

REVIEW ARTICLE

The noncanonical role of EZH2 in cancer

Jinhua Huang¹ | Hongwei Gou¹ | Jia Yao¹ | Kaining Yi¹ | Zhigang Jin¹ |
 Masao Matsuoka^{2,3}  | Tiejun Zhao^{1,3} 

¹College of Chemistry and Life Sciences, Zhejiang Normal University, Jinhua, China

²Department of Hematology, Rheumatology, and Infectious Disease, Graduate School of Medical Sciences, Faculty of Life Sciences, Kumamoto University, Kumamoto, Japan

³Laboratory of Virus Control, Institute for Frontier Life and Medical Sciences, Kyoto University, Kyoto, Japan

Correspondence

Tiejun Zhao, College of Chemistry and Life Sciences, Zhejiang Normal University, 688 Yingbin Road, Jinhua, Zhejiang 321004, China.

Email: tjzhao@zjnu.cn

Funding information

National Natural Science Foundation of China, Grant/Award Number: 31970173 and 31970755; Natural Science Foundation of Zhejiang Province, Grant/Award Number: LY21C010001 and LY21C120001

Abstract

Enhancer of zeste homolog 2 (EZH2) is the catalytic subunit of polycomb repressive complex 2 (PRC2). Dysregulation of EZH2 causes alteration of gene expression and functions, thereby promoting cancer development. The regulatory function of EZH2 varies across different tumor types. The canonical role of EZH2 is gene silencing through catalyzing the trimethylation of lysine 27 of histone H3 (H3K27me3) in a PRC2-dependent manner. Accumulating evidence indicates that EZH2 has an H3K27me3-independent function as a transcriptional coactivator and plays a critical role in cancer initiation, development, and progression. In this review, we summarize the regulation and function of EZH2 and focus on the current understanding of the noncanonical role of EZH2 in cancer.

KEYWORDS

cancer, carcinogenesis, EZH2, H3K27me3, PRC2

1 | INTRODUCTION

The polycomb group (PcG) proteins were first identified in *Drosophila* as regulatory factors that transcriptionally silence the expression of the bithorax homeobox (Hox) gene cluster.¹ It has been reported that PcG proteins interact with each other and generate two major complexes, polycomb repressive complexes 1 and 2 (PRC1 and PRC2).² PcG complexes play a synergistic role in the transcriptional suppression of target genes through methylation of lysine residues in the histone tails. PRC2 is a highly conserved complex in many species of plants and animals. It is mainly composed of four subunits: EZH2, EED, SUZ12, and RbAp46/48 (Figure 1A).³ Enhancer of zeste homolog 2 (EZH2), the core catalytic subunit of the PRC2 complex, plays a critical role in regulating a wide range of biological processes, including tumor development and malignancy, stem cell renewal and

development, immune response, and cell senescence.⁴ Numerous studies have discovered that EZH2 functions together with EED and SUZ12 and induces gene silencing mainly through trimethylation of histone H3 lysine 27 (H3K27me3).⁵⁻⁷ Recently, additional findings supporting a PRC2-independent role for EZH2 have emerged.^{8,9}

2 | EZH2 AND ITS EXPRESSION IN CANCER

Human *EZH2* gene is located on the long arm of chromosome 7 at 7q35. It contains 20 exons and encodes a protein composed of 746 amino acids.¹⁰ Sequence analysis revealed that EZH family proteins contain four homologous domains, including homologous domain I (H1 domain), homologous domain II (H2 domain), cysteine-rich

Huang and Gou contributed equally to this work.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2021 The Authors. Cancer Science published by John Wiley & Sons Australia, Ltd on behalf of Japanese Cancer Association.

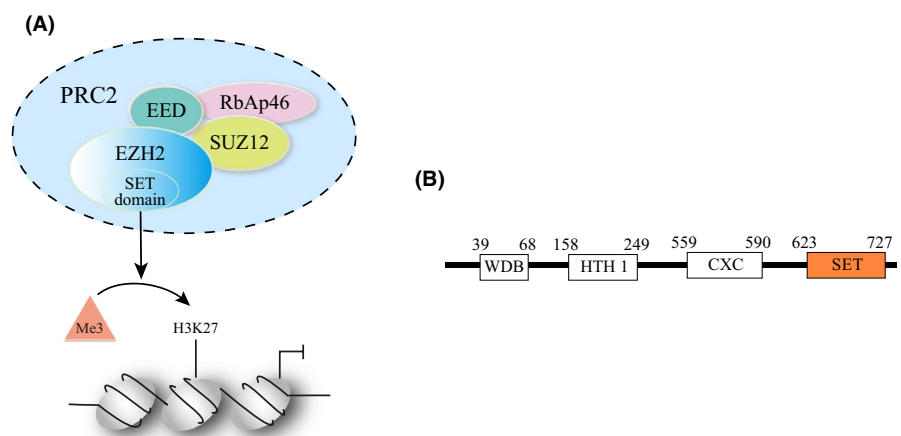
domain, and C-terminal SET domain.¹¹ EZH2 is the core catalytic subunit of PRC2, and its SET domain at the C-terminus provides the histone methyltransferase activity (Figure 1B). When the PRC2 complex is formed and recruited to the promoter region of the target genes, the SET domain of EZH2 catalyzes H3K27me₃, leading to silencing its target genes involved in cell proliferation, cell differentiation, and cancer development.¹²

