Cancer Screening and Prevention **2023** vol. 2(2) | 117–129
DOI: 10.14218/CSP.2023.00006

Review Article

The Heightened Importance of EZH2 in Cancer Immunotherapy

Xiao-Hu Lin[1,2,3], Wen-Kai Zhou[1,2,3], Cheng-Zhong Lin[2,3,4*] and Wei Cao[1,2,3,5*]

[1]Department of Oral Maxillofacial-Head and Neck Oncology, Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China; [2]National Center for Stomatology, National Clinical Research Center for Oral Diseases, Shanghai, China; [3]Shanghai Key Laboratory of Stomatology, Shanghai, China; [4]The Second Dental Center, Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China; [5]Shanghai Jiao Tong University School of Nursing, Shanghai, China

Received: March 05, 2023 | **Revised:** May 18, 2023 | **Accepted:** May 26, 2023 | **Published online:** June 20, 2023

Abstract

The transcriptional inhibitor histone methyltransferase enhancer of zeste homolog 2 (EZH2) predominantly targets genes involved in tumor suppression. EZH2 is highly expressed in a variety of human malignancies and promotes carcinogenesis and malignant transformation. Recent research has indicated that altering the tumor microenvironment by focusing on epigenetic variables can improve antitumor immunity. Recent research has also revealed that EZH2 has pleiotropic functions in immune and malignant cells. EZH2 inhibition could be a promising strategy to improve the outcomes of current immunotherapies. Based on the role of EZH2 in the immunomodulation of both immune and tumor cells, we evaluated the effect of EZH2 on tumor immunity in this review. We also highlight improvements in combined EZH2-targeted treatment and immunotherapy.

Introduction

Enhancer of zeste homolog 2 (EZH2) is a catalytic subunit of histone methyltransferase and Polycomb-repressive complex 2 (PRC2). EZH2 catalyzes the monomethylation, demethylation, and trimethylation of lysine 27 in histone H3 (H3K27me3). Histone labeling is related to tight chromatin and transcriptional inhibition.[1] The EZH2 gene is highly expressed in a wide range of cancers, including head and neck cancer,[2–4] breast cancer,[5] prostate cancer,[6] bladder cancer,[7] colorectal cancer,[8] lung cancer,[9,10] pancreatic cancer,[11] melanoma,[12] and lymphoma.[13] In addition, somatic EZH2 gene mutations were found in 22% of primary B-lymphomas, 7% of follicular lymphomas, and 12–23% of patients with myelodysplastic and myeloproliferative diseases.[13–18] Consequently, the function of EZH2 in tumors should be established based on the type of tumor. EZH2 can function as both a tumor suppressor in a small number of T-cell leukemias and myelodysplastic syndromes as well as an oncogene in the majority of solid tumors and lymphomas. Because EZH2 plays a significant role in cancer, EZH2-targeted therapy has emerged as a crucial therapeutic approach in a number of cancers.[19] Currently, a number of EZH2 inhibitors (EZH2i) have been created, including tazemetostat, which has received FDA approval for the treatment of follicular lymphoma and epithelioid sarcoma.

There is convincing evidence that EZH2 affects both immune and tumor cells in a pleiotropic manner. As a result, EZH2-targeted medications can control the antitumor immune response.[20–23] Presently, immunotherapy is regarded as the fourth pillar of cancer treatment, following radiation, chemotherapy, and surgery.[24] Malignant cells can, however, occasionally evade immune surveillance through a number of mechanisms,[25–28] proliferate quickly in the body, and potentially lead to tumors. Immunotherapies to suppress immune escape, such as immune checkpoint inhibitor therapy, chimeric antigen receptor T-cell (CAR-T) immunotherapy, and immune cell therapy, have seen significant success in re-

Keywords: EZH2; Tumor immunity; Immune checkpoint; Metabolism; Immunotherapy.

Abbreviations: AR, androgen receptor; ASCs, antibody secreting cells; CAR-T, chimeric antigen receptor T-cell; CTLA-4, cytotoxic T lymphocyte associated protein 4; DCs, dendritic cells; DN, double negative; EAF2, ELL associated factor 2; EZH2, enhancer of zeste homolog 2; EZH2i, EZH2 inhibitors; GLUT1, glucose transporter 1; H3K27, lysine 27 on histone 3; H3K27me3, trimethylation of lysine 27 of histone H3; HK2, hexokinase 2; ICB, immune checkpoint blockade; IRF, interferon regulatory factor; ISGs, interference stimulated genes; LAG-3, Lymphocyte activating gene-3; MHC, major histocompatibility complex; NK, natural kill cells; NSCLC, non-small cell lung cancer; OSCC, oral squamous cell carcinoma; PD-L1, programmed death ligand 1; PRC2, polycomb-repressive complex 2; PTEN, phosphatase and tensin homologue; RXRα, retinoid X receptor α; STAT3, signal transducer and activator of transcription 3; TAM, tumor associated macrophages; TCA, tricarboxylic acid cycle; TERT, telomerase reverse transcriptase; Tfh, T follicle helper cell; Th, T helper cell; TIM-3, T cell immunoglobulin and mucin domain-3; Tm, memory CD8+ T; TME, tumor microenvironment; Treg, T regulatory cell; YY1, Yin Yang-1.

***Correspondence to**: Chengzhong Lin and Wei Cao, National Center for Stomatology, National Clinical Research Center for Oral Diseases, Shanghai 200011, China. ORCID: https://orcid.org/0000-0002-3341-3747 (CZL) and https://orcid.org/0000-0003-2184-9380 (WC). Tel: +86-21-23271699-5211 (CZL) and +86-21-23271699-5211 (WC), Fax: +86-21-63135412 (CZL) and +86-21-63135412 (WC), E-mail: evanlin323@foxmail.com (CZL) and caowei561521@shsmu.edu.cn (WC)

How to cite this article: Lin XH, Zhou WK, Lin CZ, Cao W. The Heightened Importance of EZH2 in Cancer Immunotherapy. *Cancer Screen Prev* 2023;2(2):117–129. doi: 10.14218/CSP.2023.00006.

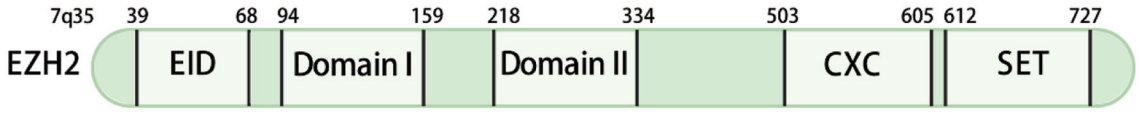

Fig. 1. Characterized domains of EZH2. Five functional domains: a C-terminal SET domain, an adjacent cysteine-rich CXC domain, domain I, domain II and an EED interaction domain (EID).

cent years with the investigation of immune escape pathways.[29–31] Nevertheless, some cancers continue to show treatment resistance; as a result, using immunotherapy in conjunction with EZH2i could be an excellent way to block tumor immunosuppression. There is evidence that EZH2 inhibition may help improve prognosis for some cancer patients and enhance the effectiveness of already-in-use immunotherapies.[32–34]

To maximize the potential of epigenetic medicines, a deeper knowledge of EZH2 in cancer immunity is needed. Here, we discuss the function of EZH2 in tumor immune regulation, including its impact on both immune and tumor cells, and the status of EZH2i in combination with anticancer immunotherapies.

Structure and action mode of EZH2

The EZH2 gene is located on chromosome 7q35 and contains five functional domains: a C-terminal SET domain, an adjacent cysteine-rich CXC domain, domain I, domain II and an EED interaction domain (EID) (Fig. 1).[1,35] The histone methyltransferase active site is located in the SET domain, and the CXC domain also participates in this activity.[36,37]

EZH2 exhibits the following modes of action: (1) Chromatin compaction is encouraged by PRC2-dependent histone methylation: H3K27 trimethylation mediated by EZH2 induces transcriptional silence of downstream genes. For instance, it has been demonstrated that the silencing of foxc1 and E-cadherin by EZH2 promotes cancer development.[38,39] (2) PRC2-dependent non-histone protein methylation: New evidence suggests that in addition to histones, EZH2 also methylates non-histone proteins, such as signal transducer and activator of transcription 3 (STAT3),[40] GATA binding protein 4,[41] and RAR-related orphan receptor α,[42] leading to their activation and thereby enhancing tumorigenicity. (3) PRC2-independent gene transactivation: EZH2 can also act as a co-activator of PRC2-independent transcription factors. For instance, EZH2 interacts physically with RelA and RelB in breast cancer to enhance NF-κB target expression and tumorigenesis.[43] Furthermore, it has been demonstrated that when EZH2 is phosphorylated at Ser21 it behaves as a transcriptional coactivator of the androgen receptor (AR) (Fig. 2).[44]

Genes directly regulated by EZH2, especially the expression of immune escape and other related genes, are presented in Table 1.[45–61]

EZH2-mediated immunomodulation in tumor cells

Immune checkpoints

Recent research has revealed a strong connection between EZH2

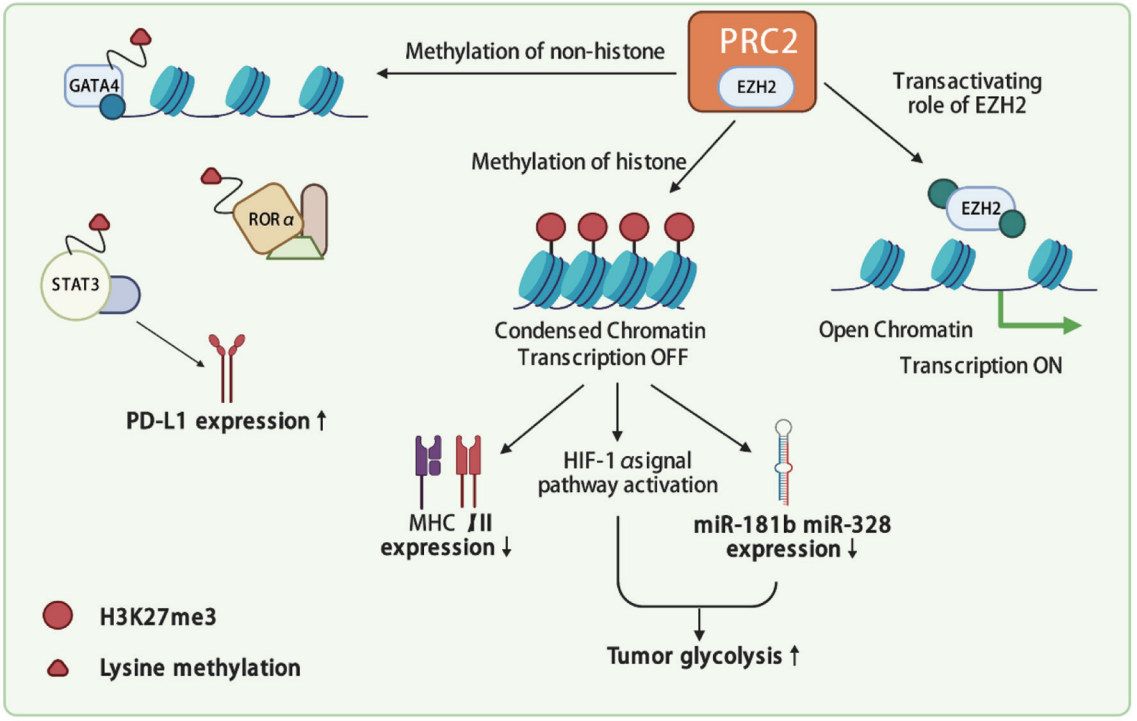

Fig. 2. Action mode of EZH2 and its effect on tumor cells. As part of PRC2, EZH2 methylates histone 3 at lysine 27 (H3K27), which contributes to transcriptional silencing, thus promoting glycolysis and reducing the expression of MHC in the tumor. EZH2 is also capable of methylating a number of non-histone protein substrates, such as STAT3, which promotes PD-L1 expression in tumor cells. In addition, EZH2 has a PRC2-independent role in transcriptional activation.

