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Review Article

Diverse involvement of EZH2 in cancer epigenetics

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Abstract: EZH2 is the catalytic subunit of Polycomb Repressor Complex 2 (PRC2) which catalyzes methylation of histone H3 at lysine 27 (H3K27me) and mediates gene silencing of target genes via local chromatin reorganization. Numerous evidences show that EZH2 plays a critical role in cancer initiation, progression and metastasis, as well as in cancer stem cell biology. Indeed, EZH2 dysregulation alters gene expression programs in various cancer types. The molecular mechanisms responsible for EZH2 alteration appear to be diverse and depending on the type of cancer. Furthermore, accumulating evidences indicate that EZH2 could also act as a PRC2-independent transcriptional activator in cancer. In this review, we address the current understanding of the oncogenic role of EZH2, including the mechanisms of EZH2 dysregulation in cancer and progresses in therapeutic approaches targeting EZH2.

Keywords: EZH2, polycomb repression, chromatin modification, histone lysine methylation, cancer, cancer stem cells

Introduction

During embryogenesis, the fertilized egg develops into a complex organism composed of many differentiated cell types. The maintenance of the differentiation status of these cell types requires a cellular memory system responsible for the stable inheritance of gene expression programs. The Polycomb group (PcG) and trithorax group (trxG) genes were discovered in Drosophila melanogaster as part of such a memory system [1, 2]. They have been identified as repressors (PcG) and activators (trxG) of genetic programs, respectively. Mutations in the PcG and trxG genes result in pleiotropic defects, of which homeotic transformations are the most apparent [3]. In vertebrates, the PcG and trxG proteins have similar roles in the maintenance of homeotic gene expression patterns. Indeed, changes in the body plan have been observed in PcG and trxG gene homolog mouse mutants [4-7]. Although primarily known for their involvement in the control of homeotic genes during the establishment of the body plan, PcG and trxG members have also been shown to be implicated in the control of various cellular processes, such as stem cell renewal and differentiation, cell fate decisions, senescence, chromosome X-inactivation in mammals, or tumorigenesis and neoplastic development [8-10].

Perturbations in local chromatin structure cause inappropriate gene expression and genomic instability, resulting in cellular transformation and malignant outgrowth. Therefore, proteins involved in chromatin organization, including Polycomb group (PcG) proteins constitute fundamental players in cancer pathogenesis [11-13].

Polycomb repression and EZH2 activity

PcG proteins have been found to interact with each other to form multimeric, chromatin-associated protein complexes of two general types: the Polycomb Repressive Complex 1 (PRC1) and PRC2 [14, 15]. These complexes post-translationally modify histone tails and are believed to cooperate in transcriptional repression of target genes by altering local, higher order chromatin structure. The PRC2 protein complex contains EZH2, a histone methyltransferase that catalyzes trimethylation of histone H3 lysine 27 (H3K27me3) [16, 17]. In addition to EZH2, core PRC2 is composed of EED, SUZ12 and RBBP4/

Table 1. EZH2 alterations and cancers

Alteration	Cancer type	References
Overexpression	Prostate, breast, bladder, ovarian, renal carcinoma, lung, liver, brain, gastric, esophageal, pancreatic, melanoma	[35, 38, 39-56, 166]
Activating mutations	Large follicular and B-cell lymphomas	[99, 100, 102, 103]
Inactivating/hypomorphic mutations	Myeloproliferative neoplasms Pediatric cancers	[115, 116, 118-120]
H3.3K27M-mediated EZH2 inhibition	Pediatric gliomas	[121-124]

