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Molecular Pathways: Protein Methyltransferases in Cancer

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Abstract

The protein methyltransferases (PMT) constitute a large and important class of enzymes that catalyze site-specific methylation of lysine or arginine residues on histones and other proteins. Site-specific histone methylation is a critical component of chromatin regulation of gene transcription—a pathway that is often genetically altered in human cancers. Oncogenic alterations (e.g., mutations, chromosomal translocations, and others) of PMTs, or of associated proteins, have been found to confer unique dependencies of cancer cells on the activity of specific PMTs. Examples of potent, selective small-molecule inhibitors of specific PMTs are reviewed that have been shown to kill cancers cells bearing such oncogenic alterations, while having minimal effect on proliferation of nonaltered cells. Selective inhibitors of the PMTs, DOT1L and EZH2, have entered phase I clinical studies and additional examples of selective PMT inhibitors are likely to enter the clinic soon. The current state of efforts toward clinical testing of selective PMT inhibitors as personalized cancer therapeutics is reviewed here. Clin Cancer Res; 19(23); 6344–50. ©2013 AACR.

Background

Posttranslational modifications of histone proteins play a critical role in defining the local structure of chromatin (the complex of DNA and histone proteins that make up chromosomes) and thereby control entire programs of gene transcription within cells (1). Both reversible small-molecule (e.g., acetylation, methylation, and phosphorylation) and protein (e.g., ubiquitination and sumoylation) modifications of histone are known to affect nucleosome compacting within chromatin and also to serve as recognition loci for binding of transcription factors, polymerases, and auxiliary proteins that either facilitate or antagonize gene transcription (2). Among these various histone modifications (Fig. 1A), methylation of lysine and arginine residues seems to play a particularly important role in control of gene transcription programs (3–5). These modifications are catalyzed by a class of group-transfer enzymes known as the protein methyltransferases (PMT; refs. 3, 6). The PMT enzyme class is composed of two distinct families of enzymes, based on their active site structures and on the amino acid to which they transfer methyl groups: the Protein lysine methyltransferases (PKMT) and the Protein arginine methyltransferases (PRMT; ref. 6). There is one exception to this general structural bifurcation of the PMT class. The enzyme DOT1L is biochemically a lysine methyltransferase, but its active site structure is most closely aligned with that of the protein arginine methyltransferases (6). On the basis of active site structure, a number of enzymes that had been previously annotated as RNA methyltransferases can also be included within the family of PRMTs, and some of these enzymes have subsequently been shown biochemically to catalyze both RNA and protein methylation (6, 7). Thus, the PMT class is composed of 96 enzymes within humans. Reaction fidelity is variable among the PMTs. In some cases, a single enzyme is responsible for site-specific methylation of a unique histone location (e.g., DOT1L methylation of H3K79; see below). In other cases, several PMTs seem to methylate a common histone site but may do so in a gene-specific manner (Fig. 1B). In yet other cases, a single enzyme may methylate multiple protein substrates.

The PMTs constitute an interesting target class for cancer therapeutics both because of the critical role these enzymes play in controlling cellular programs of gene transcription and because, increasingly, bioinformatic surveillance of cancer genome databases are showing that a number of these enzymes are implicated in specific human cancers (5, 8); in a recent review, for example, Copeland listed over 10 PMTs for which genetic alterations are found in specific human cancers (8).

PMTs may play critical roles in cancer cells in general, but their greatest value as therapeutic targets may be in cases where the oncogenic alterations of PMTs (or of other proteins that impact PMT activity; see below) associated with specific human cancers have been shown to confer to cancer cells a unique dependency on the enzymatic activity of the altered PMT for proliferation and/or survival (i.e., an addiction to PMT activity). Hence, a reasonable working hypothesis is that selective inhibition of the affected PMT would result in cell killing of cancer...
cells bearing the genetic alteration with minimal affect on nonaltered cells. This would be expected to provide a significant therapeutic index in vivo, with potent elimination of affected tumor cells and limited attendant safety concerns. Preclinical animal studies of potent, selective inhibitors of two PMTs, DOT1L (9) and EZH2 (ref. 10; see below) have borne out these expectations and have paved the way to clinical testing of PMT inhibitors in patients with cancer.