A series of studies have shown that overexpression and mutation of EZH2 were observed in many human cancers, including breast cancer, prostate cancer, endometrial cancer, melanoma, bladder cancer, colon cancer, liver cancer, lung cancer, lymphoma, etc. (Table 1).^{13–20} Overexpression of EZH2 can lead to disruptions in cell proliferation, apoptosis, migration, and invasion. High level of EZH2 is also correlated with poor prognosis in cancers.^{13,14} In addition, activating or inactivating mutations of EZH2 have been identified in a variety of cancers (Table 1).^{21–25} Point mutations at tyrosine 641 in the catalytic SET domain of EZH2 occur in diffuse large B-cell lymphomas and follicular lymphomas. Y641 mutation is proved to be a gain-of-function mutation of EZH2, which leads to the increase of the transformation from H3K27 to trimethylated form.^{21,22} Inactivating mutations of EZH2, such as deletion, nonsense and missense mutations, and frameshift, emerge in myelodysplastic syndrome (MDS), myeloproliferative neoplasms (MPN), and human T-cell acute lymphoblastic leukemia (T-ALL). It results in the loss-of-function of EZH2, and such mutated EZH2 functions as a tumor suppressor in cancer.^{24,25}

3 | METHYLASE-DEPENDENT FUNCTIONS OF EZH2 IN CANCER

EZH2 plays a vital role in epigenetic gene silencing, which usually depends on its histone methyltransferase activity. Numerous studies showed that EZH2 functions as the catalytic subunit of the PRC2 complex to catalyze H3K27me₃. Then the PRC binds to H3K27me₃ and lysine 119 of monoubiquitinated histone H2 (H2AK119-ub1) to form a ternary complex. The complex acts as a transcriptional inhibitor to mediate chromatin compaction, resulting in the silencing of its downstream target genes.²⁶

FIGURE 1 The polycomb repressive complex 2 (PRC2) complex composition, regulation function, and schematic diagram of the enhancer of zeste homolog 2 (EZH2) domains. A, The PRC2 complex contains four core subunits which include EZH2, EED, SUZ12, and RbAp46/48. PRC2 induces target genes' transcriptional repression through EZH2-mediated H3K27 trimethylation. B, EZH2 has four domains: WD-binding domain; PRC2 HTH 1 domain, CXC domain, and SET domain



The canonical role of EZH2 is mainly to silence multiple tumor suppressor genes through its histone lysine methyltransferase activity (Figure 2A). The EZH2 target genes involved in cell proliferation, cell apoptosis, and cell cycle regulation include *INK4B-ARF-INK4A*, *Bim*, *TRAIL*, *KLF2*, *MSMB*, *FOXC1*, *hDAB2IP*, etc.²⁰ In addition, EZH2 harboring an activation point mutation at residue Y641, A677, or A687 has enhanced methyltransferase activity, which results in the increase of H3K27me₃ levels (Figure 2B).^{21,22,27,28} EZH2 point mutations at residues A677 and A687 were found in non-Hodgkin's lymphoma (NHL), leading to hypertrimethylation of H3K27. The long noncoding RNAs (lncRNAs) also participate in the EZH2-mediated epigenetic regulation. Luo et al found that upregulated lncRNA H19 enhanced the metastasis of bladder cancer by binding to EZH2, and the EZH2/H19 complex subsequently inhibited the expression of E-cadherin by H3K27me₃ (Figure 2C).²⁹

A series of studies have shown that EZH2 may cooperate with other epigenetic regulators, such as DNA methyltransferases (DNMTs) and histone deacetylases (HDACs), to fulfill its epigenetic silencing function.^{30–32} Studies reported that HDAC deacetylate the lysine at H3K27 or H3K29, H3K14, and H4K8, and the ϵ -amino group of the lysine side chain is involved in the methylation process of EZH2 (Figure 2D).³³ In addition, the EZH2 could directly interact and cooperate with DNMT to control CpG methylation and silence gene transcription (Figure 2E).³² During cellular transformation, EZH2 participated in the CpG-hypermethylation modification of certain genes, thereby inducing tumorigenesis.³³

4 | PRC2-INDEPENDENT ROLES OF EZH2 IN CANCER

4.1 | EZH2 act as transcriptional activator/coactivator

Accumulating evidence shows that the function of EZH2 is independent of its role as a PRC2-associated repressor, and it acts as a transcriptional coactivator in various tumors (Figure 3A).^{34,35} In endocrine-related cancer such as breast cancer, EZH2 could activate

Status	Associated cancer type	Reference
Overexpression	Breast, prostate, endometrial, melanoma, bladder, colon, liver, lung, and lymphoma	13-20
Activating mutations	Diffuse large B-cell lymphoma and follicular lymphoma	21,22
Inactivating mutations	MDS, MPN, T-ALL	24,25

Abbreviations: MDS, myelodysplastic syndrome; MPN, myeloproliferative neoplasms; T-cell acute lymphoblastic leukemia, T-ALL.