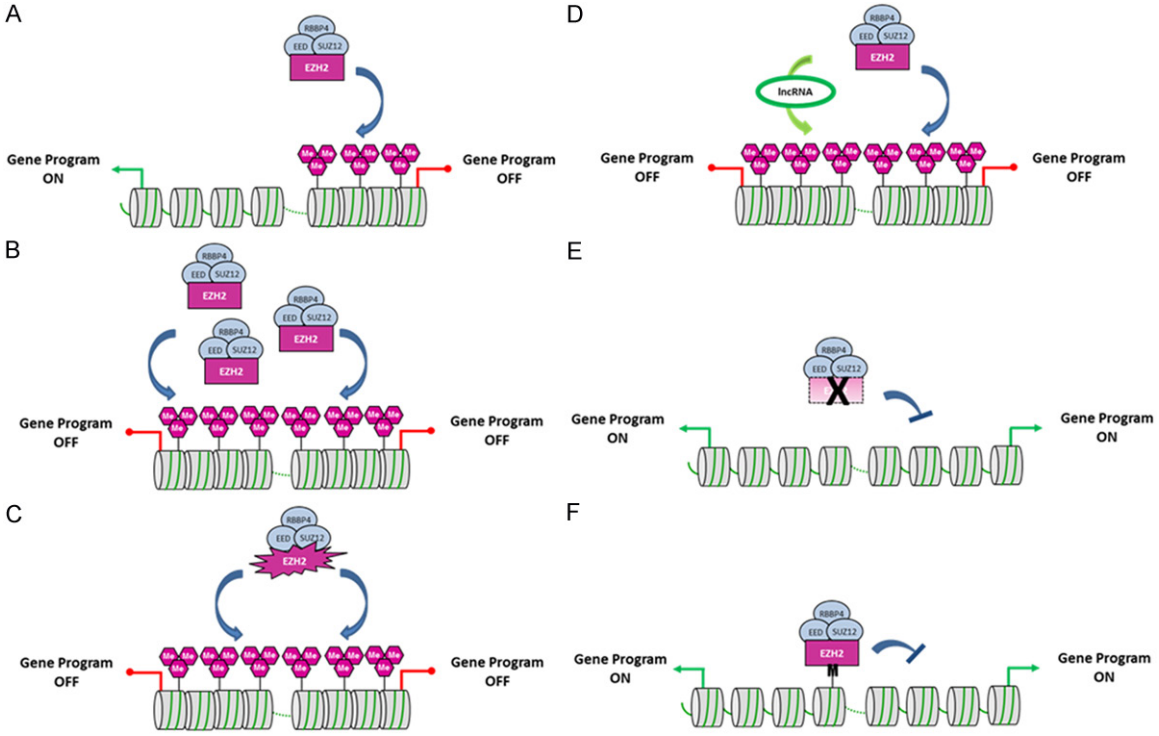

Figure 1. *Schematic representation of the diverse EZH2 dysregulations found in cancer.* A. The histone lysine methyltransferase EZH2 catalyzes H3K27 methylation at defined target genes and silences their expression. B. Overabundance of EZH2 is responsible for an increase in H3K27me3 repressive mark levels leading to the silencing of tumor suppressor genes in cancer cells. C. EZH2 bearing activating mutations at residues Y641, A677 or A687 possesses an enhanced activity leading to an increase in H3K27me3 levels. D. Overexpression of EZH2-interacting partners such as specific lncRNAs enhances recruitment of EZH2 to targets and increases H3K27me3 levels. E. EZH2 harboring an inactivating mutation or EZH2 gene deletion leads to a decrease in H3K27me3 levels and activation of EZH2 target gene programs in cancer. F. A lysine to methionine substitution at position 27 (K27M) in the gene encoding histone H3.3 (H3F3A) inhibits EZH2 activity and leads to nearly undetectable H3K27me3 repressive mark levels in pediatric gliomas. Purple hexagons represent H3K27me epigenetic marks, and M illustrates H3.3K27M mutant histones.

RbAp48/NURF55 which are required for full EZH2 histone methyltransferase activity [18, 19]. The PRC1 protein complex binds to PRC2-modified residues (H3K27me3) and monoubiquitinylates histone H2A at lysine 119 (H2AK119ub1) [20, 21]. These modifications (H3K27me3 and H2AK119ub1) cause in turn local chromatin compaction and transcriptional silencing.

EZH2 is the catalytic subunit of the PRC2 protein complex, and its C-terminal SET domain exhibits the H3K27 methyltransferase function. However, EZH2 by itself lacks enzymatic activity. Two other PRC2 components, the zinc-finger-containing protein SUZ12 and the WD40-repeat protein EED are required to maintain the integrity of the PRC2 complex and for EZH2 robust methyltransferase activity [22-24]. The fourth PRC2 core subunit, RBBP4/RbAp48 also contributes to PRC2 function but subcomplexes lacking this component retain substantial enzyme activity [18, 19]. Additional PRC2 components, such as AEBP2, PCL (PHF) and

JARID2 may function as accessory factors regulating the activity of the PRC2 protein complex. However, their role appears to be modulatory rather than essential [17, 25-28].

In the context of the PRC2 protein assembly, EZH2 SET domain performs three successive methyl transfer reactions, producing ultimately H3K27me3. This contrasts with other SET-domain protein methyltransferases whose capacity for methyl transfer appears more limited. For example, SET7/9 produces only monomethylated products (H3K4me1), whereas G9a/EHMT2 catalyzes mono- and dimethylation (H3K9me1 and H3K9me2) and SUV39H2 can di- and trimethylate monomethylated substrates (H3K9me1 methylation into H3K9me2 and H3K9me3) [29, 30].

EZH1 is a paralog of EZH2; PRC2-EZH2 and PRC2-EZH1-containing complexes control overlapping sets of target genes but act differently to maintain the repressed chromatin state [31]. Furthermore, EZH2 is mainly expressed in proliferating tissues, whereas EZH1 expression is found in dividing and differentiated cells [31, 32]. This observation suggests that EZH2-containing PRC2 complexes might establish the H3K27me3 repressive marks, whereas EZH1-containing PRC2 complexes might contribute to the restoration of the H3K27me3 methylation profile after histone demethylation or histone exchanges.