**Active site structure and enzymatic mechanism of methyl transfer**

All PMTs use S-adenosyl methionine as a universal methyl donor in much the same way that all kinases use S-adenosyl homocysteine as a universal phosphate donor.
ATP as a universal phosphate donor (Fig. 2). The crystal structures of a number of PMTs have been solved to atomic resolution and reveal a well-organized S-adenosyl methionine–binding pocket, which is conformationally distinct between the lysine and arginine methyltransferases (3, 6, 11). In both cases, however, the S-adenosyl methionine–binding pocket is juxtaposed to a long channel into which the methyl-accepting amino acid side chain binds. This arrangement allows for close proximity and molecular orbital alignment between S-adenosyl methionine and the nitrogen of lysine or arginine for facile methyl transfer. All of these enzymes transfer the methyl group directly from S-adenosyl methionine to the acceptor amino acid, so that the enzymatic reaction requires the simultaneous binding of both substrates for transfer to occur (3). These features of the enzymatic reaction of PMTs afford multiple opportunities for inhibitor interactions with the enzymes. Indeed, small-molecule PMT inhibitors of various binding modalities have been reported: S-adenosyl methionine competitive, methyl acceptor competitive, noncompetitive, etc. (5, 8). This diversity of inhibitor-binding modalities distinguishes the PMTs from other group-transfer enzymes that have been targeted for cancer therapies (e.g., the kinases) and may allow greater flexibility in choosing inhibitors that offer the greatest chance for clinical translation (12).

**Role of PMTs in regulation of gene transcription**

How site-specific methylation of histone lysines and arginines controls gene transcription is incompletely understood at present, but it is clear that methylation at specific histone sites affects downstream transcription in different ways. For example, methylation of histone H3 on lysines 4 (H3K4) and 79 (H3K79) has been implicated in the transcriptional activation of genes. In contrast, methylation at other locations, such as H3K9 and H3K27, are involved in repression of gene transcription (13). These opposing effects of methylation of different histone sites can play a critical role in cancer tumorigenesis when alterations in the location and amplitude of specific site methylation leads to aberrant activation of oncogene transcription and/or transcriptional repression of tumor suppressor genes. It is also becoming clear that the effect of site-specific methylation of histone sites on transcription cannot be understood in isolation, but depends on the context of other modifications of proximal histone sites (14). Finally, emerging data suggest that methylation of one histone site may enhance or antagonize methylation of other histone sites. For example, data in multiple myeloma cells suggest that elevated methylation of H3K36 antagonizes methylation of H3K27, and likewise diminution of H3K36 methylation may result in elevation of H3K27 methylation levels (15).

**Genetic alterations of PMT activity as drivers of cancer**

A variety of genetic alterations are seen in members of the PMT enzyme class associated with specific human cancers. Ongoing surveillance of cancer genome databases is continuing to reveal additional examples of amplifications and mutations within PMTs and within PMT-associated proteins that seem to be specific to...
individual cancer types. Over the past decade, several examples have emerged of alterations of PMT activity that seem to be critical drivers of genetically defined cancers (16). Some of these will be described here to exemplify the diversity of diver alterations observed to impinge on this enzyme class.