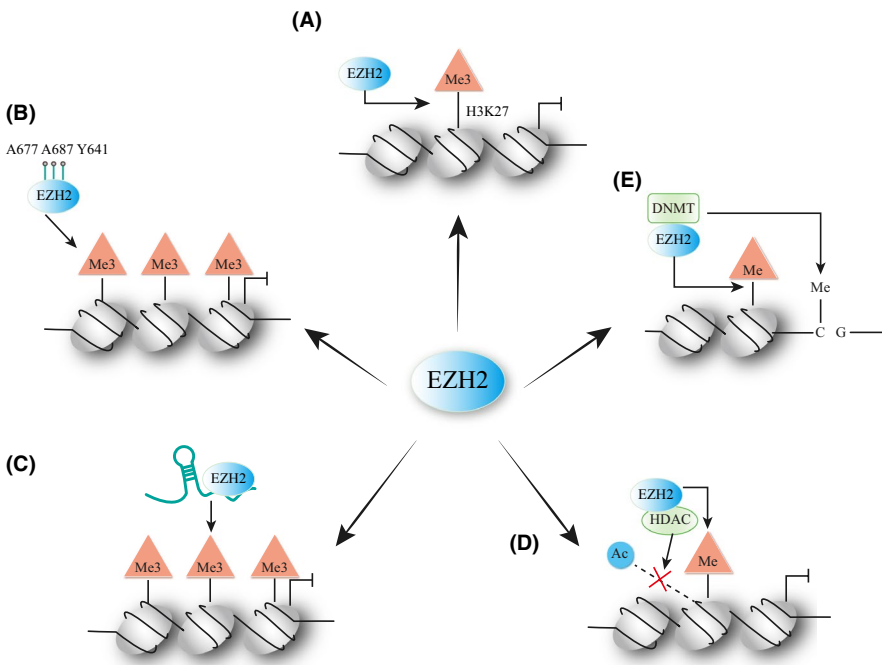


FIGURE 2 Schematic representation of the methylase-dependent functions of enhancer of zeste homolog 2 (EZH2). A, EZH2 catalyzes lysine 27 of histone H3 trimethylation (H3K27me3) and silences its target genes. B, EZH2 with gain-of-function mutations at residues Y641, A677, or A687 results in the increase of H3K27me3 levels. C, EZH2 can be recruited by long noncoding RNA (lncRNA) to its target genes and increases H3K27me3 levels via EZH2-lncRNA interaction. D, Histone deacetylase (HDAC) deacetylates H3K27 by cooperating with EZH2 so that the ϵ -amino group can be methylated by EZH2. E, EZH2 recruits DNA methyltransferase (DNMT), which methylates H3K27 of CpG DNA, leading to the silencing of related target genes

gene transcription and promote the proliferation of tumor cells independently of its methyltransferase activity.³⁵⁻³⁷ However, the molecular mechanisms differ depending on the type of cancer. In the ER⁺ breast cancer cell line MCF-7, domain I (amino acid 1-159) of EZH2 directly interacts with estrogen receptor alpha (ER α) and β -catenin to form a ternary complex. EZH2 exerts its transactivation activity to activate the promoter of c-Myc and cyclin D1 through recruiting the EZH2/ER α / β -catenin complex to its TCF/ β -catenin-binding sites.³⁵ EZH2 could also enhance the transcription of c-Myc and cyclin D1 via recruitment of EZH2 to the T-cell-specific transcription factor (TCF)/lymphoid enhancer-binding factor (LEF) binding sites in the c-Myc and cyclin D1 promoters, and this transcriptional activation is independent of H3K27me3.³⁶ In addition, EZH2 interacts with Trim28 and chromatin remodeling protein SWI/SNF and activates the expression of CXCR4 in a PRC2-independent way, resulting in promotion of mammary stem cell enrichment and maintenance.³⁸ In ER-negative basal-like breast cancer cells, EZH2 forms a ternary complex with NF- κ B subunit RelA and RelB, and activates the transcription of NF- κ B target genes such as IL6 and TNF through recruiting the EZH2/RelA/RelB complex to their promoters.³⁴ Moreover, in chronic lymphocytic leukemia (CLL) cases, EZH2

recruits MYC protein and binds to the *IGF1R* promoter to activate the transcription of *IGF1R* gene in a PRC2-independent manner, leading to the activation of its downstream PI3K/AKT signaling.³⁹ In adrenocortical carcinoma (ACC), EZH2 cooperates with transcription factor E2F1 to upregulate the expression of aggression-related genes, such as RRM2, PTTG1, and ASE1/PRC1, in a histone methyltransferase-independent manner, thereby promoting the development of ACC.⁴⁰ Avni et al reported that polycomb proteins, such as YY1, Mel-18, Ring1A, EZH2, and EED, are recruited to the promoter of interleukin-4 (IL-4) and interferon- γ (IFN- γ), and induce the transcription of IL-4 and IFN- γ cytokines in differentiated T helper cells.⁴¹ It has been reported that EZH2 can activate oncogenic signaling pathways through its binding proteins. PCNA-associated factor (PAF) is overexpressed in colon cancer, and it promotes the proliferation of cancer cells by activating the Wnt signaling pathway. Jung et al⁴² identified a protein complex composed of PAF, EZH2, and β -catenin in colon cancer cells. When the Wnt pathway is activated, PAF dissociates from PCNA and binds to β -catenin. Then, it recruits EZH2 to form a ternary complex, leading to the binding and activation of the Wnt signaling. In addition, the highly activated hypoxia-inducible factor 1- α (HIF1- α) in triple-negative breast cancer cells (TNBCs)

