Epigenetic Therapy Leaps Ahead with Specific Targeting of EZH2

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The Polycomb epigenetic silencing protein EZH2 is affected by gain-of-function somatic mutations in B cell lymphomas. Two recent reports describe the development of highly selective EZH2 inhibitors and reveal mutant EZH2 as playing an essential role in maintaining lymphoma proliferation. EZH2 inhibitors are thus a promising new targeted therapy for lymphoma.

Aberrant patterning of epigenetic marks is a fundamental hallmark of human cancer. Studies exploring genome-wide distribution of DNA methylation and histone modifications consistently reveal profound perturbations in primary human tumor specimens (Baylin and Jones, 2011). Such findings have contributed to the increasing enthusiasm for ''epigenetic therapy'' to reprogram the epigenome of tumor cells. Two recent reports have extended this paradigm to an exciting new therapeutic target, EZH2.

EZH2 is the core enzymatic subunit of an epigenetic gene-silencing complex called polycomb repressive complex 2 (PRC2). EZH2 is a SET domain histone methyltransferase that preferentially catalyzes histone 3 lysine 27 (H3K27) methylation, a repressive mark that maintains epigenetic silencing of genes (Chase and Cross, 2011). EZH2 is active only when associated with other PRC2 core components EED, SUZ12, and RbAp48 (Chase and Cross, 2011). During lymphopoiesis, EZH2 is required for developing pre-B cells to acquire a full spectrum of immunoglobulin VDJ recombinants (Su et al., 2003). However, EZH2 expression reaches its peak when mature B cells are stimulated to form germinal centers (GCs) and undergo immunoglobulin affinity maturation (Velichutina et al., 2010). GC B cells are uniquely adapted to tolerate rapid proliferation and simultaneous genotoxic stress, which enables them to generate high-affinity antibodies. GC B cells give rise to the most common types of B cell lymphomas including diffuse large B cell lymphomas (DLBCLs) and follicular lymphomas (FLs).

Remarkably, 20% of DLBCLs and 10% of FLs display heterozygous somatic mutations of EZH2 involving Y641 or A677 (Morin et al., 2010). These mutations enable EZH2 to more efficiently add a third methyl group to H3K27 (Sneeringer et al., 2010). EZH2 mutant DLBCL cells exhibit increased abundance of H3K27me3 and reduction of H3K27me1 (Morin et al., 2010). The significance of this change is unknown, but presumably would facilitate more stable or potent repression of EZH2 target genes. Until now, it has been unclear whether the relatively subtle change in the stoichiometry of methylated H3K27 in EZH2 mutant DLBCLs would exert significant influence on the malignant phenotype. Regardless of the mutation status, a majority of DLBCLs feature high expression of EZH2, likely reflecting their GC origin (Velichutina et al., 2010). Moreover, genomics studies in primary human GC B cells showed that EZH2 represses numerous proliferation checkpoint genes, suggesting a role in facilitating proliferation (Velichutina et al., 2010). Hence, it is of great interest to address whether mutations in EZH2 contribute significantly to maintain the survival of lymphoma cells or just represent subtle ''tuning'' of an epigenetic silencing mechanism that is already present.

Some of these questions can now be addressed thanks to the development of highly selective EZH2 inhibitors, an achievement with important scientific and clinical implications. Using high-throughput screening for inhibitors of the PRC2 complex followed by medicinal chemistry optimization, two research groups generated low nanomolar potency small molecule EZH2 inhibitors (Knutson et al., 2012; McCabe et al., 2012). These small molecules displayed remarkable selectivity for EZH2 and showed similar efficacy against wild-type and mutant forms of EZH2. Notably, when applied to a large panel of B cell lymphoma cell lines, EZH2 inhibitors were most effective against DLBCLs, especially those with EZH2 point mutations. The compound induced apoptotic cell death in addition to proliferation arrest in the most sensitive cell lines. EZH2 mutant DLBCL cells are therefore exquisitely dependent on EZH2 to maintain their growth and survival. Nononcogene addiction to EZH2 may also occur in at least a subset of EZH2 wild-type DLBCLs, perhaps reflecting a potential biological role for EZH2 in normal GC B cells.

In an attempt to link the biologic actions of EZH2 inhibitor to gene expression and H3k27 methylation, a series of profiling experiments were performed (McCabe et al., 2012). The most EZH2-dependent cell lines showed predominant gene upregulation after exposure to GSK126, one of the EZH2 inhibitors, associated with a heavier pretreatment burden of H3K27me3 (McCabe et al., 2012). Transcriptional response was stronger in EZH2 mutant versus wild-type cell lines. However, the logic of these associations seems to break down when comparing differentially regulated genes among the most GSK126-responsive cell lines. It would not be unreasonable to expect EZH2 mutant cell lines to display a strong signature of GSK126-induced genes given their shared biological dependence on EZH2. Yet, only 35 genes overlapped in four out of the five cell lines profiled. Independent of experimental questions, such as whether heterogeneity is a product of cell line epigenetic diversification in vitro or whether gene expression

