Review Article

EZH2 expression role in angiogenesis of prostate malignancy

Sanaz Kavoosi [1], Sanam Rezazadeh Chafjiri [2], Nastaran Asghari Moghaddam [3,*]

[1]Department of Biology, Central Tehran Branch, Islamic Azad University, Tehran, Iran; [2]Department of Biotechnology, Faculty of Basic Science, Ale-Taha Institute of Higher Education, Tehran, Iran; [3]Department of Biology, Central Tehran Branch, Islamic Azad University, Tehran, Iran

***Corresponding author:** Nastaran Asghari Moghaddam, Department of Biology, Central Tehran Branch, Islamic Azad University, Tehran, Iran. E- mail: Nas.asgharimoghaddam@iauctb.ac.ir*

DOI: 10.22034/HBB.2023.21

Received: July 20, 2023; **Accepted:** November 7, 2023

ABSTRACT

The gene Enhancer of Zeste Homolog 2 (*EZH2*) is shown to be involved in the progression of the angiogenesis in cancer including prostate cancer, also it is a well-known enzyme catalytic subunit, initially acting as a histone methyltransferase, to prevent expressing gene by trimethylating lysine at the histone H3 position. According to previous studies, it is obvious that in some cancer, such as prostate cancer, *EZH2* is overexpressed. In this review, the regulation and function of the *EZH2* gene and also signaling pathways that are regulated by this gene in angiogenesis process were reviewed. In addition, *EZH2* has been playing an important role as a target in clinical trials to provide small inhibitor molecule that classified into two groups including new chemotherapy agents and repositioning drugs with the aim of cancer therapy and malignancy.

***Keywords*:** Prostate cancer, *EZH2*, signaling pathway, angiogenesis, repositioning drugs

INTRODUCTION

Prostate cancer is a major cause of cancer-related deaths in men globally, and its prevalence is projected to rise as the population ages. The overexpression of various genes such as Enhancer of Zest Homolog 2 *(EZH2)* can lead to tumor progression and carcinogenesis. *EZH2* is an evolutionarily protected gene which is found on chromosome 7 and plays a role in cell growth, development, and the advancement of cancer [1,2]. Furthermore, it is playing a substantial role in malignant

tumors and angiogenesis as a possessed common gene that is shown in Figure1 [3,4]. *EZH2* may impact on prostate cancer advancement or halt and angiogenesis by affecting different signaling pathways, which in this paper discusses PI3K-AKT, AMPK, and RAS signaling pathways. Figure 2 shows the mechanisms of these signaling pathways, which lead to proliferation and malignancy in various cancers. Based on *EZH2* function and effect as an outstanding candidate in prostate cancer and other types of cancer treatment, several new drugs have been suggested, including Nelfinavir, Tazemetostat, Bevacizumab, Lenvatinib which can affect directly or indirectly *EZH2* gene expression [5], however, some of these drugs may be coupled with side effects or low sensitivity. Provided positioning drug management promotes the effect of drugs on target and decreases the rate of side effects that will be associated with some single drugs [6].

Prostate cancer

Prostate Cancer (PC) ranks as the second most prevalent form of cancer globally, following lung cancer. With 307,000 deaths globally, 6.6 percent of all male cancer deaths, prostate cancer ranks as the fifth leading cause of cancer-related mortality in the male population [7]. The global population is aging, and the incidence of prostate cancer is predicted to rise. Screening for prostate cancer is typically done through testing serum Prostate-Specific Antigen (PSA) levels and digital rectum inspection. However, these tests have poor sensitivity and specificity[8,9]. Prostate cancer therapies include radical prostatectomy, radical radiation, and hormone therapy with ablation, all of which have had mixed success in clinical trials [10]. Recent research on DNA methylation found multiple key genes in invasive PC. Besides, a whole altered methylation pattern is documented during prostate carcinogenesis. What has been observed reveals that overexpression of the *EZH2* gene is considered as a hallmark malignancy of progression in prostate cancers and, is linked to enhanced cell proliferation [11]. Several studies suggested the pivotal function of *EZH2* altered expression in angiogenesis mechanism of PC.

EZH2 gene

EZH2, a protein consisting of 746 amino acids and located on 7q35, is a well-known histone methyl transferase [12]. It contains several domains and is recognized as an

oncogenic stimulus in various malignancies. *EZH2* plays a crucial role in regulating gene expression in a Poly comb Repressive Complex 2 (PRC2)-dependent and -independent manner. Although *EZH2* alone is enzymatically inactive, it can convert to the catalytic subunit of PRC2 to suppress gene expression via the trimethylation of histone H3 on lysine K27 (H3K27me3) [12]. It has numerous organic operations, leading to cancer progression (Figure 1). The *EZH2* specifically plays a role in tumor malignancy in prostate, ovarian, and lung cancers [1]. *EZH2* is a two-way molecule can function as a transcriptional suppressor or activator based on its interaction with PRC2 or non-PRC2 components in various cellular regions [1]. H3K27me3 level was determined by histone methyltransferase *EZH2* and histone demethylase JMJD 3, these two proteins are upregulated in prostate cancer [13,14]. The *EZH2* expression could have been regulated through various signaling pathways.

The Figure 1 shows the change in the structure of normal prostate cells and angiogenesis, which have been switched to cancer progression and malignancy under the influence of *EZH2* gene expression.