Involvement of EZH2 in cancer

The genome-wide mapping of PcG target genes revealed more than 2,000 sites in the mouse embryonic stem cell genome [8, 33, 34]. Interaction of PcG proteins with chromatin at these loci is associated with increased levels of H3K27me3 repressive marks and Polycomb repression affects numerous genes encoding key developmental regulators and signaling proteins. These genomic studies point on the widespread roles of PRC2 and H3K27 methylation in developmental and differentiation processes of multicellular organisms, and on their implication in fundamental chromatin mechanisms that underlie stem cell regulatory circuits and cancer progression. Thus, it is not surprising that increasing evidences indicate that EZH2 deregulation is frequently observed in a variety of cancers (**Table 1**). Interestingly, different ways by which the function of EZH2 may be impaired in tumors have been described (**Figure 1**). It also appears that the type of EZH2 dysregulation often correlates with the malignancy. EZH2 overexpression is mainly found in solid tumors, whereas activating or inactivating mutations are identified in hematologic malignancies. A missense mutation Lys27Met (K27M) in the gene encoding histone H3.3 (H3F3A) is present at high frequencies in pediatric gliomas. This mutation behaves as a potent inhibitor of EZH2 activity. Thus, although mechanisms could be different, misregulation of H3K27 methylation is common in tumorigenesis and EZH2 appears to have both oncogenic and tumor suppressive functions.

EZH2 overabundance in solid cancer

Overexpression of EZH2 was first found in prostate and breast cancer in microarray studies [35, 36]. Furthermore, EZH2 overexpression is linked to aggressive and advanced metastatic stages of the disease and is strongly associated with poor clinical outcome and prognosis [36-38]. Overexpression of EZH2 has also been reported in a large number of other solid tumors such as bladder cancers [39-42], ovarian cancers [43, 44], renal carcinomas [45], small cell and non-small cell lung cancers [46-49], hepatocellular carcinomas [50], brain tumors [51], kidney cancers [52], gastric tumors [53], esophageal cancers [54], pancreatic cancers [55] or melanomas [38, 56]. EZH2 has been shown to promote cell proliferation, migration, and invasion in different in vitro cancer cell models [32, 36, 43, 57-59]. EZH2 overexpressing cells are also tumorigenic when injected into the mammary fat pads of nude mice [60], while overexpression of wild-type EZH2 in mammary epithelial cells in vivo results in epithelial hyperplasia and promotes mammary tumor initiation [61, 62]. Furthermore, the oncogenic property of EZH2 in mice correlates with its H3K27 methyltransferase activity [63].

Overabundance of EZH2 in tumor cells may result from different mechanisms. In some cases, EZH2 up-regulation is associated to gene amplification [35, 64]. Using FISH techniques, Sramaki and colleagues studied the copy number of the EZH2 gene in several prostate cancer cell lines such as LNCaP, DU145, PC-3, 22Rv1, in xenografts and in clinical tumors [64]. In contrast to early prostate cancer, in late stage tumor samples, EZH2 gene amplification was shown to correlate with its overexpression.

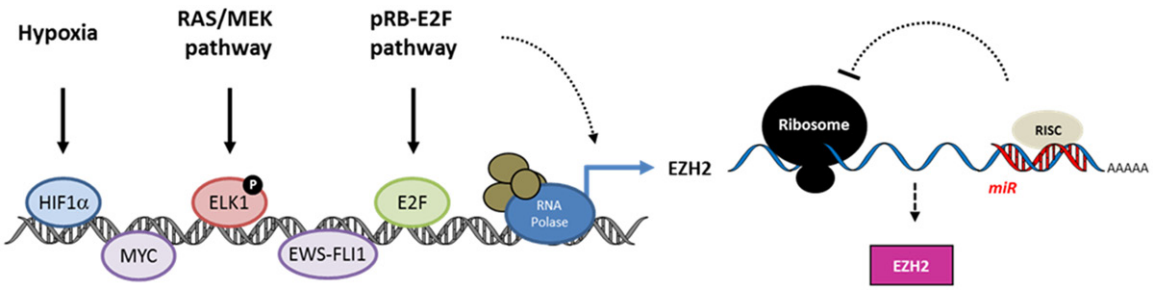

Figure 2. *Schematic representation of EZH2 overexpression controlling levels.* EZH2 overexpression in cancer cells is achieved at the transcriptional level through the binding of transcription factors to its promoter or at the post-transcriptional level via the alteration of the micro-RNA regulation.

Table 2. Regulators of EZH2 transcription

Factor/Pathway	Cancer type	References
MYC	Prostate	[69]
ETS/ERG	Prostate	[70]
E2F (pRB-E2F pathway)	Breast	[32]
	Lung	[68]
	Bladder	[66, 67]
ELK1 (MEK-ERK pathway)	Breast	[65, 76]
ATAD2/ANCCA	Breast	[73]
NF-YA	Ovarian	[71]
STAT3	Colorectal	[72]
BRAF (V600E)	Melanoma	[75]
EWS-FLI1	Ewing's sarcoma	[74]
HIF1α (Hypoxia)	Breast	[76]

