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EZH2: A breakthrough in cancer treatment

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Abstract. As one of the hot topics in cancer research, the role of EZH2 in cancer physiology has received extensive attention in recent years, and related research has become increasingly complex and in-depth. EZH2 not only regulates gene silencing through histone methylation, but also participates in tumorigenesis through other mechanisms, such as regulating cancer cell metabolism and inhibiting tumor suppressor genes. However, the EZH2 inhibitors developed in the early stage have problems such as nonspecificity, physiological toxicity and drug resistance. Therefore, the current focus of drug research and development is to improve efficiency, reduce toxicity, and seek higher specificity. This article deeply explores the mechanism of EZH2 and neuroblastoma, breast cancer bone metastasis and prostate cancer, and introduces the three development focuses of EZH2 therapeutic drugs and the therapeutic mechanism of representative drugs. In summary, it is pointed out that although EZH2 has attracted much attention in cancer research, there are still few therapeutic drugs on the market, and more drugs are still in the clinical trial stage. Future drug research and development should focus on improving specificity, safety and efficacy, and combine multiple treatment methods to better utilize EZH2 as a breakthrough in cancer treatment.

Keywords: EZH2, PRC2, neuroblastoma, prostate cancer, breast cancer

1. Introduction

Transcription is the core life activity of cells and one of the decisive factors in cell type and function. At the same time, several transcriptional disorders can be observed in cancer cells. Therefore, using the transcription process as an entry point to find the mechanism of carcinogenesis and develop treatments and drugs is a current research hotspot.

The Zeste homolog enhancer 2 (EZH2) gene encodes the EZH2 protein, which is a subunit of the Polycomb repressive complex 2 (PRC2) that catalyzes histone lysine methylation. And this complex is a member of a family of epigenetic regulators that repress transcription. EZH2 mainly catalyzes the trimethylation of lysine 27 on the histone H3 protein subunit, thereby promoting chromatin compaction and silencing of target genes such as tumor suppressor genes. In addition, researchers also observed that EZH2 has a mechanism of action independent of methylase function in regulating the occurrence of cancer, such as directly occupying the promoter to activate androgen receptor (AR) transcription to promote the formation of Castration-resistant prostate cancer [1], regulating aerobic glycolysis of cancer cells through the "miR-181b/hexokinase 2" axis to promote cancer cell proliferation [2], inhibiting IGFBP3 protein to amplify MYCN to promote tumors Growth[3] and so on. Therefore, using the various mechanisms of action of EZH2 as an entry point to develop cancer treatment drugs is a major research

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direction. Since the EZH2 inhibitors developed in the early stages have a series of problems such as non-specificity, physiological toxicity, and drug resistance, they cannot be used for clinical treatment. Therefore, the current focus of drug development is on high efficiency, high specificity, low toxicity, ease of use and so on.

This article will focus on elaborating on several detailed mechanisms of action between EZH2 and the occurrence of neuroblastoma, bone metastasis of breast cancer, and prostate cancer. It will also introduce the three current development focuses of EZH2 therapeutic drugs and explain treatment mechanism of the representative drugs. This article aims to summarize the current research status of EZH2 and the development progress of therapeutic drugs, so as to lay a new research foundation for future research.

2. Mechanism of action of EZH2 and cancer

2.1. Mechanism of action of EZH2 and neuroblastoma

Neuroblastoma is a common extracranial tumor in children. It has atypical initial symptoms, widespread metastasis after clinical symptoms, high degree of deterioration, and low five-year survival rate for advanced tumors. This article focuses on the two ways in which EZH2 promotes the growth of this cancer cell, aiming to deepen readers' understanding of this field.

EZH2 has the function of catalyzing methylation and can catalyze the trimethylation of the fourth lysine of histone H3 to generate H3K4me3. The latest research shows that H3K4me3 can inhibit the expression of tumor suppressor genes through the transcription pause-release process in cells [4]. In addition, Chen et al. used targeted shRNA transfection to inhibit the expression of EZH2 gene in three neuroblastoma cell lines and found that the activity of neuroblastoma cells with reduced EZH2 was significantly reduced. The above evidence can indicate that one of the mechanisms by which EZH2 promotes the growth of neuroblastoma is by catalyzing the generation of H3K4me3 [3].

