Collaboration between PcG Proteins and MLL Fusions in Leukemogenesis: An Emerging Paradigm

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PcG and TrxG proteins mostly with opposite transcriptional activities play key roles in normal and malignant development. In this issue of *Cancer Cell*, Tan et al. report an unexpected collaboration between CBX8 and MLL-AF9 in leukemia, revealing a far more complicated functional crosstalk between these master epigenetic regulators in oncogenesis.

Leukemia patients carrying chimeric fusion of the *mixed lineage leukemia* (*MLL*) gene remain one of the worst prognostic subgroups with poor remission and high relapse rates. MLL is the mammalian homolog of Trithorax, the founding member of trithorax group (TrxG) proteins, which function antagonistically with polycomb group (PcG) proteins to regulate expression of critical developmental genes such as *HOX* (Figure 1A). Consistently, *HOX* genes are aberrantly activated in MLL, and suppression of *HOXA9* partially compromises MLL fusion-mediated transformation (Cheung and So, 2011). Biochemical purifications of protein complexes associated with MLL fusions discovered critical epigenetic modifying enzymes including DOT1L (Okada et al., 2005) and PRMT1 (Cheung et al., 2007) that mediate methylation of specific residues on histones for transcriptional activation. Inhibition of these histone-modifying enzymes suppresses *HOX* expression and cellular transformation by MLL fusions, confirming the aberrant recruitment of transactivation complexes as a major attribute to MLL leukemogenesis. Unexpectedly, chromobox 8 (CBX8), a PcG protein, was also recurrently identified as an interacting protein with MLL fusion partners, AF9 and ENL, in spite of its putative function in transcriptional repression (García-Cuéllar et al., 2001; Monroe et al., 2010). The significance of this interaction and its role in MLL leukemogenesis are largely unknown.

PcG Protein, CBX8, As an Essential Component and Potential Target for MLL Leukemia

In this issue of *Cancer Cell*, Tan et al. (2011) have performed extensive biochemical and cell biology studies to characterize the role of CBX8 in MLL leukemia. First, they identified two highly-specific AF9 point mutants (T542A and T554A) that preferentially lost their interaction with CBX8 but not with DOT1L, AF5q31, or p-TEFb complex. Interestingly, these MLL-AF9 mutants failed to transform primary hematopoietic cells in spite of maintaining their interaction with DOT1L and p-TEFb complex. To further demonstrate the specific requirement of CBX8 in MLL leukemia, mouse bone marrow cells carrying *Cbx8* floxed/conditional alleles were employed together with wild-type MLL-AF9 in the transformation assays. MLL-AF9 was capable of transforming *Cbx8* floxed cells and induced leukemia in mice. However, an acute homozygous deletion of *Cbx8* in the first or third round of plating almost completely abolished the transformation ability and leukemogenic potential by MLL-AF9 but not E2A-HLF, confirming a specific functional requirement of CBX8 for MLL leukemogenesis.

To gain further insights into the molecular functions of CBX8 in MLL leukemia, the authors demonstrated that shRNA mediated suppression of CBX8 but not other core components of polycomb repressive complex 1 (PRC1), such as RING1b and BMI1, significantly reduced the expression of *HOXA9* and compromised MLL-AF9 transformation ability. These data suggest that the CBX8 requirement may not be directly related to the classical PRC1 function, but rather its ability to help maintain expression of MLL-AF9 downstream targets. In spite of its well-characterized repression property, CBX8 is also known to interact with TIP60, which possesses histone-acetyltransferase activity, which may explain the transactivation function of Cbx8. Consistently, suppression of TIP60 expression by shRNAs inhibited expression of *HOXA9*- and MLL-AF9-mediated transformation in vitro, suggesting a functional link between CBX8 and TIP60 in MLL leukemia. Finally, the authors further showed that CBX8 was not required for development of normal murine hematopoietic stem cells, highlighting it as a potential therapeutic target.

It is interesting to note that although CBX8, in contrast to DOT1L and PRMT1, does not contain rigid enzymatic structures as ideal docking-sites for small molecule inhibitors, a recent study shows that inhibitors can be developed to successfully target acetyl-lysine recognizing domain in the BET family for suppression of MLL leukemia (Dawson et al., 2011). Thus, the chromodomain of Cbx8 may also be a traceable target for inhibitor development if it is, indeed, required for MLL leukemia. On the other hand, the well-structured aceyltransferase domain in TIP60 could also be a good target, although its role in MLL leukemic transformation is far less clear and its functional involvement in multiple essential cellular processes also dampers the enthusiasm.

