Molecular Pathways

Aberrations of EZH2 in Cancer

Andrew Chase and Nicholas C.P. Cross

Abstract

Control of gene expression is exerted at a number of different levels, one of which is the accessibility of genes and their controlling elements to the transcriptional machinery. Accessibility is dictated broadly by the degree of chromatin compaction, which is influenced in part by polycomb group proteins. EZH2, together with SUZ12 and EED, forms the polycomb repressive complex 2 (PRC2), which catalyzes trimethylation of histone H3 lysine 27 (H3K27me3). PRC2 may recruit other polycomb complexes, DNA methyltransferases, and histone deacetylases, resulting in additional transcriptional repressive marks and chromatin compaction at key developmental loci. Overexpression of EZH2 is a marker of advanced and metastatic disease in many solid tumors, including prostate and breast cancer. Mutation of EZH2 Y641 is described in lymphoma and results in enhanced activity, whereas inactivating mutations are seen in poor prognosis myeloid neoplasms. No histone demethylating agents are currently available for treatment of patients, but 3-deazaneplanocin (DZNep) reduces EZH2 levels and H3K27 methylation, resulting in reduced cell proliferation in breast and prostate cancer cells in vitro. Furthermore, synergistic effects are seen for combined treatment with DNA demethylating agents and histone deacetylation inhibitors, opening up the possibility of refined epigenetic treatments in the future. Clin Cancer Res; 17(9); 2613–8. ©2011 AACR.

Background

DNA is wrapped around histone complexes termed nucleosomes, and gene accessibility is determined largely by the local chromatin configuration. This configuration may impact on the ability of cofactors and enhancers to bind specific DNA sequences, either via direct interactions with nucleosome components or, more generally, by influencing the degree to which DNA and/or nucleosome complexes are compacted into higher structures and, thus, the physical accessibility of specific recognition sequences. Local chromatin structure is influenced, in part, by histone usage but principally by covalent modifications to histone tails and DNA methylation. Both Pcg and Trx proteins play important roles in this process by modifying lysine residues within histone tails, resulting in repression or enhancement of transcription.

Two histone modifications, in particular, play crucial opposing roles in the epigenetic control of proliferation and differentiation. Trimethylation of histone H3 lysine 27 (H3K27me3), catalyzed by the Pcg enhancer of zeste homolog 2 (EZH2), is associated with transcriptional repression, whereas H3K4 methylation, catalyzed by the trithorax homolog myeloid-lymphoid leukemia (MLL), is associated with transcriptional activation (Fig. 1A). Importantly, many genes involved in development, stem cell maintenance, and differentiation are targets of H3K27 and H3K4 methylation. In embryonic (1, 2) and hematopoietic (3) stem cells (HSC), promoters may carry both marks, termed a ‘bivalent’ domain, and genes thus marked are thought to be poised for transcriptional activation or repression with loss of H3K27 or H3K4 methylation, respectively.

H3K27 methylation is catalyzed by the SET domain of EZH2 and requires the presence of 2 additional proteins, embryonic ectoderm development (EED) and suppressor of zeste 12 (SUZ12). These proteins, together with the histone binding proteins retinoblastoma binding protein 4 (RBBP4) and RBBP7, comprise the core components of the polycomb repressive complex 2 (PRC2; Fig. 1B). Other proteins such as PHD finger protein 1 (PHF1), which specifically stimulates H3K27 trimethylation rather than dimethylation (4), sirtuin 1 (SIRT1; ref. 5), and jumonji, AT rich interactive domain 2 (Jarid2; refs. 6, 7) have also been described in PRC2 complexes and probably serve modulating functions. A second polycomb complex, PRC1, catalyzes ubiquitination of histone H2A K119, which is also associated with a repressive chromatin structure. PRC1 consists of several proteins including chromobox homolog 2 (CBX2) or related homologs, which bind H3K27me3, ring finger protein 1A (RING1A), or RING1B, which catalyze ubiquitination and BMI1 polycomb ring finger oncoprotein (BMI1) or polycomb group ring finger 6/Mel18 (PCGF2), which modulate ubiquitination activity.

