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Review Article

Immune modulatory functions of EZH2 in the tumor microenvironment: implications in cancer immunotherapy

Xiaohai Wang[1,4], Lourdes T Brea[1], Jindan Yu[1,2,3]

[1]Division of Hematology/Oncology, Department of Medicine, [2]Robert H. Lurie Comprehensive Cancer Center, [3]Department of Biochemistry and Molecular Genetics, Northwestern University Feinberg School of Medicine, Chicago, IL, USA; [4]Department of Urology, Shanghai General Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China

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Abstract: Polycomb group protein EZH2, a histone methyltransferase, is the enzymatic subunit of the Polycomb Repressive Complex 2 (PRC2) that catalyzes histone H3 lysine 27 methylation. They are epigenetic modifiers that mediate gene repression, or epigenetic silencing. EZH2 controls developmental regulators in embryonic stem cells and is essential for cell fate determination and transition. In the last two decades, EZH2 was reported upregulated in a variety of solid tumors, including prostate cancer, and mutated in multiple hematological malignancies, such as lymphoma. EZH2 represses the expression of a plethora of tumor suppressor genes in tumor cells, thereby promoting cell cycle, cell proliferation, and cell invasion and driving cancer progression. Recently, evidence is emerging indicating important roles of EZH2 in immune cells. Here, we review EZH2 regulation of various immune cell types, the tumor microenvironment, immune responses, and cancer immunotherapies.

Keywords: EZH2, tumor microenvironment, prostate cancer

Introduction

Polycomb group proteins (PcGs) are important epigenetic regulators of stem cell and cancer biology [1, 2]. There are two major complexes: the Polycomb Repressive Complex 1 and 2 (PRC2). Histone methyltransferase Enhancer of Zeste homolog 2 (EZH2) is the catalytic subunit of PRC2 and catalyzes tri-methylation of histone H3 at lysine 27 (H3K27me3) to silence target genes. Clinical investigations have shown that EZH2 is aberrantly up-regulated in various malignant tumors, including prostate and breast cancer, and is associated with advanced stages and poor prognosis [3, 4]. EZH2 has been shown to play important roles in the development and progression of these cancers. EZH2 promotes cell survival, proliferation, epithelial to mesenchymal transition, invasion, and drug resistance of cancer cells. Moreover, new roles of EZH2 in the tumor immune microenvironment (TIME) are emerging, which will be the focus of this review. We will summarize the diverse immune modulatory functions of EZH2 in different cell types of the TIME and investigate the potential of targeting EZH2 as a cancer immunotherapy.

EZH2 regulation of immune cell types

The composition of the tumor microenvironment (TME) is complicated. It includes not only cancer cells, extracellular matrix, fibroblasts, endothelia and adipocytes, but also immune cells such as T-cells, NK cells, regulatory T (Treg) cells, tumor-associated macrophages (TAMs), and dendritic cells (DCs). Within the TME, cytokines and chemokines, together with a dynamic immunosuppressive network formed by the interaction of immune cells and tumor cells, interrupt immunotherapies and promote cancer progression across all stages of tumorigenesis [5]. EZH2 is expressed in many immune cell types and has distinct functions [6].

EZH2 in T cells

EZH2 plays critical roles in T cell response. The function of EZH2 in naive T cells is to maintain

the survival, proliferation and function of effector CD4+ and CD8+ T cells and inhibit Th1 and Th2 differentiation [7]. Low expression of EZH2 in CD8+ T cells is associated with poor survival and prognosis in patients. Similarly, another study revealed that EZH2 is crucial to the development and maintenance of T-cell memory precursors, which are correlated with enhanced tumor control [8].

The percentage of EZH2+/CD8+ T-cells is lower in the TIME than in peripheral blood and non-malignant tissues, suggesting that TME interferes with EZH2 expression in T-cells. One mechanism by which this occurs is that cancer cells restrict T-cell-mediated immunity by inhibiting EZH2 expression and limiting the glycolysis pathway, leading to reduced survival of CD8+ T-cells in the TME [8]. For example, ovarian cancer cells could suppress EZH2 expression in CD4+ and CD8+ T cells in the TME by inducing some micro-RNAs, thereby decreasing T cell survival and immune function [9]. Therefore, EZH2 inhibition may suppress survival, expansion and effect of tumor-specific effector T cells, thereby inhibiting anti-tumor immunity of TME. Zhao et al. demonstrated that targeting T-cell specific expression of EZH2 in the TME enables cancer cells to evade tumor surveillance. This ultimately increases the tumor burden and the metastatic potential in mouse models of ovarian cancer [9]. These tumor suppressive effects of EZH2 should be considered during therapeutic design of EZH2 inhibitors.