Signaling pathways in charge of regulating EZH2 in prostate cancer

To develop therapy methods, it is crucial to understand the molecular mechanisms that underlie PC. It has been proved that PI3k/Akt, AMPK, and RAS signaling pathways have affected on the expression of *EZH2* directly, although *EZH2*, itself, as a major regulator of prostate cancer could have been able to control these signaling pathways with aid of various small molecular inhibitors. One of the main signaling pathways that regulates expression of *EZH2* in prostate cancer is the Phosphoinositide-3-Kinase (PI3K) and it is a downstream target [15]. Activation and inhibition of PI3K lead to the regulation of *EZH2* expression and prostate cancer growth (Figure 2). In PC, some important downstream targets of PI3K/Akt are Raf-1, P27/Kip1 cyclin-dependent kinase inhibitor and PTEN. The viability signaling pathways from independent cells rearrange PI3K-Akt signaling by a change in PTEN expression. This is frequently observed in prostate cancer since it can increase *EZH2* expression and histone methylation, leading to transcriptional repression of target genes[15,16]. Another signaling pathway that can act as the *EZH2* regulator is AMPK-Activated Protein Kinase (AMPK) signaling pathway, which

excites SETD2 expression and is frequently downregulated in prostate cancer [17]. According to positive correlation between AMPK and *EZH2* activity, AMPK phosphorylates the histone methyltransferase *EZH2* to disrupt the interaction between *EZH2* and core components of the Poly comb Repressive Complex 2 (PRC2), leading to dependent methylation PRC2 is degraded from histone H3 to Lys27 [18]. RAS signaling pathway is another pathway that has a strong connection with *EZH2* in various cancers and malignancies. What has been discussed in Fujii's article reveals that, EIK-1 is a downstream function of the MEK-ERK pathway and is also an intronic transcription factor leading to *EZH2* induction through analysis of the *EZH2* promoter. Additionally, mutant RAS may be connected to *EZH2* through the MEK-ERK pathway. Oncogenic MEK-ERK signaling induces *EZH2* in tumorigenesis by oncogenic RAS [19].

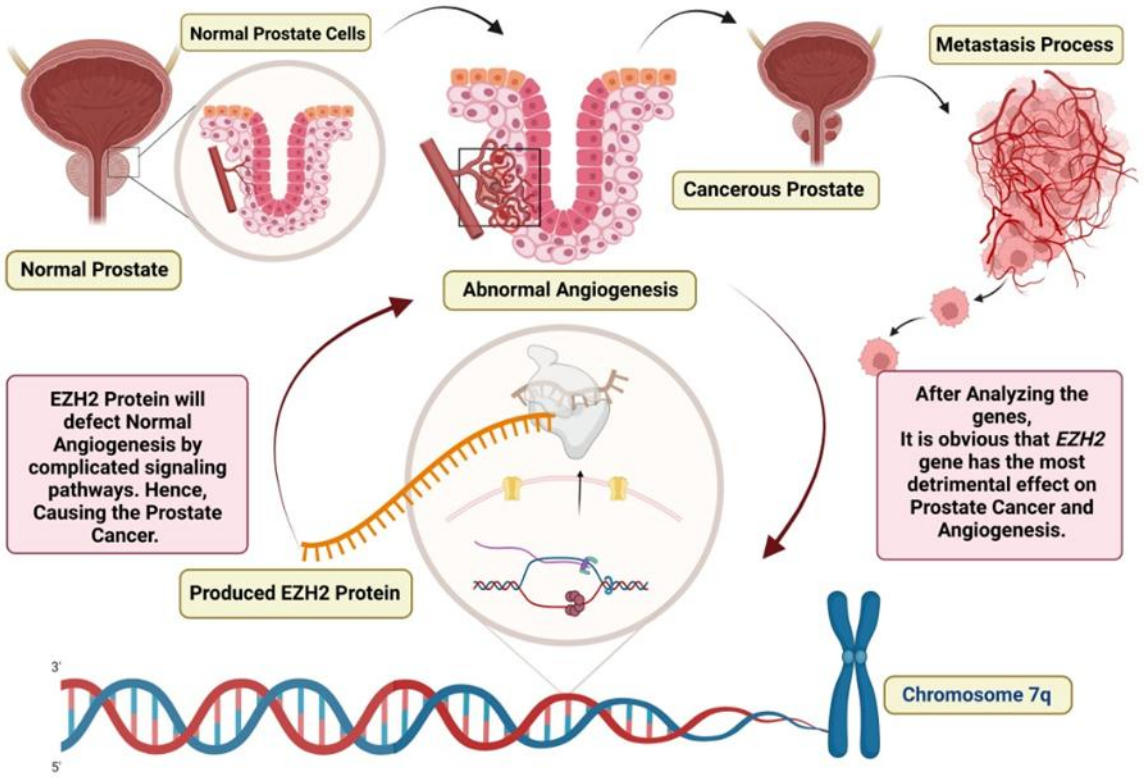

Figure 1. Angiogenesis is one of the effective factors in tumor progression and carcinogenesis. The imbalance between angiogenesis regulatory factors and changes in the expression of genes that directly and indirectly affect angiogenesis can be effective in changing the angiogenic structure and malignancy.