Table 1. Important genes directly regulated by EZH2

Gene Name	Function
BCL6[50]	EZH2 can suppress the expression of BCL6 by mediating H3K27me3 modification, thereby reducing the immune recognition and antigen presentation ability of tumor cells.
CD8A[51,52]	EZH2 can inhibit the expression of CD8A by regulating H3K27me3 modification, thereby reducing the immune recognition and clearance effect of tumor cells.
CDKN2A[53,54]	Tumor suppressor gene that inhibits cell proliferation and induces apoptosis, silenced or inactivated in many types of tumors.
CTLA4[47]	EZH2 can suppress CTLA4 expression by mediating H3K27me3 modification, thereby weakening T cell immune activity.
E-cadherin[55]	Cell adhesion molecule that promotes cell-cell adhesion and tissue structure stability.
FOXP3[56]	EZH2 can suppress FOXP3 expression by mediating H3K27me3 modification, thus reducing the number and function of regulatory T cells (Treg).
IDO1[45]	EZH2 can suppress IDO1 expression by mediating H3K27me3 modification, thereby reducing T cell activity in the tumor microenvironment.
NKG2D[48]	EZH2 can suppress NKG2D expression by mediating H3K27me3 modification, thereby reducing the sensitivity of tumor cells to NK cell attack.
PD-1[46]	EZH2 can suppress PD-1 expression by mediating H3K27me3 modification, thereby enhancing the immune evasion ability of tumor cells.
PD-L1[57]	EZH2 can suppress PD-L1 expression by mediating H3K27me3 modification, thereby reducing the attacking ability of T cells.
RUNX3[58]	Transcription factor involved in cell cycle and fate decision-making, acts as a tumor suppressor gene and is silenced or inactivated in many types of tumors.
TIMP2[59]	EZH2 can suppress the expression of TIMP2 by mediating H3K27me3 modification, while TIMP2 is a gene that inhibits tumor cell invasion and metastasis.
TIMP3[60]	Metalloproteinase inhibitor that inhibits cell migration and invasion, acts as a tumor suppressor gene and is silenced or inactivated in many types of tumors.
TNF[49]	EZH2 can suppress TNF expression by mediating H3K27me3 modification, thereby reducing the immune reaction caused when tumor cells are attacked by T cells.
VEGFA[61]	Vascular endothelial growth factor A, promotes tumor angiogenesis and cell migration, acts as an oncogene and is overexpressed in many types of tumors.

and the expression of tumor immune checkpoints. Immunohistochemical evaluation of resected lung adenocarcinoma tissue revealed a significant connection between EZH2 and programmed death ligand 1 (PD-L1) expression.[62] Studies have further demonstrated that the interaction between the chromatin remodeling SWI/SNF complex and the PRC2 complex regulates the expression of PD-L1.[45] By increasing the levels of H3K27me3 in the CD274 and interferon regulatory factor (IRF) 1 promoters without influencing the activation of the IFN-signaling pathway or the STAT1 transcription activator, EZH2 can reduce the expression of PD-L1.[63] Production of the T helper cell 1 (Th1) chemokines CXCL9/10 and the subsequent infiltration of effector T cells into tumors can be inhibited by EZH2-mediated DNA methylation linked to DNA methyltransferase DNMT1 and H3K27me3, which improves the clinical effectiveness of PD-L1 immune checkpoint blocking (ICB).[64] EZH2 inhibition can upregulate genes involved in antigen presentation, Th1 chemokine signaling, and the interferon response by activating the dsRNA-STING-ISG stress response, including STING activation-dependent PD-L1 expression. This evidence is presented in studies that show EZH2 has a negative regulatory effect on interferon-stimulated genes (ISGs).[46] Additionally, EZH2 controls the expression of PD-L1 in non-small cell lung cancer (NSCLC) [65] through increasing HIF-1.[65]

New immunological checkpoints are also important to study. A number of immunological cells express lymphocyte activating gene-3 (LAG-3), which can decrease CD8+ T cell activity and boost T regulatory cell (Treg) immunosuppressive activity. Treatment of patients with metastatic or incurable melanoma using LAG-3 targeting therapy in conjunction with anti-PD-1 therapy has been demonstrated to be beneficial in clinical trials. Numerous immune cells and other cells express the T cell immunoglobulin and mucin domain-3 (TIM-3). By promoting CD8+ T cell death, TIM-3 interacts with four ligands to inhibit antitumor immunity. Studies have demonstrated that the costimulatory molecules TIM-3/galectin9 are significantly regulated by EZH2-mediated epigenetic regulation in cervical cancer.[66] Additionally, research has demonstrated that the EZH2 inhibitors DZNep and GAK126 can suppress LAG-3 and TIM-3 by facilitating the movement of effector T cells.[67]

Major histocompatibility complex (MHC)

According to the literature, EZH2 suppresses MHC-I and MHC-II, and inhibiting EZH2 can improve the response to immune checkpoint blockade and restore the immunogenicity of some malignancies (ICB).[68] A transactivator protein called CIITA can control MHC-II molecule transcription to improve the immunological

response. It has been noted that in various tumor types, EZH2 suppresses CIITA through methylation.[69] The prosurvival EZH2 Y641 mutation in diffuse large B-cell lymphoma is also the genetic mechanism underpinning MHC-II deficiency, which results in immune surveillance evasion and poor prognosis.[70] Increased MHC-I expression has been observed *in vitro* in lung cancer cells with EZH2 gene deletion or pharmacological suppression of EZH2, which facilitates CD8+ T cell-mediated tumor cell killing.[71] Similar to prostate cancer, head and neck squamous cell carcinoma is characterized by overexpression of the MHC-I gene in response to EZH2 inhibition.[72,73]

Metabolic reprogramming and immune escape

It is generally recognized that tumor occurrence and development are primarily influenced by metabolic disorders. Recent research has suggested that EZH2 may be crucial in controlling cell metabolism. Therefore, by interfering with cellular metabolic processes, EZH2 can impact the growth and spread of malignancies. Studies have shown that tumors, even in the presence of enough tumor antigens for T-cell recognition, can suppress the activity of tumor-infiltrating T cells by competitive glucose uptake. Additionally, tumor cell glycolysis may restrict the amount of glucose that TILs may consume, leading to T-cell failure and immunological escape.

Glucose metabolism

High EZH2 expression in hepatocellular carcinoma was positively connected with Myc expression and the glycolytic signaling pathway and negatively correlated with interferon signaling, according to gene enrichment analysis.[74] By elevating the expression of H3K27me3 in the EAF2 promoter region, EZH2 can induce a transition from mitochondrial respiration to glycolysis in glioblastoma cells *in vitro*. As a result, the transcription of downstream metabolism-related genes such as hexokinase 2 (HK2), glucose transporter 1 (GLUT1), and pyruvate dehydrogenase kinase 1 is induced.[75] This inhibits the transcription of EAF2 and activates the HIF-1 signaling pathway. In NSCLC, a similar outcome has been observed. LINC00301 is substantially expressed and is directly correlated with prognosis. By controlling the EZH2/EAF2/HIF-1 axis, LINC00301 recruits EZH2 and mediates H3K27me3 in the EAF2 promoter to restrict EAF2 transcription, boosting the population of Tregs in tumors and decreasing CD8+ T cell infiltration.[76] Furthermore, in patients with oral squamous cell carcinoma (OSCC), overexpression of EZH2 promotes cell invasion and migration, as well as glycolysis-mediated epithelial-mesenchymal transition. Additional research revealed that ectopic overexpression of EZH2 promotes tumor development and glycolysis in OSCC by suppressing FoxO1 expression and increasing STAT3 phosphorylation at residue 705.[77] Furthermore, through suppressing the expression of particular miRNAs, EZH2 might indirectly activate aerobic glycolysis in cancer cells. HK2, GLUT1, and ribosomal protein S6 kinase B1 are three important glycolytic enzyme-encoding genes that exhibit favorable correlations with EZH2 expression in prostate cancer. HK2 is the downstream target of MiR-181b. By raising its H3K27me3 level, EZH2 indirectly upregulates the expression of HK2 by suppressing the activity of miR-181b.[78] When the EZH2 level is low, the glycolytic capacity and reserve in glioma cells are decreased. By attaching to the miR-328 promoter and aiding in its methylation, EZH2 inhibits miR-328 production. Additionally, it was discovered that blocking miR-328 inhibited β-catenin expression. An increase in the extracellular acidification rate, which corresponds to an increase in glycolytic capability, is caused by this EZH2/miRNA/β-catenin feed-forward loop.[79]

Although competitive uptake of glucose in the tumor microenvironment (TME) is the reason for impaired T-cell function, the levels of amino acids, glutamine, fatty acids, and other metabolites or growth factors and the expression of the corresponding transporters on the cell surface are also important factors affecting the function of immune cells.

Fatty acid metabolism

Evidence suggests that EZH2 greatly favors the synthesis of fat.[80,81] Studies have demonstrated that elevated tumor adipogenesis speeds up tumor growth in mice and impairs CD8+ T cell activity in the TME.[82] In gliomas with mutations in the telomerase reverse transcriptase (TERT) promoter, there is increased expression of EZH2. Peroxisome proliferator-activated receptor-coactivator-1, which is involved in the production of fatty acid synthase, is activated by TERT and EZH2 together. Both lipid metabolism and TERT expression are impacted by EZH2 knockout. Therefore, it is clear that EZH2 activates the TERT-EZH2 axis, which stimulates fatty acid production and lipid accumulation.[83] However, some research has indicated that EZH2 inhibition can cause fat accumulating in breast cancer and liver cell lines.[84,85] This disparity can be brought on by variations in the species or adipocyte progenitor lineage. Therefore, further research is needed to determine the possible mechanism through which EZH2 influences lipid metabolism.

Amino acid metabolism

Glutamine uptake plays an important role in many metabolic processes in T lymphocytes, including the tricarboxylic acid cycle (TCA), nucleotide synthesis, and detoxification of reactive oxygen species.[86] Studies have revealed that glutamine metabolism increases and suppresses T lymphocyte metabolism in tumors with EZH2 inactivating mutations, increasing tumor progression.[87,88] An isoenzyme known as BCAT1 catalyzes the reversible transfer of amino groups on branched chain amino acids. EZH2 inhibits BCAT1 by modifying H3K27me3 during normal hematopoiesis. By activating BCAT1, EZH2 inactivation and the carcinogenic activity of NRAS promote branched chain amino acid metabolism and mTOR signal transduction, and together they facilitate the conversion of myeloproliferative neoplasms into leukemia.[89] Accordingly, EZH2-inactivated leukemia stem cells showed active glutamine consumption and elevated expression of TCA cycle genes after EZH2 deletion.[87] Additionally, through promoting SAM production, EZH2 may control the metabolism of amino acids. Methionine is a precursor to SAM, which is necessary for tumor cells to methylate DNA and histones.[90] Methionine is a necessary amino acid that can be transported by the amino acid transporter Lat1.[91] Retinoid X receptor (RXR) derepression is caused by small molecule inhibition or knockdown of EZH2, which decreases Lat1 expression.[92] As a result, the EZH2/Lat1 positive feedback loop can promote the production of SAM, which will increase the histone methyltransferase activity of EZH2 and expedite tumor growth.

Impact of EZH2 on immune cells

It is understood that the pathogenic mechanism of cancer involves host immunological dyshomeostasis in solid tumors. Recent research demonstrated that EZH2 controls how different immune cells differentiate and function.[93] To shed light on the immunotherapeutic implications of EZH2 in immune cells, we have outlined the main functions of EZH2 in immune cells, which can be divided

into two groups: immune cells derived from common lymphoid progenitor cells and immune cells derived from common myeloid progenitor cells.

Impact of EZH2 on immune cells derived from common lymphoid progenitor cells

Hematopoietic stem cell (HSC)

EZH2 is thought to support the maintenance of HSCs by inhibiting cell cycle regulators like Cdkn2.[94,95] Defects in other parts of PRC2, such as SUZ12 and EED, may also affect HSC function.[96,97] EZH2 controls the strict regulation of thymic T lymphocytes. H3K27me3 levels in thymic progenitor cells drop due to EZH2 deficiency, and Cdkn2a expression rises as a result, increasing thymocyte block in the double-negative (DN) phase. As a result, the number of cells can be partially maintained when EZH2 and Cdkn2a are both lost. These findings imply that EZH2-induced methylation inhibits cell cycle inhibitors to control thymocyte development.[98,99]