Increased EZH2 levels in cancer may also be caused by a variety of transcriptional signals and pathways, some of them common for different cancer types while others may be more specific or limited to specific malignancies (**Figure 2**, **Table 2**). The MEK-ERK-ELK1 pathway, which is often activated in cancer, has been demonstrated to be responsible for EZH2 overexpression in triple-negative and ERBB2-overexpressing subtypes of breast cancer [65]. Upon phosphorylation ELK1 binds to three ELK1-binding motifs located within the EZH2 gene promoter and activates EZH2 transcription. The pRb-E2F signaling is another pathway involved in numerous tumors. Upon pRb/RB1 phosphorylation, E2F dissociates from the pRb-E2F complex and the activated E2F transcription factor binds to E2F-binding sites located in the EZH2 promoter to activate its transcription [32]. Overexpression of E2F or deregulation of the pRb-E2F pathway correlate with activated EZH2 expression in breast, bladder and small-cell lung cancer [32, 66-68]. A number of transcription factors involved in tumorigenesis directly bind to the EZH2 promoter and activate its mRNA expression in different cancer models. In particular, MYC and ETS transcription factors directly regulate EZH2 transcription in prostate cancer [69, 70], whereas NF-YA, STAT3 and the co-activator of the androgen receptor ATAD2/ANCCA regulate EZH2 expression in epithelial ovarian, colorectal, and breast cancer cells, respectively [71-73]. In Ewing's sarcoma, the fusion oncoprotein EWS-FLI1 induces EZH2 expression which has a key role in endothelial/neuroectodermal differentiation and tumor growth [74]. Also, expression of EZH2 was found profoundly affected by the BRAF (V600E) mutation in melanoma although the precise mechanism of regulation is not yet fully understood [75].

Hypoxia in solid tumors represents another pathway directly regulating EZH2 transcription. Chang and colleagues identified a consensus sequence for hypoxia-inducible factor-1α (HRE) within the EZH2 promoter [76]. Hypoxic microenvironment in tumors induces HIF1α binding to the HRE and transactivates EZH2 to promote breast tumor expansion (**Table 2**).

In addition, EZH2 abundance is controlled at the post-translational level by multiple microRNAs (miRs). The miR-25, -26a, -30d, -98, -101, -124, -137, -138, -144, -214 and let-7 interact with defined sequences within the EZH2 3'UTR and directly downregulate EZH2 protein abundance. The loss of control by these miR results in the up-regulation of EZH2 and appears to be involved in the aggressiveness of various cancers [57, 77-98] (**Figure 2**, **Table 3**).

Table 3. EZH2 post-transcriptional regulation by miR

MiR	Cancer Type	References
miR-25, miR-30d	Thyroid cancer	[77]
miR-26a	Lymphoma, nasopharyngeal carcinoma, breast cancer, prostate cancer	[78-80]
miR-98	Nasopharyngeal carcinoma, gastric cancer	[81, 82]
miR-101	Nasopharyngeal carcinoma, glioblastoma multiform, prostate cancer, bladder cancer, head and neck cancer, non-small cell lung cancer, melanoma	[57, 83-88]
miR-124	Hepatocellular carcinoma, gastric cancer	[89, 90]
miR-137	Melanoma	[91]
miR-138	Head and neck cancer, glioblastoma multiform, non-small cell lung cancer	[92-94]
miR-144	Bladder cancer	[98]
miR-214	Gastric cancer, hepatocellular carcinoma	[81, 95]
Let-7	Prostate cancer, nasopharyngeal carcinoma	[96, 97]