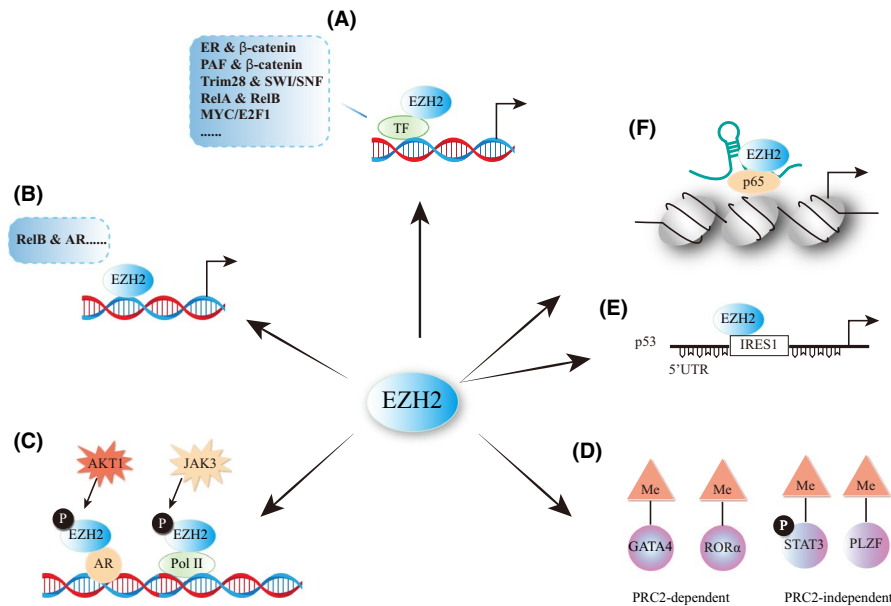


FIGURE 3 The molecular mechanisms of the role of enhancer of zeste homolog 2 (EZH2) in a polycomb repressive complex 2 (PRC2)-independent manner. A, EZH2 acts as a transcriptional coactivator by forming complexes with other transcription factors. B, EZH2 acts as a transcription factor via directly binding to the target genes' promoter. C, The phosphorylation of EZH2 turns it from a chromatin silencer to a transcriptional coactivator. D, EZH2 can methylate or phosphorylate nonhistone substrates to regulate gene silencing and transactivation in a PRC2-dependent or -independent manner. E, EZH2 binds to internal ribosome entry site 1 (IRES1) of p53 mRNA to promote the expression of p53 protein. F, EZH2 forms a complex with long noncoding RNA (lncRNA) ANRIL and p65 to activate the NF- κ B pathway

could inhibit PRC2. It led to the formation of a complex of EZH2 and forkhead box M1 (FoxM1), and occupied the promoter of matrix metalloproteinase gene (MMPs). Finally, EZH2/FoxM1 coactivates the expression of MMPs and promotes the invasion of TNBCs, independently of PRC2.⁴³

In addition to its role as a transcriptional coactivator through forming a complex with other proteins, EZH2 also acts as a transcription factor via directly binding to the target genes' promoter (Figure 3B). Lawrence et al found that EZH2 could bind to the promoter of RelB as a transcriptional activator in TNBCs and activated RelB transcription, thereby maintaining the self-renewal of breast cancer tumor-initiating cells (TNBC-TICs).⁴⁴ Moreover, Kim et al reported that EZH2 directly binds to the promoter of androgen receptor (AR) gene. It induces the transcription of AR and its downstream target genes, such as PSA, TMPRSS2, and FKBP5, in a PRC2-independent manner, which results in the development of prostate cancer.⁴⁵ Natural killer/T-cell lymphoma (NKTL) is a highly aggressive malignancy of NK cells.⁴⁶ EZH2 promotes proliferation of NKTL cells by enhancing the transcription of *Cyclin D1* gene, which is independent of its histone methyltransferase activity. Meanwhile, the EZH2 mutant deficient for histone methyltransferase activity is still able to promote cell growth.¹⁹

A series of studies have demonstrated that biochemical modification of EZH2 induced the dissociation from the PRC2 complex and converted EZH2 from a transcriptional repressor into a transcription activator in a PRC2-independent manner (Figure 3C). Yan et al found that JAK3 phosphorylated EZH2 in NKTL and then induced the release of EZH2 from the PRC2 complex, resulting in the

downregulation of the global H3K27me3 levels in lymphoma cells. The free EZH2 soon formed a complex with pol II and activated the expression of genes related to DNA replication, cell cycle regulation, and tumor cell invasion, leading to the development of NKTL.⁴⁷ Meanwhile, the threonine residues at position 367 of EZH2 could be phosphorylated by p38 α in invasive ER-breast cancer cells and led to the cytoplasmic localization of EZH2. Cytoplasmic pEZH2 (T367) potentiated a PRC2-independent interaction with cytoskeletal regulatory proteins and promoted breast cancer metastasis.⁴⁸ In castration-resistant prostate cancer (CRPC), EZH2 acts as a transcriptional activator to induce carcinogenesis. Xu et al reported that Ser21 of EZH2 was phosphorylated by AKT1. After phosphorylation, EZH2 formed a complex with AR and exerted its oncogenic functions in prostate cancer, independently of its polycomb repressive activity.⁴⁹