CD4+ T cells

CD4+ Th cells typically coordinate the activation of immune responses by differentiating into multiple lineages, such as Th1, Th2, Th17, and T follicular helper cell (Tfh) subsets, which each play a specific function in antitumor immunity. DNA methylation and histone modification are carefully coordinated to control the flexibility of CD4+ T cell differentiation.[100,101] Following T cell-specific EZH2 deletion, CD4+ T cells displayed changes in H3K27me3 levels and the expression of distinctive transcription factors such T-bet, STAT2 and GATA-3, which increased cytokine production and aided in the differentiation of CD4+ T cells into effector Th1 and Th2 cells.[102,103] Additionally, EZH2-deficient Th1 and Th2 cells secreted more Th1 and Th2 cytokines, such as IFN-γ, IL-4, and IL-13, indicating that EZH2 often inhibits the expression of particular cytokines. EZH2 increases survival rates and maintains the tumor immune response by inhibiting the expression of apoptosis-related target genes, such as FAS, TNFR1, DR4, and Mlkl1 in effector CD4+ T cells.[104–107] Recent research has also demonstrated that expression of the major Th17 transcription factor ROR is increased in mouse embryonic fibroblasts following EZH2 deletion, indicating that EZH2-mediated ROR methylation can promote breakdown of ROR and prevent Th17 cell differentiation.[42] However, only a very modest increase in IL-17 production and ROR expression was found in EZH2 mutant CD4+ T cells cultivated under Th17 induction conditions.[104] This indicates that controlling Th17 differentiation may not be possible only through EZH2's epigenetic mechanism. The ability of B cells to undergo somatic hypermutation, affinity maturation, and differentiation into plasma cells and memory B cells can all be increased by Tfh cells, a distinct subgroup of CD4+ T cells.[108] Tfh cells are essential for triggering a defense against infection and antibody response.[109,110] H3K27ac rather than H3K27me3 is connected with the promoter of the distinctive Tfh transcription factor BCL6, which suggests that EZH2 may not be an important player in these processes.[111] The fact that T cell factor 1 recruits EZH2 to directly activate BCL6 transcription and that BCL6 requires EZH2 to be phosphorylated at Ser21 for it to work suggests an unexpected purpose for EZH2 in controlling the fate of Tfh cells.[112,113] Additionally, EZH2 reduces Cdkn2a expression via controlling H3K27me3, which impacts the proliferation and death of Tfh cells.[112] The impact of EZH2 inhibition on Tfh differentiation in various cancer types needs to be further investigated in light of the mounting evidence that the development of ectopic tertiary lymphoid structures containing Tfh cells may be a favorable prognostic sign during immunotherapy.[110]

Tregs

Tregs maintains immune tolerance and internal environmental stability by inhibiting the inflammatory response and play an important role in inhibiting antitumor immunity.[114–117] Tregs are typically CD4+ T cell subsets that express Foxp3, a transcription factor that is crucial for the differentiation and operation of Tregs.[118,119] Silencing of genes typically produced by CD4+ T effector (Teff) cells is linked to the H3K27me3 alteration in the Foxp3 binding site, which EZH2 can support.[120] EZH2 is essential for Treg activation in addition to controlling Treg differentiation. Studies have demonstrated that the expression of EZH2 is much higher in activated Tregs than in dormant or quiescent Tregs.[120] In comparison to normal tissues, there are more Tregs present among tumor-infiltrating tumors,[121] and EZH2 expression was simultaneously elevated in these Tregs.[122,123] Recent research has demonstrated that, following the activation of the costimulatory receptor CD28, EZH2 is the chromatin modification that is most strongly increased in mouse Treg cells. Its expression aids in suppressing the phenotype of CD4+ Teff cells and stabilizing the functional phenotype of activated Tregs.[123] In summary, targeting EZH2 expression in Tregs in tumors may be a potentially efficient way to improve antitumor immunity.[119,122,124] To illustrate the positive potential of EZH2 inhibitor and anti-cytotoxic T lymphocyte associated protein 4 (CTLA-4) therapy, suppression of EZH2 expression in Tregs can enhance the antitumor response caused by anti CTLA-4 treatment.[47]

CD8+ T cells

The proliferation of CD8+ T cells, which differentiate into enough CD8+ T effector cells to significantly reduce the number of tumor cells expressing antigens or into long-lived memory CD8+ T (Tm) cells to rapidly react to repeatedly presented antigens, is stimulated by antigens produced by cancer cells.[125,126] It has been demonstrated that the amount of EZH2 expression in renal cell carcinoma is correlated with a high density of CD8+ T cells.[127,128] Immature CD8+ T cells with EZH2 deficiency exhibit decreased proliferation and elevated apoptosis in response to antigen stimulation.[129–131] Additionally, EZH2 controls how immature CD8+ T cells differentiate. After TCR activation, EZH2-deficient CD8+ naive T cells showed impaired memory cell differentiation.[132] According to mounting evidence, EZH2 apparently has a significant impact on T-cell exhaustion in addition to its immunological editing effects. When patients with solid tumors experience this condition, the clinical outcomes are typically not favorable.[133–135] A versatile zinc finger transcription factor called Yin Yang-1 (YY1) is engaged in numerous cellular and molecular processes. It recruits EZH2 to inhibit the expression of IL-2. The dysregulation of exhausted T cells is characterized by persistent T-cell activation, which upregulates YY1 and EZH2, epigenetically silencing IL-2.[135,136]

B cells

B lymphocytes are the primary effector cells of humoral immunity.[137,138] High T cell and B cell numbers are viewed as indicators of successful therapeutic outcomes.[139,140] EZH2 actively alters the epigenome at various B-cell development phases. While remaining at a low expression level in dormant and immature B cells, EZH2 is strongly expressed in proliferating B cells, such as pre-B cells and germinal center (GC) B cells.[141–143] ZH2 inhibits germline Ig

transcription and takes part in variable (V), diversity (D), and joining (J) recombination in pre-B cells.[144] By blocking the cell cycle inhibitors Cdkn1a and Cdkn1b, EZH2 stimulates the growth of GC B cells, but it inhibits their final differentiation into antibody-secreting cells (ASCs).[142,143,145,146] EZH2 is therefore required for B cells to respond to immunological activation. According to the literature, the EZH2 inhibitor EPZ-6438 decreases pre-B cell and B cell proliferation, speeds up the transcriptional modifications that mediate the differentiation of B cells into plasma cells associated with the induction of plasma cell maturation, and increases immunoglobulin secretion.[147] Additionally, the total limit on B-cell proliferation is abolished by the EZH2 Y641 mutation in follicular lymphoma, leading to malignant proliferation.[148] The therapeutic benefit is lessened by the fact that EZH2 suppression in hepatocellular carcinoma cells can encourage B cells to differentiate into IgG+ plasma cells, which have a tumor-promoting effect.[149] This finding shows that consideration should be paid to B cell interference and the requirement for a combined B-cell deletion treatment during EZH2 inhibitor therapy.

NK cells

NK cells are natural lymphocytes that actively engage in the body's immune response. They have powerful cytolytic activity, the ability to recognize and manifest cytotoxicity toward cancer cells, and the ability to resist the growth of tumors and microbial infections.[150–153] The phenotypic change, proliferation, activation, and cytotoxic activity of NK cells are all significantly influenced by EZH2. According to the literature, EZH2 deletion or functional inhibition considerably boosts the quantity and caliber of NK cells.[131,154] By directly boosting NK-cell killing in hepatocellular cancer, EZH2 inhibition can upregulate MHC I polypeptide-related sequences.[48,155] Interestingly EZH2 expression is inherently downregulated by NK cells in prostate cancer cells, demonstrating the anticancer effect of NK cells on EZH2 inhibition rather than on direct tumor cell killing.[156] Additionally, EZH2 inhibition induces enhanced expression of NKG2D, CD122, TLRs, and granzymes necessary for tumor cell elimination, which in turn boosts the activity of mature NK cells.[157] As a result, the ability of EZH2 inhibitors to control NK-mediated death has gained more and more attention. Oncogenes implicated in antigen processing, antigen presentation, and NK cell-mediated cytotoxicity are activated when EZH2 inhibitors and DNA methyltransferase inhibitors are combined.[158]

Impact of EZH2 on immune cells derived from common myeloid progenitor cells

Tumor-associated macrophages (TAMs)

The survival and growth of tumor cells can be boosted by TAMs, and they can also foster an immunosuppressive microenvironment that aids in the development of tumors. TAMs can be divided into two groups: M1 type, which has anticancer effects, and M2 type, which has tumor-promoting effects.[159] Inflammatory chemokines and cytokines released from tumor cells have an impact on TAM polarization.[160] When PTEN is lost on chromosome 10 in gliomas, EZH2 inhibits miR-454-3p and increases N(6)-methyladenosine (m6A) modification of PTEN, which causes TAM polarization toward the M2 type.[161] When EZH2 is inhibited in glioblastoma multiforme cells, macrophages cocultured with microglia can repolarize from the M2 phenotype to the M1 phenotype, which enhances the phagocytic ability of microglia.[162–166] By releasing cytokines such as IL-8, macrophage inflammatory protein-3, and IL-1, M2 TAMs stimulate the development of glioma cells.[161,167,168] In lung cancer, EZH2 mediates H3K27me3 in the CCL2 enhancer region and inhibits the infiltration of M1 TAMs in the TME, thus promoting tumor development, and these effects can be reversed by epigenetic inhibitors.[169] Additionally, lung cancer cells that express EZH2 are more likely to produce CCL5, which can attract M2 TAMs and facilitate metastasis and macrophage infiltration.[33,170] By targeting hepatocyte growth factor and macrophage migration inhibitory factor, EZH2-mediated suppression of the miR-144/miR-451a cluster boosts antitumor immunity and promotes M1 polarization of TAMs.[171] Additionally, it has been demonstrated that miR-17, which is carried by bone marrow stem cell-derived extracellular vesicles, affects the EZH2/trail axis to reduce macrophage inflammatory responses.[172] The aforementioned findings demonstrate that the detrimental impact of EZH2 inhibitors on macrophage function should be taken into account.

Dendritic cells (DCs)

In contrast to monocytes, which can only differentiate from common myeloid progenitor cells, DCs can differentiate from both common lymphoid progenitor cells and myeloid progenitor cells. The primary job of DCs is to present antigens. *In vivo* tests demonstrated that EZH1 compensated for EZH2 loss in mature DCs.[173] But according to other research, EZH2 inhibition can lessen the inflammatory response mediated by DCs and minimize liver damage by increasing the expression of the tumor suppressor gene RUNT-related transcription factor 1 in bacteria-induced liver injury.[174,175] Additionally, the recruitment of EZH2 by the active form of STAT5B modulates IRF4 and IRF8 expression to generate tolerogenic DC function.[176] As a result, little is known about how EZH2 might affect DC activity and how that might affect tumor immunity.[173,177] Understanding the impact of EZH2 on DCs is essential given the significance of DCs in anticancer immunity.

The multiple functions of EZH2 in different immune cells are shown in Figure 3.

Combinations of EZH2 inhibition and immunotherapy

Surface EZH2 is a crucial regulator of cancer immune editing because of the regulatory effects of EZH2 on immune and tumor cells that have been previously discussed. To enhance the therapeutic efficacy and circumvent the drawbacks of monotherapy, it is worthwhile to weigh the benefits of combining immunotherapies with clinically available EZH2 inhibitors.

According to recent research, EZH2 inhibitor therapy and ICB therapy can overcome medication resistance that develops during treatment. Combination therapy for prostate cancer that includes an immune checkpoint inhibitor and an EZH2 inhibitor can lessen the prostate cancer resistance to PD-1 inhibitors and boost the effectiveness of prostate cancer immunotherapy by inhibiting EZH2.[46] The Th1 chemokines CXCL9 and CXCL10 were increased in a mouse model of human ovarian cancer following EZH2 inhibition, increasing the infiltration of effector T cells and enhancing the therapeutic effects of PD-L1 ICB treatment and adoptive T-cell infusion in tumor-bearing mice.[178] Additionally, anti-CTLA-4 therapy increased the expression of EZH2 in melanoma cells and decreased immunogenicity and antigen presentation in a mouse melanoma model. Melanoma growth was shown to be greatly slowed by EZH2 suppression and anti-CTLA-4 therapy.[49] According to the study, anti-CTLA-4 therapy impacted the function of T cells by increasing EZH2 expression in peripheral T cells. The

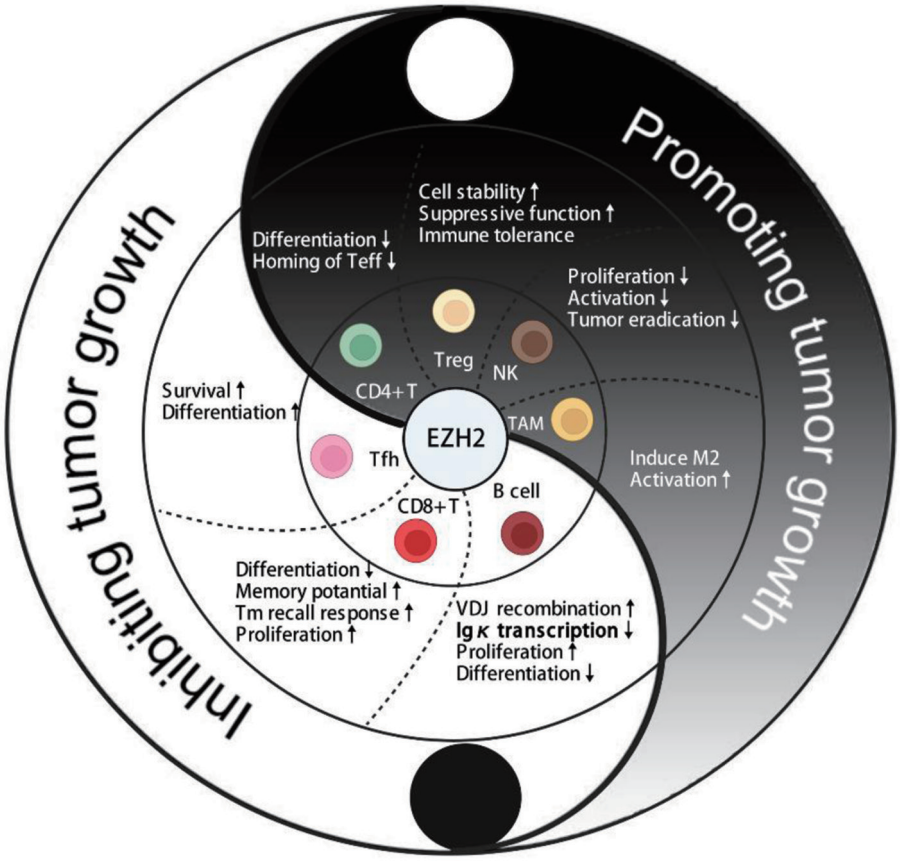

Fig. 3. EZH2 has multiple functions in different immune cells. EZH2 mediates the activation, differentiation, proliferation, and phenotypic transformation of a variety of immune cells, plays a role in tumor promotion and tumor inhibition, and finally regulates cancer cell proliferation, tumor growth, and invasion.