An Emerging Paradigm for Collaboration between PcG Proteins and MLL Fusions in Leukemia

In addition to the potential therapeutic implication, this study also highlights an interesting biological question and recent challenge in understanding the functional relationship between PcG and TrxG proteins in cancer. In solid tumors, TrxG proteins such as SNF5, BRM, and BRG1

may act as tumor suppressors to counteract the oncogenic functions of PcG proteins, such as EZH2 and BMI1, which are frequently activated in various cancers (Figure 1B). However, a recent study has shown that BMI1 collaborates with MLL fusion for the development of leukemic stem cells (Smith et al., 2011). BMI1, as a major gatekeeper for cellular senescence, is required for transformation mediated by a number of leukemia associated transcription factors. Nonetheless, MLL fusion-mediated activation of *Hox* expression can compensate for the lack of *Bmi1*, in part by suppressing *Ink4a/Arf* expression. Consistently, forced expression of *Hoxa9* can also suppress cellular senescence induced by BMI1-dependent leukemia-associated transcription factors such as AML1-ETO and PLZF-RARα in the absence of *Bmi1* (Smith et al., 2011). In contrast to the classical antagonistic relationship, BMI1 and MLL/HOX may actually cooperate to suppress the major checkpoint guarded by *Cdkn2a/2b* loci to promote oncogenic transformation (Figure 1C). The current study by Tan et al. (2011) further reinforces this idea by making a direct biochemical link between PcG and MLL fusion proteins where CBX8 forms a part of the MLL fusion transcriptional complex to help activate expression of downstream targets such as *HOX* genes (Figure 1C). In contrast to BMI1, the CBX8-mediated transactivation of *HOX* by MLL fusions seems to be independent of the normal PRC1 functions, because specific knockdown of other PRC1 components did not have any significant effect on *HOX* gene expression or transformation by MLL-AF9. This suggests the presence of multiple intersecting points between MLL fusions and different members of PcG proteins in mediating acute leukemogenesis. So far, these two studies focus on components of PRC1. It is very possible that members of PRC2 such as EZH2, which is mutated in myeloid malignancies (Ernst et al., 2010; Nikoloski et al., 2010), may also be interacting with TrxG proteins for leukemogenesis. Another major outstanding question is: what are the factors that determine if PcG proteins should cooperate or antagonize with TrxG proteins for oncogenic transformation (Figure 1)? It is clear that tissue specific transcription factors and interacting cofactors, which respectively dictate the DNA binding specificity and composition of the transcriptional complexes associated with PcG/TrxG proteins, can play a key role in determining the nature and outcome of their functional interactions. On the other hand, TrxG proteins consist of a diverse group of proteins with different molecular functions. In contrast to MLL protein with histone-modification property, TrxG proteins such as SNF5 directly associated with nucleosome-remodelling activity act in an antagonistic way against the oncogenic function of PRC in solid tumors (Wilson et al., 2010). However, this has not been investigated in leukemia, which has a totally different set of tissue specific transcription factors and interactomes. Thus future studies examining the functional interaction between different classes of TrxG and PcG proteins will give unique insights into this important issue. Nevertheless, these recent studies reveal a far more complicated crosstalk between PcG and TrxG proteins in oncogenesis, and functional cooperation between these proteins emerges as a new theme in leukemogenesis.

