

Polycomb Group Proteins Are Key Regulators of Keratinocyte Function

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The Polycomb group (PcG) proteins are epigenetic suppressors of gene expression that function through modification of histones to change chromatin structure and modulate gene expression and cell behavior. Recent studies show that PcG proteins are expressed in epidermis, that their levels change during differentiation and in disease states, and that PcG expression is regulated by agents that influence cell proliferation and survival. The results indicate that PcG proteins regulate keratinocyte cell-cycle progression, apoptosis, senescence, and differentiation. These proteins are expressed in progenitor cells, in the basal layer, and in suprabasal keratinocytes, and the level, timing, and distribution of expression suggest that the PcG proteins have a central role in maintaining the balance between cell survival and death in multiple epidermal compartments. Additional studies indicate an important role in skin cancer progression.

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INTRODUCTION

Polycomb group genes

The role of epigenetic regulation in modulating cell function is an area of intense interest. The Polycomb group (PcG) genes encode a family of evolutionarily conserved epigenetic regulators that were discovered in *Drosophila* as repressors of the genes that control body segmentation. In mammalian

systems PcG proteins regulate genes involved in development, differentiation, and survival through epigenetic (for example, chromatin modification) mechanisms (Orlando, 2003; Valk-Lingbeek *et al.*, 2004). Gene silencing by PcG proteins involves the sequential action of two polycomb repressor complexes—PRC2 and PRC1. The PRC2 protein complex includes four core proteins—Ezh2, Suz12, eed, and RBAP48 (Figure 1). The catalytic subunit of this complex is Ezh2, a methyltransferase that methylates H3-K27 through its SET domain-encoded catalytic site (Simon and Lange, 2008). The complex contains three noncatalytic subunits, including Suz12, eed (embryonic ectoderm development), and RBAP48 (retinoblastoma-binding protein p48). Suz12 and eed are required for optimal Ezh2 histone methyltransferase activity. The complex is not invariant and alternative subunits can be substituted including Ezh1 for Ezh2, RBAP46 for RBAP48, and there are several eed variants derived from a common gene (Simon and Kingston, 2009). Interaction of Suz12 and eed with Ezh2 results in a 1,000-fold increase in Ezh2 catalytic activity, showing that the full complex is required for optimal trimethylated histone H3 lysine K27 (H3K27me3) formation (Cao and Zhang, 2004; Pasini *et al.*, 2004).

The PRC1 protein complex includes a core of four proteins including Ring1B, Bmi-1, PH1, and CBX (Simon and Kingston, 2009). The catalytic subunit of this complex, Ring1B, ubiquitinylates H2A-K119 and is optimally active in association with Bmi-1 (Li *et al.*, 2006). An important role of the CBX protein is interaction with H3K27me3 to anchor the PRC1 complex to chromatin (Hatano *et al.*, 2010). The PRC1 complex is also not invariant and proteins can be substituted including Ring1 for Ring1B, MEL18, or NSPC1 for Bmi-1, CBX2, 6, 7, or 8 for CBX, and PH2 for PH1 (Orlando, 2003; Levine *et al.*, 2004; Sparmann and van Lohuizen, 2006; Simon and Kingston, 2009). PRC1 has been proposed to ubiquitinylate H2A-K119 as part of the process leading to a closed chromatin state. However, recent findings suggest that a second complex, BCOR, which consists of Ring1B, Ring1, NSPC1, FBXL10, and BCOR, may also have an important role in H2A-K119 ubiquitinylate (Simon and Lange, 2008; Simon and Kingston, 2009).

