Editorial

New insights into the mechanisms of EZH2's promotion of oncogenesis

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EZH2 is an important enzymatic subunit of the epigenetic regulator polycomb repressive complex 2 (PRC2), which includes EED and SUZ12 subunits. EZH2 functions as a methyltransferase that targets the lysine 27 of histone H3, leading to trimethylation (H3K27me3), a mechanism of post-translational modification that leads to transcriptional repression of PRC2 target genes (1).

EZH2 is expressed in stem cells and proliferating cells and is down regulated in differentiated cells. It is overexpressed in a wide range of non-hematopoietic and hematopoietic neoplasms, and its overexpression is associated with tumor cell proliferation, metastasis, and poor prognosis. Neoplasms with increased EZH2 expression include a range of carcinomas, including breast, non-small cell lung, prostate, hepatocellular, ovarian, colorectal, renal and endometrial carcinomas, glioblastoma multiforme, other solid tumors, as well as hematopoietic neoplasms, including aggressive B-cell lymphomas, plasma cell neoplasms, myeloid neoplasms, including acute myeloid leukemia, a range of T-cell lymphomas, as well as histiocytic and dendritic cell neoplasms (2-5).

EZH2 is able to promote oncogenesis by several distinct mechanisms (*Table 1*). One mechanism is for EZH2 to function as a transcriptional repressor of tumor suppressor genes through its methyltransferase activity, which is increased in a wide range of neoplasms, due to increased EZH2 expression. EZH2 overexpression in various neoplasms has been shown to be due to various mechanisms, including intracytoplasmic oncogenic signaling molecules, transcription factors, gene amplification, gain of function mutations, and other mechanisms. For example, in a subset of follicular lymphomas and diffuse large B-cell lymphomas of germinal center B-cell type, a change of amino acid tyrosine 641 (Y641) has been identified as a recurrent somatic mutation in the *EZH2* gene, leading to increased enzymatic activity (6). The oncogenic role of the Y641 mutation was further confirmed in an engineered mouse model in which conditional expression of mutant EZH2 in germinal center B-cells induced germinal center hyperplasia and promoted lymphomagenesis in cooperation with BCL2 overexpression (13). In addition to the Y641 mutation, A687V and A677G mutations have been identified as gain of function mutations of *EZH2* in B-cell lymphomas (14,15). Several studies have shown that a significant subset of PRC2 target genes are repressed in various tumors. Small molecule inhibitors of EZH2, which selectively block EZH2 methyltransferase activity and reduce global H3K27 methylation, have been shown to block tumor cell proliferation and induce cell cycle arrest and apoptosis in B cell lymphomas (7,8). Interestingly, EZH2 can also methylate non-histone proteins such as STAT3, which functions as a transcription factor, leading to increased STAT3 activity and tumor growth in glioblastoma stem-like cells (9). AKT signaling results in EZH2 phosphorylation at S21, and is required for EZH2-STAT3 interaction and STAT3 methylation (9).

Another mechanism by which EZH2 promotes oncogenesis is through loss of its tumor suppressor function. Frequent homozygous and heterozygous *EZH2* deletions or inactivating mutations have been found

Table 1 Mechanisms of EZH2 promotion of tumorigenesis

EZH2 function	Mechanism	Neoplasm (example)	Possible treatment	Reference(s)
Methyltransfersase activity leading to transcriptional repression of tumor suppressor genes	*EZH2* mutations leading to increased EZH2 activity (as well as other mechanisms of increased EZH2 production)	Diffuse large B-cell lymphoma of germinal center B-cell type	Specific EZH2 inhibitors targeting its enzymatic activity	(6-8)
EZH2-mediated methylation of STAT3 leading to its increased activity as a transcription factor promoting tumor cell growth	Phosphorylation of EZH2 at S21 by AKT signaling	Glioblastoma	Specific EZH2 inhibitors targeting its enzymatic activity	(7-9)
Loss of EZH2 tumor suppressor function	Deletions or inactivating mutations of *EZH2*	T-cell acute lymphoblastic leukemia	Targeted therapeutics to block oncogenic pathways activated as a result of decreased EZH2 activity, e.g., NOTCH1	(10)
EZH2 switch to a transcriptional activator of growth and survival genes implicated in cancer	JAK3 phosphorylation of EZH2 at Y244	NK/T-cell lymphoma	Pharmacological inhibition of JAK3 activity or of phosphorylated EZH2	(11,12)