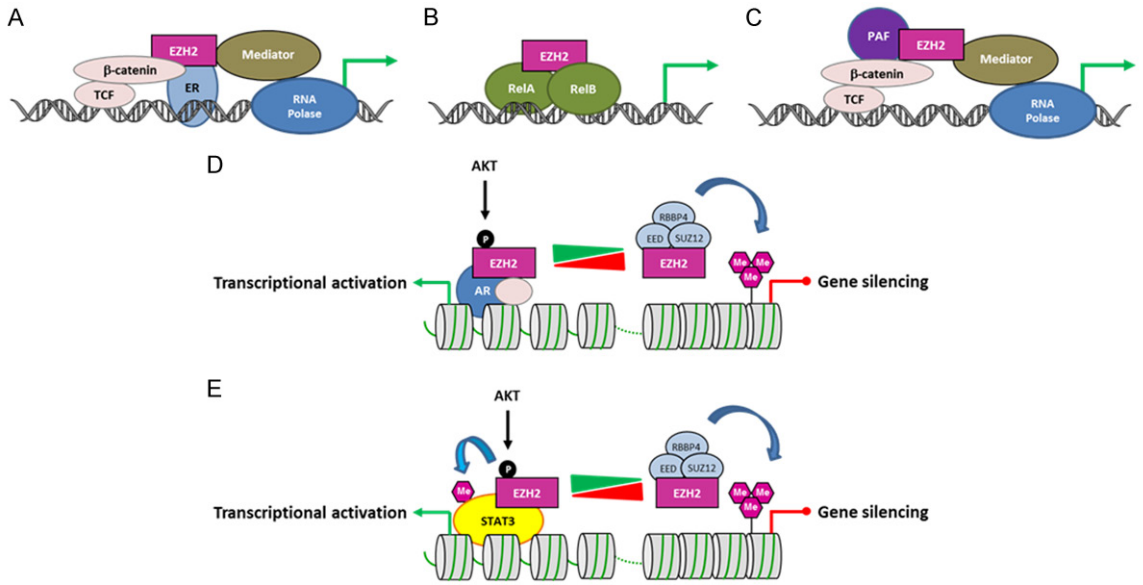

Figure 3. *PRC2-independent transcriptional activation by EZH2 in cancer.* A. In ER-positive breast cancer cells, EZH2 interacts with β-catenin and ER, and functionally enhances gene expression. B. In ER-negative breast cancer cells, EZH2 interacts with RELA/RELB to stimulate NF-κB target gene expression. C. In colorectal cancer cells, EZH2 forms a complex with β-catenin and PAF to promote transcription. D. In castration-resistant prostate cancer, AKT1-mediated phosphorylation of EZH2 at serine 21 allows EZH2 to interact with the AR at target genes to activate transcription. The AKT pathway then acts as a molecular switch changing EZH2 function from a chromatin silencer to a transcriptional co-activator of the AR. This transcriptional activation function is methyltransferase activity-dependent. E. AKT1-mediated phosphorylation of EZH2 at serine 21 also facilitates STAT3 methylation and activation in glioblastoma stem cells. ER: estrogen receptor; TCF: T-cell factor; AR: androgen receptor; PAF: PCNA-associated factor; RNA Polase: RNA polymerase II.

Altogether, different studies suggest that the diverse mechanisms involved in EZH2 overabundance depend on the cell context. However, EZH2 functions as an oncogenic factor in the majority of solid tumors and regardless of the molecular mechanisms involved, EZH2 overabundance leads to higher levels of H3K27me3 repressive epigenetic marks that would be responsible in turn, for the silencing of tumor suppressor genes in cancer cells (**Figure 1B**).

EZH2 activating mutations in lymphoma

In about 7% of large follicular lymphomas and 22% of diffuse B-cell lymphomas, recurrent somatic mutations were identified at tyrosine 641 (Y641) within the catalytic SET domain of EZH2 [99]. Initially reported to be a loss-of-function mutation [99], it has been demonstrated that these mutations shift the methylation capacity of EZH2 [100]. Indeed, EZH2-Y641 mutants have reduced H3K27 mono- and

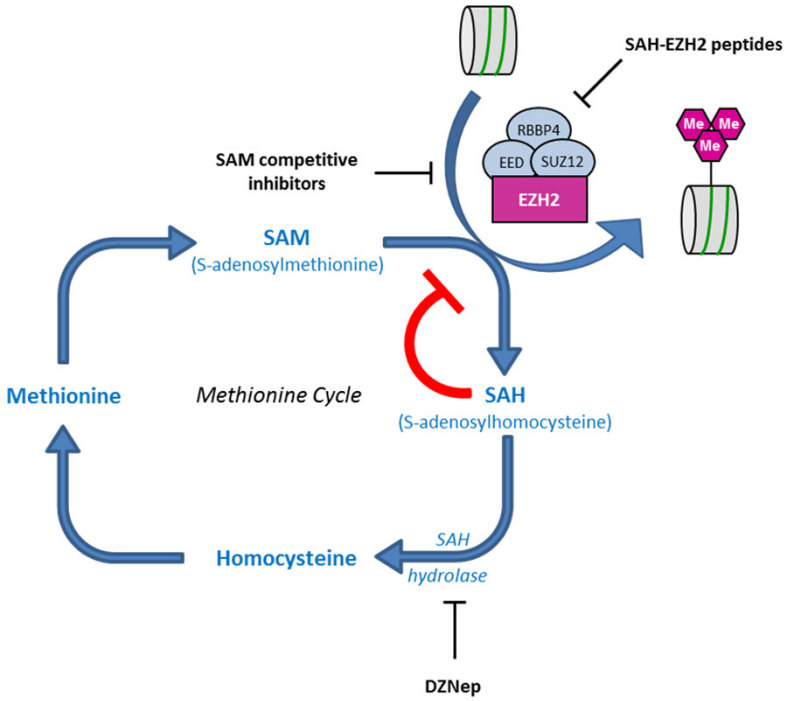

Figure 4. *Methionine cycle and mode of action of several EZH2 inhibitors.* DNZep is an inhibitor of SAH hydrolase leading to an accumulation of SAH which in turn inhibits EZH2 activity, as well as other cellular methltransferases. SAM competitors such as EPZ005687, EPZ-6438, EI1, UNC1999 and GSK126 bind to the SAH-binding pocket of EZH2 to prevent the recruitment of the SAM methyl donor. SAH-EZH2 peptides disrupt the PRC2 assembly required for full EZH2 activity.

dimethylation activities, but aberrantly elevated trimethyltransferase activity. Because of their reduced H3K27me1/2 activities, EZH2-Y641 mutant alleles are invariably found at heterozygous forms together with the wild-type EZH2 allele in lymphomas. In agreement with these observations, transgenic mice expressing the EZH2-Y641F mutant in lymphocytes displayed a global increase in trimethylated H3K27 in spleen cells and developed lymphomas when combined with Eμ-Myc expression [101]. Similar to the Y641 mutation, mutations within the EZH2 SET domain at residues Alanine 677 (A677G) and Alanine 687 (A687V) are drivers of H3K27 hypertrimethylation [102-104]. Thus, EZH2-Y641, EZH2-A677 and EZH2-A687 are oncogenic mutants responsible for an excess of H3K27me3 repressive marks impairing gene expression programs in lymphomas (**Figure 1C**).

