PERSPECTIVE

A complex Polycomb issue: the two faces of EZH2 in cancer

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In the April 1, 2012, issue of *Genes & Development*, Simon and colleagues (pp. 651–656) demonstrated that the disruption of *Ezh2* in mice is sufficient to cause T-acute lymphoblastic leukemia (T-ALL). Moreover, in concert with concurrent studies, the authors revealed that similar mechanisms are involved in human T-ALL. These data contrast with previous findings showing that increased EZH2 activity promotes cancer.

Polycomb genes were originally discovered in *Drosophila* as a result of their essential role in establishing orderly body segmentation by silencing homeobox genes. Now, Polycomb group proteins are recognized as key components in repressive multiprotein complexes that modify chromatin to silence large numbers of genes in all higher eukaryotic cells (for review, see Simon and Kingston 2009; Margueron and Reinberg 2011). In mammalian cells, two main Polycomb-repressive complexes (PRCs) have been defined: PRC1 and PRC2. Both PRCs repress hundreds of genes in embryonic stem (ES) cells, including a plethora of developmentally important transcriptional regulators (Boyer et al. 2006). The majority of target genes in ES cells are jointly occupied and repressed by PRC1 and PRC2, in part because PRC2 has the capacity to generate a histone modification, trimethyl histone 3 Lys 27 (H3K27me3), that can serve to recruit PRC1 by binding H3K27me3 via a chromodomain in a polycomb group CBX family member protein. However, a substantial proportion of PRC2 targets are not occupied by PRC1 (Ku et al. 2008), and PRC1 also has the capacity to bind genes independently of PRC2 (Boyer et al. 2006). Moreover, even at promoters jointly bound by PRC1 and PRC2, they appear to repress genes concurrently, but independently (Leeb et al. 2010). The notion of independent actions of PRC1 and PRC2 may be particularly important for understanding their functions beyond development; for example, in early hematopoiesis, gene targeting experiments suggest that PRC1 and PRC2 functionally oppose each other. Specifically, loss of Bmi1 (PRC1) severely compromises stem cell function, while heterozygous loss of several PRC2 components was reported to enhance stem and progenitor cell activity (Lessard et al. 1999; Lessard and Sauvageau 2003; Majewski et al. 2010).

The mechanisms by which Polycomb complexes repress target genes are incompletely understood, but involve covalent modifications of histone tails catalyzed by PRC1 (histone 2A Lys 119 ubiquitination [H2AK119ub1]) and PRC2 (H3K27me2,me3), as well as direct chromatin compaction (Simon and Kingston 2009; Margueron and Reinberg 2011). As an example of the latter, the PRC1 core constituent Ring1b retains the capacity to compact chromatin and repress genes even after mutation of its catalytic domain. Efficient H3K27 methylation requires the cooperation of several PRC2 core components, including Ezh2, the enzyme that catalyzes the reaction via its Set domain, and cofactors Suz12 and Eed. Knockout of either of these genes in ES cells results in severe global reduction of H3K27me3. Jarid2, a more recently identified PRC2-associated molecule, is important for recruiting PRC2 to promoters but is not required for maintaining global H3K27 methylation, although it has a minor but definite role in maintaining this mark at selected promoters (Shen et al. 2009).

All core components of PRC2 are strictly required for embryonic development, and their loss is usually lethal at the stage of gastrulation, when the basic body architecture is established (Simon and Kingston 2009; Margueron and Reinberg 2011). In contrast, mice remain viable and show no acute morbidity after the loss of Ezh2 in hematopoietic stem cells (Mochizuki-Kashio et al. 2011). Likewise, loss of Ezh2 in the skin does not result in major abnormalities (Ezhkova et al. 2011). In both cases, the absence of Ezh2 appears to be partially compensated by its homolog, Ezh1 (Ezhkova et al. 2011; Mochizuki-Kashio et al. 2011). Notably, however, there is a more stringent requirement for Ezh2 activity at distinct bottlenecks of differentiation, as loss of Ezh2 is associated with blocks in early B-cell and T-cell development (Su et al. 2005) that are ostensibly not compensated by Ezh1.