Chen et al. [3] used a cancer cell line encyclopedia to generate a prediction model and found that neuroblastoma cell lines amplified by the oncoprotein "super transcription factor" MYCN expressed higher levels of EZH2. In addition, Chen et al. also found that IGFBP3 protein levels in MYCN-unamplified neuroblastoma cell lines were generally higher than those in MYCN-amplified neuroblastoma cell lines [3]. Through controlled experiments in mice, it was found that overexpression of IGFBP3 protein can inhibit the activity of neuroblastoma cells and improve the survival rate of mice. Therefore, IGFBP3 protein inhibits the expression of MYCN and thereby inhibits cancer cell proliferation. At the same time, Chen et al. conducted RNA sequencing and found that EZH2 can enrich and combine with IGFBP3 protein to render the protein unable to function [5]. After inhibiting EZH2, the transcription level of IGFBP3 protein increased significantly. The above studies indicate that another mechanism by which EZH2 promotes neuroblastoma growth is by inhibiting IGFBP3 protein to amplify MYCN to promote tumor growth.

Compared with previous studies that mostly focused on the role of MYCN in transcription, the innovation of Chen et al. is to reveal the direct connection between MYCN overexpression and tumor suppressor programs, laying the foundation for subsequent research on MYCN and cancer.

2.2. Mechanism of action of EZH2 and breast cancer bone metastasis

According to statistics from the American Cancer Society in 2019 [5], breast cancer is the most common cancer among women around the world. In its late stages, bone metastasis is prone to occur, significantly reducing the patient's five- and ten-year survival. At the same time, bone metastasis can also lead to pathological osteolysis, spinal compression and other symptoms, seriously affecting the patient's life. Therefore, it is extremely important to understand the mechanism of breast cancer bone metastasis.

In the experiment of Zhang et al. [6], the researchers first constructed an EZH2 knockdown breast cancer cell line and injected it into the left ventricle of nude mice. Another group of nude mice was injected with a control cell line. As a result, nude mice with the injection of EZH2 knockdown breast cancer cell lines showed longer bone metastasis-free period and overall survival. Apparently, EZH2 can

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promote breast cancer bone metastasis. Interestingly, Zhang et al. [6] found that bone metastasis in cancer model mice was not affected after treatment with methyltransferase inhibitors, indicating that EZH2 does not cause breast cancer bone metastasis through its methyltransferase activity.

Previous studies have shown that metastatic breast cancer cells secrete parathyroid hormone-like hormone (PTHLH) to activate osteolysis [7], and the cytokine IL-8 can also regulate osteolysis that occurs when breast cancer bone metastases [8]. Zhang et al. [6] measured through qRT-PCR technology and found that EZH2 knockout inhibited the expression of PTHLH and IL-8, indicating that EZH2 can mediate the vicious cycle of breast cancer bone metastasis through PTHLH and IL-8. PTHLH is a downstream gene of transforming growth factor TGFβ and is regulated by the p-Smad2/Gli2 transcription factor complex [9]. Therefore, in order to explore how EZH2 activates Smad2 signaling, Zhang et al. [6] detected changes in protein expression of two groups of EZH2 knockout cancer cell lines under TGFβ treatment. As a result, the amount of kinases in the two groups of cell lines changed significantly, especially the local focal adhesion kinase FAK, which regulates cancer cell survival and affects cancer occurrence, decreased. In addition, if FAK is knocked down, the level of pS465/467-Smad2 will also be inhibited [6]. This suggests that activation of FAK kinase by EZH2 increases the phosphorylation of the transcription factor complex pS465/467-Smad2 in breast cancer cells and activates the TGFβ/Smad2/PTHLH pathway, thereby promoting breast cancer bone metastasis.

Since FAK is a key mediator of EZH2 promoting breast cancer bone metastasis, it is not difficult to see that developing FAK inhibitors for clinical treatment is a feasible measure for the treatment of breast cancer bone metastasis. At the same time, compared with directly inhibiting EZH2, which plays multiple roles in cells, this measure can be speculated to have fewer side effects and higher specificity.