therapeutic impact can be greatly increased by combining treatment with an EZH2 inhibitor in mouse models.[47] Recent research has demonstrated that EZH2 inhibitors increase the immunological checkpoint PD-1 in malignant pleural mesothelioma, and it is thought that using EZH2 inhibitors and PD-1 blockers together can increase macrophage toxicity and hence increase the effectiveness of immunotherapy.[179] According to the aforementioned research, the combination of EZH2 inhibitor therapy and ICB therapy will have significant clinical implications, particularly in malignancies that do not respond to or are resistant to ICB medications. Another study found that the combination of EZH2 inhibitor medication with CAR-T therapy can enhance the therapeutic efficacy of CAR-T therapy by boosting the expression of tumor-associated antigens in Ewing sarcoma.[180]

In conclusion, combinations with various immunotherapies should be further researched to determine the best therapeutic approach and any potential side effects of the EZH2 inhibitor medication in combination with immunotherapy. Further ongoing clinical trials are summarized in Table 2.

Future perspectives

Tumor immunotherapy has made tremendous advances, but there are still many obstacles in the way of achieving the larger social objective of "curing cancers." Tumors, on the one hand, are inherently complex, adaptable, and heterogeneous. In contrast, immunotherapy primarily controls the tumor immune microenvironment rather than tumor cells. A complicated network of connections forms between tumor cells and distinct non-tumor cells, and there are many different impacting elements.

EZH2 plays a significant role in the control of immune and tumor cells by controlling their activation, activation, proliferation, and differentiation. Predicting clinical responses may be made easier with a thorough understanding of the pleiotropic effects of EZH2i on patients. Understanding the unique TME alterations brought on by EZH2i and the indication specificity it induces may also help to rationally combine immunotherapies. Similar to current immunotherapeutic approaches, EZH2i may have various impacts on the TME in terms of both the type of malignancy and the individual. When planning collaborative trials, this potential must be taken into account. In contrast to systemic injection of EZH2 inhibitors, which generally inhibit EZH2, targeted and tailored treatment targeting particular cell types with low toxicity is emerging. For instance, targeted EZH2 inhibitors and nanoparticles made of biomaterials, engineered medicinal materials, or chemical compounds can precisely control the expression of EZH2 in particular cell types. By focusing on EZH2, this strategy is anticipated to increase the impact of cancer immunotherapy. Additionally, there is developing data from the combined testing of EZH2i and ICB treatment, which may be used to inform the design of future combination therapies.

Therefore, it is essential to develop new anticancer therapy

Table 2. Ongoing clinical trials of EZH2 inhibitors

Drug(s)	Disease(s)	Phase
Tazemetostat	Mesothelioma; BAP1 loss of function	II (NCT02860286)
Tazemetostat	Malignant rhabdoid tumors (MRTs); Rhabdoid tumors of the kidney (RTKs); Atypical teratoid rhabdoid tumors (ATRTs); Selected tumors with rhabdoid features; Synovial sarcoma; INI1-negative tumors; Malignant rhabdoid tumor of ovary; Renal medullary carcinoma; Epithelioid sarcoma; Poorly differentiated chordoma	II (NCT02601950)
Tazemetostat	Rhabdoid tumors; INI1-negative tumors; Synovial sarcoma; Malignant rhabdoid tumor of ovary	II (NCT02601937)
Tazemetostat	B-cell lymphomas (Phase 1); Diffuse large B-cell lymphoma (Phase 2); Follicular lymphoma (Phase 2); Transformed follicular lymphoma; Primary mediastinal large B-cell lymphoma	I/II (NCT01897571)
SHR2554	Relapsed or refractory mature lymphoid neoplasms	I (NCT03603951)
SHR2554; SHR1701	Lymphoma	I/II (NCT04407741)
Dabrafenib mesylate; Tazemetostat hydrobromide; Trametinib dimethyl sulfoxide	Clinical stage IV cutaneous melanoma; Metastatic malignant neoplasm in the central nervous system; Metastatic melanoma; Pathologic stage IV cutaneous melanoma	I/II (NCT04557956)
Tazemetostat; Rituximab	Follicular lymphoma	II (NCT04762160)
Tafasitamab; Lenalidomide; Tazemetostat; Acalabrutinib; Daratumumab; Hyaluronidase-Fihj; Pomalidomide; Dexamethasone	Relapsed/refractory hematologic malignancies	I/II (NCT05205252)
CPI-1205	B-cell lymphoma	I (NCT02395601)
CPI-1205; Enzalutamide; Abiraterone/prednisone	Metastatic castration-resistant prostate cancer	I/II (NCT03480646)
Pyrotinib with capecitabine; AR inhibitor combined with everolimus or CDK4/6 inhibitor, or EZH2 inhibitor	Triple-negative breast cancer	I/II (NCT03805399)
SHR7390; Famitinib; SHR3162; Pyrotinib; Capecitabine; SHR1210; Everolimus; Nab paclitaxel; SHR2554; SHR3680; SHR6390; SHR1701; SERD; AI; VEGFi	Breast cancer	II (NCT04355858)

approaches targeting EZH2 in a range of human cancers. Future research concentrating on the immunoregulatory effects of EZH2 in tumors will give a platform for in-depth knowledge of the pathogenic processes of EZH2.

Conclusions

EZH2 plays a complex role in both promoting and inhibiting anti-tumor immune responses. On one hand, EZH2 is overexpressed in various cancers and promotes tumor growth by suppressing immune surveillance and enhancing immune evasion mechanisms. On the other hand, targeting EZH2 has been shown to enhance anti-tumor immune responses by increasing T cell infiltration, inducing immune checkpoint inhibitor expression, and promoting antigen presentation. Therefore, EZH2 inhibitors may have therapeutic potential as immunomodulatory agents for treating cancer patients by reprogramming the TME and enhancing anti-tumor immunity.

Acknowledgments

None.

References

[1] Simon JA, Lange CA. Roles of the EZH2 histone methyltransferase in cancer epigenetics. Mutat Res 2008;647(1-2):21–29. doi:10.1016/j.mrfmmm.2008.07.010, PMID:18723033.

[2] Wu K, Jiang Y, Zhou W, Zhang B, Li Y, Xie F, *et al*. Long Noncoding RNA RC3H2 Facilitates Cell Proliferation and Invasion by Targeting MicroRNA-101-3p/EZH2 Axis in OSCC. Mol Ther Nucleic Acids 2020;20:97–110. doi:10.1016/j.omtn.2020.02.006, PMID:32163895.

[3] Cao W, Feng Z, Cui Z, Zhang C, Sun Z, Mao L, *et al*. Up-regulation of enhancer of zeste homolog 2 is associated positively with cyclin D1 overexpression and poor clinical outcome in head and neck squamous cell carcinoma. Cancer 2012;118(11):2858–2871. doi:10.1002/cncr.26575, PMID:21989926.

[4] Cao W, Younis RH, Li J, Chen H, Xia R, Mao L, *et al*. EZH2 promotes malignant phenotypes and is a predictor of oral cancer development in patients with oral leukoplakia. Cancer Prev Res (Phila) 2011;4(11):1816–1824. doi:10.1158/1940-6207.CAPR-11-0130, PMID:21697275.

[5] Zhang L, Qu J, Qi Y, Duan Y, Huang YW, Zhou Z, *et al*. EZH2 engages TGFβ signaling to promote breast cancer bone metastasis via integrin β1-FAK activation. Nat Commun 2022;13(1):2543. doi:10.1038/s41467-022-30105-0, PMID:35538070.

[6] Yi Y, Li Y, Li C, Wu L, Zhao D, Li F, *et al*. Methylation-dependent and -independent roles of EZH2 synergize in CDCA8 activation in prostate cancer. Oncogene 2022;41(11):1610–1621. doi:10.1038/s41388-022-