EZH2 in Treg cells

It has been demonstrated that tumor cells can secrete soluble factors, such as TGFβ, VEGF and GM-CSF in the TIME, and convert tumor-infiltrating CD4+ T cells to Foxp3+ Tregs, which negatively impact anti-tumor immunity [10, 11]. EZH2-mediated epigenetic program was found critical for the recruitment and immunosuppressive function of activated Tregs [12]. The expression of EZH2 is upregulated in tumor-infiltrating Treg cells as compared to the effector T cells or Treg cells in the peripheral blood. Goswami et al. [13] showed that ipilimumab, a human monoclonal immunoglobulin G1 antibody that blocks cytotoxic T lymphocyte associated protein 4 (CTLA-4), increases EZH2 expression in human T cells across various tumor types. Specific targeting of EZH2 in the Treg cells results in an increased anti-cancer immune response and improved tumor control. For example, genetic depletion of EZH2 in the FoxP3cre/EZH2$^{fl/fl}$ mice or the use of EZH2 inhibitor CPI-1205 elicit phenotypic and functional alterations of Tregs. This leads to enhanced effector-like T cell responses and effectivity of anti-CTLA-4 therapy. Therefore, it is important to investigate the regulation of EZH2 expression in Tregs in the TME.

EZH2 in natural killer (NK) cells

NK cells, as a component of the innate immune system, are able to respond quickly to a wide variety of pathological challenges, including foreign, infected, or cancerous cells, in the absence of antigen presentation by MHC-I [14]. Some studies have demonstrated an epigenetic mechanism regulating NK cell development and NK-based cancer immunotherapies. Inhibition of EZH2 expression or activity in hematopoietic stem and progenitor cells increases NK precursors and mature progeny, which display up-regulated IL-2 receptor β (also termed IL-15R, CD122), NK cell-activating receptor NKG2D, Toll-like receptors, and granzymes of NK cells. This leads to increased cell proliferation, activation and cytotoxicity against tumor cells [6, 15, 16].

EZH2 in dendritic cells (DCs)

DCs are antigen-presenting cells that function as an important interface between innate and adaptive immune systems [17]. Tian et al. [18] reported that EZH2 overexpression plays an important role in the tumorigenesis of a majority of histiocytic and dendritic neoplasms, such as histiocytic sarcoma, follicular dendritic cell sarcoma, Langerhans cell histiocytosis, and interdigitating dendritic cell sarcoma. Additionally, Gunawan et al. [19] demonstrated that EZH2 regulates integrin signaling and adhesion dynamics of DCs to promote the development of experimental autoimmune encephalomyelitis. Donas et al. [20] found that inhibition of H3K27 demethylation induced tolerogenic DCs to inhibit inflammation and the development of experimental autoimmune encephalomyelitis. These studies indicate a potential role of EZH2 in DC function. How EZH2 regulates the function of DCs and influences DC-based cancer immunotherapies should be further investigated.

EZH2 in tumor-associated macrophages (TAM)

Another innate immune cell type in the TIME are macrophages, so called tumor-associated macrophages (TAMs). TAMs can promote tumor progression directly by favoring tumor cell proliferation and survival and indirectly by creating an immunosuppressive microenvironment [21]. Qiao et al. demonstrated that IFN-γ-induced macrophage activation is facilitated by EZH2-mediated H3K27me3 and subsequent silencing of genes, such as MERTK and PPARG, which could otherwise promote deactivation of macrophages by M2 stimuli [22]. However, the mechanism by which dysregulated EZH2 in TAM contributes to tumor progression is still unknown.