2.3. Mechanism of action of EZH2 and prostate cancer

According to 2023 statistics from the American Cancer Society [10], prostate cancer is a very common type of cancer in men and the sixth leading cause of death among patients with malignant tumors, and its incidence rate is increasing year by year. Its symptoms include sexual dysfunction, difficulty urinating, etc. About half of prostate cancer patients have gene fusions between the transcription regulator ERG and the serine protease TMPRSS2, which is one of the most common gene rearrangements in human cancers [11]. So it can be seen that clarifying the pathogenesis of prostate cancer has an important impact on cancer research at the genetic level.

In the experiments of Zoma et al. [12], the researchers found that ERG contains a histone centered on lysine 362 (K362), which is the EZH2 recognition motif. In subsequent sets of experiments to exclude the influence of EZH2, no intracellular ERG methylation was observed. This result shows that EZH2 can exert its methyltransferase activity to catalyze the methylation of K362 of ERG.

Next, Zoma et al. [12] hypothesized that methylation of K362 would affect the binding of ERG to DNA. In order to test this conjecture, they first used molecular dynamics simulation technology to compare self-inhibitory ERG and active ERG, and found that the α4 helix in the active ERG concept showed a state that easily binds to DNA. When examining the conformation of the double mutant ERG in which both K362 and E412 were mutated, they found that the double mutant showed a conformation similar to active ERG and easy to bind to DNA, but the ERG with only K362 mutation showed a conformation that was not conducive to DNA binding. Therefore, it can be found that after K362 is methylated, it changes its relationship with other amino acids, inducing secondary changes in conformation, thereby promoting the combination of ERG and DNA to exert transcriptional activity. Then, Zoma et al. [12] constructed mice with prostate-specific expression of ERG and PTEN gene deletion. After culturing for a period of time, they found that the prostate size of the mice increased and invasive lesions occurred; after treatment with EZH2 inhibitors, the mice Prostate tumor volume was significantly reduced. Based on the above results, it can be concluded that methylation of ERG by EZH2 enhances ERG transcriptional activity, thereby promoting the growth of prostate tumors.

It is worth noting that the experiments by Zoma et al. demonstrated the mechanism of EZH2 and ERG and proved that this mechanism promotes the growth of prostate cancer. However, the mechanism

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of how ERG induces tumorigenesis and growth remains unknown. Therefore, how ERG uses its transcriptional activity to promote cancer cell proliferation may be a future research direction.

3. EZH2 as a cancer therapeutic target

3.1. Start with the methyltransferase activity of EZH2

It is not difficult to see from the above mechanisms that the methyltransferase activity of EZH2 promotes cancer. Therefore, it is a feasible solution to use this as an entry point to develop cancer inhibitors. The earliest widely used inhibitor is 3-Deazaneplanocin A (DZNep), which acts as a global histone methylation inhibitor and can inhibit S-adenosyl-L-homocysteine (SAH) hydrolase so that cellular SAH levels will increase, thereby indirectly inhibiting EZH2 [13]. However, because DZNep is not specific and has problems such as short plasma half-life and toxicity [13], it cannot be used as a clinical treatment drug and its application range is small. Therefore, in recent years, researchers have been committed to developing inhibitors with high specificity and low toxicity. One of the more prominent ones is GSK126, whose key structure is 2-pyridone, which can competitively occupy the site of the methyl donor S-adenosylmethionine (SAM) in EZH2, making EZH2 unable to function as a methyltransferase [14]. However, clinical trials of GSK126 showed that it had insufficient activity and short half-life, which prevented further clinical testing [14]. The inhibitor EPZ005678 developed at the same time also has the above defects and cannot be used clinically [14]. With the advancement of technology, researchers have developed an EZH2-specific inhibitor called Tazemetostat. Its mechanism is similar to GSK126, but it has better pharmacokinetic properties and has been proven to significantly inhibit tumors in clinical trials as well as good safety and body tolerance [14]. On January 23, 2020, Tazemetostat, as the world's first EZH2 inhibitor drug, was approved by the FDA for the treatment of epithelioid sarcoma patients over 16 years old who are not suitable for surgical resection. This event highlights the considerable therapeutic prospects of Tazemetostat. At the same time, ease of clinical administration is also the direction for the development of EZH2 inhibitors, such as the first orally available inhibitor UNC1999, (R)-OR-S1 and (R)-OR-S2 produced by Daiichi Sankyo, EPZ-6438 and so on. In addition, EZH2-specific inhibitors also include EI1, CPI-1205, ZLD1039, etc. [13,14]. It can be seen that inhibitors of EZH2 methyltransferase activity are currently a hot development focus (Table 1).