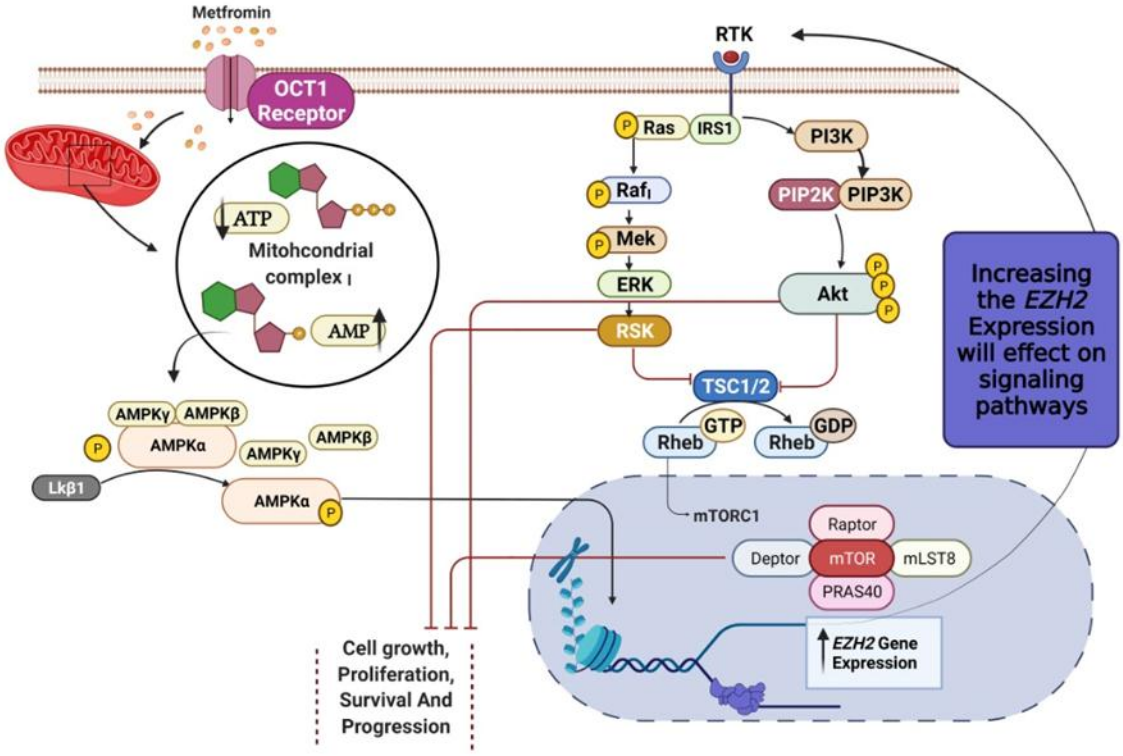

Figure 2. Signaling pathways play an essential and decisive role in cell destiny. Increased expression of *EZH2* by acting on receptors that activate or inhibit the components of signaling pathways, and these three signaling pathways ultimately lead to cell growth, survival, and progression.

Angiogenesis and EZH2

Angiogenesis, a complex and intricate phenomenon, encompasses a series of stages that facilitate the growth and development of blood vessels and is regulated by the balance between angiogenesis factors whose activity is strongly modulated by gene dose and anti-angiogenesis factors. [3,4,20]. Multiple growth factors, Vascular Endothelial Growth Factor (VEGF), Transforming Growth Factor (TGF) α, Epithelial Growth Factor (EGF), derived growth factor Platelet-Derived Growth Factor (PDGF), and Nitric Oxide Synthase (NOS) induce angiogenesis [7,21]. VEGF is the major tumor angiogenesis regulators and is the most important target of hypoxic factor 1 (HIF-1/2) [3]. Furthermore, there is an intriguing correlation between HIF-1 and

EZH2, but there is a lack of sufficient studies regarding hypoxia, HIF-1, and *EZH2* in relation to tumor configuration and progression. Prostate cancer is one of the cancers that HIF-1α could have positively affected *EZH2* expression [22] [3]. The HIF-1α expression also was upregulated by VEGF instigation [22]. VEGF-A plays a crucial role in the regulation of angiogenesis and vascular permeability through the activation of two receptors, namely VEGFR-1 and VEGFR-2 [23]. Among these receptors, VEGFR-2 stands out as a distinctive member of the receptor tyrosine kinase family, exhibiting high activity in response to VEGF stimulation. Its activation leads to the fine-tuning of angiogenesis, as well as the promotion of mitogenesis and permeability in vascular endothelial cells. Previous research has indicated a close association between the overexpression of *EZH2* and H3K27me3 and the augmentation of VEGF, indeed, *EZH2* may affect the expression of VEGF. As well, VEGF is widely acknowledged as a significant regulatory factor in the facilitation of angiogenesis by *EZH2*, while also serving as a downstream target of *EZH2* during the progression of peritoneal angiogenesis [22,23].

Angiogenesis in prostate cancer

Pro-angiogenic factors in PC include growth factor-translocating beta (TGF-β), VEGFs, FGFs, ILs, and various metalloproteinases [24,25], that expression of VEGFA and its receptors in prostate cancer, implicated in androgen regulation, hence co-regulates angiogenesis [26]. Although the mechanisms regulating peritoneal angiogenesis remain undisclosed, a compilation of evidence suggests a correlation between heightened *EZH2* signaling and the occurrence of pathological angiogenesis. In addition, based on Shi's article, the prohibition of *EZH2* repressed VEGF generation through the Wnt1/β-catenin and STAT3 signaling pathways [23]. The presence of heightened levels of TGFβ1 in the prostate cancer tissues, urine, and serum of individuals diagnosed with prostate cancer has been linked to an increased risk of angiogenesis metastasis and unfavorable clinical outcomes [27]. TGF-β indirectly regulates VEGFA in PC via the activity of SMAD and the activity of the SRC/focal adhesion protein kinase B (PKB or Akt)/(FAK) kinase signaling pathways [28]. Prostate cancer angiogenesis is also regulated by increased dissociation of Cancer-Associated Fibroblasts (CAFs),

thereby enhancing tumor angiogenesis through VEGFA [29], which has been classified as a regulative function is observed in the process of *EZH2* facilitating angiogenesis, while an *EZH2* downstream target remains pending during the angiogenesis process and tumorigenesis [23].