EZH2 regulation of TIME/immune response

There are two main immune regulatory functions of EZH2 in tumor cells. One is the maintenance of chronic inflammation, and the other the establishment of tumor immune tolerance [23]. Chronic inflammation can induce epigenetic reprogramming and promote oncogenic transformation. Conversely, genetic and epigenetic changes in tumor cells can generate an inflammatory microenvironment that further supports tumor progression [24]. For example, EZH2-dependent transcriptional activation of IL-6/TNF and repression of IFNGR1 promote tumorigenesis through the maintenance of chronic inflammation [25]. Chronic inflammation upregulates EZH2 through NF-κB activation and promotes tumorigenesis [26]. In prostate cancer cells, the EZH2 complex switches from a repressor to an activator, as a result of EZH2 phosphorylation at S21, which is mediated by PI3K-AKT signaling. EZH2 in turn activates NF-κB target genes, such as IL-6, IL-8 and TNF [27]. These events result in the activation of the chronic inflammation/NF-κB/EZH2 signaling loop and promote cancer progression. Cholangiocarcinoma (CCA) is another lethal cancer associated with EZH2-regulated chronic inflammation. Knockdown of EZH2 regulates the STAT3 signaling pathway by restoring the function of tumor suppressor miR-124 and induces autophagy-related cell death. Thus, the EZH2-STAT3 signaling axis may be a potential therapeutic target in chronic inflammation-related CCA [28].

Chronic immune cells in the TME not only interact intimately with tumor cells to promote oncogenic activity, but also fail to mount an effective anti-tumor immune response [29]. Interferon-γ receptor 1 (IFNGR1) is directly repressed by EZH2 in a MYC-dependent manner in a subset of metastatic prostate cancers [30]. EZH2 knockdown or EZH2 inhibition restored the expression of IFNGR1. The combination of EZH2 inhibitor with IFN-γ treatment can strongly activate IFN-JAK-STAT1 tumor suppressor signaling and robust apoptosis. Thus, EZH2-inactivated IFN signaling may represent a potential therapeutic target for patients with advanced prostate cancer driven by MYC.

Peng et al. [31] showed that in ovarian tumor EZH2 and DNA methyltransferase 1 (DNMT1) are negatively associated with tumor-infiltrating CD8+ T cells and patient outcomes. EZH2-mediated H3K27me3 and DNMT1-mediated DNA methylation repress the secretion of T helper 1 (Th1)-type chemokines CXCL9 and CXCL10, and subsequently obstruct effector T-cell trafficking to the TME. Treatment with epigenetic modulators increases the infiltration of effector T cells, slows down tumor progression, and improves the therapeutic efficacy of PD-L1 checkpoint blockade and adoptive T-cell transfusion in tumor-bearing mice. A similar effect of EZH2 was also observed in colorectal cancer [32]. Inhibition of EZH2 in colorectal cancer cells increased CXCL9 and CXCL10 expression and augmented the infiltration of effector T cells in TIME. Zingg et al. [33] showed that during anti-CTLA-4 or IL-2 immunotherapy in mouse models, T cell infiltration and T cell dependent tumor necrosis factor-α (TNF-α) production in TIME result in EZH2 upregulation in melanoma cells. This EZH2 upregulation, in turn, silences essential immune-related genes, including those involved in melanocyte lineage, MHC-I, antigen processing and presentation machinery, immunoproteasome, and T-cell-attractant chemokines, through methylation of H3K27. This eventually represses tumor immunogenicity and T-cell infiltration, leading to treatment failure. EZH2 inhibition using GSK503 or tumor cell-specific RNA interference can reverse this resistance. Hence, the combination of EZH2 inhibition with anti-CTLA-4 and IL-2 immunotherapy can augment the infiltration of IFN-γ-producing $PD\text{-}1_{low}$ CD8+ T cells in TIME and improve tumor control.

EZH2 as a target for immunotherapy in various cancer

In recent years, cancer immunotherapy has undergone great advances. Several cancer immunotherapeutic strategies have been establi-

shed and validated for the treatment of aggressive cancers [34]. However, a good percentage of patients do not respond to these therapies while some others relapse rapidly after initial response [35, 36]. Epigenetic reprogramming, especially through the activity of EZH2, is involved in a variety of escape mechanisms. The inherent reversibility of epigenetic modifications makes this mechanism an attractive therapeutic target [6]. More and more EZH2-targeting drugs have been reported and shown to exhibit anti-tumor effects in various malignancies in *in vitro* biochemical/cellular assays, *in vivo* preclinical model experiments, and in clinical trials in patients [6]. Additionally, EZH2 inhibitors such as Tazemetostat (also known as EPZ-6438 or E7438, Epizyme, Inc) [4, 37], CPI-1205 (Constellation Pharmaceuticals) [38], GSK2816126 (also known as GSK126, GlaxoSmithKline) [39], PF-06821497 (Pfizer) [40, 41], and SHR-2554 (Jiangsu HengRui Medicine Co., Ltd.) (Source: Clinicaltrials.gov; accessed 17 October 2018) are currently in clinical trials to treat many malignant tumors, both hematologic tumors and solid tumors, including rhabdoid tumors, Sarcoma, Nervous System Neoplasm, Hepatocellular Carcinoma and castration resistant prostate cancer (CRPC). In one of these clinical trials, the effects of tazemetostat on tumor immune priming will be assessed in epithelioid sarcoma (ES) (NCT02601950) (data not shown).