Table 1. Comparative analysis of properties of methyltransferase inhibitors

Inhibitor	Mechanism	Features
deazaneplanocin A (DZNep)	Inhibits S-adenosyl-L-homocysteine (SAH) hydrolase	Non-specific, Short half-life, Toxic
GSK126	Competitive inhibition of S-adenosylmethionine (SAM)	Specific, Insufficient activity
Tazemetostat	Competitive inhibition of S-adenosylmethionine (SAM)	Specific, High safety, Good pharmacokinetic properties
UNC1999	Competitive inhibition of S-adenosylmethionine (SAM)	Specific, Oral
EPZ-6438	Competitive inhibition of S-adenosylmethionine (SAM)	Specific, Oral
CPI-1205	Indole-based inhibition of EZH2	Specific, Oral

3.2. Start with the structure of PRC2

EZH2 is the catalytic subunit of PRC2, so starting with PRC2 to destroy the interaction between subunits or destroy its overall structure is another option for cancer treatment. Researchers developed PROTAC EED Degrader-1 and PROTAC EED Degrader-2 through proteolysis-targeted chimera technology (PROTAC). Both of them can inhibit the EZH2 subunit from binding to EED and SUZ12 subunits, thereby inhibiting EZH2 from functioning as a methyltransferase [15]. Researchers have also developed EZH2-stabilized α-helix (SAH-EZH2) peptides that specifically disrupt the EED subunit, and drugs such as astemizole and AZD9291 that disrupt the structure of the EZH2-EED complex [14]. They all

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block the interaction between PRC2 subunits and prevent EZH2 from exerting catalytic activity. In addition, MAK683/EED226 developed by Novartis can directly bind to the subunit on PRC2 to change its overall conformation, thus preventing EZH2 from exerting catalytic activity. Compared with directly inhibiting the catalytic activity of EZH2, cancer inhibitory drugs developed based on the structure of PRC2 have more diverse action sites. Therefore, it can be speculated that this method is more difficult for cancer cells to develop drug resistance and has the potential for long-term treatment.

3.3. Multimodality combined treatment

Nowadays, multiple methods are usually used to treat cancer in clinical practice. Therefore, the possible relationship between EZH2 inhibitory drugs and other treatment methods is also a focus worthy of discussion and research. It has been found that the EZH2 inhibitor EPZ-6438 combined with traditional chemotherapy for non-Hodgkin lymphoma (NHL) can prevent the occurrence of NHL, and combined with the glucocorticoid receptor agonist (Grag) can inhibit the proliferation of NHL; researchers also observed that the combined use of the EZH2 inhibitor GSK126 and the chemotherapy drug etoposide can enhance the inhibitory effect on prostate cancer cells [13]. Similar synergism also occurs when EZH2 inhibitors are used in combination with monoclonal antibodies. In addition, the advantages of multi-modal combination therapy are not only reflected in enhancing the anti-cancer and tumor suppressor effects, but also in reducing drug resistance of cancer cells. For example, research by Zhou et al. [16] showed that using an EZH2 inhibitor to inhibit EZH2 can enhance the presentation of head and neck squamous cell carcinoma cells as antigens in the immune response, thus avoiding the resistance of cancer cells to PD-1 treatment. It can be seen that in order to adapt to the mutation-prone characteristics of cancer cells and to comply with modern medical treatment methods, the benefits or risks brought by combined treatment are factors that must be considered clinically.

4. Conclusion

It is not difficult to find that EZH2 is a hot topic in current cancer research. Research to reveal the mechanism of its interaction with cancer, and the number of therapeutic drugs developed through each mechanism are increasing year by year. However, the number of EZH2 therapeutic drugs currently approved for marketing is scarce, and more drugs are still in or even suspended from clinical trials due to factors such as efficacy and side effects. Therefore, future drug development should focus on aspects such as high specificity, strong safety, and high efficacy, and be prepared to combine various treatment methods, so as to better utilize the potential of EZH2 as a breakthrough in cancer treatment. It is foreseeable that more related drugs will be developed and approved for marketing in the future, which will not only bring good news to cancer patients, but also be a highlight in the history of epigenetics.

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