4.2 | Modification of nonhistone substrates by EZH2

EZH2 can also methylate or phosphorylate nonhistone substrates to regulate multiple cellular activities in a PRC2-dependent or -independent manner (Figure 3D). It has been reported that EZH2 directly methylated transcription factors such as GATA4 and ROR α .^{50,51} However, the underlying mechanism and function were unknown. Ji Min Lee et al screened the proteins which have a similar sequence to the domain methylated in histone H3K27 and named the amino acid sequence "R-K-S". Studies showed that EZH2 could

induce the methylation of lysine in the R-K-S sequence of the ROR α transcription factor. Subsequently, methylated ROR α was recognized by the DDB1/DCAF1/CUL4 ubiquitin ligase complex, leading to ubiquitination and degradation of the tumor suppressor protein ROR α .⁵¹ Zheng et al found that overexpression of EZH2 in oral squamous cell carcinoma (OSCC) patients enhances cell migration and invasion, as well as glycolysis-mediated epithelial-mesenchymal transition (EMT). Further studies indicated that ectopic overexpression of EZH2 elevated the phosphorylation of STAT3 at the 705 residue, downregulated FoxO1 expression, and thereby promoted tumor growth and glycolysis of OSCC.⁵² Similarly, AKT1-mediated phosphorylation of EZH2 at Ser21 in glioblastoma allows EZH2 to bind and induce methylation of STAT3 at K180 in a PRC2-independent manner. It thus enhances the activity of STAT3 by increasing phosphorylation of STAT3 at Y705 in glioma stem cells (GSCs), thereby promoting gene transcription and clonogenic growth of GSCs.⁵³ In addition, PLZF is an important transcription factor which regulates the function of NKT cells. EZH2 directly induces the methylation of PLZF protein, which leads to its ubiquitination and degradation. It is thus indicated that EZH2 may be involved in controlling the development of NKT cells and maintaining immune homeostasis.⁵⁴

4.3 | EZH2 acts as RNA-binding protein

EZH2 has been shown to interact with lncRNAs or mRNAs to promote the expression of oncogenes. A recent study indicated that EZH2 directly bound to the internal ribosome entry site 1 (IRES1) of p53 mRNA 5'UTR (Figure 3E). It finally enhanced the stability of p53 mRNA and promoted the translation of p53 protein. In addition, EZH2 elevated the expression of p53 GOF mutant protein in a histone methyltransferase-independent manner, leading to the growth and metastasis of cancer cells.⁵⁵ We previously reported that the expression of lncRNA ANRIL was significantly enhanced in HTLV-1-infected cell lines and clinical adult T-cell leukemia (ATL) samples. Knockdown of ANRIL inhibited the proliferation of ATL cells and induced cell apoptosis. A further study revealed that ANRIL associated with EZH2 and p65 protein to form a ternary complex, which finally resulted in the activation of the NF- κ B signaling pathway in an EZH2 methyltransferase-independent manner (Figure 3F).⁵⁶

4.4 | Regulatory function of EZH2 at other cellular levels

In addition to the regulation at transcriptional level, EZH2 also functions in other manners. A recent study has shown that EZH2 has a noncatalytic function in overcoming cisplatin resistance in small cell lung cancer (SCLC) by promoting nucleotide excision repair (NER). Further experiments demonstrated that EZH2 interacted with DDB1-DDB2 and stabilized DDB2 by inhibiting its ubiquitination, thereby promoting DDB2 localization to cyclobutane pyrimidine

dimer (CPD) crosslinks to govern their repair. Such an effect was independent of the methyltransferase activity of EZH2.

In addition to its known roles in transcriptional activation, EZH2 may also silence gene expression by regulating gene promoter activity, resulting in inactivation of tumor suppressor. In MYC-driven prostate cancer, EZH2 inhibits the expression of IFNGR1 by binding to the promoter of interferon- γ receptor 1 (IFNGR1). This leads to the suppression of the IFN-JAK-STAT1 signaling pathway thereby promoting cancer development. Further research confirmed that the function of EZH2 does not depend on its H3K27 methylase activity.⁵⁸

In short, numerous evidences support the hypothesis that EZH2 acts as a "bifunctional molecule". In different organisms or in different tissues, EZH2 and other subunits of the PRC2 complex exhibit distinct patterns of behavior in different cancer types. It is suggested that EZH2 could act as a transcriptional inhibitor or a transcriptional activator.⁵⁹

5 | EZH2 AS A CANCER THERAPEUTIC TARGET

Given that EZH2 plays a vital role in cancer cell proliferation, migration, invasion, and other cellular processes, EZH2 is considered to be a potential therapeutic target in cancer. The anticancer therapy which targets EZH2 is mainly achieved by inhibiting the methyltransferase activity of EZH2. S-adenosyl methionine (SAM) is a universal methyl group donor which carries an activated methyl and participates in biological methylation reactions.⁶⁰ Multiple EZH2-specific SAM-competitive inhibitors, such as GSK126, GSK343, GSK926, EI1, EPZ-005687, EPZ-011989, EPZ-6438, UNC-1999, and CPI-169, have been developed.²⁰

However, Kim et al reported that EZH2 enzymatic inhibitors could not completely suppress the oncogenic activity of EZH2 in SWI/SNF mutant cancers unless they were capable of disrupting the protein interactions of the PRC2 complex.⁶¹ It indicated that dependence upon EZH2 for cancer progression can be derived from both methylase-dependent and methylase-independent functions of EZH2. Thus, further research is required for the development of the novel EZH2 inhibitors for the blockade of the catalytic and noncatalytic activity of EZH2. Recently, Zhou et al performed two high-throughput screening (HTS) campaigns targeting the catalytic and noncatalytic activity of EZH2. They identified several compounds which can efficiently act against the catalytic EZH2 Y641F mutant (gain-of-function mutation of EZH2) or EZH2-EED interaction, respectively.⁶²