According to the hierarchical model of PRC recruitment first developed through genetic studies in Drosophila (8),
Figure 1. A, PRC2 catalyzes H3K27me associated with transcriptional repression, whereas myeloid-lymphoid leukemia catalyzes methylation of H3K4 associated with transcriptional activation. In bivalent domains, both marks are present and associated with a low level of transcription; genes thus marked are thought to be poised for later transcriptional repression or activation. B, the PRC2 core components are EZH2, SUZ12, EED, and RBBP4 or RBBP7. C, PRC2 is recruited to DNA, resulting in H3K27me3. This mark facilitates recruitment of PRC1, which ubiquitinates H2AK119, DNMT, and HDACs, which contribute to chromatin compaction and transcriptional repression. D, Levels of EZH2 and H3K27me3 can be indirectly reduced by treatment with DZNep, resulting in transcriptional derepression.
PRC2 is first recruited to target genes and catalyzes methylation of H3K27. This step is followed by recruitment of PRC1 and ubiquitination of H2AK119 (Fig. 1C). This model would predict that targets of PRC1 and PRC2 largely overlap, as has indeed been shown by RNA interference (RNAi) depletion studies in human embryonic fibroblasts (9). In HSCs, however, there is evidence for opposing roles for PRC1 and PRC2 (10–13). BMI1 is required for HSC repopulation (10, 12), whereas reduced levels of Suz12, Ezh2, or Eed result in increased HSC-progenitor proliferation (13). In addition, genes known to be repressed by Ezh2, or Eed result in increased HSC-progenitor proliferation (13). It is currently not known whether the differential effects of PRC1 and PRC2 seen in HSC also occur in other tissues.

Although PRC2 in Drosophila is recruited to defined Polycomb Response Elements upstream of target genes, similar motifs have not been identified in vertebrates. It has been suggested that recruitment may occur via intermediary molecules, with evidence for involvement of the long noncoding RNA hox transcript antisense RNA (HOTAIR; refs. 14, 15), nuclear inhibitor of protein Ser/Thr phosphatase-1 (NIPPP1; ref. 16), Pho repressive complex (PhoRC; refs. 8, 17), and Jarid2 (18).

It is clear that activating and repressive histone methylation marks do not act in isolation but are intimately coordinated with the action of other epigenetic modifications (Fig. 1C). EZH2 can bind the DNA methyltransferases DNMT1, DNMT3A, and DNMT3B (19), which can result in DNA methylation, at least in certain circumstances (20). Knockdown of EZH2 can result in reexpression of genes with H3K27 methylation and minimal DNA methylation but not genes that are hypermethylated (21). Recruitment of histone deacetylase (HDAC) proteins by PRC2 has been suggested as a further mechanism for transcriptional repression. PRC2 can bind HDAC via EED (22), and treatment of nonerythroid cells with the HDAC inhibitor trichostatin A results in depletion of PRC2 and induction of α-globin expression, a locus that is normally tightly repressed and marked by hypoacetylation and the presence of PRC2 and H3K27me3 (23).

**EZH2 and cancer**

Overexpression of EZH2 was first linked to cancer by microarray studies of prostate cancer (24) and breast cancer (25). In prostate cancer, EZH2 overexpression is associated with aggressive and metastatic disease. Similarly in breast cancer, EZH2 expression is elevated in invasive carcinoma and metastases, and increased expression is strongly associated with a poor clinical outcome (25). Overexpression of EZH2 has also been described in other cancers including bladder (26–28), gastric (29), lung (30), and hepatocellular carcinoma (31). Deletions of microRNA-101, a negative regulator of EZH2 expression, have been described in prostate cancer, thus providing a mechanism for EZH2 overexpression (32). Experimental support for the oncogenic action of EZH2 has been provided by induction of anchorage-independent colony growth and promotion of invasion in vitro by overexpression of EZH2 in the breast epithelial cell line H16N2 (25). In addition, ectopic EZH2 increased the proliferation of mouse embryonic fibroblasts (33).