02208-x, PMID:35094010.
[7] Vantaku V, Putluri V, Bader DA, Maity S, Ma J, Arnold JM, *et al*. Correction: Epigenetic loss of AOX1 expression via EZH2 leads to metabolic deregulations and promotes bladder cancer progression. Oncogene 2020;39(40):6387–6392. doi:10.1038/s41388-020-1283-7, PMID:32820250.
[8] Yu J, Yang K, Zheng J, Zhao P, Xia J, Sun X, *et al*. Activation of FXR and inhibition of EZH2 synergistically inhibit colorectal cancer through cooperatively accelerating FXR nuclear location and upregulating CDX2 expression. Cell Death Dis 2022;13(4):388. doi:10.1038/s41419-022-04745-5, PMID:35449124.
[9] Cao W, Ribeiro Rde O, Liu D, Saintigny P, Xia R, Xue Y, *et al*. EZH2 promotes malignant behaviors via cell cycle dysregulation and its mRNA level associates with prognosis of patient with non-small cell lung cancer. PLoS One 2012;7(12):e52984. doi:10.1371/journal.pone.0052984, PMID:23300840.
[10] Hu FF, Chen H, Duan Y, Lan B, Liu CJ, Hu H, *et al*. CBX2 and EZH2 cooperatively promote the growth and metastasis of lung adenocarcinoma. Mol Ther Nucleic Acids 2022;27:670–684. doi:10.1016/j.omtn.2021.12.032, PMID:35070495.
[11] April-Monn SL, Andreasi V, Schiavo Lena M, Sadowski MC, Kim-Fuchs C, Buri MC, *et al*. EZH2 Inhibition as New Epigenetic Treatment Option for Pancreatic Neuroendocrine Neoplasms (PanNENs). Cancers (Basel) 2021;13(19):5014. doi:10.3390/cancers13195014, PMID:34638497.
[12] Emran AA, Fisher DE. Dual Targeting with EZH2 Inhibitor and STING Agonist to Treat Melanoma. J Invest Dermatol 2022;142(4):1004–1006. doi:10.1016/j.jid.2021.09.028, PMID:35131084.
[13] Lue JK, Amengual JE. Emerging EZH2 Inhibitors and Their Application in Lymphoma. Curr Hematol Malig Rep 2018;13(5):369–382. doi:10.1007/s11899-018-0466-6, PMID:30112706.
[14] Morin RD, Johnson NA, Severson TM, Mungall AJ, An J, Goya R, *et al*. Somatic mutations altering EZH2 (Tyr641) in follicular and diffuse large B-cell lymphomas of germinal-center origin. Nat Genet 2010;42(2):181–185. doi:10.1038/ng.518, PMID:20081860.
[15] Sneeringer CJ, Scott MP, Kuntz KW, Knutson SK, Pollock RM, Richon VM, *et al*. Coordinated activities of wild-type plus mutant EZH2 drive tumor-associated hypertrimethylation of lysine 27 on histone H3 (H3K27) in human B-cell lymphomas. Proc Natl Acad Sci U S A 2010;107(49):20980–20985. doi:10.1073/pnas.1012525107, PMID:21078963.
[16] Ernst T, Chase AJ, Score J, Hidalgo-Curtis CE, Bryant C, Jones AV, *et al*. Inactivating mutations of the histone methyltransferase gene EZH2 in myeloid disorders. Nat Genet 2010;42(8):722–726. doi:10.1038/ng.621, PMID:20601953.
[17] Nikoloski G, Langemeijer SM, Kuiper RP, Knops R, Massop M, Tönnissen ER, *et al*. Somatic mutations of the histone methyltransferase gene EZH2 in myelodysplastic syndromes. Nat Genet 2010;42(8):665–667. doi:10.1038/ng.620, PMID:20601954.
[18] Ntziachristos P, Tsirigos A, Van Vlierberghe P, Nedjic J, Trimarchi T, Flaherty MS, *et al*. Genetic inactivation of the polycomb repressive complex 2 in T cell acute lymphoblastic leukemia. Nat Med 2012;18(2):298–301. doi:10.1038/nm.2651, PMID:22237151.
[19] Wang X, Cao W, Zhang J, Yan M, Xu Q, Wu X, *et al*. A covalently bound inhibitor triggers EZH2 degradation through CHIP-mediated ubiquitination. EMBO J 2017;36(9):1243–1260. doi:10.15252/embj.201694058, PMID:28320739.
[20] Wang X, Brea LT, Yu J. Immune modulatory functions of EZH2 in the tumor microenvironment: implications in cancer immunotherapy. Am J Clin Exp Urol 2019;7(2):85–91. PMID:31139703.
[21] Zhang T, Gong Y, Meng H, Li C, Xue L. Symphony of epigenetic and metabolic regulation-interaction between the histone methyltransferase EZH2 and metabolism of tumor. Clin Epigenetics 2020;12(1):72. doi:10.1186/s13148-020-00862-0, PMID:32448308.
[22] Shao FF, Chen BJ, Wu GQ. The functions of EZH2 in immune cells: Principles for novel immunotherapies. J Leukoc Biol 2021;110(1):77–87. doi:10.1002/JLB.1RU0520-311R, PMID:33040370.
[23] Sun S, Yu F, Xu D, Zheng H, Li M. EZH2, a prominent orchestrator of genetic and epigenetic regulation of solid tumor microenvironment and immunotherapy. Biochim Biophys Acta Rev Cancer 2022;1877(2):188700. doi:10.1016/j.bbcan.2022.188700, PMID:35217116.
[24] Wolchok J. Putting the Immunologic Brakes on Cancer. Cell 2018;175(6):1452–1454. doi:10.1016/j.cell.2018.11.006, PMID:30500529.
[25] Starzer AM, Preusser M, Berghoff AS. Immune escape mechanisms and therapeutic approaches in cancer: the cancer-immunity cycle. Ther Adv Med Oncol 2022;14:17588359221096219. doi:10.1177/17588359221096219, PMID:35510032.
[26] Gong X, Karchin R. Pan-Cancer HLA Gene-Mediated Tumor Immunogenicity and Immune Evasion. Mol Cancer Res 2022;20(8):1272–1283. doi:10.1158/1541-7786.MCR-21-0886, PMID:35533264.
[27] Ting Koh Y, Luz García-Hernández M, Martin Kast W. Tumor Immune Escape Mechanisms. In: Teicher BA (ed). Cancer Drug Resistance. Totowa (NJ): Humana Press; 2006:577–602. doi:10.1007/978-1-59745-035-5_31.
[28] Sharma P, Hu-Lieskovan S, Wargo JA, Ribas A. Primary, Adaptive, and Acquired Resistance to Cancer Immunotherapy. Cell 2017;168(4):707–723. doi:10.1016/j.cell.2017.01.017, PMID:28187290.
[29] Benboubker V, Boivin F, Dalle S, Caramel J. Cancer Cell Phenotype Plasticity as a Driver of Immune Escape in Melanoma. Front Immunol 2022;13:873116. doi:10.3389/fimmu.2022.873116, PMID:35432344.
[30] Hegde PS, Karanikas V, Evers S. The Where, the When, and the How of Immune Monitoring for Cancer Immunotherapies in the Era of Checkpoint Inhibition. Clin Cancer Res 2016;22(8):1865–1874. doi:10.1158/1078-0432.CCR-15-1507, PMID:27084740.
[31] Guerrouahen BS, Maccalli C, Cugno C, Rutella S, Akporiaye ET. Reverting Immune Suppression to Enhance Cancer Immunotherapy. Front Oncol 2019;9:1554. doi:10.3389/fonc.2019.01554, PMID:32039024.
[32] Duan R, Du W, Guo W. EZH2: a novel target for cancer treatment. J Hematol Oncol 2020;13(1):104. doi:10.1186/s13045-020-00937-8, PMID:32723346.
[33] Kang N, Eccleston M, Clermont PL, Latarani M, Male DK, Wang Y, *et al*. EZH2 inhibition: a promising strategy to prevent cancer immune editing. Epigenomics 2020;12(16):1457–1476. doi:10.2217/epi-2020-0186, PMID:32938196.
[34] Eich ML, Athar M, Ferguson JE 3rd, Varambally S. EZH2-Targeted Therapies in Cancer: Hype or a Reality. Cancer Res 2020;80(24):5449–5458. doi:10.1158/0008-5472.CAN-20-2147, PMID:32978169.
[35] 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(3):174–180. doi:10.1038/sj.ejhg.5200439, PMID:10780782.
[36] Laible G, Wolf A, Dorn R, Reuter G, Nislow C, Lebersorger A, *et al*. Mammalian homologues of the Polycomb-group gene Enhancer of zeste mediate gene silencing in Drosophila heterochromatin and at S. cerevisiae telomeres. EMBO J 1997;16(11):3219–3232. doi:10.1093/emboj/16.11.3219, PMID:9214638.
[37] Tan JZ, Yan Y, Wang XX, Jiang Y, Xu HE. EZH2: biology, disease, and structure-based drug discovery. Acta Pharmacol Sin 2014;35(2):161–174. doi:10.1038/aps.2013.161, PMID:24362326.
[38] Du J, Li L, Ou Z, Kong C, Zhang Y, Dong Z, *et al*. FOXC1, a target of polycomb, inhibits metastasis of breast cancer cells. Breast Cancer Res Treat 2012;131(1):65–73. doi:10.1007/s10549-011-1396-3, PMID:21465172.
[39] Cao Q, Yu J, Dhanasekaran SM, Kim JH, Mani RS, Tomlins SA, *et al*. Repression of E-cadherin by the polycomb group protein EZH2 in cancer. Oncogene 2008;27(58):7274–7284. doi:10.1038/onc.2008.333, PMID:18806826.
[40] Xu Z, Sun Y, Guo Y, Qin G, Mu S, Fan R, *et al*. NF-YA promotes invasion and angiogenesis by upregulating EZH2-STAT3 signaling in human melanoma cells. Oncol Rep 2016;35(6):3630–3638. doi:10.3892/or.2016.4761, PMID:27109360.
[41] He A, Shen X, Ma Q, Cao J, von Gise A, Zhou P, *et al*. PRC2 directly methylates GATA4 and represses its transcriptional activity. Genes Dev 2012;26(1):37–42. doi:10.1101/gad.173930.111, PMID:22215809.
[42] Lee JM, Lee JS, Kim H, Kim K, Park H, Kim JY, *et al*. EZH2 generates a methyl degron that is recognized by the DCAF1/DDB1/CUL4 E3 ubiquitin ligase complex. Mol Cell 2012;48(4):572–586. doi:10.1016/j.molcel.2012.09.004, PMID:23063525.
[43] Lee ST, Li Z, Wu Z, Aau M, Guan P, Karuturi RK, *et al*. Context-specific regulation of NF-κB target gene expression by EZH2 in breast cancers.

Mol Cell 2011;43(5):798–810. doi:10.1016/j.molcel.2011.08.011, PMID:21884980.
[44] Xu K, Wu ZJ, Groner AC, He HH, Cai C, Lis RT, *et al*. EZH2 oncogenic activity in castration-resistant prostate cancer cells is Polycomb-independent. Science 2012;338(6113):1465–1469. doi:10.1126/science.1227604, PMID:23239736.
[45] Jancewicz I, Szarkowska J, Konopinski R, Stachowiak M, Swiatek M, Blachnio K, *et al*. PD-L1 Overexpression, SWI/SNF Complex Deregulation, and Profound Transcriptomic Changes Characterize Cancer-Dependent Exhaustion of Persistently Activated CD4(+) T Cells. Cancers (Basel) 2021;13(16):4148. doi:10.3390/cancers13164148, PMID:34439305.
[46] Morel KL, Sheahan AV, Burkhart DL, Baca SC, Boufaied N, Liu Y, *et al*. EZH2 inhibition activates a dsRNA-STING-interferon stress axis that potentiates response to PD-1 checkpoint blockade in prostate cancer. Nat Cancer 2021;2(4):444–456. doi:10.1038/s43018-021-00185-w, PMID:33899001.
[47] Goswami S, Apostolou I, Zhang J, Skepner J, Anandhan S, Zhang X, *et al*. Modulation of EZH2 expression in T cells improves efficacy of anti-CTLA-4 therapy. J Clin Invest 2018;128(9):3813–3818. doi:10.1172/JCI99760, PMID:29905573.
[48] Bugide S, Green MR, Wajapeyee N. Inhibition of Enhancer of zeste homolog 2 (EZH2) induces natural killer cell-mediated eradication of hepatocellular carcinoma cells. Proc Natl Acad Sci U S A 2018;115(15):E3509–E3518. doi:10.1073/pnas.1802691115, PMID:29581297.
[49] Zingg D, Arenas-Ramirez N, Sahin D, Rosalia RA, Antunes AT, Haeusel J, *et al*. The Histone Methyltransferase Ezh2 Controls Mechanisms of Adaptive Resistance to Tumor Immunotherapy. Cell Rep 2017;20(4):854–867. doi:10.1016/j.celrep.2017.07.007, PMID:28746871.
[50] Chen X, Cao G, Wu J, Wang X, Pan Z, Gao J, *et al*. The histone methyltransferase EZH2 primes the early differentiation of follicular helper T cells during acute viral infection. Cell Mol Immunol 2020;17(3):247–260. doi:10.1038/s41423-019-0219-z, PMID:30842630.
[51] Li Z, Duan Y, Ke Q, Wang M, Cen H, Zhu X. Gene set-based identification of two immune subtypes of diffuse large B cell lymphoma for guiding immune checkpoint blocking therapy. Front Genet 2022;13:1000460. doi:10.3389/fgene.2022.1000460, PMID:36276947.
[52] Rai S, Inoue H, Sakai K, Hanamoto H, Matsuda M, Maeda Y, *et al*. Decreased expression of T-cell-associated immune markers predicts poor prognosis in patients with follicular lymphoma. Cancer Sci 2022;113(2):660–673. doi:10.1111/cas.15224, PMID:34837284.
[53] Hernández-Verdin I, Kirasic E, Wienand K, Mokhtari K, Eimer S, Loiseau H, *et al*. Molecular and clinical diversity in primary central nervous system lymphoma. Ann Oncol 2023;34(2):186–199. doi:10.1016/j.annonc.2022.11.002, PMID:36402300.
[54] Versemann L, Patil S, Steuber B, Zhang Z, Kopp W, Krawczyk HE, *et al*. TP53-Status-Dependent Oncogenic EZH2 Activity in Pancreatic Cancer. Cancers (Basel) 2022;14(14):3451. doi:10.3390/cancers14143451, PMID:35884510.
[55] Du L, Fakih MG, Rosen ST, Chen Y. SUMOylation of E2F1 Regulates Expression of EZH2. Cancer Res 2020;80(19):4212–4223. doi:10.1158/0008-5472.CAN-20-1259, PMID:32816857.
[56] Hou S, Clement RL, Diallo A, Blazar BR, Rudensky AY, Sharpe AH, *et al*. FoxP3 and Ezh2 regulate Tfr cell suppressive function and transcriptional program. J Exp Med 2019;216(3):605–620. doi:10.1084/jem.20181134, PMID:30705058.
[57] Böttcher M, Bruns H, Völkl S, Lu J, Chartomatsidou E, Papakonstantinou N, *et al*. Control of PD-L1 expression in CLL-cells by stromal triggering of the Notch-c-Myc-EZH2 oncogenic signaling axis. J Immunother Cancer 2021;9(4):e001889. doi:10.1136/jitc-2020-001889, PMID:33931470.
[58] Wang C, Liu Z, Woo CW, Li Z, Wang L, Wei JS, *et al*. EZH2 Mediates epigenetic silencing of neuroblastoma suppressor genes CASZ1, CLU, RUNX3, and NGFR. Cancer Res 2012;72(1):315–324. doi:10.1158/0008-5472.CAN-11-0961, PMID:22068036.
[59] Chien YC, Liu LC, Ye HY, Wu JY, Yu YL. EZH2 promotes migration and invasion of triple-negative breast cancer cells via regulating TIMP2-MMP-2/-9 pathway. Am J Cancer Res 2018;8(3):422–434. PMID:29636998.
[60] Shin YJ, Kim JH. The role of EZH2 in the regulation of the activity of matrix metalloproteinases in prostate cancer cells. PLoS One 2012;7(1):e30393. doi:10.1371/journal.pone.0030393, PMID:22272343.
[61] Geng J, Li X, Zhou Z, Wu CL, Dai M, Bai X. EZH2 promotes tumor progression via regulating VEGF-A/AKT signaling in non-small cell lung cancer. Cancer Lett 2015;359(2):275–287. doi:10.1016/j.canlet.2015.01.031, PMID:25633838.
[62] Toyokawa G, Takada K, Tagawa T, Hamamoto R, Yamada Y, Shimokawa M, *et al*. A Positive Correlation Between the EZH2 and PD-L1 Expression in Resected Lung Adenocarcinomas. Ann Thorac Surg 2019;107(2):393–400. doi:10.1016/j.athoracsur.2018.08.056, PMID:30343006.
[63] Xiao G, Jin LL, Liu CQ, Wang YC, Meng YM, Zhou ZG, *et al*. EZH2 negatively regulates PD-L1 expression in hepatocellular carcinoma. J Immunother Cancer 2019;7(1):300. doi:10.1186/s40425-019-0784-9, PMID:31727135.
[64] Chen X, Pan X, Zhang W, Guo H, Cheng S, He Q, *et al*. Epigenetic strategies synergize with PD-L1/PD-1 targeted cancer immunotherapies to enhance antitumor responses. Acta Pharm Sin B 2020;10(5):723–733. doi:10.1016/j.apsb.2019.09.006, PMID:32528824.
[65] Zhao Y, Wang XX, Wu W, Long H, Huang J, Wang Z, *et al*. EZH2 regulates PD-L1 expression via HIF-1α in non-small cell lung cancer cells. Biochem Biophys Res Commun 2019;517(2):201–209. doi:10.1016/j.bbrc.2019.07.039, PMID:31331645.
[66] Zhang L, Tian S, Pei M, Zhao M, Wang L, Jiang Y, *et al*. Crosstalk between histone modification and DNA methylation orchestrates the epigenetic regulation of the costimulatory factors, Tim-3 and galectin-9, in cervical cancer. Oncol Rep 2019;42(6):2655–2669. doi:10.3892/or.2019.7388, PMID:31661141.
[67] Wu X, Gu Z, Chen Y, Chen B, Chen W, Weng L, *et al*. Application of PD-1 Blockade in Cancer Immunotherapy. Comput Struct Biotechnol J 2019;17:661–674. doi:10.1016/j.csbj.2019.03.006, PMID:31205619.
[68] Piunti A, Meghani K, Yu Y, Robertson AG, Podojil JR, McLaughlin KA, *et al*. Immune activation is essential for the antitumor activity of EZH2 inhibition in urothelial carcinoma. Sci Adv 2022;8(40):eabo8043. doi:10.1126/sciadv.abo8043, PMID:36197969.
[69] Truax AD, Thakkar M, Greer SF. Dysregulated recruitment of the histone methyltransferase EZH2 to the class II transactivator (CIITA) promoter IV in breast cancer cells. PLoS One 2012;7(4):e36013. doi:10.1371/journal.pone.0036013, PMID:22563434.
[70] Ennishi D, Takata K, Béguelin W, Duns G, Mottok A, Farinha P, *et al*. Molecular and Genetic Characterization of MHC Deficiency Identifies EZH2 as Therapeutic Target for Enhancing Immune Recognition. Cancer Discov 2019;9(4):546–563. doi:10.1158/2159-8290.CD-18-1090, PMID:30705065.
[71] Burr ML, Sparbier CE, Chan KL, Chan YC, Kersbergen A, Lam EYN, *et al*. An Evolutionarily Conserved Function of Polycomb Silences the MHC Class I Antigen Presentation Pathway and Enables Immune Evasion in Cancer. Cancer Cell 2019;36(4):385–401.e8. doi:10.1016/j.ccell.2019.08.008, PMID:31564637.
[72] Zhao L, Rao X, Huang C, Zheng R, Kong R, Chen Z, *et al*. Epigenetic reprogramming of carrier free photodynamic modulator to activate tumor immunotherapy by EZH2 inhibition. Biomaterials 2023;293:121952. doi:10.1016/j.biomaterials.2022.121952, PMID:36502580.
[73] Zhou L, Mudianto T, Ma X, Riley R, Uppaluri R. Targeting EZH2 Enhances Antigen Presentation, Antitumor Immunity, and Circumvents Anti-PD-1 Resistance in Head and Neck Cancer. Clin Cancer Res 2020;26(1):290–300. doi:10.1158/1078-0432.CCR-19-1351, PMID:31562203.
[74] Guo B, Tan X, Cen H. EZH2 is a negative prognostic biomarker associated with immunosuppression in hepatocellular carcinoma. PLoS One 2020;15(11):e0242191. doi:10.1371/journal.pone.0242191, PMID:33180829.
[75] Pang B, Zheng XR, Tian JX, Gao TH, Gu GY, Zhang R, *et al*. EZH2 promotes metabolic reprogramming in glioblastomas through epigenetic repression of EAF2-HIF1α signaling. Oncotarget 2016;7(29):45134–45143. doi:10.18632/oncotarget.9761, PMID:27259264.
[76] Sun CC, Zhu W, Li SJ, Hu W, Zhang J, Zhuo Y, *et al*. FOXC1-mediated LINC00301 facilitates tumor progression and triggers an immune-suppressing microenvironment in non-small cell lung cancer by regulating the HIF1α pathway. Genome Med 2020;12(1):77. doi:10.1186/s13073-020-00773-y, PMID:32878637.
[77] Zheng M, Cao MX, Luo XJ, Li L, Wang K, Wang SS, *et al*. EZH2 promotes invasion and tumour glycolysis by regulating STAT3 and FoxO1 signal-