Importantly, EZH2 can lead to distinct and opposite effects on tumor cells, Treg cells, and T-cells in anti-tumor immunity. For example, although inhibition of EZH2 contributes to reversing immune resistance, it might also reduce T cell survival in the TIME [9, 31-33]. In addition, the role of EZH2 on immune cells might depend on the tumor type, the treatment used and the TME, because systemic inhibition of EZH2 did not affect T-cell proliferation and effector functions in mouse models of melanoma [33]. Thus, the use of EZH2 inhibitors with the goal to inhibit tumor growth will likely simultaneously alter the functions of immune cells in the TIME. Although EZH2 inhibitors might provide an attractive treatment in cancers with high EZH2 expression and activation, such approaches might have unpredictable effects on anti-tumor immunity, which should be taken into consideration with the systemic use of epigenetic therapies.

Interestingly, the study of EZH2 inhibitors *in vitro* and in various pre-clinical models showed their capacity to regulate different pathways and molecules involved in the interaction of the immune system with cancer cells. For example, recently, immune-checkpoint blockers, including anti-CTLA4 antibody, anti-PD1 (PDCD1) antibody and anti-PDL1 (CD274) antibody, are emerging as a new class of cancer therapeutics that augment antitumor immunity [42]. Long et al. [43] identified that miR-26a expression is elevated in CTLs responding to TME secretome stimulation. Elevated miR-26a subsequently inhibits EZH2, which impairs CTL function, indicating miR-26a-EZH2 axis as a novel target to improve the efficacy of CTL-based cancer immunotherapy. Combining EZH2 inhibitors with immune-checkpoint blockers seems to be a potential and reasonable strategy in cancer therapy. These efforts are currently being translated through clinical trials of epigenetic-modifying drugs in combination with immune checkpoint inhibitors, such as in NCT03525795, a study of CPI-1205 with Ipilimumab (a monoclonal antibody that works to activate the immune system by targeting CTLA-4) in patients with advanced solid tumors previously treated with PD-1 or PD-L1 Inhibitors.

As EZH2 is overexpressed in several cancers, it has been suggested that EZH2 can function as a tumor-associated antigen (TAA). Indeed, studies have identified an immunogenic epitope of EZH2 in lung cancer, which is recognized by CD4 T-cells and could serve as a potent immunogenic target inducing both CD4 and CD8 T-cell anti-tumor responses [44]. In prostate cancer, although targeting the androgen receptor signaling is an effective strategy [45], androgen ablation increases the levels of EZH2 in prostate cancer [46]. EZH2 could be a promising target in specific immunotherapy of prostate cancer patients, particularly in those with metastases or castration-resistant cancer. Along this line, EZH2-derived peptides that can be used for peptide-based anti-cancer vaccine to reactivate CTLs for cancer patients with HLA-A2, -A24 or -A3 molecules have been reported [47, 48].

Conclusions and future perspectives

In summary, EZH2 is a methyltransferase and the catalytic subunit of PRC2 that mediates

H3K27me3. EZH2 acts as an oncogene that promotes the development and progression of a variety of human cancers. In addition, EZH2 plays complicated roles in tumor immunity, which should be the focus of future studies. Therapeutic targeting of EZH2 thus poses new challenges and systematic application of EZH2 inhibitors should consider potential implications on host and tumor immunity. It may be advantageous to combine EZH2 inhibitors with immunotherapies in some cancers, which warrants further investigation.

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Disclosure of conflict of interest

None.

Address correspondence to: Dr. Jindan Yu, Division of Hematology/Oncology, Department of Medicine, Northwestern University Feinberg School of Medicine, Chicago, IL, USA; Robert H. Lurie Comprehensive Cancer Center, Northwestern University Feinberg School of Medicine, 303 E. Superior St. Lurie 5-117, Chicago, IL 60611, USA. Tel: 312-503-1761; Fax: 312-503-0189; E-mail: jindan-yu@northwestern.edu

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