EZH2 in PC from regulative and functional points of view

EZH2 overexpression is frequently observed in metastatic prostate cancer, and it is also detected in aggressive tumors associated with a significant likelihood of recurrence following radical prostatectomy [30]. Phosphorylation of *EZH2* by AKT results in the reduction of *EZH2* effect on H3; thus, it is catalytic activity is affected. In certain cellular mechanisms, the functionality of EZH2 might be influenced by collaborative transcription factors, like Androgen Receptor (AR) in PC which is evidenced by recent genome-wide studies of AR [31]. It assumes a significant role in prostate cancer progression by blocking the transcription of tumor/metastasis suppressor genes [2]. It has been confirmed that *EZH2* negative regulation and loss of RKIP (RAF-1 metastasis inhibitor protein) in prostate cancer progression and metastasis, the spread of cancer cells from one part of the body to another, can disrupt various cell signaling pathways. These pathways include RAF-1, GPCR, MEK, ERK, NF-KB [32] GSK-A functions as a competitive agonist within the methyltransferase system by replacing the naturally occurring layer, which significantly reduces the trimethylation of H3K27 [33]. *EZH2* has emerged as a promising contender for molecular targets in the management of prostate cancer, offering a novel avenue to regulate the disease via epigenetic mechanisms. However, the drug target has posed a formidable challenge due to its nature as a transcription factor [2].

Effective new drugs in prostate cancer

In recent times, the pathological adaptation mechanism of *EZH2* has been elucidated in several diseases, including prostate cancer. A considerable number of small molecule *EZH2* inhibitors have been discovered, and a subset of these compounds have progressed to different phases of clinical trials, in the following we mentioned some of these small molecule inhibitors which could be considered as an agent in the treatment of PC. Albeit, the progression of *EZH2* inhibitors has been coupled with some problems including short *in vivo* half-life,

little clinical efficacy, and preclinical drug continuity. A multitude of *EZH2* inhibitors has entered clinical trials since 2012 [5] .

Tazemetostat

Tazverik, also known as Tazemetostat, has been approved by the FDA and is marketed by Epizyme Inc. It is a powerful and specific inhibitor of *EZH2*, an enzyme responsible for methyltransferase activity. Tazemetostat is currently being evaluated in phase 1/2 clinical trials for various hematological and solid tumors, showcasing its potential therapeutic efficacy [5,34]. Some common side effects of Tazemetostat include congestion, runny nose, sore throat, sneezing, bleeding, unusual bruising, abnormal fatigue and weakness. The activity of both natural and mutated forms of *EZH2* can be effectively prohibited through inhibition. Specifically, inhibiting *EZH2* leads to the prevention of methylation of Histone H3 Lysine 27 (H3K27). This decrease in histone methylation results in altered gene expression patterns associated with cancer pathways, ultimately leading to reduced proliferation of tumor cells in *EZH2* mutated cancer cells [5]. What has been mentioned above reveals that prohibition of *EZH2* with aid of Tazemetostat can be beneficial to control cancer progression.

Cabozantinib

Cabozantinib, marketed as Cometriq and Cabometyx, is a pharmaceutical compound manufactured by Exelixis Inc. This small-molecule inhibitor is primarily designed to target VEGFR2, tyrosine kinases c-Met, and possesses the ability to effectively inhibit RET and AXL [35]. Cabozantinib is an anticancer drug that is using in phase 2 of clinical trials and to treat some cancers including renal cell carcinoma, medullary, hepatocellular carcinoma, prostate cancer, brain cancers, etc. The most common noxious effects of Cabozantinib can be included fungal infections, anxiety, loss of albumin, low blood pressure, tinnitus, etc. According to Shi's study, the expression of VEGF has had a direct connection with the expression of *EZH2*, indeed, the overexpression of *EZH2* can enhance activation of VEGF; Thus, cabozantinib is able to inhibit *EZH2* and VEGF for preventing malignancies [23,35]. As shown above, it is concluded It has been observed that the expression of EZH2 and the function of VEGF are positively correlated, which can explain the cabozantinib suppressive activity.

GSK126

GSK126 (GSK2816126A) is a small molecule that progressed by GlaxoSmithKline and selectively inhibits the enzymatic activity of *EZH2* methyltransferase by competitively binding to the co-substrate S-Adenosyl Methionine (SAM). This compound is currently being investigated in a phase 1 clinical trial for lymphoma, where it has shown potential in overcoming drug resistance to *EZH2* inhibitors (EZH2i) by activating the Insulin-like Growth Factor-1 Receptor (IGF-1R) and Phosphatidylinositol 3 Kinase (PI3K) pathways, and MEK. Treatment by this drug has been associated with common side effects including intense noxious reactions, fatigue and nausea symptoms [36]. One effective method to diminish cell growth and migration in various types of prostate cancer cells was by removing *EZH2*, although *EZH2* inhibitors, containing GSK126 was minimally effective when used alone in CRPC cells. However, combining it with chemotherapy and radiotherapy is a possible way to enhance its effectiveness [5,36]. What has been discussed reveals that GSK126 can act as an *EZH2* inhibitor to decrease proliferation and migration in various cancers.

Bevacizumab

Bevacizumab, marketed as Avastin and manufactured by Genentech, Inc., is a monoclonal antibody that functions as an angiogenesis inhibitor. Its primary target is VEGF-A, new-made vessels. By impeding the growth of these vessels, Bevacizumab effectively hinders angiogenesis. Bevacizumab also is a medicine using in phase 1 of clinical trials and to treat several sorts of cancers and a particular eye disease. This drug is coupled with some common side effects among cancer patients including high blood pressure, headache, rash, and nose bleeds [37]. Based on the direct relationship amongst the *EZH2* expression and VEGF, VEGF prohibition can repress *EZH2* and malignancy [23]. By binding to VEGF-A, bevacizumab is expected to exert its effects extracellularly. However, in certain instances, such as cervical and breast cancer, the drug can be internalized by cells through constitutive endocytosis [38-40]. Due to the relationship described above, Bevacizumab may be an appropriate candidate to repress cancer progression that depend on DORG activation.