**Indirect chromosomal translocation leading to ectopic PMT activity.** MML-rearranged leukemia is a devastating form of acute leukemia that affects patients from infancy through adulthood and is also prevalent among secondary leukemia associated with the use of etoposide (and other topoisomerase II inhibitors; ref. 17). This disease is universally associated with a chromosomal translocation (11q23) affecting the MLL gene that encodes for the PMT MLL, which normally catalyzes the methylation of the H3K4 residue (18, 19). As a result of the translocation, the MLL protein loses its enzymatic active site (hence, PMT activity) and is fused to any of a number of proteins within the AF or ENL protein families (20, 21). Gene localization is conferred by recognition elements within the N-terminal region of the MLL protein, and these are retained within the various fusion proteins. Hence, the MLL fusion proteins localize to the same gene locations as wild-type MLL, but lack the ability to catalyze histone methylation at H3K4. It has been recognized for some time that this chromosomal translocation plays a causal role in MML-rearranged leukemia, but the molecular mechanism of pathogenesis was not clear. Early attention focused on the loss of PMT activity associated with the fusion proteins that results from the translocation, but this was hard to reconcile with the residual H3K4 methylation activity of the wild-type MLL protein resulting from the second, unaffected allele. In 2005, Okada and colleagues (22) showed that another enzyme, DOT1L, binds to AF10, one of the various fusion partner proteins associated with the MLL translocation, and thereby recruits DOT1L to ectopic gene locations at which the MLL fusion proteins reside. Subsequent studies revealed that other AF and ENL fusion partners were also able to directly, or indirectly recruit DOT1L to MLL fusion–bound genes (23–25). The resulting ectopic localization of DOT1L catalyzes H3K79 methylation of genes otherwise under control of the MLL-catalyzed H3K4 methylation, including proleukemogenic genes such as HOX9 and MEIS1 (22, 26, 27). Methylation of H3K79 is a transcriptional activation mark. Thus, the ectopic localization of DOT1L, due to the universal chromosomal translocation in MML-rearranged leukemia, results in elevated transcription of a program of leukemogenic genes that in turn drive this proliferative disease. It has been hypothesized that MML-rearranged leukemia is uniquely dependent on DOT1L enzymatic activity and that selective inhibition of DOT1L would lead to specific cell killing of MML-rearranged leukemia cells. This hypothesis was confirmed by gene silencing approaches (26) and by the use of potent and selective small-molecule inhibitors of DOT1L (see below; refs. 28–30).

**Direct chromosomal translocations involving PMTs.** WHSC1 (also referred to as MMSET or NSD2) is a PMT that catalyzes the dimethylation of H3K36 (31, 32). Methyltransferase activity of the wild-type MLL protein resulting from the fusion proteins that results from the translocation, but attention focused on the loss of PMT activity associated with the translocation, and thereby recruits DOT1L to ectopic

**Point mutations affecting PMT activity.** The most well-studied example of tumorigenic point mutations affecting PMT activity is that of mutations within the catalytic domain of EZH2 in germlinal center diffuse large B-cell lymphoma and follicular lymphoma subsets of non-Hodgkin lymphoma (NHL; 36–38). EZH2 (or the closely related EZH1) is the catalytic subunit of a multiprotein complex known as PRC2 (polycomb repressive complex 2) that is responsible for mono-, di-, and trimethylation of the H3K27 site. Trimethylated H3K27 is a translational repressive mark, and hypertrimethylation of H3K27 has always been found to lead to tumorigenesis in various human cancers, presumably due to the transcriptional repression of tumor suppressor genes. Mutations at Tyr641 or Ala677 have been found to occur heterozygously in NHL patient groups (36, 37) and have been shown to be change-of-function mutations with respect to the substrate specificity of the enzyme (38). The wild-type enzyme is most active in placing the third methyl group on. In the context of disease methyltransferase activity of the wild-type MLL protein resulting from the fusion proteins that results from the translocation, but attention focused on the loss of PMT activity associated with the translocation, and thereby recruits DOT1L to ectopic

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**Synthetic lethal associations with PMT activity.** In addition to histone methylation, gene expression is also regulated by remodeling of nucleosomes in an ATP-dependent manner. Of the ATP-dependent chromatin remodelers, SWI/SNF complexes are emerging as *bona fide* tumor suppressors, as specific inactivating mutations in several SWI/SNF subunits are found in human cancers (41). For instance, the INI1 (also known as SMARCB1 or hSNF5) subunit is inactivated in nearly all malignant rhabdoid tumors, aggressive cancers of young children with no effective therapy (42). Tumorigenesis in INI1-deficient malignant rhabdoid tumors can be completely suppressed by tissue specific codeletion of EZH2, suggesting an antagonistic relationship between PRC2 and SWI/SNF activities (43). Recently, Knutson and colleagues (44) have shown that potent, selective inhibitors of EZH2 are effective in eradicating INI1-deficient malignant rhabdoid tumors in cell culture and in a mouse xenograft model. Hence, although the tumorigenic driver of malignant rhabdoid tumors occurs in a pathway distinct from any PMT, it nevertheless creates a synthetic lethal relationship that confers a unique sensitivity to selective EZH2 inhibition in this disease.

