Protein Methyltransferases as Targets for Personalized Cancer Therapeutics

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Background

A variety of molecular mechanisms work together to effect strict control of gene transcription in human cells. Paramount among these mechanisms is a collection of post-translational modifications of chromatin that facilitate conformational transitions between transcriptionally permissive and suppressive chromatin structures (Figure 1; refs. 1, 2). Each of these modifications is catalyzed by a specific set of enzymes (Figure 2). Not surprisingly, dysregulation of chromatin modification leads to pathologic alterations in gene transcription, hence to human disease. Among chromatin modifying enzymes, the protein methyltransferases (PMTs) stand out due to the large size of the class, and the disease association of many of these enzymes (3).

The PMTs have thus emerged as a novel target class for oncology indications where specific genetic alterations, affecting PMT activity, drive cancer tumorigenesis. This target class has proved quite druggable; small molecule inhibitors (PMTi) of several PMT enzymes have been identified that display a diversity of chemical structures and target binding modalities. Here we present two examples of PMTs that are genetically altered in specific human cancers and use these to illustrate the breadth of genetic alterations seen among the PMT targets that directly impact cancer pathogenesis.

Discussion

PMTs in Human Cancers

The PMT target class is composed of two distinct enzyme families: the protein arginine methyltransferases (PRMTs) and protein lysine methyltransferases (PKMTs) (1–3). The two families are distinguished not only by the amino acid side chain nitrogen atoms that are methylated, but also by the architecture of the enzyme active sites for the two families, both at the amino acid sequence level and at the three-dimensional tertiary structure level.

Among this putatively large target class are a number of enzymes for which genetic alterations have been identified in association with specific forms of human cancer, as summarized in Table 1. Two enzymes are presented below that exemplify the diversity of genetic alterations that may impact PMT activity in a pathogenic manner, leading to human cancers: DOT1L and EZH2.
TARGETING THE EPIGENOME TO TREAT CANCER

Fig. 1. Cartoon illustrating the conformational states of chromatin that impact gene transcription. The transition between these conformational states is effected, in part, through post-translational modifications of the histone proteins that make up the core protein structures of chromatin.

DOT1L

MLL-rearranged leukemia is a genetically distinct form of acute leukemia that affects people at all stages of life, from infancy to late adulthood; patients with MLL have a relatively poor prognosis and are not well treated by currently available therapies. A universal hallmark of this disease is a chromosomal translocation affecting the MLL gene on chromosome 11q23 (4). Normally, the MLL gene encodes for a PKMT that catalyzes the methylation of lysine 4 of histone H3 (H3K4) at specific gene loci (4). Gene localization is conferred by specific recognition elements within MLL, external to the catalytic domain (4). In the disease-linked translocations, the catalytic domain (SET domain) is lost and the remaining MLL protein is fused to a variety of partners, including members of the AF and ENL family of proteins such as Euchromatin - Transcriptionally Permissive

Heterochromatin - Transcriptionally Repressive

Fig. 2. (Left) Cartoon illustrating the various forms of covalent modification of the histone proteins and DNA of chromatin. (Right) pie chart illustrating the number of enzymes in the major classes of chromatin-modifying enzymes found in humans. Numbers in parentheses indicate the number of putative enzymes for each class. The data used to construct this pie chart was taken from (15). Abbreviations: HAT, histone acetyltransferase; HDAC, histone deacetylase; PKMT, protein lysine methyltransferase; PRMT, protein arginine methyltransferase; KDM, lysine demethylase; RDI, arginine deiminase; DNMT, DNA methyltransferase.
AF4, AF9, AF10 and ENL (4). Thus, the MLL gene translocation is a common feature of MLL-rearranged leukemia and is thought to play a causal role in this malignancy.

DOT1L catalyzes the methylation of histone H3 on lysine 79 (H3K79); H3K79 methylation is an activating mark with respect to gene transcription. DOT1L forms high-affinity complexes with the various MLL-fusion proteins via the fusion partner domains (i.e., AF4, AF9, AF10, ENL, etc.). As a consequence of the localization elements within the MLL portion of the fusion protein, the fusion-protein-DOT1L complex results in ectopic gene localization of the DOT1L enzyme, where it catalyzes H3K79 methylation and consequent gene activation. Armstrong and colleagues have shown that ectopic H3K79 methylation, resulting from MLL fusion protein recruitment of DOT1L, leads to enhanced expression of a number of leukemogenic genes, including HOXA9 and MEIS1 (5). Hence, while DOT1L is not genetically altered in the disease per se, its mis-located enzymatic activity is a direct consequence of the chromosomal translocation affecting MLL-rearranged leukemia patients; thus, DOT1L has been proposed to be a catalytic driver of leukemogenesis in this disease (6).