High noon: cancer genetics meets epigenetics at H3K27

Over the last decade, many independent studies established that EZH2 is overexpressed in several very com-

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mon solid tumor types, and high expression is associated with aggressive cases and progression (Varambally et al. 2002; Bracken et al. 2003), consistent with a straightforward role of EZH2 as an oncogene. In fact, disruption of PRC2/EZH2 either pharmacologically or genetically was shown to interfere with the growth of cancer cells expressing high levels of EZH2 in human cancer cell lines (Tan et al. 2007; Wilson et al. 2010) and in mice (Wilson et al. 2010). However, mutations implicating PRC2 or its mark, H3K27me3, in cancer were unknown until 3 years ago. In 2009, Van Haaften et al. (2009) discovered the presence of inactivating somatic mutations of UTX, an H3K27 demethylase (Agger et al. 2009), in many types of human cancer, including multiple myeloma (10%), esophageal cancer (8%), and renal cancer (1.4%). Furthermore, the investigators demonstrated that reintroduction of UTX into cancer cells decreased H3K27me3 at Polycomb target genes and reduced cell proliferation (van Haaften et al. 2009). The idea that lack of UTX functionally resembles excess of EZH2 in dysregulating H3K27me3 in cancer was subsequently corroborated by more in-depth studies (Herz et al. 2010; Wang et al. 2010). Very recently, UTX mutations were also found in 20% of transitional cell bladder cancers (Gui et al. 2011). In keeping with these findings, it was shown that the only other H3K27 demethylase, JMDJ3 (aka KDM6B), is also expressed at a lower than expected level in cancer (Agger et al. 2009; Barradas et al. 2009).

Directly implicating a core PRC2 component in cancer, Morin et al. (2010) discovered recurrent Tyr 641 mutations in the catalytic EZH2 Set domain in two types of lymphomas that arise from germinal center B cells (germinal center-type diffuse large B-cell lymphoma [GCB-DLBCL]: 21.7%; follicular lymphoma [FL]: 7.2%). Initially, these mutations were thought to impair the overall enzymatic activity of EZH2 (Morin et al. 2010), but later on they were shown to modulate substrate specificity and increase H3K27me3 in a coordinated interplay with the remaining wild-type *EZH2* allele (Sneeringer et al. 2010). A similar mutation in the SET domain of *Drosophila* PRC2 was also shown to increase H3K27me3 and cause inappropriate Polycomb target gene silencing (Stepanik and Harte 2012).

All of these initial results fit the simple assumption that increased or unchecked PRC2 activity hypermethylates H3K27, repressing the expression of tumor suppressor genes. The picture became more complicated as the genetic integrity of PRC2 and the H3K27 demethylases were scrutinized in malignant myeloid diseases (i.e., disorders of hematopoietic stem cells or progenitors of red blood cells, granulocytes, and platelets, such as myelodysplastic syndromes [MDSs], myeloproliferative disorder [MPD], and acute myeloid leukemia [AML]) (for review, see McDevitt 2012). Three large independent studies revealed that homozygous and heterozygous *EZH2* deletions or inactivating mutations are common in MDSs (~6%) and certain subtypes of MPD (up to ~12%) (Ernst et al. 2010; Makishima et al. 2010; Nikoloski et al. 2010). It has since been shown that other PRC2 components (*SUZ12*, *EED*, and *JARID2*) are also subject to mutations and deletions in MDSs, although at lower frequencies (Score et al. 2012). Moreover, inactivating mutations of *EZH2* in MDSs and myelofibrosis (a subtype of MPD) predict poor survival (Bejar et al. 2011; Guglielmelli et al. 2011). Collectively, these analyses demonstrate that loss rather than overexpression of PRC2 contributes to oncogenesis in the context of malignant myeloid diseases, presumably by the derepression of oncogenes due to insufficient H3K27 methylation.