3-deazaneplanocin A (DZNep), which is an inhibitor of S-adenosylhomocysteine, acts as a competitive EZH2 inhibitor. At present, DZNep is widely used in *in vivo* studies and shows effective anticancer activities in a variety of cancers. Tan et al reported that DZNep could effectively deplete cellular levels of PRC2 component EZH2, which led to apoptotic cell death in cancer cells.⁶³ Novel strategies to inhibit EZH2 by protein degradation have been

developed. Proteolysis-targeted chimeras (PROTACs) are molecules designed to recruit a target protein to an E3 ligase, thereby inducing ubiquitination and degradation of specific pathological proteins.⁶⁴ Accumulated PROTAC compounds targeting EZH2 for degradation, such as AM29-117A, MS1943, PROTAC 1, and GNA002, have been developed.⁶⁵ Using these compounds, researchers claimed to induce the degradation of EZH2, resulting in broad and striking effects on cancer cell growth. Thus, it is expected that multiple approaches which target both the catalytic and noncatalytic activity of EZH2 represent a wealth of therapeutic options for cancer patients.

6 | CONCLUSION

Numerous studies demonstrated that overexpression of EZH2 is associated with the invasive growth and poor prognosis of many human cancers. EZH2 participates in gene expression regulation mainly through epigenetic machinery. Studies in the last decade have shown that EZH2 could also act as a PRC2-independent transcriptional activator. Herein, we summarized multiple methylation-independent functions of EZH2 in cancer, including transcriptional activation, modification of nonhistone substrates, and post-transcriptional regulation. Moreover, the diverse involvement of EZH2 is mainly dependent on cellular context and cancer types.

Therefore, future studies focusing on the regulation of EZH2 will provide insights into pathogenesis and are necessary for the development of novel anticancer therapeutic strategies targeting EZH2 in a variety of human cancers. Given the emerging noncanonical role of EZH2, it will be important to develop drugs that depend on its enzymatic activity and nonenzymatic function.

ACKNOWLEDGMENTS

This work was supported by grants from the National Natural Science Foundation of China to TZ (No. 31970173) and ZJ (No. 31970755), and grants from the Natural Science Foundation of Zhejiang Province to TZ (No. LY21C010001) and ZJ (No. LY21C120001).

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

ORCID

Masao Matsuoka  <https://orcid.org/0000-0002-0473-754X>

Tiejun Zhao  <https://orcid.org/0000-0002-8266-8076>

REFERENCES

- Morey L, Helin K. Polycomb group protein-mediated repression of transcription. *Trends Biochem Sci*. 2010;35:323-332.
- Sauvageau M, Sauvageau G. Polycomb group proteins: Multifaceted Regulators of Somatic Stem Cells and Cancer. *Cell Stem Cell*. 2010;7:299-313.
- O'Meara MM, Simon JA. Inner workings and regulatory inputs that control Polycomb repressive complex 2. *Chromosoma*. 2012;121:221-234.
- Han Li C, Chen Y. Targeting EZH2 for cancer therapy: progress and perspective. *Curr Protein Pept Sci*. 2015;16:559-570.
- Simon JA, Kingston RE. Mechanisms of polycomb gene silencing: knowns and unknowns. *Nat Rev Mol Cell Biol*. 2009;10:697-708.
- Cao R, Wang L, Wang H, et al. Role of histone H3 lysine 27 methylation in polycomb-group silencing. *Science*. 2002;298:1039-1043.
- Cao R, Zhang Y. SUZ12 is required for both the histone methyltransferase activity and the silencing function of the EED-EZH2 complex. *Mol Cell*. 2004;15:57-67.
- Völkel P, Dupret B, Le Bourhis X, Angrand P-O. Diverse involvement of EZH2 in cancer epigenetics. *Am J Transl Res*. 2015;7:175-193.
- Yamaguchi H, Hung MC. Regulation and Role of EZH2 in Cancer. *Cancer Res Treat*. 2014;46:209-222.
- Cardoso C, Mignon C, Hetet G, Grandchamps B, Fontes M, Colleaux L. The human *EZH2* gene: genomic organisation and revised mapping in 7q35 within the critical region for malignant myeloid disorders. *Eur J Hum Genet*. 2000;8:174-180.
- Margueron R, Li G, Sarma K, et al. Ezh1 and Ezh2 maintain repressive chromatin through different mechanisms. *Mol Cell*. 2008;32:503-518.
- Christofides A, Karantanos T, Bardhan K, Boussiotis VA. Epigenetic regulation of cancer biology and anti-tumor immunity by EZH2. *Oncotarget*. 2016;7:85624-85640.
- Kleer CG, Cao Q, Varambally S, et al. EZH2 is a marker of aggressive breast cancer and promotes neoplastic transformation of breast epithelial cells. *Proc Natl Acad Sci USA*. 2003;100:11606-11611.
- Cebria F, Kobayashi C, Umehara Y, et al. The polycomb group protein EZH2 is involved in progression of prostate cancer. *Nature*. 2002;419:620-624.
- Bachmann IM, Halvorsen OJ, Collett K, et al. EZH2 expression is associated with high proliferation rate and aggressive tumor subgroups in cutaneous melanoma and cancers of the endometrium, prostate, and breast. *J Clin Oncol*. 2006;24:268-273.
- Arisan S, Buyuktuncer ED, Palavan-Unsal N, Caskurlu T, Cakir OO, Ergenekon E. Increased expression of EZH2, a polycomb group protein, in bladder carcinoma. *Urol Int*. 2005;75:252-257.
- Ferraro A, Boni T, Pintzas A. EZH2 regulates cofilin activity and colon cancer cell migration by targeting ITGA2 gene. *PLoS One*. 2014;9:e115276.
- Zhang H, Qi J, Reyes JM, et al. Oncogenic deregulation of EZH2 as an opportunity for targeted therapy in lung cancer. *Cancer Discov*. 2016;6:1006-1021.
- Yan J, Ng SB, Tay JL, et al. EZH2 overexpression in natural killer/T-cell lymphoma confers growth advantage independently of histone methyltransferase activity. *Blood*. 2013;121:4512-4520.
- Kim KH, Roberts CW. Targeting EZH2 in cancer. *Nat Med*. 2016;22:128-134.
- Morin RD, Johnson NA, Severson TM, et al. Somatic mutations of EZH2 (Y641) in follicular and diffuse large B-cell lymphomas of germinal-center origin. *Nat Genet*. 2010;42:181-185.
- Bodor C, O'Riain C, Wrench D, et al. EZH2 Y641 mutations in follicular lymphoma. *Leukemia*. 2011;25:726-729.
- McCabe MT, Ott HM, Ganji G, et al. EZH2 inhibition as a therapeutic strategy for lymphoma with EZH2-activating mutations. *Nature*. 2012;492:108-112.
- Ernst T, Chase AJ, Score J, et al. Inactivating mutations of the histone methyltransferase gene *EZH2* in myeloid disorders. *Nat Genet*. 2010;42:722-726.
- Nikoloski G, Langemeijer SM, Kuiper RP, et al. Somatic mutations of the histone methyltransferase gene *EZH2* in myelodysplastic syndromes. *Nat Genet*. 2010;42:665-667.
- Gan L, Yang Y, Li Q, Feng Y, Liu T, Guo W. Epigenetic regulation of cancer progression by EZH2: from biological insights to therapeutic potential. *Biomark Res*. 2018;6:10.