Increased recruitment of EZH2 at chromatin in cancer

An excess of H3K27me3 repressive marks in cancer can also result from an increase of PRC2 recruitment at chromatin (**Figure 1D**). In particular, long non-coding RNAs (lncRNAs) have emerged as potential factors involved in PRC2 recruitment. HOTAIR is one of these lncRNAs interacting with EZH2 and playing an oncogenic role in cancer [105-109]. Overexpression of HOTAIR increases invasiveness and metastatic potential of epithelial cancer cells and induces relocalization of the PRC2 complex which binds to target genes in a pattern similar to that observed in embryonic fibroblasts, whereas HOTAIR knockdown decreases cancer invasiveness, especially in cells expressing high levels of PRC2 proteins [106]. Other lncRNAs, such as HEIH, PCAT-1, H19 or linc-UBC1 have been shown to interact with EZH2 and to be involved in cancer [110-113].

EZH2 null-mutations in malignant myeloid disorders

Mutations in EZH2 have been identified in about 10%-23% of various subtypes of myelodysplastic syndromes and myeloproliferative neoplasms, as well as in 13% of myelofibrosis [114-118]. Both monoallelic and biallelic mutations were identified, and almost all are predicted to inactivate the methyltransferase activity of EZH2. In particular mutations found in myeloid disorders include missense mutations, frameshift mutations, premature stop codons, and spliceosomal mutations. In addition to EZH2 mutation, decreased EZH2 expression was found to be associated with hemizygous deletion (7/del7q) involving the EZH2 gene. EZH2 loss-of-function mutations are associated with reduced H3K27me3 methylation and derepression of EZH2 target genes, which may contribute to leukemogenesis (**Figure 1E**). Consistent with these findings, deletion of Ezh2 alone is sufficient to induce myelodysplastic syndrome/myeloproliferative neoplasm-like diseases in mice [119].

Furthermore, EZH2-inactivating mutations are also primarily detected in pediatric cancers including core binding factor acute myeloid leukemia and T-lineage acute lymphoblastic leukemia [120].

Altogether, these studies outline the tumor suppressor function of EZH2 in myeloid malignancies, in addition to its role as an oncogene in other cancer types.

H3.3K27M mutation-dependent inhibition of EZH2 in high-grade pediatric gliomas

Sequencing studies of high-grade pediatric gliomas, including glioblastoma multiforms (GBM) and diffuse intrinsic pontine gliomas (DIPG) identify recurrent heterozygous Lysine to Methionine substitutions at position 27 (K27M) in the gene encoding histone H3.3 (H3F3A) [121, 122]. H3.3K27M mutation occurs in 70-80% of midline GBM and DIPG in young children and confers a dismal prognosis [122-124]. The mutation also leads to nearly undetectable H3K27me3 repressive mark levels in gliomas [125-128]. Furthermore, it has been shown that H3.3K27M peptides bind to EZH2 and interfere with its methyltransferase activity, potentially as methionine mimics the structure of monomethyl lysine [127] (**Figure 1F**). Since H3.3 is a variant of canonical histone H3 which contributes to a minority of total histone H3 in gliomas, H3.3K27M behaves like a powerful dominant negative inhibitor of EZH2 activity. Interestingly, EZH2 protein and remaining H3K27me3 repressive marks were also shown to be locally increased at hundreds of gene loci in glioma cells expressing H3.3K27M [128]. Therefore, H3.3K27M mutation alters gene expression programs and the global epigenetic landscape which may drive to tumorigenesis in gliomas.

PRC2-independent function of EZH2 in cancer

EZH2 is primarily known for its role in gene silencing through H3K27 trimethylation. However, several studies reveal that EZH2 may also function as a transcriptional activator in different cancer models [129-131] (**Figure 3**).

In breast cancer cells, EZH2 has been reported to act as a transcriptional activator but its mechanism of action depends on the cell type [129, 130]. In estrogen receptor-positive, luminal-like MCF-7 breast cancer cells, EZH2 links physically the estrogen receptor α to the Wnt signaling components β-catenin and TCF at target gene promoters. By this way, EZH2 activates Cyclin D1 (CCND1) and MYC transcription, independently of its methyltransferase activity [129] (**Figure 3A**). By contrast, in estrogen receptor-negative, basal like MDA-MB-231 breast cancer cells, EZH2 interacts with the NF-κB components RELA and RELB to activate transcription of several NF-κB target genes, such as the TNF and IL6 genes [130] (**Figure 3B**). Thus, EZH2 could act as a transcriptional repressor via its H3K27 histone methyltransferase activity or, as a transcriptional activator through different molecular mechanisms, in promoting breast tumorigenesis.

The PCNA-associated factor PAF (KIAA0101) is overexpressed in colon cancers and is required for cancer cell proliferation via Wnt signaling activation. Jung and colleagues [131] identified a PAF-EZH2-β-catenin protein complex involved in Wnt target gene transactivation in colon cancer cells. Upon Wnt signaling activation, PAF dissociates from PCNA, binds to β-catenin and recruits EZH2 at Wnt target genes to induce their expression independently of EZH2's enzymatic activity (**Figure 3C**).