Intriguingly, myeloid malignancies are also subject to inactivating mutations and deletions of *UTX* that should increase H3K27me3 (van Haaften et al. 2009; Jankowska et al. 2011). It remains to be resolved why loss-of-function mutations of both *EZH2* and *UTX* contribute to malignant pathogenesis in the same lineage despite an opposite presumed impact on H3K27me3. Pertinent to this issue, the spectrum of target genes of EZH2 and UTX is not identical. In fact, only 11% of the genes occupied by UTX also bear H3K27me3, the mark of EZH2 (Wang et al. 2010). Moreover, it is possible that UTX and JMJD3, the other H3K27 demethylase, act redundantly at some EZH2 targets but not others. Illustrating a distinct biology, UTX and JMJD3 have been shown to have different roles in regulating proliferation and cell cycle control (Agger et al. 2009; Barradas et al. 2009; Wang et al. 2010). Similarly, UTX mutations were not found in the type of lymphoma that harbor EZH2Tyr641 mutations (Morin et al. 2010). Thus, while loss of function of UTX or JMJD3 and gain of function of EZH2 are all expected to increase H3K27me3, the overall biological impact of these changes may be opposite in the context of some cells because distinct, large groups of target genes are affected. Alternatively, it is possible that demethylase-independent functions of UTX and JMJD3 contribute to their distinct biology (Miller et al. 2010). Regardless of the explanation, at present, the overall data suggest that the divergent expected impact of PRC2 and *UTX* mutations on H3K27me3 in myeloid malignancies is unusual. In most cases, the impact of oncogenic mutations on H3K27me3 levels appears to follow the same direction in a specific tissue or tumor type.

We now learn from the study of Simon et al. (2012) in the April 1, 2012, issue of *Genes & Development* that loss of *Ezh2* in HSCs is sufficient to cause aggressive T-acute lymphoblastic leukemia (T-ALL) in mice. After the conditional disruption of the Ezh2 gene in mouse hematopoietic stem cells, T-ALL occurred with almost complete penetrance within 3 mo to 1 yr (Simon et al. 2012). While the leukemic cells did express Ezh1, levels of H3K27me3 were markedly reduced in the leukemic cells, indicating compromised PRC2 activity (Simon et al. 2012). Mouse T-ALL often shares similar pathogenetic mechanisms with human T-ALL, including a dominant role of Notch signaling (Kindler et al. 2008; Aster et al. 2011). To clarify whether PRC2 is similarly involved in the pathogenesis of human T-ALL, the investigators analyzed a limited number of clinical T-ALL samples and provided evidence in favor of such a role, including a missense mutation in *JARID2* and two mutations in *DNMT3A*, which has been linked to PRC2 activity (Simon et al. 2012). The inves-

tigators also demonstrated decreased expression of EZH2 and increased expression of JMJD3 (aka KDM6B), which (like decreased EZH2) should decrease H3K27me3 (Agger et al. 2009). These clinical data were suggestive, but not entirely sufficient to close the case. Fortuitously, two other concurrent studies provided more extensive genetic evidence for an involvement of PRC2 in clinical T-ALL. Ntziachristos et al. (2012) examined 68 adult T-ALL samples and reported mutations in PRC2 core components in 25% of the cases (*EZH2*: 18%; *SUZ12*: 7%). Intriguingly, these investigators also showed in a murine model that Notch signaling, the major known pathway driving human T-ALL (Aster et al. 2011), involves diminishing PRC2 activity at target genes (Ntziachristos et al. 2012). In *Drosophila*, Polycomb-mediated repression is required upstream of Notch to suppress its tumorigenic activity, so it is tempting to speculate that Notch signaling and Polycomb silencing connect at multiple levels in T-ALL (Martinez et al. 2009). Finally, Zhang et al. (2012) revealed that the frequency of PRC2 alterations is even higher in a pediatric subtype of T-ALL, known as early T-cell precursor (ETP) ALL (42% combined *SUZ12*, *EZH2*, and *EED* mutations or deletions; $n = 55$), and also found mutations in non-ETP pediatric T-ALL, albeit at a lower frequency (Zhang et al. 2012). Together, these three studies establish PRC2 as major tumor suppressor in T-ALL.

Collectively, the genetic evidence implicating PRC2, and the demethylases antagonizing it, in a wide spectrum of malignancies strongly points to the pivotal importance of H3K27 in cancer. Moreover, very recently, this residue was found to be subject to very distinct, recurrent mutations in three types of aggressive pediatric brain tumors (Schwartzentruber et al. 2012; Wu et al. 2012). Remarkably, pediatric diffuse intrinsic pontine gliomas (DIPGs) featured mutations leading to lysine-to-methionine changes in residue 27 of the histone 3 tail in 78% of the cases (H3.1: 18%; H3.3: 60%) (Wu et al. 2012). Such mutations directly link H3K27 with human cancer, and likely a lot can be learned from studying how they promote cancer.