27. Majer CR, Jin L, Scott MP, et al. A687V EZH2 is a gain-of-function mutation found in lymphoma patients. *FEBS Lett.* 2012;586:3448-3451.
28. McCabe MT, Graves AP, Ganji G, et al. Mutation of A677 in histone methyltransferase EZH2 in human B-cell lymphoma promotes hypermethylation of histone H3 on lysine 27 (H3K27). *Proc Natl Acad Sci USA.* 2012;109:2989-2994.
29. Luo M, Li Z, Wang W, Zeng Y, Liu Z, Qiu J. Long non-coding RNA H19 increases bladder cancer metastasis by associating with EZH2 and inhibiting E-cadherin expression. *Cancer Lett.* 2013;333:213-221.
30. Vlag JVD, Otte AP. Transcriptional repression mediated by the human polycomb-group protein EED involves histone deacetylation. *Nat Genet.* 1999;23:474-478.
31. Tie F, Furuyama T, Prasad-Sinha J, Jane E, Harte PJ. The Drosophila Polycomb Group proteins ESC and E(Z) are present in a complex containing the histone-binding protein p55 and the histone deacetylase RPD3. *Compang Biologist.* 2001;128:275-286.
32. Vire E, Brenner C, Deplus R, et al. The Polycomb group protein EZH2 directly controls DNA methylation. *Nature.* 2006;439:871-874.
33. Simon JA, Lange CA. Roles of the EZH2 histone methyltransferase in cancer epigenetics. *Mutat Res.* 2008;647:21-29.
34. Lee ST, Li Z, Wu Z, et al. Context-specific regulation of NF- κ B target gene expression by EZH2 in a breast cancers. *Mol Cell.* 2011;43:798-810.
35. Shi B, Liang J, Yang X, et al. Integration of estrogen and Wnt signaling circuits by the polycomb group protein EZH2 in breast cancer cells. *Mol Cell Biol.* 2007;27:5105-5119.
36. Deb G, Thakur VS, Gupta S. Multifaceted role of EZH2 in breast and prostate tumorigenesis: epigenetics and beyond. *Epigenetics.* 2013;8:464-476.
37. Chan KM, Fang D, Gan H, et al. The histone H3.3K27M mutation in pediatric glioma reprograms H3K27 methylation and gene expression. *Genes Dev.* 2013;27:985-990.
38. Li J, Xi Y, Li W, et al. TRIM28 interacts with EZH2 and SWI/SNF to activate genes that promote mammosphere formation. *Oncogene.* 2017;36:2991-3001.
39. Kosalai ST, Morsy MHA, Papakonstantinou N, et al. EZH2 up-regulates the PI3K/AKT pathway through IGF1R and MYC in clinically aggressive chronic lymphocytic leukaemia. *Epigenetics.* 2019;14:1125-1140.
40. Tabbal H, Septier A, Mathieu M, et al. EZH2 cooperates with E2F1 to stimulate expression of genes involved in adrenocortical carcinoma aggressiveness. *Br J Cancer.* 2019;121:384-394.
41. Jacob E, Hod-Dvorai R, Schif-Zuck S, Avni O. Unconventional association of the polycomb group proteins with cytokine genes in differentiated T helper cells. *J Biol Chem.* 2008;283:13471-13481.
42. Jung HY, Jun S, Lee M, et al. PAF and EZH2 induce Wnt/ β -catenin signaling hyperactivation. *Mol Cell.* 2013;52:193-205.
43. Mahara S, Lee PL, Feng M, Tergaonkar V, Chng WJ, Yu Q. HIF1- α activation underlies a functional switch in the paradoxical role of Ezh2/PRC2 in breast cancer. *Proc Natl Acad Sci USA.* 2016;113:E3735-E3744.
44. Lawrence CL, Baldwin AS. Non-canonical EZH2 transcriptionally activates RelB in triple negative breast cancer. *PLoS One.* 2016;11:e0165005.
45. Kim J, Lee Y, Lu X, et al. Polycomb- and methylation-independent roles of EZH2 as a transcription activator. *Cell Rep.* 2018;25:2808-2820.
46. Lee J, Suh C, Park YH, et al. Extranodal natural killer T-cell lymphoma, nasal-type: a prognostic model from a retrospective multicenter study. *J Clin Oncol.* 2006;24:612-618.