**Clinical–Translational Advances**  

**Preclinical in vivo activity of selective PMT inhibitors**  

Selective inhibitors of several PMTs have been described, and compounds targeting two particular PMTs, DOT1L and EZH2, have been reported to show dramatic antitumor activity in rodent models of disease (9, 10, 40, 44). The DOT1L tool compound EPZ004777 was the first PMT inhibitor reported to show antitumor effects in a disseminated mouse model of MLL-rearranged leukemia (28). Subsequent pharmacologic optimization within this chemotype series resulted in the clinical candidate DOT1L inhibitor EPZ-5676, which was also shown to eradicate MLL-rearranged tumors (>100% tumor growth inhibition) in a rat xenograft model (9, 45). Two distinct EZH2 inhibitors have been shown to display significant, dose-dependent antitumor activity in mouse xenograft models of EZH2 mutant–bearing NHL tumors (60%–100% tumor growth inhibition in various tumor models) and of INI1-deficient malignant rhabdoid tumors (>100% tumor growth inhibition; refs. 10, 40, 44). In all of these studies, the compounds were well tolerated by the rodents at doses that resulted in significant tumor growth inhibition, with no acute toxicity or weight loss observed. These results provide a strong foundation for the clinical testing of DOT1L and EZH2 inhibitors in genetically defined patients with cancer and also portend the general use of PMT inhibitors as personalized cancer therapeutics. Also, in all of these studies, the investigators were able to show a correlative relationship between tumor growth inhibition and diminution of the relevant histone methyl mark in tumor tissue as well as in surrogate tissues such as skin and peripheral blood mononuclear cells. The ability to measure drug-induced loss of site-specific histone methylation in surrogate tissue by noninvasive means provides a cogent basis for pharmacodynamics assessment of target engagement in clinical trials.

**Clinical trials**  

At the time of this writing, two PMT inhibitors have entered phase I human clinical trials: the DOT1L inhibitor EPZ-5676 and the EZH2 inhibitor EPZ-6438. The primary goal of both of these trials is to assess the safety of the drugs and to establish the maximum tolerated dose for each.

The EPZ-5676 phase I trial is divided into two parts: a dose-escalation portion to establish maximum tolerated dose and an expansion cohort of patients treated at the maximum tolerated dose. To accelerate dose escalation, eligibility is not restricted to genetically defined patients; patients with hematologic malignancies (mainly acute myelogenous leukemia and acute lymphocytic leukemia) are eligible for this portion of the trial. Once the maximum tolerated dose has been established, the expansion cohort study will begin exclusively in patients with leukemia involving the MLL rearrangement at 11q23. In this manner, the drug may be tested as early as possible in the genetically defined patients for whom it was designed.

The EZH2 inhibitor EPZ-6438 is being studied in a phase I trial of relapsed or refractory malignancies that have failed all standard therapy. Again, the primary goal here is to establish the safety and define the maximum tolerated dose of the drug. As with the EPZ-5676 trial, a secondary objective is to test EPZ-6438 as early as possible in the genetically defined patients for whom the drug was designed. Hence, once the maximum tolerated dose has been established, a phase IIa trial is planned exclusively in NHL patients bearing mutations in EZH2.

No data have yet been reported for either of these trials. Nevertheless, these trials represent a true watershed in the development of PMT inhibitors as personalized cancer therapeutics. The cancer community will be looking forward with great interest toward the outcomes of these trials. It is clear that these trials represent the vanguard of a much larger array of PMT inhibitors that will be entering clinical trials in the near future for testing against genetically defined groups of patients with cancer.

**Disclosure of Potential Conflicts of Interest**  

R.A. Copeland has an ownership interest (including patents) in Epzyme Inc. and is a consultant/advisory board member of Mirana.

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