A remarkable connection has been described between PRC2 occupancy and the aberrant methylation of CpG islands at promoters seen in some cancers (34–36). Promoters found to have aberrant CpG island hypermethylation were also found to be enriched for PRC2 and H3K27 methylation. These sites were also found to be precisely those occupied by PRC2 in stem cells. Because PRC2 can recruit DNMTs (19), it seems that PRC2 may act as a platform for aberrant de novo promoter methylation, although the precise contexts in which this occurs is still unclear.

**EZH2 mutations**

Recent work has identified acquired EZH2 mutations in lymphoma and myeloid neoplasms. In lymphoma, a heterozygous missense mutation at amino acid Y641, within the SET domain, was initially identified by high throughput transcriptome sequencing. Various heterozygous mutations at Y641 were subsequently found in 7% of follicular lymphomas and 22% of diffuse large cell B-cell lymphomas of germinal center origin (37). Mutations elsewhere in EZH2 were not seen. Although initially thought to result in a loss of catalytic activity, more detailed analysis showed that Y641 mutants actually result in a gain of function. Specifically, normal EZH2 displays greatest catalytic activity for monomethylation of H3K27 and relatively weak efficiency for the subsequent (mono- to di- and di- to trimethylation) reactions. Remarkably, Y641 mutants display very limited ability to do the first methylation reaction but have enhanced catalytic efficiency for the subsequent reactions (38). Heterozygous Y641 mutants, thus, work in conjunction with wild-type EZH2 to increase levels of H3K27me3 and may be functionally equivalent to EZH2 overexpression.

In myeloid neoplasms, mutations have been described largely in poor prognosis myelodysplasia-myeloproliferative neoplasms (10 to 13%), myelofibrosis (13%), and various subtypes of myelodysplastic syndromes (6%; refs. 39, 40). In contrast to the mutation of a single residue and gain of function seen in lymphoma, mutations were spread throughout the gene and comprised missense, nonsense, and premature stop codons, some of which were homozygous. Because the catalytic SET domain lies at the far C terminus, all nonsense and stop codon mutations would be predicted to result in loss of histone methyltransferase activity and, thus, are clearly different functionally from Y641 mutants. The fact that both activating and inactivating mutations of EZH2 are associated with malignancy is remarkable and reflects the complex role that PcG genes play in cell fate decisions (41).

Mutations of other PRC2 components have not been reported, although SUZ12 (also known as JJAZ1) fuses to juxtaposed with another zinc finger 1 (JAZF1) in
endometrial stromal tumors (42). In addition, mutations have been reported in a growing number of genes encoding functionally related epigenetic components. For example, inactivating mutations of ubiquitously transcribed tetratricopeptide repeat gene on X chromosome (UTX), an H3K27 demethylase, are seen in a wide range of malignancies and may also be functionally equivalent to EZH2 overexpression (43). Inactivating mutations of the PRC2 downstream substrate DNMT3A are seen in acute myeloid leukemia (AML; ref. 44), and other PcG members such as ASXL1 (additional sex combs-like 1) are mutated in myeloid malignancies (45).