ling in human OSCC cells. J Cell Mol Med 2019;23(10):6942–6954. doi:10.1111/jcmm.14579, PMID:31368152.
[78] Tao T, Chen M, Jiang R, Guan H, Huang Y, Su H, *et al*. Involvement of EZH2 in aerobic glycolysis of prostate cancer through miR-181b/HK2 axis. Oncol Rep 2017;37(3):1430–1436. doi:10.3892/or.2017.5430, PMID:28184935.
[79] Wang Y, Wang M, Wei W, Han D, Chen X, Hu Q, *et al*. Disruption of the EZH2/miRNA/β-catenin signaling suppresses aerobic glycolysis in glioma. Oncotarget 2016;7(31):49450–49458. doi:10.18632/oncotarget.10370, PMID:27385092.
[80] Wang L, Jin Q, Lee JE, Su IH, Ge K. Histone H3K27 methyltransferase Ezh2 represses Wnt genes to facilitate adipogenesis. Proc Natl Acad Sci U S A 2010;107(16):7317–7322. doi:10.1073/pnas.1000031107, PMID:20368440.
[81] Wan D, Liu C, Sun Y, Wang W, Huang K, Zheng L. MacroH2A1.1 cooperates with EZH2 to promote adipogenesis by regulating Wnt signaling. J Mol Cell Biol 2017;9(4):325–337. doi:10.1093/jmcb/mjx027, PMID:28992292.
[82] Ringel AE, Drijvers JM, Baker GJ, Catozzi A, García-Cañaveras JC, Gassaway BM, *et al*. Obesity Shapes Metabolism in the Tumor Microenvironment to Suppress Anti-Tumor Immunity. Cell 2020;183(7):1848–1866.e26. doi:10.1016/j.cell.2020.11.009, PMID:33301708.
[83] Ahmad F, Patrick S, Sheikh T, Sharma V, Pathak P, Malgulwar PB, *et al*. Telomerase reverse transcriptase (TERT) - enhancer of zeste homolog 2 (EZH2) network regulates lipid metabolism and DNA damage responses in glioblastoma. J Neurochem 2017;143(6):671–683. doi:10.1111/jnc.14152, PMID:28833137.
[84] Vella S, Gnani D, Crudele A, Ceccarelli S, De Stefanis C, Gaspari S, *et al*. EZH2 down-regulation exacerbates lipid accumulation and inflammation in in vitro and in vivo NAFLD. Int J Mol Sci 2013;14(12):24154–24168. doi:10.3390/ijms141224154, PMID:24351808.
[85] Hayden A, Johnson PW, Packham G, Crabb SJ. S-adenosylhomocysteine hydrolase inhibition by 3-deazaneplanocin A analogues induces anti-cancer effects in breast cancer cell lines and synergy with both histone deacetylase and HER2 inhibition. Breast Cancer Res Treat 2011;127(1):109–119. doi:10.1007/s10549-010-0982-0, PMID:20556507.
[86] Johnson MO, Siska PJ, Contreras DC, Rathmell JC. Nutrients and the microenvironment to feed a T cell army. Semin Immunol 2016;28(5):505–513. doi:10.1016/j.smim.2016.09.003, PMID:27712958.
[87] Li M, Melnick AM. An "EZ" Epigenetic Road to Leukemia Stem Cell Metabolic Reprogramming? Cancer Discov 2019;9(9):1158–1160. doi:10.1158/2159-8290.CD-19-0737, PMID:31481404.
[88] Papathanassiu AE, Ko JH, Imprialou M, Bagnati M, Srivastava PK, Vu HA, *et al*. BCAT1 controls metabolic reprogramming in activated human macrophages and is associated with inflammatory diseases. Nat Commun 2017;8:16040. doi:10.1038/ncomms16040, PMID:28699638.
[89] Gu Z, Liu Y, Cai F, Patrick M, Zmajkovic J, Cao H, *et al*. Loss of EZH2 Reprograms BCAA Metabolism to Drive Leukemic Transformation. Cancer Discov 2019;9(9):1228–1247. doi:10.1158/2159-8290.CD-19-0152, PMID:31189531.
[90] Wang Z, Yip LY, Lee JHJ, Wu Z, Chew HY, Chong PKW, *et al*. Methionine is a metabolic dependency of tumor-initiating cells. Nat Med 2019;25(5):825–837. doi:10.1038/s41591-019-0423-5, PMID:31061538.
[91] Cormerais Y, Massard PA, Vucetic M, Giuliano S, Tambutté E, Durivault J, *et al*. The glutamine transporter ASCT2 (SLC1A5) promotes tumor growth independently of the amino acid transporter LAT1 (SLC7A5). J Biol Chem 2018;293(8):2877–2887. doi:10.1074/jbc.RA117.001342, PMID:29326164.
[92] Dann SG, Ryskin M, Barsotti AM, Golas J, Shi C, Miranda M, *et al*. Reciprocal regulation of amino acid import and epigenetic state through Lat1 and EZH2. EMBO J 2015;34(13):1773–1785. doi:10.15252/embj.201488166, PMID:25979827.
[93] Li Y, Goldberg EM, Chen X, Xu X, McGuire JT, Leuzzi G, *et al*. Histone methylation antagonism drives tumor immune evasion in squamous cell carcinomas. Mol Cell 2022;82(20):3901–3918.e7. doi:10.1016/j.molcel.2022.09.007, PMID:36206767.
[94] Mochizuki-Kashio M, Mishima Y, Miyagi S, Negishi M, Saraya A, Konuma T, *et al*. Dependency on the polycomb gene Ezh2 distinguishes fetal from adult hematopoietic stem cells. Blood 2011;118(25):6553–6561. doi:10.1182/blood-2011-03-340554, PMID:22042701.
[95] Cordero FJ, Huang Z, Grenier C, He X, Hu G, McLendon RE, *et al*. Histone H3.3K27M Represses p16 to Accelerate Gliomagenesis in a Murine Model of DIPG. Mol Cancer Res 2017;15(9):1243–1254. doi:10.1158/1541-7786.MCR-16-0389, PMID:28522693.
[96] Xie H, Xu J, Hsu JH, Nguyen M, Fujiwara Y, Peng C, *et al*. Polycomb repressive complex 2 regulates normal hematopoietic stem cell function in a developmental-stage-specific manner. Cell Stem Cell 2014;14(1):68–80. doi:10.1016/j.stem.2013.10.001, PMID:24239285.
[97] Lee SC, Miller S, Hyland C, Kauppi M, Lebois M, Di Rago L, *et al*. Polycomb repressive complex 2 component Suz12 is required for hematopoietic stem cell function and lymphopoiesis. Blood 2015;126(2):167–175. doi:10.1182/blood-2014-12-615898, PMID:26036803.
[98] Jacobsen JA, Woodard J, Mandal M, Clark MR, Bartom ET, Sigvardsson M, *et al*. EZH2 Regulates the Developmental Timing of Effectors of the Pre-Antigen Receptor Checkpoints. J Immunol 2017;198(12):4682–4691. doi:10.4049/jimmunol.1700319, PMID:28490575.
[99] Wang C, Oshima M, Sato D, Matsui H, Kubota S, Aoyama K, *et al*. Ezh2 loss propagates hypermethylation at T cell differentiation-regulating genes to promote leukemic transformation. J Clin Invest 2018;128(9):3872–3886. doi:10.1172/JCI94645, PMID:30080177.
[100] Liu H, Li P, Wei Z, Zhang C, Xia M, Du Q, *et al*. Regulation of T cell differentiation and function by epigenetic modification enzymes. Semin Immunopathol 2019;41(3):315–326. doi:10.1007/s00281-019-00731-w, PMID:30963214.
[101] Zhu J. T Helper Cell Differentiation, Heterogeneity, and Plasticity. Cold Spring Harb Perspect Biol 2018;10(10):a030338. doi:10.1101/cshperspect.a030338, PMID:28847903.
[102] Zhang Y, Kinkel S, Maksimovic J, Bandala-Sanchez E, Tanzer MC, Naselli G, *et al*. The polycomb repressive complex 2 governs life and death of peripheral T cells. Blood 2014;124(5):737–749. doi:10.1182/blood-2013-12-544106, PMID:24951427.
[103] Hong J, Lee JH, Zhang Z, Wu Y, Yang M, Liao Y, *et al*. PRC2-Mediated Epigenetic Suppression of Type I IFN-STAT2 Signaling Impairs Antitumor Immunity in Luminal Breast Cancer. Cancer Res 2022;82(24):4624–4640. doi:10.1158/0008-5472.CAN-22-0736, PMID:36222718.
[104] Tumes DJ, Onodera A, Suzuki A, Shinoda K, Endo Y, Iwamura C, *et al*. The polycomb protein Ezh2 regulates differentiation and plasticity of CD4(+) T helper type 1 and type 2 cells. Immunity 2013;39(5):819–832. doi:10.1016/j.immuni.2013.09.012, PMID:24238339.
[105] Yang XP, Jiang K, Hirahara K, Vahedi G, Afzali B, Sciume G, *et al*. EZH2 is crucial for both differentiation of regulatory T cells and T effector cell expansion. Sci Rep 2015;5:10643. doi:10.1038/srep10643, PMID:26090605.
[106] Xiao XY, Li YT, Jiang X, Ji X, Lu X, Yang B, *et al*. EZH2 deficiency attenuates Treg differentiation in rheumatoid arthritis. J Autoimmun 2020;108:102404. doi:10.1016/j.jaut.2020.102404, PMID:31952907.
[107] Yang Y, Liu K, Liu M, Zhang H, Guo M. EZH2: Its regulation and roles in immune disturbance of SLE. Front Pharmacol 2022;13:1002741. doi:10.3389/fphar.2022.1002741, PMID:36313363.
[108] Craft JE. Follicular helper T cells in immunity and systemic autoimmunity. Nat Rev Rheumatol 2012;8(6):337–347. doi:10.1038/nrrheum.2012.58, PMID:22549246.
[109] Bindea G, Mlecnik B, Tosolini M, Kirilovsky A, Waldner M, Obenauf AC, *et al*. Spatiotemporal dynamics of intratumoral immune cells reveal the immune landscape in human cancer. Immunity 2013;39(4):782–795. doi:10.1016/j.immuni.2013.10.003, PMID:24138885.
[110] Helmink BA, Reddy SM, Gao J, Zhang S, Basar R, Thakur R, *et al*. B cells and tertiary lymphoid structures promote immunotherapy response. Nature 2020;577(7791):549–555. doi:10.1038/s41586-019-1922-8, PMID:31942075.
[111] Choi YS, Yang JA, Yusuf I, Johnston RJ, Greenbaum J, Peters B, *et al*. Bcl6 expressing follicular helper CD4 T cells are fate committed early and have the capacity to form memory. J Immunol 2013;190(8):4014–4026. doi:10.4049/jimmunol.1202963, PMID:23487426.
[112] Li F, Zeng Z, Xing S, Gullicksrud JA, Shan Q, Choi J, *et al*. Ezh2 programs T(FH) differentiation by integrating phosphorylation-dependent activation of Bcl6 and polycomb-dependent repression of p19Arf. Nat Commun 2018;9(1):5452. doi:10.1038/s41467-018-07853-z, PMID:30575739.
[113] Cao W, Shen Q, Lim MY. Editorial: "Non-Coding RNAs in Head and Neck Squamous Cell Carcinoma". Front Oncol 2021;11:785001. doi:10.3389/