47. Yan J, Li B, Lin B, et al. EZH2 phosphorylation by JAK3 mediates a switch to noncanonical function in natural killer/T-cell lymphoma. *Blood.* 2016;128:948-958.
48. Anwar T, Arellano-García C, Ropa J, et al. p38-mediated phosphorylation at T367 induces EZH2 cytoplasmic localization to promote breast cancer metastasis. *Nat Commun.* 2018;9:2801.
49. Xu K, Wu ZJ, Groner AC, et al. EZH2 oncogenic activity in castration-resistant prostate cancer cells is polycomb-independent. *Science.* 2012;338:1465-1469.
50. He A, Shen X, Ma Q, et al. PRC2 directly methylates GATA4 and represses its transcriptional activity. *Genes Dev.* 2012;26:37-42.
51. Lee JM, Lee JS, Kim H, et al. EZH2 generates a methyl degron that is recognized by the DCAF1/DDB1/CUL4 E3 ubiquitin ligase complex. *Mol Cell.* 2012;48:572-586.
52. Zheng M, Cao MX, Luo XJ, et al. EZH2 promotes invasion and tumour glycolysis by regulating STAT3 and FoxO1 signalling in human OSCC cells. *J Cell Mol Med.* 2019;23:6942-6954.
53. Kim E, Kim M, Woo DH, et al. Phosphorylation of EZH2 activates STAT3 signaling via STAT3 methylation and promotes tumorigenicity of glioblastoma stem-like cells. *Cancer Cell.* 2013;23:839-852.
54. Vasanthakumar A, Xu D, Lun AT, et al. A non-canonical function of Ezh2 preserves immune homeostasis. *EMBO Rep.* 2017;18:619-631.
55. Zhao Y, Ding L, Wang D, et al. EZH2 cooperates with gain-of-function p53 mutants to promote cancer growth and metastasis. *EMBO J.* 2019;38:e99599.
56. Song Z, Wu W, Chen M, et al. Long noncoding RNA ANRIL supports proliferation of adult T-cell Leukemia cells through cooperation with EZH2. *J Virol.* 2018;92:e00909-e00918.
57. Koyen AE, Madden MZ, Park D, et al. EZH2 has a non-catalytic and PRC2-independent role in stabilizing DDB2 to promote nucleotide excision repair. *Oncogene.* 2020;39:4798-4813.
58. Wee ZN, Li Z, Lee PL, Lee ST, Lim YP, Yu Q. EZH2-mediated inactivation of Irfn- γ -JAK-STAT1 signaling is an effective therapeutic target in MYC-driven prostate cancer. *Cell Rep.* 2014;8:204-216.
59. Yan KS, Lin CY, Liao TW, et al. EZH2 in cancer progression and potential application in cancer therapy: A Friend or Foe? *Int J Mol Sci.* 2017;18(6):1172.
60. Karas KN. S-Adenosyl methionine in the therapy of depression and other psychiatric disorders. *Drug Dev Res.* 2016;77:346-356.
61. Kim KH, Kim W, Howard TP, et al. SWI/SNF-mutant cancers depend on catalytic and non-catalytic activity of EZH2. *Nat Med.* 2015;21:1491-1496.
62. Zhou Y, Du DH, Wang J, et al. Identification of catalytic and non-catalytic activity inhibitors against PRC2-EZH2 complex through multiple high-throughput screening campaigns. *Chem Biol Drug Des.* 2020;96(4):1024-1051.
63. Tan J, Yang X, Zhuang L, et al. Pharmacologic disruption of Polycomb-repressive complex 2-mediated gene repression selectively induces apoptosis in cancer cells. *Genes Dev.* 2007;21:1050-1063.
64. Li X, Song Y. Proteolysis-targeting chimera (PROTAC) for targeted protein degradation and cancer therapy. *J Hematol Oncol.* 2020;13:50.
65. Wimalasena VK, Wang T, Sigua LH, Durbin AD, Qi J. Using chemical epigenetics to target cancer. *Mol Cell.* 2020;78:1086-1095.

How to cite this article: Huang J, Gou H, Yao J, et al. The noncanonical role of EZH2 in cancer. *Cancer Sci.* 2021;112: 1376-1382. <https://doi.org/10.1111/cas.14840>