Modification of chromatin by PRC2 and PRC1 is a coordinated sequential process. It is initiated by interaction of PRC2 with chromatin through a mechanism that is not well understood. Polycomb response elements have been identified in *Drosophila* chromatin. Polycomb response elements serve as DNA binding sites for “recruiter proteins”, including PHO and PHOL, to recruit the PRC2 complex to chromatin

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Abbreviations: cdk, cyclin-dependent kinase; H2A-K119-Ub, ubiquitinylated histone H2A lysine 119; H3K27me3, tri-methylated histone H3 lysine K27; PcG, Polycomb group genes; SCC, squamous cell carcinoma

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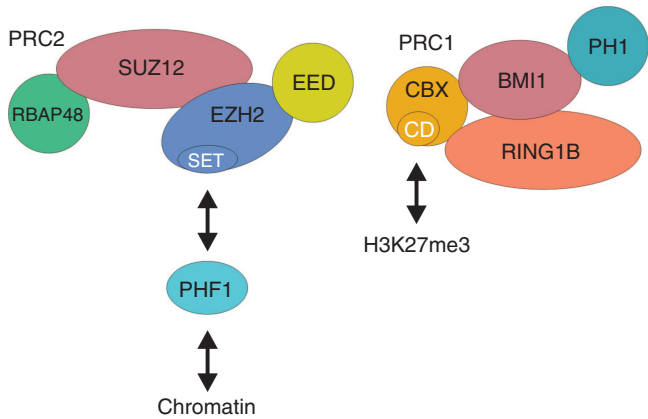


Figure 1. Polycomb complexes. The PRC2 complex consists of four core proteins including RBAP48, SUZ12, EZH2, and EED. EZH2 is the methyltransferase responsible for methylation of lysine 27 of histone H3, and this methylation activity resides in the SET domain. Interaction with other proteins in the complex is required for EZH2 activity. PHF1 is a polycomb protein that is not part of the core complex, but functions to increase EZH2 activity and is also involved in PRC2 complex interaction with chromatin. The PRC1 complex comprises four core proteins including RING1B, CBX, Bmi-1, and PH1. RING1B is an E3 ubiquitin ligase that specifically ubiquitinylates lysine 119 of histone H2A. Bmi-1 interacts directly with RING1B to enhance Ring1B ubiquitin ligase activity. CBX encodes the domain (chromodomain, CD) responsible for anchoring the PRC1 complex to methylated lysine 27 of histone H3.

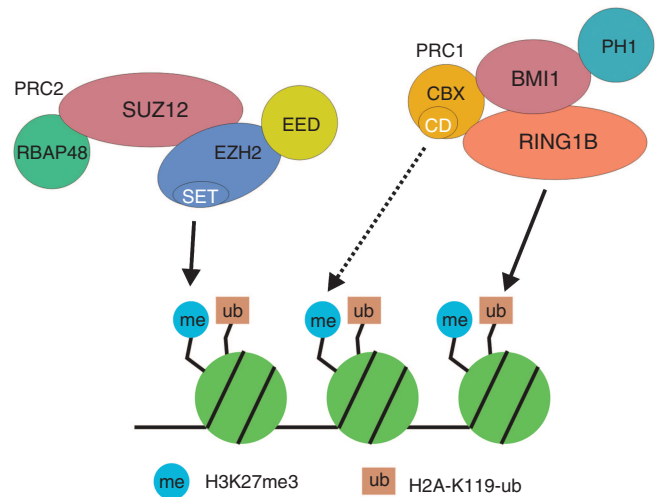


Figure 2. Simplified description of PcG regulation of transcription. The PRC2 complex is recruited to chromatin, and the Ezh2 protein of this complex, a histone methyltransferase, catalyzes formation of H3K27me3 through its SET catalytic site. H3K27me3 then functions as a binding site for the CBX protein of the PRC1 complex. CBX encodes a chromodomain site that has a high affinity for H3K27me3. The interaction of CBX with H3K27me3 anchors the PRC1 complex to chromatin, and the RING1B subunit of the complex, an E3 ubiquitin ligase, catalyzes formation of H2A-K119-ub. Ultimately these events lead to chromatin folding and compaction and cessation of transcription. The dashed line indicates binding interaction and the solid lines indicate covalent modifications.

(Brown *et al.*, 1998, 2003; Mihaly *et al.*, 1998). Polycomb response elements have not been well described in mammalian cells, but YY1 and Oct4 have been proposed to serve as recruiter proteins in specific contexts (Caretto *et al.*, 2004; Endoh *et al.*, 2008), and noncoding RNA may also function as part of the recruiting complex (Rinn *et al.*, 2007; Pandey *et al.*, 2008).

