullrich A, Chait BT, Tarakhovskiy A (2003) Ezh2 controls B cell development through histone H3 methylation and Igh rearrangement. *Nat Immunol* 4:124–131
- Su IH, Dobenecker MW, Dickinson E, Oser M, Basavaraj A, Marqueron R, Viale A, Reinberg D, Wulfsberg C, Tarakhovskiy A (2005) Polycomb group protein ezh2 controls actin polymerization and cell signaling. *Cell* 121:425–436

- Takada Y, Isono K, Shinga J, Turner JM, Kitamura H, Ohara O, Watanabe G, Singh PB, Kamijo T, Jenuwein T, Burgoyne PS, Koseki H (2007) Mammalian Polycomb Scmh1 mediates exclusion of Polycomb complexes from the XY body in the pachytene spermatocytes. *Development* 134:579–590
- Takahara Y, Tomotsune D, Shirai M, Katoh-Fukui Y, Nishii K, Motaleb MA, Nomura M, Tsuchiya R, Fujita Y, Shibata Y, Higashinakagawa T, Shimada K (1997) Targeted disruption of the mouse homologue of the Drosophila polyhomeotic gene leads to altered anteroposterior patterning and neural crest defects. *Development* 124:3673–3682
- Tan J, Jones M, Koseki H, Nakayama M, Muntean AG, Maillard I, Hess JL (2011) CBX8, a Polycomb Group Protein, Is Essential for MLL-AF9-Induced Leukemogenesis. *Cancer Cell* 20:563–575
- Tanaka S, Miyagi S, Sashida G, Chiba T, Yuan J, Mochizuki-Kashio M, Suzuki Y, Sugano S, Nakaseko C, Yokote K, Koseki H, Iwama A (2012) Ezh2 augments leukemogenicity by reinforcing differentiation blockage in acute myeloid leukemia. *Blood* 120:1107–1117
- Tavares L, Dimitrova E, Oxley D, Webster J, Poot R, Demmers J, Bezstarosti K, Taylor S, Ura H, Koide H, Wutz A, Vidal M, Elderkin S, Brockdorff N (2012) RYBP-PRC1 complexes mediate H2A ubiquitylation at polycomb target sites independently of PRC2 and H3K27me3. *Cell* 148:664–678
- Thiel AT, Feng Z, Pant DK, Chodosh LA, Hua X (2013) The trithorax protein partner menin acts in tandem with EZH2 to suppress C/EBPalpha and differentiation in MLL-AF9 leukemia. *Haematologica* 98:918–927
- Tiacci E, Grossmann V, Martelli MP, Kohlmann A, Haferlach T, Falini B (2012) The corepressors BCOR and BCORL1: two novel players in acute myeloid leukemia. *Haematologica* 97:3–5
- Tokimasa S, Ohta H, Sawada A, Matsuda Y, Kim JY, Nishiguchi S, Hara J, Takihara Y (2001) Lack of the Polycomb-group gene rae28 causes maturation arrest at the early B-cell developmental stage. *Exp Hematol* 29:93–103
- Trimarchi JM, Fairchild B, Wen J, Lees JA (2001) The E2F6 transcription factor is a component of the mammalian Bmi1-containing polycomb complex. *Proc Natl Acad Sci USA* 98:1519–1524
- Trojer P, Cao AR, Gao Z, Li Y, Zhang J, Xu X, Li G, Losson R, Erdjument-Bromage H, Tempst P, Farnham PJ, Reinberg D (2011) L3MBTL2 protein acts in concert with PcG protein-mediated monoubiquitination of H2A to establish a repressive chromatin structure. *Mol Cell* 42:438–450
- Ueda T, Sanada M, Matsui H, Yamasaki N, Honda ZI, Shih LY, Mori H, Inaba T, Ogawa S, Honda H (2012) EED mutants impair polycomb repressive complex 2 in myelodysplastic syndrome and related neoplasms. *Leukemia* 26:2557–2560
- van den Boom V, Rozenveld-Geugien M, Bonardi F, Malanga D, van Gosliga D, Heijink AM, Viglietto G, Morrone G, Fusetti F, Vellenga E, Schuringa JJ (2013) Nonredundant and locus-specific gene repression functions of PRC1 paralog family members in human hematopoietic stem/progenitor cells. *Blood* 121:2452–2461
- van der Lugt NM, Domen J, Linders K, van Roon M, Robanus-Maandag E, te Riele H, van der Valk M, Deschamps J, Sofroniew M, van Lohuizen M (1994) Posterior transformation, neurological abnormalities, and severe hematopoietic defects in mice with a targeted deletion of the bmi-1 proto-oncogene. *Genes Dev* 8:757–769
- van Gosliga D, Schepers H, Rizo A, van der Kolk D, Vellenga E, Schuringa JJ (2007) Establishing long-term cultures with self-renewing acute myeloid leukemia stem/progenitor cells. *Exp Hematol* 35:1538–1549
- van Kemenade FJ, Raaphorst FM, Blokzijl T, Fieret E, Hamer KM, Satijn DP, Otte AP, Meijer CJ (2001) Coexpression of BMI-1 and EZH2 polycomb-group proteins is associated with cycling cells and degree of malignancy in B-cell non-Hodgkin lymphoma. *Blood* 97:3896–3901