Lenvatinib

Lenvatinib sold under the brand name Lenvima, and was enlarged by Eisai Company. It serves as a potent inhibitor of the VEGFR1, VEGFR2, and VEGFR3 kinases, effectively targeting multiple kinases simultaneously. It is anti-cancer medicine for using in the phase 1 clinical trials and treating certain sorts of Thyroid cancer and other cancers [41]. It is imagined that the principal reason for hypertension is VEGFR2 inhibition. The most common side effect is hypertension followed by diarrhea and fatigue. Moreover, other common side effects can be included hypotension, decreased appetite, muscle, and bone pain, and thrombocytopenia [42,43]. In addition, Lenvatinib through prohibition of tyrosine kinase family can affect *EZH2* in cancers that have been associated with overexpression of *EZH2* [41,42]. As mentioned above, inhibiting tyrosine kinase family can be therapeutic potential to influence *EZH2* expression in many cancers with aid of Lenvatinib.

Repositioned drugs

By repurposing drugs make us able to identify and develop new therapeutic indications to existing drugs. Reprofiling drugs has been associated with significant merits that are obvious based on comprehensive studies and analyses done regarding the information on these drugs, including safety, side effects, toxicity, therapy doses, and drug production. This approach can be beneficial to reduce probe time and final inquiries in pre-clinical and clinical tests. The number of repurposing drugs has climbed rapidly, especially in the oncology area, because not only decreases chemotherapy adverse effects, and promotes cancer patient's life quality, but also ameliorates patients' survival. The main aim of repositioning drugs depends on identifying drugs, but these drugs must not have impressive negative effects [43,44]. Lourenço's article introduced combined treatments for cancers by utilizing repositioned drugs. He deduced that, especially in prostate cancer, several commonly prescribed medications appear to have the potential to serve as suitable candidates for an integrated approach to treat. This method relies on the reality that carcinogenesis in tumors may share the same signaling pathways; thus, drugs against a cancer can be helpful in other cancer types. Then, in combined treatment, a chemotherapy drug can be utilized with repositioned drugs, in PC, some drugs would be appropriate

candidates as repurposed drugs and combination treatment involved in various cancer types because of shared signaling pathways [6]. In continue, we summarized some promising repositioned drugs against PC, which were rediscovered in recent years.

Nelfinavir

Nelfinavir sold under the brand name Viracept and developed by Agouron Pharmaceuticals. It also categorized as an antiviral drug, which has potential therapeutic effects for various conditions, involving in cancer, and also can operate through several mechanisms. These mechanisms include activating caspase-9 and caspase-6, inhibiting angiogenesis and androgen receptor activation, inducing apoptosis and autophagy, inhibiting regulated intramembrane proteolysis, and inhibiting STAT3 and AKT [6,43]. It is used in the phases 1 and 2 clinical trials and in the treatment of HIV/AIDS. Based on strong evidence Nelfinavir has been identified as a promising option for the treatment of prostate cancer, with potential benefits in both monotherapy and associated with radiotherapy or chemotherapy. Treatment by nelfinavir is associated with common side effects including Flatulence, diarrhea, or abdominal pain [6]. The *EZH2* could facilitate methylation of STAT3 lysine 180 via *EZH2* S21 phosphorylation [23]. This effect can consider targeted therapy for repressing *EZH2* and eventually STAT3 signaling [5]. In addition to what has been cited, Nelfinavir can be applicable to prohibit methylation of some genes and signaling pathways which are related to malignancy.

Itraconazole

Itraconazole sold under the brand name Sporanox, Sporaz, Orungal and developed by Janssen Pharmaceuticals. It is also well-known as the antifungal agent that binds to VDAC1, and clogs the action devoted to mitochondrial metabolism. Furthermore, it barricades cell proliferation via mTOR and VEGFR2 inhibition and AMPK activation which these actions lead to inhibit angiogenesis [6]. Itraconazole has been probed as an anticancer agent in the phase 1 of clinical trials for those suffering from prostate cancer, basal cell carcinoma, non-small cell lung cancer, which has been coupled with manifold side effects that are common, including diarrhea, rash, abdominal pain, headache, and nausea. Some signaling pathways including mTOR and VEGFR2 inhibition and

AMPK have had a correlation with *EZH2* and can promote sensitizer for *EZH2*-targeting cancer therapy [6,18]. As mentioned above, Itraconazole can affect specifically on various signaling pathways to repress cell proliferation in malignancies.

Valproic acid

Valporic acid sold under the brand name Depakote, Epilim, and Convulex, which developed by Beverly S. Burton, also considered an antiepileptic and anticonvulsant drug. It can inhibit Class I Histone Deacetylases (HDACI), and according to the effect that it has had on the DNA region can suppress essential anticancer genes, which are coupled with these enzymes. Hence, inhibition by valproic acid leads to stopping the silencing of these genes [6]. Valproic acid control gene expression through noncoding RNAs [45]. It is in the phase 2 clinical trial for treating epilepsy and bipolar disorder and intercepting migraine headaches [46]. This medication is linked to typical adverse reactions such as, vomiting, nausea, somnolence, dry mouth, and somnolence [6,43,47]. Besides, treatment by valproic acid leads to decreased *EZH2* expression, but also increases the expression of P21 and PTEN impressively [45]. That has been mentioned above shows that valproic acid affects histone deacetylases and DNA regions that are allocated to anticancer genes for suppressing them, which can consider as a targeted therapy.