The first step in polycomb complex-mediated chromatin modification is performed by PRC2. The Ezh2 protein of the PRC2 complex catalyzes trimethylation of Lys₂₇ of histone H3 (H3K27me3). In the next step, the CBX protein of the PRC1 complex binds to H3K27me3 to anchor the PRC1 complex at this site and the PRC1 Ring1B protein catalyzes ubiquitinylation of histone H2A at lysine K119 (Figure 2; Fischle *et al.*, 2003; Cao *et al.*, 2005). These events lead to chromatin compaction-mediated suppression of transcription. The mechanism of transcription repression is not well defined, but the chromatin compaction may block transcription factor–DNA interaction or inhibit transcript elongation. Recent findings suggest that inhibition of transcript elongation may be a key mechanism (Stock *et al.*, 2007; Zhou *et al.*, 2008). The combination of PRC2/PRC1 action results in the stable suppression of gene expression and the resulting gene silencing is associated with increased cell proliferation/survival and decreased senescence and differentiation (Jacobs and van Lohuizen, 2002; Orlando, 2003).

PcG proteins are important in maintaining stem cell survival during development (Lessard and Sauvageau, 2003; Molofsky *et al.*, 2003; Iwama *et al.*, 2004), but they also function in adult organisms. For example, knockout mice

lacking the PRC1 complex Bmi-1 protein lose stem cells after birth (Lessard and Sauvageau, 2003; Molofsky *et al.*, 2003; Leung *et al.*, 2004), Bmi-1-deficient fibroblasts show slowed proliferation and enhanced senescence (Jacobs *et al.*, 1999b) and cells with elevated Bmi-1 expression show increased cell proliferation (Lessard and Sauvageau, 2003; Yu *et al.*, 2007). Bmi-1 is also important in disease, as it is overexpressed in many tumor types (Vonlanthen *et al.*, 2001; Breuer *et al.*, 2004; Kim *et al.*, 2004a,b) and collaborates with various oncogenes to enhance cell proliferation and survival (Haupt *et al.*, 1993; Jacobs *et al.*, 1999a,b; Dimri *et al.*, 2002; Datta *et al.*, 2007; Haga *et al.*, 2007; Brunner *et al.*, 2008).

The prototypic mode of Bmi-1 action is suppression of gene expression. The *Ink4a/Arf* locus was one of the first targets identified (Bracken *et al.*, 2007). This locus encodes two tumor suppressor genes called p16^{Ink4a} and p14^{Arf} (p19^{Arf} in mouse) that are transcribed in independent reading frames (Jacobs *et al.*, 1999b). This locus has a key role in control of cell-cycle progression. p16^{Ink4a} inhibits the kinase activity of the cyclin D-dependent kinases, Cdk4 and Cdk6, and prevents their association with cyclins. This ultimately leads to reduced pRB phosphorylation and reduced cell-cycle progression through G₁ (Serrano *et al.*, 1993). p16^{Ink4a} levels are normally reduced under conditions of cell-cycle progression. Consistent with this role, cells in p16^{Ink4a} knockout mice are more susceptible to malignant progression (Krimpenfort *et al.*, 2001; Sharpless *et al.*, 2001). p14^{Arf} promotes cell-cycle arrest by interacting with MDM2 and reducing its ability to target p53 for degradation. This also ultimately leads to cell-cycle arrest (Weber *et al.*, 1999; Lowe

and Sherr, 2003). Thus, in the presence of Bmi-1, p16^{Ink4a} and p14^{Arf} levels are reduced thereby permitting the cells to proliferate. As noted below, the *Ink4a/Arf* locus is an important target gene in keratinocytes, but other target genes have also been identified.