Manipulating the H3K27 cancer switch for therapy

The impressive, recent crescendo of genetic data implicating molecular machinery that methylates H3K27 in cancer begs for the development of strategies to manipulate it for clinical benefit. The system involves four enzymes that may eventually be amenable to targeting by small molecule drugs: the lysine methyltransferases EZH1 and EZH2, and the lysine demethylases UTX (KDM6A) and JMJD3 (KDM6B) (Copeland et al. 2009). Of these, EZH2 is the best-studied candidate therapeutic target. A small molecule, 3-deazaneplanocin A, that inhibits EZH2 by indirect mechanisms has been shown to interfere with the growth of many human tumor cell lines, but likely inhibits histone methylation very broadly (Tan et al. 2007). Notably, however, knockdown of EZH2 RNA in human malignant rhabdoid tumors inhibits tumor growth, and genetic disruption of *Ezh2* in mice prevented manifestation of tumors driven by *Snf5* loss (Wilson et al. 2010). Thus, there is solid support for the idea that inhibition of EZH2 is associated with anti-tumor activity in certain settings. In contrast, there are little data demonstrating anti-tumor activity resulting from inhibiting the other three enzymes affecting H3K27 methylation. However, efforts to explore the impact of interfering with each of these enzymes can be rationalized. Because of the redundant activity of EZH1 and EZH2 (Shen et al. 2009; Ezhkova et al. 2011; Mochizuki-Kashio et al. 2011), interference with both enzymes may prove synergistic. JMJD3 was found to be overexpressed in cases of Hodgkin's lymphoma and T-ALL and is associated with the loss of H3K27me3 at derepressed target genes that may contribute to pathogenesis (Anderton et al. 2011; Simon et al. 2012). Finally, depletion of UTX was recently reported to severely impair proliferation of human leukemia cell lines (Liu et al. 2012).

The clinical genetic data also point to dangers and challenges in developing agents aimed at modulating H3K27 methylation. The robust frequency of *EZH2* loss-of-function mutations in the myeloid and T-cell malignancies raises concerns that strategies aimed at decreasing H3K27 methylation may cause or promote cancer. Similar considerations apply to strategies aimed at increasing H3K27 methylation because *UTX* is subject to loss-of-function mutations in such a broad spectrum of cancers. It remains difficult, however, to gauge how valid these concerns are because we still lack an understanding of the precise pathogenetic impact of the recently discovered mutations. Surprisingly, there is a scarcity of data from mammalian model systems that might shed light on the role of PRC2 in cancer. In an early study, the Sauvageau group (Lessard et al. 1999) had reported that adult mice bearing a hypomorphic allele for the PRC2 component Eed developed severe lymphoid and myeloid disorders with aging. Moreover, such mice developed malignant lymphomas upon mutagenesis (Richie et al. 2002). However, until recently, there were no data suggesting that impeding PRC2 alone causes frank malignancy. Now, Simon et al. (2012) unequivocally demonstrate that disruption of *EZH2* is sufficient to cause T-ALL in mice, and three concurrent studies implicate PRC2 in human T-ALL (Ntziachristos et al. 2012; Simon et al. 2012; Zhang et al. 2012). Together, these studies represent an important landmark. It is now clear that PRC2 loss not only contributes to cancer development, but can be sufficient to set the stage for eventual full transformation.

Should concerns about the pro-oncogenic effects of EZH2 inhibition halt or slow down efforts to use it for developing novel therapeutic strategies? Au contraire. However, the potential for such adversity must be considered when moving forward. Small molecule inhibitors of EZH2 may have powerful beneficial effects without ever promoting malignancy in a clinical setting. In contrast to disruption in mice, pharmacologic inhibitors are unlikely to completely shut down activity and may not reach all target cells, and it may not be necessary to administer them in the long-term. Moreover, in the setting of very specific mutations, like the Tyr 641 mutations in lymphoma, it may be possible to develop agents with higher affinity for mutant isoforms, so normal EZH2 is

affected less. It is possible that pro-oncogenic effects will be observed only in the context of specific disease entities, and it is prudent to incorporate this possibility in the design of clinical studies that will hopefully emerge. Finally, it will be very important to push research on the H3K27 cancer switch in preclinical studies at all levels, from yeast to mice. While this cannot entirely predict what will happen clinically, it will help integrate the data that may emerge on this complex issue. This glass is far more than half full.

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