Clinical-Translational Advances

The therapeutic modulation of epigenetic marks has led to improvements in treatment of some cancers, for example, the use of DNA demethylating agents azacitidine and decitabine for myelodysplastic syndrome (46, 47) and the HDAC inhibitor suberoylanilide hydroxamic acid (vorinostat) for cutaneous T-cell lymphoma (48). In contrast to DNA demethylating agents and HDAC inhibitors, no therapies that directly target histone methylation are clinically available, despite the fact that there is experimental evidence for a potential therapeutic benefit for this approach. Most experimental work on the inhibition of EZH2 activity has used the carbocyclic adenosine analog 3-dezaadenosine (DZNep), a derivative of the naturally occurring antibiotic neplanocin-A, in which ribose is replaced by a cyclopentyl ring (49). DZNep inhibits S-adenosylhomocysteine hydrolase, a component of the methionine cycle, resulting in accumulation of the inhibitory S-adenosylhomocysteine with a knock-on disruption of methylation of substrates by EZH2 (Fig. 1D). The effect of DZNep upon histone methylation is, therefore, global rather than EZH2 specific (50). A comparison of DZNep action and specific knockdown of EZH2 by targeted RNAi has shown interesting differences; DZNep reduces levels of EZH2 protein but not the mRNA, whereas RNAi reduces both protein and mRNA levels. Both strategies result in loss of H3K27 methylation but differ somewhat in the pattern of genes that are reexpressed (51). Inhibition of EZH2 by either DZNep or RNAi leads to reduced proliferation in a subset of breast cancer cell lines (51) and reduced proliferation and invasiveness in prostate cancer cell lines (24, 51, 52).

After initial work on DZNep as a single agent histone methylation inhibitor, in vitro experimental work progressed to the use of combinatorial approaches to modify histone methylation, acetylation, and DNA methylation. In breast cancer cell lines, DZNep treatment and HDAC inhibition result in synergistic effects upon proliferation, apoptosis, differentiation, and cell cycle arrest (53). Chromatin immunoprecipitation experiments have shown differential effects on gene reexpression patterns with different combinatorial approaches with DZNep, DNA demethylating agents, and HDAC inhibitors (54). In AML, combined treatment with DZNep and the HDAC inhibitor panobinostat induced a synergistic apoptotic effect upon primary leukemia cells compared with normal cells (55). The complexity of the interactions between different epigenetic marks is highlighted by recent work showing downregulation of BMI1 and EZH2, by treatment with the HDAC inhibitors valproic acid or sodium butyrate by mechanisms that are currently unclear (56).

The concept of “synthetic lethality” refers to the dependency of a tumor on the presence of either 1 of 2 genes, but with resulting cell death if both are lost. Recently, a possible “synthetic lethal” relationship between EZH2 and BRCA1 was described. BRCA1 plays a role in differentiation of breast stem cells in addition to a well-documented role in DNA repair. In a mouse model of basal-type tumors (which are generally poorly differentiated and do not express ERBB2, estrogen, or progesterone receptors and are, therefore, without rational therapeutic targets), EZH2 expression was found to be higher in BRCA1-deficient tumors compared with those that were wild-type. Remarkably, knockdown of EZH2 by RNAi or by treatment with DZNep was 20 times more efficient at killing BRCA1-deficient cells (57). Further work has shown that EZH2 knockdown can result in increased BRCA1 expression with a concomitant contribution to reduced proliferation (58).

Although the development of histone demethylating agents is an area of great interest, currently no compounds are approved for treatment or in clinical trials. However, it is clear that modulation of EZH2 levels and H3K27 methylation can be achieved by therapies primarily directed at DNA methylation or histone acetylation. Although there is an obvious rationale for inhibiting EZH2 function in malignancies in which this gene is overactive as a consequence of overexpression or Y641 mutation, the finding of inactivating mutations in myeloid neoplasms suggests that this approach will need to be employed with caution. Understanding how to effectively combine current treatments with future histone demethylating agents will be a major challenge in the coming years.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received November 24, 2010; revised January 10, 2011; accepted January 11, 2011; published OnlineFirst March 2, 2011.

References


Aberrations of EZH2 in Cancer


Clinical Cancer Research

Aberrations of EZH2 in Cancer
Andrew Chase and Nicholas C.P. Cross

Clin Cancer Res 2011;17:2613-2618. Published OnlineFirst March 2, 2011.

Updated version
Access the most recent version of this article at:
doi:10.1158/1078-0432.CCR-10-2156

Cited articles
This article cites 56 articles, 21 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/17/9/2613.full#ref-list-1

Citing articles
This article has been cited by 41 HighWire-hosted articles. Access the articles at:
http://clincancerres.aacrjournals.org/content/17/9/2613.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, use this link
http://clincancerres.aacrjournals.org/content/17/9/2613.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.