PcG genes in epidermis

Recent studies have begun to focus on the role of the PcG gene products in epidermis, with a particular emphasis on *Bmi-1*. The epidermis is a multilayered tissue that includes three major functional compartments—undifferentiated cells that retain proliferative potential, differentiating cells that are viable but not able to proliferate, and terminally differentiated dead cells. Maintaining the relative size of these compartments requires that the cell proliferation rate in the basal layer, the tendency of cells to survive versus differentiate in the suprabasal layers, and terminal cell death in the cornified layer be coordinately controlled and balanced (Eckert et al., 1997). Recent studies suggest that the PcG gene products regulate these processes. As noted above, Bmi-1 is a component of the PRC1 PcG complex that functions in the nucleus to modify chromatin and suppress gene expression (Cohen et al., 1996; Aoto et al., 2008). Indeed, Bmi-1 is localized in the nucleus of proliferating keratinocytes in culture (Silva et al., 2006; Lee et al., 2008) and has been detected in the cytoplasm of quiescent keratinocytes (Silva et al., 2006). These findings are consistent with the fact that Bmi-1 has a nuclear localization signal and that inactive Bmi-1 can shuttle to the cytoplasm (Cohen et al., 1996). Bmi-1 was originally characterized as a stem cell maintenance protein that is required for efficient renewal of hematopoietic stem cells (Jacobs et al., 1999a; Molofsky et al., 2003; Park et al., 2003), and leukemic and neuronal stem cells (Lessard and Sauvageau, 2003; Molofsky et al., 2003). However, Bmi-1 does not appear to function exclusively as a stem cell regulator in epidermis. If this were the case, one would expect expression to be restricted to epidermal stem cells compartments. However, the available evidence suggests that Bmi-1 is expressed in multiple epidermal layers, both basal and suprabasal, and is not strictly confined to stem cells (Ressler et al., 2006; Reinisch et al., 2007; Balasubramanian et al., 2008; Lee et al., 2008; Shaw and Martin, 2009). These findings suggest that Bmi-1 is likely to function in multiple epidermal cell layers.

Bmi-1: role in cell survival, proliferation, and senescence

Recent studies have investigated the role of Bmi-1 in regulating keratinocyte senescence. Senescence is a process whereby aging cells gradually lose proliferative potential (Barrandon and Green, 1987). Senescence has the physiological role of limiting the long-term survival of self-renewing cells during aging. A hallmark of senescence is increased expression of products of the *Ink4a/Arf* locus, p16^{Ink4a} and p14^{Arf} (p19^{Arf} in mouse cells) (Krishnamurthy et al., 2004). Dellambra and co-workers, using the holoclone/merclone/paraclone clonogenic assay system (Barrandon and Green, 1987), have measured the expression of Bmi-1 as keratinocytes undergo progressive restriction in replicative potential.

These studies reveal that Bmi-1 level is high in holoclones (cells with high proliferative potential) and low in paraclones (cells with limited potential) and that there is an inverse relationship between p16^{Ink4a} level and Bmi-1 content. Thus, cells that have high proliferative potential have high Bmi-1 and low p16^{Ink4a} (Silva et al., 2006; Cordisco et al., 2010). This relationship is also observed in aging epidermis, as Bmi-1 levels are high in keratinocytes isolated from young individuals and lower in cells isolated from older individuals (Ressler et al., 2006; Cordisco et al., 2010). Bmi-1 levels are also reduced in keratinocytes from young xeroderma pigmentosum group c, trichothiodystrophy, and progeria patients as compared to keratinocytes isolated from healthy young individuals (Cordisco et al., 2010). In addition, keratinocytes derived from photo-aged epidermis have lower Bmi-1 level than keratinocytes collected from nonexposed epidermis of the same patient (Cordisco et al., 2010). Treatment with trichostatin A results in replicative senescence and this is associated with reduced Bmi-1 level (Cordisco et al., 2010). These studies imply that Bmi-1 has a role in controlling keratinocyte senescence via regulation of the *Ink4a/Arf* locus.