fonc.2021.785001, PMID:35004307.
[114] Cortez JT, Montauti E, Shifrut E, Gatchalian J, Zhang Y, Shaked O, *et al*. CRISPR screen in regulatory T cells reveals modulators of Foxp3. Nature 2020;582(7812):416–420. doi:10.1038/s41586-020-2246-4, PMID:32499641.
[115] Di Pilato M, Kim EY, Cadilha BL, Prüßmann JN, Nasrallah MN, Seruggia D, *et al*. Targeting the CBM complex causes T(reg) cells to prime tumours for immune checkpoint therapy. Nature 2019;570(7759):112–116. doi:10.1038/s41586-019-1215-2, PMID:31092922.
[116] Delgoffe GM, Woo SR, Turnis ME, Gravano DM, Guy C, Overacre AE, *et al*. Stability and function of regulatory T cells is maintained by a neuropilin-1-semaphorin-4a axis. Nature 2013;501(7466):252–256. doi:10.1038/nature12428, PMID:23913274.
[117] Nakagawa H, Sido JM, Reyes EE, Kiers V, Cantor H, Kim HJ. Instability of Helios-deficient Tregs is associated with conversion to a T-effector phenotype and enhanced antitumor immunity. Proc Natl Acad Sci U S A 2016;113(22):6248–6253. doi:10.1073/pnas.1604765113, PMID:27185917.
[118] Kitagawa Y, Sakaguchi S. Molecular control of regulatory T cell development and function. Curr Opin Immunol 2017;49:64–70. doi:10.1016/j.coi.2017.10.002, PMID:29065384.
[119] Plitas G, Konopacki C, Wu K, Bos PD, Morrow M, Putintseva EV, *et al*. Regulatory T Cells Exhibit Distinct Features in Human Breast Cancer. Immunity 2016;45(5):1122–1134. doi:10.1016/j.immuni.2016.10.032, PMID:27851913.
[120] Arvey A, van der Veeken J, Samstein RM, Feng Y, Stamatoyannopoulos JA, Rudensky AY. Inflammation-induced repression of chromatin bound by the transcription factor Foxp3 in regulatory T cells. Nat Immunol 2014;15(6):580–587. doi:10.1038/ni.2868, PMID:24728351.
[121] Saito T, Nishikawa H, Wada H, Nagano Y, Sugiyama D, Atarashi K, *et al*. Two FOXP3(+)CD4(+) T cell subpopulations distinctly control the prognosis of colorectal cancers. Nat Med 2016;22(6):679–684. doi:10.1038/nm.4086, PMID:27111280.
[122] Wang D, Quiros J, Mahuron K, Pai CC, Ranzani V, Young A, *et al*. Targeting EZH2 Reprograms Intratumoral Regulatory T Cells to Enhance Cancer Immunity. Cell Rep 2018;23(11):3262–3274. doi:10.1016/j.celrep.2018.05.050, PMID:29898397.
[123] DuPage M, Chopra G, Quiros J, Rosenthal WL, Morar MM, Holohan D, *et al*. The chromatin-modifying enzyme Ezh2 is critical for the maintenance of regulatory T cell identity after activation. Immunity 2015;42(2):227–238. doi:10.1016/j.immuni.2015.01.007, PMID:25680271.
[124] De Simone M, Arrigoni A, Rossetti G, Gruarin P, Ranzani V, Politano C, *et al*. Transcriptional Landscape of Human Tissue Lymphocytes Unveils Uniqueness of Tumor-Infiltrating T Regulatory Cells. Immunity 2016;45(5):1135–1147. doi:10.1016/j.immuni.2016.10.021, PMID:27851914.
[125] Chang JT, Wherry EJ, Goldrath AW. Molecular regulation of effector and memory T cell differentiation. Nat Immunol 2014;15(12):1104–1115. doi:10.1038/ni.3031, PMID:25396352.
[126] Kaech SM, Cui W. Transcriptional control of effector and memory CD8+ T cell differentiation. Nat Rev Immunol 2012;12(11):749–761. doi:10.1038/nri3307, PMID:23080391.
[127] Zhao E, Maj T, Kryczek I, Li W, Wu K, Zhao L, *et al*. Cancer mediates effector T cell dysfunction by targeting microRNAs and EZH2 via glycolysis restriction. Nat Immunol 2016;17(1):95–103. doi:10.1038/ni.3313, PMID:26523864.
[128] Eichenauer T, Simmendinger L, Fraune C, Mandelkow T, Blessin NC, Kluth M, *et al*. High level of EZH2 expression is linked to high density of CD8-positive T-lymphocytes and an aggressive phenotype in renal cell carcinoma. World J Urol 2021;39(2):481–490. doi:10.1007/s00345-020-03200-4, PMID:32303902.
[129] Chen G, Subedi K, Chakraborty S, Sharov A, Lu J, Kim J, *et al*. Ezh2 Regulates Activation-Induced CD8(+) T Cell Cycle Progression via Repressing Cdkn2a and Cdkn1c Expression. Front Immunol 2018;9:549. doi:10.3389/fimmu.2018.00549, PMID:29632530.
[130] Gray SM, Amezquita RA, Guan T, Kleinstein SH, Kaech SM. Polycomb Repressive Complex 2-Mediated Chromatin Repression Guides Effector CD8(+) T Cell Terminal Differentiation and Loss of Multipotency. Immunity 2017;46(4):596–608. doi:10.1016/j.immuni.2017.03.012, PMID:28410989.
[131] Yin J, Leavenworth JW, Li Y, Luo Q, Xie H, Liu X, *et al*. Ezh2 regulates differentiation and function of natural killer cells through histone methyltransferase activity. Proc Natl Acad Sci U S A 2015;112(52):15988–15993. doi:10.1073/pnas.1521740112, PMID:26668377.
[132] He S, Liu Y, Meng L, Sun H, Wang Y, Ji Y, *et al*. Ezh2 phosphorylation state determines its capacity to maintain CD8(+) T memory precursors for antitumor immunity. Nat Commun 2017;8(1):2125. doi:10.1038/s41467-017-02187-8, PMID:29242551.
[133] Emran AA, Chatterjee A, Rodger EJ, Tiffen JC, Gallagher SJ, Eccles MR, *et al*. Targeting DNA Methylation and EZH2 Activity to Overcome Melanoma Resistance to Immunotherapy. Trends Immunol 2019;40(4):328–344. doi:10.1016/j.it.2019.02.004, PMID:30853334.
[134] Koss B, Shields BD, Taylor EM, Storey AJ, Byrum SD, Gies AJ, *et al*. Epigenetic Control of Cdkn2a.Arf Protects Tumor-Infiltrating Lymphocytes from Metabolic Exhaustion. Cancer Res 2020;80(21):4707–4719. doi:10.1158/0008-5472.CAN-20-0524, PMID:33004350.
[135] Balkhi MY, Wittmann G, Xiong F, Junghans RP. YY1 Upregulates Checkpoint Receptors and Downregulates Type I Cytokines in Exhausted, Chronically Stimulated Human T Cells. iScience 2018;2:105–122. doi:10.1016/j.isci.2018.03.009, PMID:30428369.
[136] Philip M, Fairchild L, Sun L, Horste EL, Camara S, Shakiba M, *et al*. Chromatin states define tumour-specific T cell dysfunction and reprogramming. Nature 2017;545(7655):452–456. doi:10.1038/nature22367, PMID:28514453.
[137] Mizukami M, Hanagiri T, Yasuda M, Kuroda K, Shigematsu Y, Baba T, *et al*. Antitumor effect of antibody against a SEREX-defined antigen (UOEH-LC-1) on lung cancer xenotransplanted into severe combined immunodeficiency mice. Cancer Res 2007;67(17):8351–8357. doi:10.1158/0008-5472.CAN-06-3889, PMID:17804751.
[138] Shi JY, Gao Q, Wang ZC, Zhou J, Wang XY, Min ZH, *et al*. Margin-infiltrating CD20(+) B cells display an atypical memory phenotype and correlate with favorable prognosis in hepatocellular carcinoma. Clin Cancer Res 2013;19(21):5994–6005. doi:10.1158/1078-0432.CCR-12-3497, PMID:24056784.
[139] Kinoshita T, Muramatsu R, Fujita T, Nagumo H, Sakurai T, Noji S, *et al*. Prognostic value of tumor-infiltrating lymphocytes differs depending on histological type and smoking habit in completely resected non-small-cell lung cancer. Ann Oncol 2016;27(11):2117–2123. doi:10.1093/annonc/mdw319, PMID:27502728.
[140] Tokunaga R, Naseem M, Lo JH, Battaglin F, Soni S, Puccini A, *et al*. B cell and B cell-related pathways for novel cancer treatments. Cancer Treat Rev 2019;73:10–19. doi:10.1016/j.ctrv.2018.12.001, PMID:30551036.
[141] Su IH, Basavaraj A, Krutchinsky AN, Hobert O, Ullrich A, Chait BT, *et al*. Ezh2 controls B cell development through histone H3 methylation and Igh rearrangement. Nat Immunol 2003;4(2):124–131. doi:10.1038/ni876, PMID:12496962.
[142] Béguelin W, Popovic R, Teater M, Jiang Y, Bunting KL, Rosen M, *et al*. EZH2 is required for germinal center formation and somatic EZH2 mutations promote lymphoid transformation. Cancer Cell 2013;23(5):677–692. doi:10.1016/j.ccr.2013.04.011, PMID:23680150.
[143] Guo M, Price MJ, Patterson DG, Barwick BG, Haines RR, Kania AK, *et al*. EZH2 Represses the B Cell Transcriptional Program and Regulates Antibody-Secreting Cell Metabolism and Antibody Production. J Immunol 2018;200(3):1039–1052. doi:10.4049/jimmunol.1701470, PMID:29288200.
[144] Mandal M, Powers SE, Maienschein-Cline M, Bartom ET, Hamel KM, Kee BL, *et al*. Epigenetic repression of the Igk locus by STAT5-mediated recruitment of the histone methyltransferase Ezh2. Nat Immunol 2011;12(12):1212–1220. doi:10.1038/ni.2136, PMID:22037603.
[145] Scharer CD, Barwick BG, Guo M, Bally APR, Boss JM. Plasma cell differentiation is controlled by multiple cell division-coupled epigenetic programs. Nat Commun 2018;9(1):1698. doi:10.1038/s41467-018-04125-8, PMID:29703886.
[146] Caganova M, Carrisi C, Varano G, Mainoldi F, Zanardi F, Germain PL, *et al*. Germinal center dysregulation by histone methyltransferase EZH2 promotes lymphomagenesis. J Clin Invest 2013;123(12):5009–5022. doi:10.1172/JCI70626, PMID:24200695.
[147] Herviou L, Jourdan M, Martinez AM, Cavalli G, Moreaux J. EZH2 is overexpressed in transitional preplasmablasts and is involved in human plasma cell differentiation. Leukemia 2019;33(8):2047–2060. doi:10.1038/s41375-019-0392-1, PMID:30755708.