Daigle et al. (7) reported the first evidence that inhibition of a PMT could lead to selective cancer cell killing and resultant in vivo efficacy in a cancer animal model. They reported studies of EPZ004777, a potent (K, 300 pM) and selective (≥1200-fold over other PMTs) DOT1L inhibitor. The compound blocked H3K79 methylation in leukemia cells regardless of whether or not the cells harbored MLL gene translocations. However, despite the ability to affect intracellular H3K79 methylation in all cells, the compound was selectively cytotoxic for leukemia cells containing the MLL gene translocations, with minimal effects on non-translocated cells (Figure 3A). Daigle et al. (7) tested the effects of EPZ004777 in a highly
aggressive, disseminated mouse model of MLL-rearranged leukemia. The compound was administered by continuous i.v. administration at three concentrations of 50, 100 and 150 mg/mL for 14 days, and survival was monitored over the course of 30 days. At all doses administered, statistically significant survival advantages over the vehicle-dosed animals were achieved (Figure 3B). As illustrated in Figure 1B, the extent of survival increased with increasing dose of compound. These data represent the first in vivo evidence that selective PMT inhibition can lead to anti-tumor efficacy.

**EZH2**

EZH2 is the catalytic subunit of a multi-protein PKMT complex known as Polycomb Repressive Complex 2 (PRC2). PRC2 catalyzes the mono-, di- and tri-methylation of H3K27. Trimethylation of H3K27 is a transcriptionally repressive mark that silences affected genes. Elevated levels of H3K27me3 (hypertrimethylation) are often seen at tumor suppressor gene loci in a variety of human cancers (8). Several mechanisms for effecting H3K27 hypertrimethylation have been documented in different human cancers where they are thought to be tumorigenic. For example, amplication of PRC2 genes, including EZH2, is found in breast and other solid cancers. In myeloma, loss-of-function mutations have been identified in the histone demethylase UTX, which removes methyl groups from the H3K27 site. The loss of demethylase activity at H3K27 should result in a hypertrimethylated phenotype and consequent transcriptional repression of tumor suppressor genes, in an equivalent manner to elevation of the PRC2 methyltransferase. Yet another mechanism for elevating tumorigenic H3K27me3 is by over-expression of the auxiliary protein PHF19/PCL3, a protein that enhances the ability of PRC2 to trimethylate H3K27 (9).
Most recently, Sneeringer et al. (10) and subsequently Yap et al. (11) have presented an unusual molecular mechanism of H3K27 hypertrimethylation associated with a specific human cancer. Subsets of patients with non-Hodgkin lymphoma, had been found to be heterozygous for point mutations at tyrosine 641 within the catalytic domain of EZH2 (Y641F, Y641N, Y641S and Y641H); these patients were found to express both the wild type and mutant enzymes. While wild type EZH2 is most efficient at performing the first, mono-methylation reaction - and its catalytic efficiency wanes with each subsequent methylation reaction - all of the mutant enzymes display the exact opposite pattern of substrate utilization; the mutants are essentially unable to perform the first methylation reaction, but once presented with the mono-methylated H3K27 precursor, they are much more efficient than wild type enzyme in performing the di- and especially tri-methylation reactions. Hence, the hypertrimethylated H3K27 phenotype, associated with patient heterozygosity for mutant EZH2, is the result of a uniquely pathogenic coupling of enzymatic activity between the wild type and mutant enzymes: the wild type enzyme performs the first methylation reaction and the mutants then accelerate the subsequent transformations to the tumorigenic H3K27me3 species.

Future Directions

The field of cancer therapeutics has undergone a continuous evolution of focus from early days of indiscriminant cytotoxics, such as mustard gas derivatives, to molecularly targeted agents, such as the various kinase inhibitors in clinical use today. Thus today the concept of personally-targeted therapeutics resonates well in this era of advanced genetic, proteomic, biochemical and biological understanding of cancer cells.

Increasingly, oncologists are moving away from descriptions of patient’s disease based solely on tissue of origin or site of lesion (e.g., breast cancer, prostate cancer, and the like), and moving instead towards more genetic descriptors based on increased understanding of the alterations that drive individual cancers. Contemporary examples of how this sea-change is affecting therapeutic strategies include the development of vemurafenib to specifically treat melanoma patients carrying the B-Raf V600E mutation (12, 13) and of crizotinib to specifically treat lung cancer patients harboring a chromosomal translocation of the protein kinase ALK (14). As described here, the PMTs seem exquisitely well poised to become the next wave of clinical targets for personally-targeted therapeutics in cancer.

In this article we have introduced the PMTs as a target class for cancer drug discovery. Specific genetic alterations affecting PMT function are known in certain human cancers, where they appear to be drivers of tumorigenesis. Hence, targeting of these specific PMTs is likely to be a cogent approach towards personalized cancer therapeutics. We discussed as examples of this approach, the genetic alterations affecting DOT1L in MLL-rearranged leukemia and EZH2 in non-Hodgkin lymphoma. As efforts towards more complete cancer genomic analysis continue, additional examples of genetic alterations in PMT function are likely to emerge. These enzymes may thus serve as important targets of therapeutic intervention for specific cancers in which the genetic alteration drives tumorigenesis.
References