Keratinocyte survival and proliferation are also influenced by Bmi-1. Treatment with chemopreventive agent or keratinocyte differentiating/apoptosis-inducing agent reduces Bmi-1 expression and the expression of other PcG proteins, and this is associated with reduced survival of normal and transformed keratinocytes (Lee et al., 2008; Balasubramanian et al., 2010). Bmi-1 overexpression in keratinocytes enhances cell number which is associated with increased expression of cdk2 (Lee et al., 2008), Bmi-1 expression extends the replicative life span of keratinocyte-like cells derived from human embryonic stem cells (Dabelsteen et al., 2009) and Bmi-1 siRNA reduces proliferation of the SCC-13 skin cancer cells (Balasubramanian et al., 2010). In this context, Bmi-1 may serve as a prosurvival protein (Lee et al., 2008; Balasubramanian et al., 2010).

Agents that regulate Bmi-1 level in keratinocytes

Given that Bmi-1 regulates keratinocyte survival, it is perhaps not surprising that various extrinsic and intrinsic processes such as cell aging (Silva et al., 2006) and treatment with growth regulatory agents may influence Bmi-1 level (Balasubramanian et al., 2010; Cordisco et al., 2010). Treating with agents that alter keratinocyte survival modulates Bmi-1 level. Hydrogen peroxide treatment reduces Bmi-1 level (Cordisco et al., 2010) as does expression of Ha-Ras(G12V), a constitutively active form of Ha-ras (Cordisco et al., 2010). Potential therapeutic agents also influence Bmi-1 expression. Treatment of keratinocytes with (–)-epigallocatechin-3-gallate (EGCG), which is the major bioactive chemopreventive agent in green tea (Ahmad and Mukhtar, 1999), reduces Bmi-1 level in keratinocytes (Lee et al., 2008; Balasubramanian et al., 2010). This reduction in Bmi-1 level is associated with reduced cell survival and proliferation, and reduced levels of cdk1, cdk2, cdk4, cdk6, cyclin D1, and cyclin E. In addition to reducing Bmi-1 level, EGCG treatment also reduces expression of the PRC2

complex proteins, Ezh2 and Suz12, and reduces histone H3 lysine K27 trimethylation (H3K27me₃; Balasubramanian *et al.*, 2010). Ezh2, a component of the PRC2 complex, is the methyltransferase that specifically methylates H3-K27 to form H3K27me₃ (Kirmizis *et al.*, 2004; Wang *et al.*, 2004; Kuzmichev *et al.*, 2005). These findings indicate that treatment with EGCG reduces function of both the PRC2 and PRC1 complexes. An interesting finding is that overexpression of Bmi-1, a PRC1 complex protein, is associated with increased expression of Ezh2, a PRC2 complex protein, suggesting that Bmi-1 somehow functions to maintain expression of the enzyme that prepares its chromatin (H3K27me₃) binding site (Balasubramanian *et al.*, 2010).

Impact of Bmi-1 on apoptosis

It has been appreciated for several years that Bmi-1 functions to enhance cell survival by regulating cell-cycle-related events. However, less has been studied regarding the impact of Bmi-1 on apoptosis. Recent studies show that challenging keratinocytes with okadaic acid or EGCG results in activation of apoptosis as measured by accumulation of sub-G₁ cells and accumulation of cleaved caspases 3 and 9 and PARP (Lee *et al.*, 2008; Balasubramanian *et al.*, 2010). Forced expression of Bmi-1 reverses the okadaic acid/EGCG-associated activation of apoptosis (Lee *et al.*, 2008; Balasubramanian *et al.*, 2010). This finding indicates that Bmi-1 influences a broad range of responses in keratinocytes and the effects cannot be solely explained by Bmi-1-dependent enhancement of cell proliferation or inhibition of senescence. The mechanism of regulation of apoptosis by Bmi-1 is likely to be indirect but has not been investigated.