EZH2 is oncogenic and functions as a transcriptional activator in castration-resistant prostate cancer (CRPC) [132]. However in contrast to what was described in breast cancer cells and in colon cancer cells, the transcriptional properties of EZH2 in CRPC rely on its methyltransferase activity, but do not require the other PRC2 components. It has been suggested that EZH2-mediated transcriptional activation may occur through the methylation of the androgen receptor or other associated proteins. Furthermore, Xu et al. [132] reported that AKT1-mediated phosphorylation of EZH2 at serine 21 (S21) allows the methyltransferase to interact with the androgen receptor at many target genes. Thus, the AKT pathway acts as a molecular switch changing EZH2 function from a chromatin silencer to a transcriptional co-activator of the androgen receptor (**Figure 3D**). A similar situation has been described in glioblastoma where AKT1-mediated phosphorylation of EZH2 at S21 enhances STAT3 activation via its EZH2-mediated trimethylation at lysine 180 [133] (**Figure 3E**).

The methyltransferase activity of EZH2 is required for EZH2-dependent gene activation in CRPC and in glioblastoma, indicating that EZH2 can methylate non-histone substrates. In this regard, it is worth noting that previous studies showed that EZH2 indeed methylates non-histone proteins such as the transcription factors GATA4 and RORα, although the role of these methylations in cancer remains to be explored [134, 135].

Post-translational modifications of EZH2 in cancer

The discovery that AKT1-mediated EZH2 phosphorylation at S21 converts EZH2 silencing activity into an activating effector [60, 132, 133] underlines the important role cell signaling may have on EZH2 activity control. There is growing evidence showing that EZH2 activity and stability are tightly regulated by multiple post-translational modifications.

In addition to phosphorylation at S21, EZH2 could be phosphorylated at multiple threonine residues [136-139]. In particular, cyclin-dependent kinases (CDK1/2) phosphorylate EZH2 at T350 and T492. T350 phosphorylation promotes the interaction between EZH2 and lncRNAs such as HOTAIR [137]. Thus, CDK-mediated phosphorylation positively impacts EZH2 action by enhancing its recruitment at chromatin. In contrast, T492 phosphorylation reduces the methyltransferase activity of EZH2 by disrupting PRC2 assemblies [138]. However, phosphomimic change at the corresponding mouse Ezh2 residue (T487D) does not seem to have an effect on methyltransferase activity nor on PRC2 assembly [137]. In another study, Wu and Zhang [139] reported that CDK1-mediated phosphorylation at T350 and T492 promotes EZH2 ubiquitinylation and subsequent degradation of the protein by the proteasome. These discrepancies might reflect that distinct regulatory mechanisms control EZH2 activity in different cell types.

Finally, recent experiments suggest that glycosylation may also affect EZH2 stability and H3K27me3 levels [140]. EZH2 interacts with O-linked N-acetylglucosamine (GlcNAc) transferase (OGT) and the methyltransferase is O-GlcNAcylated at serine 75 (S75) in vivo. Furthermore, OGT knockdown specifically downregulates EZH2 protein stability and greatly reduces H3K27me3 levels. EZH2-S75A mutants also exhibit a reduction in stability. This OGT-EZH2 axis might then explain in part how the dysregulation of OGT is implicated in cancer.

Involvement of EZH2 in cancer stem cell biology

According to the cancer stem cell (CSC) hypothesis, the CSCs which represent a small fraction of the tumor population are the only tumor-initiating clones. CSCs have unlimited self-renewal capacities and the ability to differentiate in many cancer cell types. They are resistant to chemotherapy [141] and thought to be responsible for metastatic spreading [142]. Thus, deciphering the cellular regulations involved in CSC biology is central to the development of efficient anti-cancer opportunities.

EZH2 is a crucial factor playing a role in the maintenance of self-renewal of adult and embryonic stem cells [13, 143, 144], while EZH2 expression is reduced in differentiated cells. Since EZH2 overexpression is linked to cancer initiation and metastasis and considering that high-grade tumors are enriched with a high content of CSCs, it is proposed that EZH2 expression could favor the transition of dormant progenitors and/or differentiated cells into a more aggressive stem-cell like phenotype. Indeed, EZH2 expression has been shown to be involved in breast CSCs formation and expansion that promote cancer progression [76]. Similarly, EZH2 expression is crucial for glioblastoma CSC self-renewal and tumor-initiating capacity [145-147].

Anti-cancer strategies targeting EZH2

Numerous experimental evidences indicate that EZH2 plays a prominent role in carcinogenesis, from tumor initiation to metastasis, in various cancer types. EZH2 has thus emerged as a potential cancer therapeutic target. Indeed, knock-down of EZH2 inhibits cancer cell proliferation and decreases in vivo tumor growth in xenograft models [148, 149].

The search for pharmacological inhibitors of EZH2 activity also yielded several promising molecules. Among them, 3-deazaneplanocin A (DNZep) has been shown to exhibit significant anti-tumor activity against different cancer

models, such as breast, prostate, lung, brain, colorectal and liver cancer cells [150-152]. Indeed, DNZep treatment reduces EZH2 protein and H3K27me3 levels, reactivates PRC2 target genes and is responsible for apoptosis of cancer cells, but not of normal cells [150]. Interestingly, DNZep has also been shown to abrogate self-renewal and tumor-initiating capacities of glioblastoma cancer stem cells, ovarian cancer stem cell-like populations and prostate cancer stem cells [59, 146, 153, 154]. However, DNZep is an S-adenosylhomocysteine (SAH) hydrolase inhibitor causing intracellular SAH levels to increase. Accumulation of SAH subsequently inhibits diverse methyltransferases including EZH2, by a feed-back loop mechanism (**Figure 4**). DNZep is therefore not selective and specific to EZH2 [155, 156].