[148] Béguelin W, Teater M, Meydan C, Hoehn KB, Phillip JM, Soshnev AA, *et al*. Mutant EZH2 Induces a Pre-malignant Lymphoma Niche by Reprogramming the Immune Response. Cancer Cell 2020;37(5):655–673. e11. doi:10.1016/j.ccell.2020.04.004, PMID:32396861.

[149] Wei Y, Lao XM, Xiao X, Wang XY, Wu ZJ, Zeng QH, *et al*. Plasma Cell Polarization to the Immunoglobulin G Phenotype in Hepatocellular Carcinomas Involves Epigenetic Alterations and Promotes Hepatoma Progression in Mice. Gastroenterology 2019;156(6):1890–1904.e16. doi:10.1053/j.gastro.2019.01.250, PMID:30711627.

[150] Li L, Li W, Wang C, Yan X, Wang Y, Niu C, *et al*. Adoptive transfer of natural killer cells in combination with chemotherapy improves outcomes of patients with locally advanced colon carcinoma. Cytotherapy 2018;20(1):134–148. doi:10.1016/j.jcyt.2017.09.009, PMID:29056549.

[151] Böttcher JP, Bonavita E, Chakravarty P, Blees H, Cabeza-Cabrerizo M, Sammicheli S, *et al*. NK Cells Stimulate Recruitment of cDC1 into the Tumor Microenvironment Promoting Cancer Immune Control. Cell 2018;172(5):1022–1037.e14. doi:10.1016/j.cell.2018.01.004, PMID:29429633.

[152] Noman MZ, Berchem G, Janji B. Targeting autophagy blocks melanoma growth by bringing natural killer cells to the tumor battlefield. Autophagy 2018;14(4):730–732. doi:10.1080/15548627.2018.1427398, PMID:29368981.

[153] Guerra N, Tan YX, Joncker NT, Choy A, Gallardo F, Xiong N, *et al*. NKG2D-deficient mice are defective in tumor surveillance in models of spontaneous malignancy. Immunity 2008;28(4):571–580. doi:10.1016/j.immuni.2008.02.016, PMID:18394936.

[154] Zhong J, Yang X, Chen J, He K, Gao X, Wu X, *et al*. Circular EZH2-encoded EZH2-92aa mediates immune evasion in glioblastoma via inhibition of surface NKG2D ligands. Nat Commun 2022;13(1):4795. doi:10.1038/s41467-022-32311-2, PMID:35970825.

[155] Ramakrishnan S, Granger V, Rak M, Hu Q, Attwood K, Aquila L, *et al*. Inhibition of EZH2 induces NK cell-mediated differentiation and death in muscle-invasive bladder cancer. Cell Death Differ 2019;26(10):2100–2114. doi:10.1038/s41418-019-0278-9, PMID:30692641.

[156] Lin SJ, Chou FJ, Li L, Lin CY, Yeh S, Chang C. Natural killer cells suppress enzalutamide resistance and cell invasion in the castration resistant prostate cancer via targeting the androgen receptor splicing variant 7 (ARv7). Cancer Lett 2017;398:62–69. doi:10.1016/j.canlet.2017.03.035, PMID:28373004.

[157] Arenas-Ramirez N, Sahin D, Boyman O. Epigenetic mechanisms of tumor resistance to immunotherapy. Cell Mol Life Sci 2018;75(22):4163–4176. doi:10.1007/s00018-018-2908-7, PMID:30140960.

[158] Tiffen J, Gallagher SJ, Filipp F, Gunatilake D, Emran AA, Cullinane C, *et al*. EZH2 Cooperates with DNA Methylation to Downregulate Key Tumor Suppressors and IFN Gene Signatures in Melanoma. J Invest Dermatol 2020;140(12):2442–2454.e5. doi:10.1016/j.jid.2020.02.042, PMID:32360600.

[159] Mantovani A, Schioppa T, Porta C, Allavena P, Sica A. Role of tumor-associated macrophages in tumor progression and invasion. Cancer Metastasis Rev 2006;25(3):315–322. doi:10.1007/s10555-006-9001-7, PMID:16967326.

[160] Pathria P, Louis TL, Varner JA. Targeting Tumor-Associated Macrophages in Cancer. Trends Immunol 2019;40(4):310–327. doi:10.1016/j.it.2019.02.003, PMID:30890304.

[161] Qi B, Yang C, Zhu Z, Chen H. EZH2-Inhibited MicroRNA-454-3p Promotes M2 Macrophage Polarization in Glioma. Front Cell Dev Biol 2020;8:574940. doi:10.3389/fcell.2020.574940, PMID:33363140.

[162] Saha D, Martuza RL, Rabkin SD. Macrophage Polarization Contributes to Glioblastoma Eradication by Combination Immunovirotherapy and Immune Checkpoint Blockade. Cancer Cell 2017;32(2):253–267.e5. doi:10.1016/j.ccell.2017.07.006, PMID:28810147.

[163] Hambardzumyan D, Gutmann DH, Kettenmann H. The role of microglia and macrophages in glioma maintenance and progression. Nat Neurosci 2016;19(1):20–27. doi:10.1038/nn.4185, PMID:26713745.

[164] Mosser DM, Edwards JP. Exploring the full spectrum of macrophage activation. Nat Rev Immunol 2008;8(12):958–969. doi:10.1038/nri2448, PMID:19029990.

[165] Yin Y, Qiu S, Li X, Huang B, Xu Y, Peng Y. EZH2 suppression in glioblastoma shifts microglia toward M1 phenotype in tumor microenvironment. J Neuroinflammation 2017;14(1):220. doi:10.1186/s12974-017-0993-4, PMID:29132376.

[166] Qiu S, Huang D, Yin D, Li F, Li X, Kung HF, *et al*. Suppression of tumorigenicity by microRNA-138 through inhibition of EZH2-CDK4/6-pRb-E2F1 signal loop in glioblastoma multiforme. Biochim Biophys Acta 2013;1832(10):1697–1707. doi:10.1016/j.bbadis.2013.05.015, PMID:23707559.

[167] Xu Y, Liao C, Liu R, Liu J, Chen Z, Zhao H, *et al*. IRGM promotes glioma M2 macrophage polarization through p62/TRAF6/NF-κB pathway mediated IL-8 production. Cell Biol Int 2019;43(2):125–135. doi:10.1002/cbin.11061, PMID:30288851.

[168] Lu J, Xu Z, Duan H, Ji H, Zhen Z, Li B, *et al*. Tumor-associated macrophage interleukin-β promotes glycerol-3-phosphate dehydrogenase activation, glycolysis and tumorigenesis in glioma cells. Cancer Sci 2020;111(6):1979–1990. doi:10.1111/cas.14408, PMID:32259365.

[169] Zheng Y, Wang Z, Wei S, Liu Z, Chen G. Epigenetic silencing of chemokine CCL2 represses macrophage infiltration to potentiate tumor development in small cell lung cancer. Cancer Lett 2021;499:148–163. doi:10.1016/j.canlet.2020.11.034, PMID:33253790.

[170] Xia L, Zhu X, Zhang L, Xu Y, Chen G, Luo J. EZH2 enhances expression of CCL5 to promote recruitment of macrophages and invasion in lung cancer. Biotechnol Appl Biochem 2020;67(6):1011–1019. doi:10.1002/bab.1875, PMID:31855281.

[171] Zhao J, Li H, Zhao S, Wang E, Zhu J, Feng D, *et al*. Epigenetic silencing of miR-144/451a cluster contributes to HCC progression via paracrine HGF/MIF-mediated TAM remodeling. Mol Cancer 2021;20(1):46. doi:10.1186/s12943-021-01343-5, PMID:33658044.

[172] Su Y, Song X, Teng J, Zhou X, Dong Z, Li P, *et al*. Mesenchymal stem cells-derived extracellular vesicles carrying microRNA-17 inhibits macrophage apoptosis in lipopolysaccharide-induced sepsis. Int Immunopharmacol 2021;95:107408. doi:10.1016/j.intimp.2021.107408, PMID:33915488.

[173] Gunawan M, Venkatesan N, Loh JT, Wong JF, Berger H, Neo WH, *et al*. The methyltransferase Ezh2 controls cell adhesion and migration through direct methylation of the extranuclear regulatory protein talin. Nat Immunol 2015;16(5):505–516. doi:10.1038/ni.3125, PMID:25751747.

[174] Wang Y, Wang Q, Wang B, Gu Y, Yu H, Yang W, *et al*. Inhibition of EZH2 ameliorates bacteria-induced liver injury by repressing RUNX1 in dendritic cells. Cell Death Dis 2020;11(11):1024. doi:10.1038/s41419-020-03219-w, PMID:33262329.

[175] Wang Q, Zheng J, Zou JX, Xu J, Han F, Xiang S, *et al*. S-adenosylhomocysteine (AdoHcy)-dependent methyltransferase inhibitor DZNep overcomes breast cancer tamoxifen resistance via induction of NSD2 degradation and suppression of NSD2-driven redox homeostasis. Chem Biol Interact 2020;317:108965. doi:10.1016/j.cbi.2020.108965, PMID:32001260.

[176] Zerif E, Khan FU, Raki AA, Lullier V, Gris D, Dupuis G, *et al*. Elucidating the Role of Ezh2 in Tolerogenic Function of NOD Bone Marrow-Derived Dendritic Cells Expressing Constitutively Active Stat5b. Int J Mol Sci 2020;21(18):6453. doi:10.3390/ijms21186453, PMID:32899608.

[177] Doñas C, Carrasco M, Fritz M, Prado C, Tejón G, Osorio-Barrios F, *et al*. The histone demethylase inhibitor GSK-J4 limits inflammation through the induction of a tolerogenic phenotype on DCs. J Autoimmun 2016;75:105–117. doi:10.1016/j.jaut.2016.07.011, PMID:27528513.

[178] Peng D, Kryczek I, Nagarsheth N, Zhao L, Wei S, Wang W, *et al*. Epigenetic silencing of TH1-type chemokines shapes tumour immunity and immunotherapy. Nature 2015;527(7577):249–253. doi:10.1038/nature15520, PMID:26503055.

[179] Hamaidia M, Gazon H, Hoyos C, Hoffmann GB, Louis R, Duysinx B, *et al*. Inhibition of EZH2 methyltransferase decreases immunoediting of mesothelioma cells by autologous macrophages through a PD-1-dependent mechanism. JCI Insight 2019;4(18):128474. doi:10.1172/jci.insight.128474, PMID:31534051.

[180] Kailayangiri S, Altvater B, Lesch S, Balbach S, Göttlich C, Kühnemundt J, *et al*. EZH2 Inhibition in Ewing Sarcoma Upregulates G(D2) Expression for Targeting with Gene-Modified T Cells. Mol Ther 2019;27(5):933–946. doi:10.1016/j.ymthe.2019.02.014, PMID:30879952.