Metformin

Fortamet is a brand name for Metformin, Glucophage, and Glumetza, and developed by Bristol-Myers Squibb Company. It categorized as an antidiabetic drug, which is peculiar to the biguanide group. In type 2 diabetes treatment program, it is considered the first line approach. Metformin has showed pleasant results in prostate cancer treatments in phase 2 of clinical trials since it upgrades apoptosis in prostate cancer cell lines, and the PI3K signaling pathway can be down-regulated, leading to a reduction in growth, proliferation, and differentiation of tumor cells [42,47]. On the other hand, Metformin commonly causes adverse effects such as diarrhea, nausea, and abdominal pain [48]. It has the ability to inhibit the growth of prostate cancer, which is associated with the activation of Adenosine Monophosphate-Activated Protein Kinase (AMPK). This is because AMPK blocks the cell cycle and ultimately prevents cell growth [7,47].

One potential role of *EZH2* may be mediate the downregulation of H3K27me3 triggered by AMPK. The reduction of *EZH2* in the AMPK pathway results in decreased levels of H3K27me3. As well as, it has been proven that prohibition of AMPK induces H3K27me3 in *EZH2*. Indeed, results revealed that AMPK might adjust H3K27me3 by modifying the methyltransferase activity of PRC2/EZH2 [18]. In addition to what has been mentioned above, Metformin is a targeted drug to influence on PI3K and AMPK signaling pathways for repressing cell proliferation, migration, and angiogenesis.

Table 1. Lists of drugs having an impressive effect on prostate cancer treatment

	Trade name	**Formula**	**Class of drug**	**Common side effect**	**Function**	**Signaling pathway**
Nelfinavir	Viracept	$C_{32}H_{45}N_3O_4S$	protease inhibitor(PIs)	insulin resistance, hyperglycemia lipodystrophy[50]	Inducing apoptosis and necrosis Inducing cell-protective mechanism Response of unfolded protein[49]	Akt/Pkb inhibition Endoplasmic reticulum stress activation unfold protein response [48]
Itraconazole	Sporanox	$C_{35}H_{38}Cl_2N_8O_4$	Antifunga (inhibitor)	nausea diarrhea abdomen nal pain rash headache[56, 57]	Anticancer agents against prostate cancer , basal cell carcinoma, non-small cell lung cancer [50]. Examining activity in the second phase of the trial in men with non-small cell lung cancer when it was combined with the chemotherapy agent, pemetrexed[54, 55]	inhibit the hedgehog signaling pathway inhibit angiogenesis[52, 53]

Valporic acid	Depakote	C8H16O2	an antiepileptic and anticonvulsant [61]	nausea vomiting somnolence dry mouth[60]	Affecting GABA (gamma-aminobutyric acid) levels Preventing voltage-gated sodium channels Prohibiting histone deacetylases[59]	P21/PTEN pathway[45]
Metformin	Glucophage	C4H11N5	a biguanide anti-hyperglycemic agent[61]	diarrhea cramps nausea vomiting increased flatulence[64]	Prohibiting the mitochondrial respiratory chain (complex I) Activity AMP-activated protein kinase (AMPK) Inhibiting glucagon-induced increase in cyclic adenosine monophosphate (cAMP) by decreasing protein kinase A (PKA) activation [62, 63]	AMPK signaling pathway[62]

CONCLUSION

The present study demonstrated the impact of *EZH2* on angiogenesis in prostate cancer progression. *EZH2* overexpression has been linked to the development of malignancy in diverse types of cancer; hence, an innovative approach to disease control through epigenetic mechanisms can be achieved by discovering diminutive inhibitors targeting *EZH2*. Although several drugs such as Nelfinavir, Itraconazole, Valporic acid, and Metformin are not recognized as the mainstay of cancer treatment, they have been shown potential efficacy in cancer treatment as monotherapy or associated with additional medications, every one of which has different mechanism. Nowadays, the repositioned drugs are intended as a potentially effective therapeutic approach for various types of cancer, especially prostate cancer in this study.

REFERENCES

[1]. Gan, L., *et al.*, Epigenetic regulation of cancer progression by EZH2: from biological insights to therapeutic potential. *Biomark. Res*., 2018. 6(1): 1-10.

[2]. Yang, Y.A. and J. Yu, EZH2, an epigenetic driver of prostate cancer. *Pro . cell*, 2013. 4(5): 331-41.

[3]. Manuelli, V., *et al.*, Regulation of redox signaling in HIF-1-dependent tumor angiogenesis. *FEBS J*, 2021. 5413–25.

[4]. Bergers, G. and D. Hanahan, Modes of resistance to anti-angiogenic therapy. *Na Rev Cancer*, 2008. 8(8): 592-603.

[5]. Duan, R., W. Du, and W. Guo, EZH2: a novel target for cancer treatment. *J Hematol Oncol*, 2020. 13(1): 1-12.

[6]. Lourenço, T. and N. Vale, Pharmacological efficacy of repurposing drugs in the treatment of prostate cancer. *Int. J. Mol. Sci*., 2023. 24(4): 4154.

[7]. Nordby, Y., Angiogenic biomarkers in prostate cancer-A study into the prognostic significance of angiogenesis related growth factor ligands and receptors and miR-205 in a cohort of Norwegian prostatectomy patients. *Uro.*, 2018. 23: 2578.

[8]. Vendrami, C.L., *et al*., The utility of prostate specific antigen density, prostate health index, and prostate health index density in predicting positive prostate biopsy outcome is dependent on the prostate biopsy methods. *Uro*, 2019. 129: 153-59.

[9]. Giunchi, F., M. Fiorentino, and M. Loda, The metabolic landscape of prostate cancer. *Eur. Urol. Oncol.*, 2019. 2(1): 28-36.

[10]. Melegh, Z. and S. Oltean, Targeting angiogenesis in prostate cancer. *Int. J. Mol. Sci.*, 2019. 20(11): 2676.