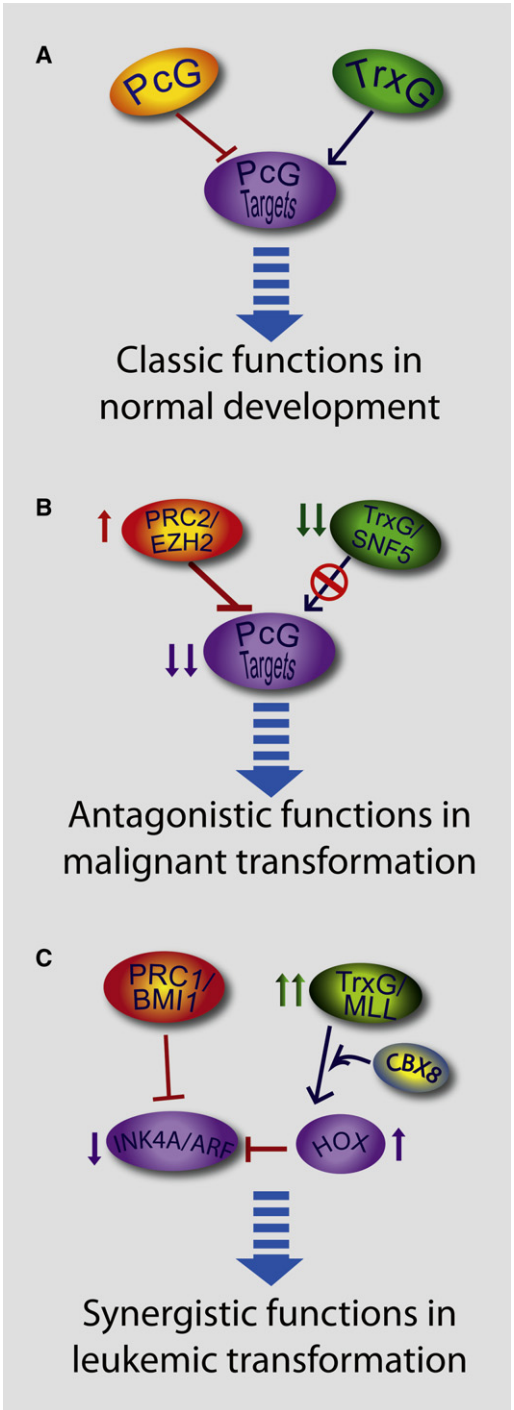

Figure 1. Functional Crosstalk between PcG and TrxG Proteins in Normal and Malignant Development

(A) PcG and TrxG proteins with opposite transcriptional activities coregulate the expression of PcG targets to maintain transcription programs for normal development.

(B) Chromatin remodelling TrxG proteins such as SNF5 function as tumor suppressors against PcG/EZH2 proteins by activating the expression of PcG targets.

(C) Histone modifying TrxG proteins such as MLL may collaborate with PcG proteins to (1) activate *HOX* gene expression and (2) suppress expression of *INK4A/ARF* loci for leukemogenic transformation.

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Platelets Alter Tumor Cell Attributes to Propel Metastasis: Programming in Transit

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Metastasis of epithelial tumors critically depends on acquisition of a disseminating phenotype that allows tumor cells to colonize distant organs. In this issue of *Cancer Cell*, Labelle et al. demonstrate that an epithelial-mesenchymal-like transition can be induced by interaction between platelets and tumor cells.

Host cell types provide cues that regulate tumor metastasis, essentially at all stages of tumor progression. Cells disseminating from primary epithelial tumors use the bloodstream and lymphatics to colonize distant sites. The impact of tumor-host interactions encountered within the bloodstream has been explored far less than that occurring in primary tumors and distant lesions. There are primarily three reasons: microenvironments within the vasculature are difficult to access, hard to define, and transient in nature. While cancer patients often present with conditions indicating activation of platelets and the coagulation system, the role of platelets in cancer dissemination is only partially understood (Erpenbeck and Schön, 2010; Gay and Felding-Habermann, 2011). In this issue of *Cancer Cell*, Labelle et al. (2011) demonstrate a novel role for platelets that profoundly impacts the ability of blood borne tumor cells to seed distant metastases. Direct platelet signaling to tumor cells leads to enhanced metastasis through platelet release of transforming growth factor β (TGF-β) which induces epithelial-mesenchymal-like transition in tumor cells and is critically enhanced through direct tumor cell-platelet contact.

Platelets are key contributors to hemostasis, leukocyte trafficking during inflammation, and maintenance of vessel stability. A hallmark of platelet function is the prevention of hemorrhage and perpetuation of coagulation to form and stabilize blood clots. Platelets are implicated in supporting metastasis through coherence with tumor cells, formation of heteroaggregates that also include leukocytes (Läubli et al., 2006), and proteins of the coagulation system that provide a transient microenvironment, which supports tumor cell survival and protection from immune elimination (Palumbo et al., 2005).

As the links between platelets, coagulation, and tumor metastasis coalesce, platelet-specific factors and recipient signaling mechanisms on tumor cells, important for malignancy, are still being resolved in mouse models. Labelle et al. (2011) now identify specific platelet factor and signaling pathways evoked in tumor cells that critically support metastasis. By conditioning tumor cells with platelets in vitro, the authors elicit colon and breast carcinoma cells to become more invasive and mesenchymal-like and ultimately more aggressive in an experimental lung metastasis model. This enhanced metastasis shows that the initial exposure to platelets can reprogram tumor cells. Thus, these results extend beyond documented contributions of platelets to tumor cell arrest, survival, and immune evasion en route to metastasis (Figure 1). Signaling factors released from platelet granules could directly affect tumor cell survival, proliferation, or invasiveness during metastasis. In a definitive and beautifully-executed experiment, Labelle et al. (2011) knockout expression of TGF-β1 specifically in megakaryocytes, and hence in platelets, by generating a TGF-β1 floxed/platelet factor 4 cre mouse model (*Pf4-cre*$^{+}$*; TGFβ1*$^{fl/fl}$). The dramatically diminished metastasis seen in mice deficient in platelet TGF-β1 suggests that tumor cell behavior is altered as a result of platelet activation and release of alpha granules. A lack of TGF-β1 in platelets also delayed tumor cell extravasation in