Other PcG proteins in epidermis

Other PcG proteins have also been studied in epidermis including the PRC2 complex components, Ezh2 and Suz12. Ezh2 expression is inversely correlated with the differentiation status of keratinocytes (Ezhkova *et al.*, 2009). Ezhkova *et al.* observed high-level Ezh2 expression in keratinocytes maintained in low calcium medium and reduced levels in cells differentiated by treatment with high calcium. Moreover, strong Ezh2 expression is observed in epidermal progenitor cells and the level gradually declines as the cells differentiate. It is interesting that Ezh2 levels, although reduced, are still detected in suprabasal epidermal layers (Ezhkova *et al.*, 2009). These findings suggest that Ezh2 may participate in regulating the survival/differentiation balance in multiple epidermal compartments. Chromatin immunoprecipitation analysis reveals extensive H3K27me₃ modification of chromatin in differentiation marker genes in basal progenitor cells, which is associated with gene silencing. Thus, Ezh2 appears to have a role in suppressing differentiation-associated gene expression in basal epidermis (Ezhkova *et al.*, 2009). In addition, loss of Ezh2 from the promoter region of differentiation-associated genes is inversely associated with accumulation of AP1 transcription factors at these sites and gene activation (Ezhkova *et al.*, 2009). Additional studies show increased Ezh2, Suz12 level, and H3K27me₃ formation in immortalized/transformed skin cancer cell lines

including A431, SCC-13, and HaCaT cells (Balasubramanian *et al.*, 2010), and that Ezh2 knockdown reduces H3K27me₃ formation and this is associated with reduced SCC-13 cell proliferation and survival. Ezh2 is an example of a PRC2 protein that is overexpressed in tumors (Varambally *et al.*, 2002; Raaphorst *et al.*, 2003; Raman *et al.*, 2005; Sudo *et al.*, 2005; Saramaki *et al.*, 2006).

In addition to PcG proteins, which are involved in H3K27me₃ formation, a class of demethylases have recently been described that specifically erase this mark (Lan *et al.*, 2008; Nottke *et al.*, 2009; Mosammamarast and Shi, 2010). Thus, the PcG protein Ezh2 promotes H3K27me₃ formation, whereas the Jumanji C domain-containing H3K27me₃-specific demethylases, UTX and JMJD3, remove this mark. A recent study by Khavari and colleagues show that H3K27me₃ marks the promoter of many genes that are expressed during differentiation and that loss of this mark is observed when these genes are expressed during calcium-dependent differentiation (Sen *et al.*, 2008). The authors suggest that epigenetic derepression by JMJD3 enhances keratinocyte differentiation. These findings again emphasize the importance of epigenetic modification in regulating the balance between keratinocyte survival and differentiation.

PcG proteins in skin cancer

Several studies have examined the role of PcG proteins in the pathogenesis of skin cancer. Bmi-1 overexpression in HaCaT cells causes malignant transformation as measured by colony formation in soft agar and tumor formation in severe combined immunodeficient mice (Wang *et al.*, 2009). Moreover, Bmi-1 expression is observed at elevated levels in squamous cell carcinoma (SCC) tumors (Reinisch *et al.*, 2007; Balasubramanian *et al.*, 2010). Cultured skin cancer cells express elevated Bmi-1, Suz12, and Ezh2 levels as compared to normal cells and this is associated with increased H3K27me₃ (Balasubramanian *et al.*, 2010). Chemopreventive agents suppress proliferation of tumor cells and this is associated with reduced expression of cdk1, cdk2, cdk4, and cyclins D1, E, and A. The level of the cyclin-dependent kinase (cdk) inhibitors p21 and p27 is increased in these cells (Balasubramanian *et al.*, 2010). As noted above, these chemopreventive agent-associated changes are reversed by forced overexpression of Bmi-1. Chemopreventive agent treatment also activates apoptosis in skin cancer cells as evidenced by increased caspase and PARP cleavage and appearance of sub-G₁ cells. It is interesting that these effects are also reversed by forced Bmi-1 expression (Balasubramanian *et al.*, 2010). A potentially meaningful observation is that forced Bmi-1 expression restores Ezh2 levels in EGCG-treated SCC-13 cells, suggesting that feedback regulation functions to assure an appropriate balance of PRC1 and PRC2 complex proteins in cells (Balasubramanian *et al.*, 2010). This may be important as Bmi-1 is not likely to be functional in the absence of Ezh2 and Bmi-1 chromatin binding site is synthesized by Ezh2. Bmi-1 also provides protection when normal keratinocytes are challenged with the differentiation/apoptosis promoting agent, okadaic acid, by inhibiting okadaic acid-dependent apoptosis

(Lee *et al.*, 2008). It is also important to note that PcG protein function is also altered in other skin cancer types. For example, PcG protein levels are elevated in basal cell carcinoma (Reinisch *et al.*, 2007) and melanoma (Bachmann *et al.*, 2006; Mihic-Probst *et al.*, 2007).