- van Lohuizen M, Verbeek S, Scheijen B, Wientjens E, van der Gulden H, Berns A (1991) Identification of cooperating oncogenes in E mu-myc transgenic mice by provirus tagging. *Cell* 65:737–752

- Vandamme J, Volkel P, Rosnoblet C, Le Faou P, Angrand PO (2011) Interaction proteomics analysis of polycomb proteins defines distinct PRC1 complexes in mammalian cells. *Mol Cell Proteomics* 10:M110
- Villa R, Pasini D, Gutierrez A, Morey L, Occhionorelli M, Vire E, Nomdedeu JF, Jenuwein T, Pelicci PG, Minucci S, Fuks F, Helin K, Di CL (2007) Role of the polycomb repressive complex 2 in acute promyelocytic leukemia. *Cancer Cell* 11:513–525
- Visser HP, Gunster MJ, Kluijn-Nelemans HC, Manders EM, Raaphorst FM, Meijer CJ, Willemze R, Otte AP (2001) The Polycomb group protein EZH2 is upregulated in proliferating, cultured human mantle cell lymphoma. *Br J Haematol* 112:950–958
- Voncken JW, Roelen BA, Roefs M, de Vries S, Verhoeven E, Marino S, Deschamps J, van Lohuizen M (2003) Rnf2 (Ring1b) deficiency causes gastrulation arrest and cell cycle inhibition. *Proc Natl Acad Sci USA* 100:2468–2473
- Waldron T, De Dominicis M, Soliera AR, Audia A, Iacobucci I, Lonetti A, Martinelli G, Zhang Y, Martinez R, Hyslop T, Bender TP, Calabretta B (2011) c-Myb and its target Bmi1 are required for p190BCR/ABL leukemogenesis in mouse and human cells. *Leukemia* 26:644–653
- Wang H, Wang L, Erdjument-Bromage H, Vidal M, Tempst P, Jones RS, Zhang Y (2004) Role of histone H2A ubiquitination in Polycomb silencing. *Nature* 431:873–878
- Warner JK, Wang JC, Takenaka K, Doulatov S, McKenzie JL, Harrington L, Dick JE (2005) Direct evidence for cooperating genetic events in the leukemic transformation of normal human hematopoietic cells. *Leukemia* 19:1794–1805
- Whitcomb SJ, Basu A, Allis CD, Bernstein E (2007) Polycomb Group proteins: an evolutionary perspective. *Trends Genet* 23:494–502
- Wu X, Johansen JV, Helin K (2013) Fbxl10/Kdm2b recruits polycomb repressive complex 1 to CpG islands and regulates H2A ubiquitylation. *Mol Cell* 49:1134–1146
- Xu F, Li X, Wu L, Zhang Q, Yang R, Yang Y, Zhang Z, He Q, Chang C (2011) Overexpression of the EZH2, RING1 and BMI1 genes is common in myelodysplastic syndromes: relation to adverse epigenetic alteration and poor prognostic scoring. *Ann Hematol* 90:643–653
- Yamamoto Y, Tsuzuki S, Tsuzuki M, Handa K, Inaguma Y, Emi N (2010) BCOR as a novel fusion partner of retinoic acid receptor alpha in a t(X;17)(p11;q12) variant of acute promyelocytic leukemia. *Blood* 116:4274–4283
- Yong AS, Stephens N, Weber G, Li Y, Savani BN, Eniafe R, Keyvanfar K, Kurlander R, Rezvani K, Barrett AJ (2011) Improved outcome following allogeneic stem cell transplantation in chronic myeloid leukemia is associated with higher expression of BMI-1 and immune responses to BMI-1 protein. *Leukemia* 25:629–637
- Zeisig BB, Milne T, Garcia-Cuellar MP, Schreiner S, Martin ME, Fuchs U, Borkhardt A, Chanda SK, Walker J, Soden R, Hess JL, Slany RK (2004) Hoxa9 and Meis1 are key targets for MLL-ENL-mediated cellular immortalization. *Mol Cell Biol* 24:617–628
- Zhang J, Sarge KD (2009) Identification of a polymorphism in the RING finger of human Bmi-1 that causes its degradation by the ubiquitin-proteasome system. *FEBS Lett* 583:960–964
- Zhang J, Ding L, Holmfeldt L, Wu G, Heatley SL, Payne-Turner D, Easton J, Chen X, Wang J, Rusch M, Lu C, Chen SC, Wei L, Collins-Underwood JR, Ma J, Roberts KG, Pounds SB, Ulyanov A, Beckscfort J, Gupta P, Huether R, Kriwacki RW, Parker M, McGoldrick DJ, Zhao D, Alford D, Espy S, Bobba KC, Song G, Pei D, Cheng C, Roberts S, Barbato MI, Campana D, Coustan-Smith E, Shurtleff SA, Raimondi SC, Kleppe M, Cools J, Shimano KA, Hermiston ML, Doulatov S, Eppert K, Laurenti E, Notta F, Dick JE, Basso G, Hunger SP, Loh ML, Devidas M, Wood B, Winter S, Dunsmore KP, Fulton RS, Fulton LL, Hong X, Harris CC, Dooling DJ, Ochoa K, Johnson KJ, Obenauer JC, Evans WE, Pui CH, Naeve CW, Ley TJ, Mardis ER, Wilson RK, Downing JR, Mullighan CG (2012) The genetic basis of early T-cell precursor acute lymphoblastic leukaemia. *Nature* 481:157–163