in myeloid malignancies, including myelodysplastic syndromes, myeloproliferative neoplasms, and myelodysplastic/myeloproliferative neoplasms, such as chronic myelomonocytic leukemia (16-19), and are predictive of poor survival (20,21). In addition, inactivation of the *EZH2* gene through loss of function mutations and gene deletions leading to inactivation of the PRC2 complex were found in a significant number of cases of T-cell acute lymphoblastic leukemia (T-ALL), suggesting that loss rather than overexpression of EZH2 may also contribute to tumorigenesis in this T-cell neoplasm (10). Decreased EZH2 activity in T-ALL was found to be associated with oncogenic *NOTCH1* mutations and activation of the NOTCH signaling pathway (10). In mice, a high frequency of T-ALL occurred following biallelic deletion of *Ezh2*, demonstrating the tumor suppressive function of EZH2, and biallelic deletion of *Ezh2* was also found to induce a myelodysplastic/myeloproliferative-like disease in mice (22,23). A different mechanism of decreased EZH2 activity occurs in pediatric gliomas, where EZH2 activity is decreased as a result of mutations in the histone *H3F3A* gene, which results in the synthesis of a mutant histone H3.3 protein that appears to bind to EZH2 and inhibit its methyltransferase activity (24).

A third mechanism by which EZH2 promotes oncogenesis is by switching to a transcriptional activator independent of its methyltransferase activity as part of the PRC2 complex, a noncanonical function of EZH2. Yan and coworkers found that EZH2 is overexpressed in NK/T-cell lymphoma as a result of MYC-mediated suppression of expression of tumor suppressor micro RNAs (miRNAs) miR-26 and miR-101 (11). EZH2 overexpression was able to promote proliferation of NK cells and NK/T-cell lymphoma cells in a manner that was independent of its methyltransferase activity, since an EZH2 mutant lacking methyltransferase activity was able to promote cell growth of EZH2-depleted cells (11). Conversely, depletion of EZH2 led to inhibition of cell growth. Furthermore, Yan and coworkers were also able to show that EZH2 acts as a direct transcriptional activator of cyclin D1 in NK/T-cell lymphoma (11). EZH2 action as a transcriptional activator able to stimulate cell growth may also occur in breast, colon, and other carcinomas. For example, EZH2 appears to serve as a transcriptional activator in estrogen receptor-positive breast cancer by interacting with estrogen receptor α and β-catenin, which enhances gene activation in the estrogen and Wnt pathways and promotes cell proliferation (25). In other, triple negative breast cancer cells, EZH2 serves as a coactivator of RelA and RelB to promote expression of NF-κB target genes (26). In colon cancer, EZH2 activates Wnt target genes independent of its methyltransferase activity, after physically associating with proliferating cell nuclear antigen-associated factor (PAF) and binding to the β-catenin transcriptional complex (12).

In a recent publication, Yan and coworkers identified the mechanism that leads to the switch in EZH2 function from methyltransferase to transcriptional activator in NK/T-cell lymphoma cells. They found that JAK3

phosphorylates EZH2 at Y244, which leads to EZH2's activity as a transcriptional activator which associates with RNA polymerase II, promotes cell growth, and inhibits EZH2's methyltransferase function and resultant epigenetic gene silencing (27). This noncanonical function of EZH2 includes the activation of 93 target genes, including *CCND1*, *RAD51C*, *PMSD1*, *KRAS*, and *MAPK15*. Treatment of NK tumor cells with JAK3 kinase inhibitor PF956980, but not with an inhibitor of EZH2 methyltransferase activity, led to decreased expression of noncanonical target genes, but not canonical target genes, as well as decreased tumor cell growth and viability (27).

These recent findings demonstrate a novel mechanism by which EZH2 is switched from a gene repressor to a gene activator, through JAK3-mediated posttranslational modification. These findings suggest that JAK3 inhibition, or inhibition of phosphorylated EZH2, may serve as an effective treatment for neoplasms such as NK/T-cell lymphoma in which EZH2 serves as a gene activator following phosphorylation by JAK3. These recent results also indicate that specific inhibitors of EZH2 methyltransferase activity will be insufficient to treat malignant neoplasms in which EZH2 functions as a gene activator independent of its role in PRC2 as a methyltransferase and could interfere with EZH2's growth regulatory and tumor suppressor function. Since JAK3 is increased in expression in a number of other malignant neoplasms that have increased EZH2 expression, including colon and breast carcinomas, JAK3 may also function to phosphorylate EZH2 in those neoplasms, causing it to switch to a transcriptional activator (27). If so, JAK3 inhibition, or inhibition of phosphorylated EZH2, may be a useful treatment for those neoplasms as well. As multiple mechanisms of EZH2-mediated oncogenesis have been discovered, so too the number of possible treatments for malignant neoplasms that overexpress EZH2 has grown (*Table 1*).

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Footnote

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