[11]. Bhagirath, D., *et al.*, Epigenetics of prostate cancer and novel chemopreventive and therapeutic approaches, in Epigenetics of Cancer Prevention. 2019, *Els.* 287-308.

[12]. Al Khatib, M.O., *et al.*, Benefits and Challenges of Inhibiting EZH2 in Malignant Pleural Mesothelioma. *Cancers*, 2023. 15(5): 1537.

[13]. Daures, M., *et al.*, A new metabolic gene signature in prostate cancer regulated by JMJD3 and EZH2. *Oncotarget*, 2018. 9(34): 23413.

[14]. Park, J.M., *et al.*, USP44 promotes the tumorigenesis of prostate cancer cells through EZH2 protein stabilization. *Mol. Cells*, 2019. 42(1): 17.

[15]. Lee, S.H., et al., Crosstalking between androgen and PI3K/AKT signaling pathways in prostate cancer cells. *J. Biol. Chem.*, 2015. 290(5): 2759-68.

[16]. Zeng, J., *et al.*, Targeting EZH2 for cancer therapy: From current progress to novel strategies. *Eur. J. Med. Chem.*, 2022: 114419.

[17]. Yuan, H., *et al.*, SETD2 restricts prostate cancer metastasis by integrating EZH2 and AMPK signaling pathways. *Cancer cell*, 2020. 38(3): 350-65.

[18]. Wan, L., et al., Phosphorylation of EZH2 by AMPK suppresses PRC2 methyltransferase activity and oncogenic function. *Mol. cell*, 2018. 69(2): 279-91.

[19]. Fujii, S., *et al.*, RAS oncogenic signal upregulates EZH2 in pancreatic cancer. *Biochem. Biophys. Res. Commun.*, 2012. 417(3): 1074-79.

[20]. Kavousi, S., *et al.*, Specific signaling pathways and drugs play key role in regulation of angiogenesis. *Health Biotech Biopharm.*, 2020. 4(1): 7-37

[21]. Semenza, G.L., Hypoxia-inducible factors in physiology and medicine. *Cell*, 2012. 148(3): 399-408.

[22]. Papale, M., *et al.*, EZH2, HIF-1, and their inhibitors: an overview on pediatric cancers. *Front. Pediatr.*, 2018. 6: 328.

[23]. Shi, Y., et al., Inhibition of EZH2 suppresses peritoneal angiogenesis by targeting a VEGFR2/ERK1/2/HIF-1α-dependent signaling pathway. *J. Pathol.*, 2022. 258(2): 164-78.

[24]. Bilusic, M. and Y.-N. Wong, Anti-angiogenesis in prostate cancer: knocked down but not out. *Asian J. Androl.*, 2014. 16(3): 372.

[25]. Mateus, P.A.M., et al., Association of anti-inflammatory and antiangiogenic therapies negatively influences prostate cancer progression in TRAMP mice. *Prostate*, 2019. 79(5): 515-35.

[26]. Sarkar, C., *et al.*, Angiogenesis inhibition in prostate cancer: An update. *Cancers*, 2020. 12(9): 2382.

[27]. Principe, D.R., *et al.*, TGF-β: duality of function between tumor prevention and carcinogenesis.

JNCI: J. Natl. Cancer Inst., 2014. 106(2).

[28]. Mirzoeva, S., C.A. Franzen, and J.C. Pelling, Apigenin inhibits TGF-β-induced VEGF expression in human prostate carcinoma cells via a Smad2/3-and Src-dependent mechanism. *Mol. Carcinog.*, 2014. 53(8): 598-609.

[29]. Ji, H., *et al.*, Inhibition of transforming growth factor beta/SMAD signal by MiR-155 is involved in arsenic trioxide-induced anti-angiogenesis in prostate cancer. *Cancer. Sci*, 2014. 105(12): 1541-49.

[30]. Varambally, S., *et al.*, The polycomb group protein EZH2 is involved in progression of prostate cancer. *Nature*, 2002. 419(6907): 624-29.

[31]. Zhao, J.C., *et al.*, Cooperation between Polycomb and androgen receptor during oncogenic transformation. *Genome Res*, 2012. 22(2): 322-31.

[32]. Ren, G., *et al.*, Polycomb protein EZH2 regulates tumor invasion via the transcriptional repression of the metastasis suppressor RKIP in breast and prostate cancer. *Cancer Res*, 2012. 72(12): 3091-3104.

[33]. Kong, D., *et al.*, Loss of let-7 up-regulates EZH2 in prostate cancer consistent with the acquisition of cancer stem cell signatures that are attenuated by BR-DIM. *PloS one*, 2012. 7(3): 33729.

[34]. Knutson, S.K., *et al.*, Synergistic anti-tumor activity of EZH2 inhibitors and glucocorticoid receptor agonists in models of germinal center non-Hodgkin lymphomas. *PloS one*, 2014. 9(12): 111840.

[35]. Fisher, M.J., *et al.*, Cabozantinib for neurofibromatosis type 1–related plexiform neurofibromas: a phase 2 trial. *Nature. Med*, 2021. 27(1): 165-73.

[36]. Negri, A., *et al.*, Simultaneous administration of EZH2 and BET inhibitors inhibits proliferation and clonogenic ability of metastatic prostate cancer cells. *J. Enzyme Inhib. Med. Chem.*, 2023. 38(1): 2163242.

[37]. Semenza, G.L., A new weapon for attacking tumor blood vessels. N. *Eng. J. Med.*, 2008. 358(19): 2066-67.

[38]. Los, M., J.M. Roodhart, and E.E. Voest, Target practice: lessons from phase III trials with bevacizumab and vatalanib in the treatment of

advanced colorectal cancer. *Oncologist*, 2007. 12(4): 443-50.