More recently, high-throughput screens performed with the PRC2 complex led to the discovery of potent compounds that selectively inhibit EZH2 enzymatic activity [157-161]. These molecules including EPZ005687, EPZ-6438, EI1, UNC1999 and GSK126, all act as S-adenosylmethionine (SAM) competitive inhibitors (**Figure 4**).

The compound EPZ005687 has an equilibrium dissociation constant (Ki) value of 24 nM and has a 50-fold selectivity for EZH2 against the closely related EZH1 enzyme and more than 500-fold selectivity against 15 other protein methyltransferases [157]. Interestingly, EPZ-005687 can also inhibit the H3K27 methylation activities of lymphoma cells harboring heterozygous EZH2-Y641 and EZH2-A677 mutations, killing these cells with minimal effects on the proliferation of wild-type cells [157]. EPZ-6438, with a Ki value of 2.5 nM, possesses a superior potency and drug-like properties, including good oral bioavailability in EZH2-mutant xenograft mice models [158]. The molecule is currently undergoing a phase I clinical trial in patients with advanced solid tumors or refractory B-cell lymphoma [158].

EI1 (Ki of about 13 nM), UNC1999 an orally bioavailable compound in mice, but non-selective for EZH1 and GSK126, the most potent EZH2 inhibitor (Ki of about 0.5-3 nM) [159-161], as well as EPZ005687 and EPZ-6438, all bind to the SAM pocket of the EZH2 catalytic SET domain and selectively inhibit its H3K27 methyltransferase activity. However, the inhibitors are only effective in killing cell lines harboring gain-of-function EZH2 mutations (EZH2-Y641, EZH2-A677), although all of these compounds induce a decrease in H3K27me3 levels in both EZH2-mutated and wild-type cancer cells. This indicates that the SAM competitive inhibitors may be more beneficial to patients with lymphoma rather than with other cancer types.

Since the enzymatic activity of EZH2 requires its association with other components of the PRC2 complex, such as EED and SUZ12, a strategy disrupting PRC2 assembly has been successfully developed to inhibit EZH2 methyltransferase activity [162]. A hydrocarbon-stapled peptide that mimics the α-helical EED-binding domain of EZH2 (SAH-EZH2 peptide) was shown to disrupt the interaction between EZH2 and EED (**Figure 4**). Indeed, the SAH-EZH2 peptide reduces the H3K27 methyltransferase activity of the PRC2 complex and leads to growth arrest and differentiation of MLL-AF9 leukemia cells which are dependent on PRC2 activity. The antiproliferative activity of SAH-EZH2 was also extended to EZH2-dependent B-cell lymphomas; the inhibitory peptide reduces H3K27 trimethylation, EZH2 protein levels, as well as cancer cell viability. Thus, disruption of the PRC2 complex may represent an alternative and complementary strategy for selectively arresting the proliferation of at least some EZH2-dependent cancers. It is also worth noting that the SAH-EZH2 peptide also dissociates EZH1-EED complexes, in addition to EZH2-EED assemblies [162].

In addition, some natural chemopreventive agents have been shown to effectively inhibit EZH2 and reactivate PRC2-silenced target genes in various cancer models. These compounds include epigallocatechin-3-gallate (EGCG), a major component of green tea and dietary omega-3 polyunsaturated fatty acids (omega-3 PUFAs) which both reduce EZH2 levels by increasing proteasomal degradation [163, 164]. Curcumin, a natural ingredient of turmeric also decreases cancer proliferation by modulating EZH2 levels, but in that case down-regulation of EZH2 expression is mediated by the MAPK pathway rather than by the increase of protein degradation [165].

Conclusion

A large set of experimental data have established that EZH2 acts as a key player in tumori-

genesis. However, the molecular mechanisms involved in EZH2 dysregulation in cancer appear to be diverse. EZH2 overexpression is mainly found in solid tumors and activating mutations are found in B-cell lymphomas while inactivating mutations are often identified in myelodysplastic syndromes and myeloproliferative neoplasms. Finally a missense mutation in the gene encoding histone H3.3 (H3F3A) inhibiting EZH2 activity is present at high frequencies in pediatric gliomas. Thus, EZH2 functions as an oncogene in solid tumors and lymphomas whereas it behaves like a tumor suppressor gene in myeloid disorders and in pediatric glioblastomas. The oncogenic role of EZH2 mainly depends on its ability to repress gene expression programs via H3K27 methylation and chromatin compaction. However, studies in certain cancers revealed that the oncogenic function of EZH2 could also result of its action as a PRC2-independent transcriptional activator. Then, EZH2 could be involved in cancer through multiple mechanisms and could be regulated by different pathways depending on cellular context and cancer type. A better understanding of the regulatory network involving EZH2 is consequently required for the development of novel anti-cancer therapeutic strategies.

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Disclosure of conflict of interest

None to disclose.

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