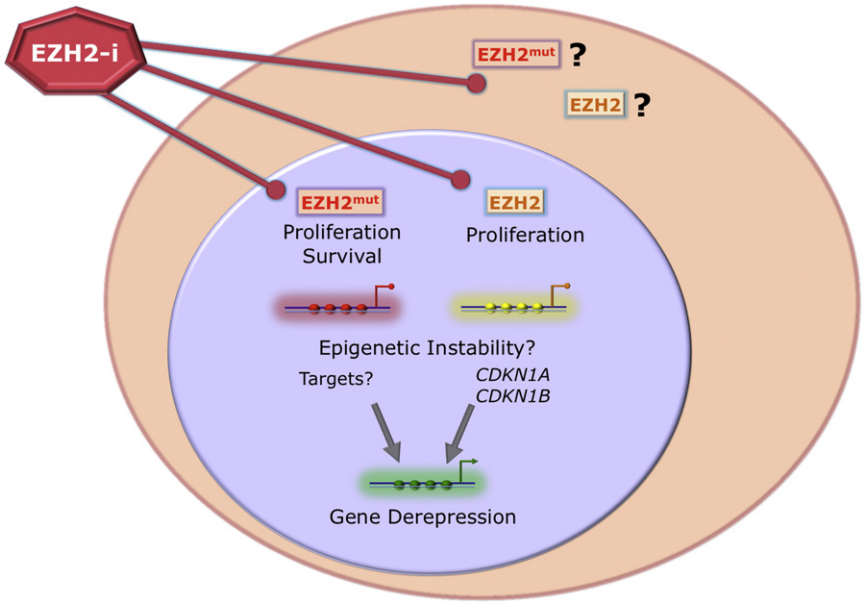

Figure 1. EZH2 Inhibitors Target Mutant and Wild-type EZH2 in DLBCL Cells with Equal Potency

EZH2 may form an active PRC2 complex in both nucleus and cytoplasm, although its cytoplasmic function in B cells is unknown. Mutant EZH2 mediates both survival and proliferation, whereas wild-type might be mostly linked to proliferation. Mutant EZH2 more strongly represses chromatin (red) than wild-type EZH2 (yellow) by inducing greater levels of H3K27me3. Wild-type EZH2 represses proliferation-associated genes such as *CDKN1A* and *CDKN1B*, whereas the target genes of mutant EZH2 may be different or more variable. Mutant EZH2, and perhaps wild-type EZH2 to a lesser extent, may contribute to epigenetic instability such as more heterogeneous distribution of H3K27me3. Newly-described EZH2 inhibitors (EZH-i) epigenetically reprogram DLBCL cells with mutant or wild-type EZH2 (green), leading to the derepression of target genes and perhaps alleviation of epigenetic instability manifested as proliferation arrest and cell death.

microarrays have the necessary dynamic range to robustly capture changes in low abundance transcripts, it is interesting to consider the implications of these results. For example, might it be possible that EZH2 mutation enables a state of "epigenetic instability" whereby H3K27 patterning evolves stochastically such that tumors in individual patients may silence different sets of genes, any combination of which might have similar effects in facilitating transformation? There is a precedent for such a notion in that proliferating GC B cells display a greater degree of cytosine methylation heterogeneity than resting B cells (Shaknovich et al., 2011). Likewise, it is conceivable that proliferation in GC B cells could also drive H3K27 methylation heterogeneity. Alternatively, it is possible that only a small number of genes contribute in a meaningful way to the oncogenic effects of mutant EZH2, in which case, the nonoverlapping genes may represent mostly noise. It is also intriguing to consider whether gene expression and/or H3K27 methylation changes might be a red herring. For example, PRC2 complex is also localized to cytoplasm, and in T cells, may regulate processes such as actin polymerization (Su et al., 2005). Might the effect of EZH2 inhibitors be related to nonepigenetic mechanisms? These provocative results challenge the field to perform functional assays and modeling to resolve these questions (Figure 1).

Regardless of these deeper mechanistic questions, the report by McCabe et al. (2012) has major translational implications. Even though EZH2 is an essential protein during development, mice tolerated the drug without apparent toxicity. Most importantly, GSK126 displayed an extremely powerful anti-lymphoma effect on human DLBCL cell line xenografts, with complete growth inhibition at lower doses and tumor eradication at higher doses of drug (McCabe et al., 2012). Tumor eradication is not easily achievable in DLBCL xenograft studies, raising the possibility that mutant EZH2 might play a role in self-renewal of putative lymphoma propagating cells. EZH2 is known to play a key role in the self-renewal of stem cells (Chase and Cross, 2011), so it is conceivable that, in addition to growth arrest and apoptosis, EZH2 inhibitors might promote the extinction of EZH2-dependent lymphomagenic clones. These data provide a compelling case for testing EZH2 inhibitors in clinical trials for patients with DLBCL harboring mutant EZH2 and/or high H3K27 methylation levels. Finally, in addition to DLBCL, EZH2 is also mutated in FL and is overexpressed in solid tumors including prostate and breast cancer (Chase and Cross, 2011). Therefore, the rational translation of EZH2 inhibitor therapy holds great promise toward improved efficacy and reduced toxicity for patients with these diseases.

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