Processes in wound healing are related to processes that control development and abnormal regulation observed in cancer cells. It is interesting that three major components of the PRC2 complex, *eed*, *Ezh2*, and *Suz12*, are reduced in epidermis during wound healing (Shaw and Martin, 2009). Reexpression of these proteins is observed approximately 10 cells back from the wound edge, and it is proposed that PcG proteins may be involved in silencing the repair genes after completion of wound healing. The loss of these PcG proteins, as expected, is associated with reduced levels of H3K27me3 in the healing epidermis (Shaw and Martin, 2009). Epidermal growth factor receptor and the *c-myc* genes are markers of the wound healing process. Using a cell culture model, Shaw and Martin (2009) showed that forced expression of *eed* resulted in reduced epidermal growth factor receptor and *c-myc* gene expression. These results appear to conflict with the idea that PcG protein expression correlates with enhanced cell survival and increased expression of survival genes. These findings, however, highlight the potential complexity of this regulation, and suggest that the role of the PcG proteins may be context dependent.

CONCLUSION

The findings described above support several conclusions regarding the function of PcG proteins in epidermis. First, expression of *Bmi-1* and other PcG proteins is not strictly confined to stem cells in normal epidermis or in cancer cells. A gradient appears to exist such that basal cells express more *Bmi-1* (for example) than suprabasal cells; however, expression is observed in a wide range of cells in the progenitor, basal, and suprabasal layers. This suggests that although *Bmi-1* expression, and the expression of other PcG proteins, correlates inversely with survival potential, they are not strictly stem-cell-specific proteins (Lee *et al.*, 2008; Ezhkova *et al.*, 2009; Cordisco *et al.*, 2010). Second, *Bmi-1* and other PcG proteins enhance expression of pro-proliferation cell-cycle regulatory proteins and suppress differentiation and apoptosis (Silva *et al.*, 2006; Lee *et al.*, 2008; Ezhkova *et al.*, 2009). Moreover, in general, loss of the H3K27me3 mark is associated with expression of differentiation marker genes and reduced cell proliferation/survival. Third, *Bmi-1* and other PcG proteins are expressed at higher levels in tumors, both SCC (Reinisch *et al.*, 2007; Balasubramanian *et al.*, 2010), basal cell carcinoma (Reinisch *et al.*, 2007) and melanoma (Bachmann *et al.*, 2006; Mihic-Probst *et al.*, 2007), although the *Bmi-1* finding is somewhat controversial for melanoma, as one report describes reduced expression with clinical progression in malignant melanoma (Bachmann *et al.*, 2008). Moreover, forced *Bmi-1* expression in HaCaT cells causes transformation (Wang *et al.*, 2009). This suggests that overexpression of these proteins may be one mechanism whereby tumor cells escape death through a mechanism that involves modulation of cell cycle and apoptotic processes

(Lee *et al.*, 2008; Balasubramanian *et al.*, 2010). Thus, although it is possible that *Bmi-1* could be restricted to maintaining survival of tumor stem cells, the ubiquitous distribution of *Bmi-1* in skin tumor cells suggests a broader role in skin cancer (Balasubramanian *et al.*, 2008). Fourth, PcG protein expression declines in aging epidermis, showing that loss of PcG protein expression is associated with keratinocyte senescence both *in vivo* and in cell culture models (Ressler *et al.*, 2006; Cordisco *et al.*, 2010). Fifth, *Bmi-1* and other PcG proteins are likely important targets for cancer prevention, as the level of these proteins is reduced in cells treated with chemopreventive agent and the action of chemopreventive agent is antagonized by overexpression of these proteins (Balasubramanian *et al.*, 2008, 2010).

CONFLICT OF INTEREST

The authors state no conflict of interest.

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