[39]. Karpinska, A., *et al*., Cellular uptake of Bevacizumab in cervical and breast cancer cells revealed by single-molecule spectroscopy. *J. Phys. Chem. Lett.,* 2023. 14(5): 1272-78.

[40]. Tehrani, N.S., *et al.,* Pathogenesis, significant biomarkers and treatment strategies in ovarian cancer. *Health Biotech Biopharm,* 2020. 4(2): 1-27

[41]. Matsui, J., *et al.,* Multi-kinase inhibitor E7080 suppresses lymph node and lung metastases of human mammary breast tumor MDA-MB-231 via inhibition of vascular endothelial growth factor-receptor (VEGF-R) 2 and VEGF-R3 kinase. *Clin. Cancer Res.,* 2008. 14(17): 5459-65.

[42]. Haberfeld, H., Austria-codex. Beč, Österreichischer Apothekerverlag, 2015.

[43]. Bahmad, H.F., *et al*., Overcoming drug resistance in advanced prostate cancer by drug repurposing. *Med.Sci*, 2022. 10(1): 15.

[44]. Duarte, D. and N. Vale, Antidepressant drug sertraline against human cancer cells. *Bio,* 2022. 12(10): 1513.

[45]. Sajadpoor, Z., *et al*., Valproic acid promotes apoptosis and cisplatin sensitivity through downregulation of H19 noncoding RNA in ovarian A2780 cells. *Appl Biochem Biotech,* 2018. 185: 1132-44.

[46]. Carr, R.B. and K. Shrewsbury, Hyperammonemia due to valproic acid in the psychiatric setting. *Am. J. Psychiatry*, 2007. 164(7): 1020-1027.

[47]. Turanli, B., *et al*., Drug repositioning for effective prostate cancer treatment. *Front.*, 2018: 500.

[48]. Koltai, T., Nelfinavir and other protease inhibitors in cancer: mechanisms involved in anticancer activity. *F1000 Res,* 2015. 4.

[49]. Musarrat, F., *et al.,* The anti-HIV drug nelfinavir mesylate (Viracept) is a potent inhibitor of cell fusion caused by the SARSCoV-2 spike (S) glycoprotein warranting further evaluation as an antiviral against COVID-19 infections. *J. Med. Virol.*, 2020. 92(10): 2087-95.

[50]. Shim, J.S. and J.O. Liu, Recent advances in drug repositioning for the discovery of new anticancer

drugs. *Int. J of Bio Sci*, 2014. 10(7): 654.

[51]. Zhang, K.E., *et al.*, Circulating metabolites of the human immunodeficiency virus protease inhibitor nelfinavir in humans: structural identification, levels in plasma, and antiviral activities. *AAC*, 2001. 45(4): 1086-93.

[52]. Aftab, B.T., *et al.*, Itraconazole inhibits angiogenesis and tumor growth in non–small cell lung cancer. *Cancer Res*, 2011. 71(21): 6764-72.

[53]. Aftab, B.T., *et al.*, Itraconazole and arsenic trioxide inhibit hedgehog pathway activation and tumor growth associated with acquired resistance to vismodegib. *AACR*. 2013.

[54]. Antonarakis, E.S., *et al.*, Repurposing itraconazole as a treatment for advanced prostate cancer: a noncomparative randomized phase II trial in men with metastatic castration-resistant prostate cancer. *Oncologist*, 2013. 18(2): 163-73.

[55]. Ip, K.H.K. and K. McKerrow, Itraconazole in the treatment of basal cell carcinoma: A case-based review of the literature. *Australas. J. Dermatol.*, 2021. 62(3): 394-97.

[56]. Fischer, J. and C.R. Ganellin, Analogue-based drug discovery.*CI*, 2010. 32(4): 12-15.

[57]. Kim, B.-K., *et al.*, Prospective Study on Prophylactic Micafungin Sodium against Invasive Fungal Disease during Neutropenia in Pediatric & Adolescent Patients Undergoing Autologous Hematopoietic Stem Cell Transplantation. *Children*, 2022. 9(3): 372.

[58]. Trevor, A.J., *et al.*, Pharmacology examination & board review. *M-H.*, 2010. 191-95.

[59]. Mohamed, N.A.A., Effects of antiepileptic drug (valporic acid) on complete blood count parameters among patients attending eltigain elmahi neurological and psychological hospital-Khartoum Stata-Sudan. *HAO.*, 2018, Hussam Ali Osman.

[60]. Rout, S. and S.K. Manna, Effect of valproic acid treatment on hepatocytes *in vitro.*

[61]. Olsen, K., E. Taubøll, and L. Gjerstad, valproate is an effective, well-tolerated drug for treatment of status epilepticus/serial attacks in adults. *Acta Neurol. Scand.*, 2007. 115: 51-54.

[62]. Madiraju, A.K., *et al.,* Metformin suppresses gluconeogenesis by inhibiting mitochondrial glycerophosphate dehydrogenase. *Nature,* 2014. 510(7506): 542-46.

[63]. LaMoia, T.E., *et al.*, Metformin, phenformin, and galegine inhibit complex IV activity and reduce glycerol-derived gluconeogenesis. *Proc. Natl. Acad. Sci.*, 2022. 119(10): 2122287119.

[64]. Lipska, K.J., C.J. Bailey, and S.E. Inzucchi, Use of metformin in the setting of mild-to-moderate renal insufficiency. *Diabetes care,* 2011. 34(6): 1431-37.

[65]. De Silva, V.A., *et al.,* Metformin in prevention and treatment of antipsychotic induced weight gain: a systematic review and meta-analysis. BMC psychiatry, 2016. 16(1): 1-10.