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Review

Functional and therapeutic significance of EZH2 in urological cancers

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ABSTRACT

The enhancer of zeste homolog 2 (EZH2) is a core subunit of the polycomb repressor complex 2 (PRC2), which is overexpressed in numerous cancers and mutated in several others. Notably, EZH2 acts not only a critical epigenetic repressor through its role in histone methylation, it is also an activator of gene expression, acting through multiple signaling pathways in distinct cancer types. Increasing evidence suggests that EZH2 is an oncogene and is central to initiation, growth and progression of urological cancers. In this review, we highlight the critical role of EZH2 as a master regulator of tumorigenesis in the prostate, bladder and the kidney through epigenetic control of transcription as well as a modulation of various critical signaling pathways. We also discuss the promise and challenges for EZH2 inhibitors as future anticancer therapeutics, some of which are currently in clinical trials.

INTRODUCTION

Human cancer genome sequencing has revealed that various histone modifying genes that encode chromatin regulators are frequently mutated in a wide variety of cancers [1-3]. Covalent epigenetic modifications at enhancers and promoters of genes regulate critical genomic and biological processes like gene expression and cell fate specification [3-9]. There is increasing evidence that the chromatin modifier EZH2 is associated with cancer [10-21]. EZH2 is one of the core enzymatic subunits of PRC2, a highly conserved protein complex that methylates lysine27 of histone H3 (H3K27) to promote transcriptional silencing of many genes [22-25]. EZH2 is overexpressed in many cancers. Also, many gain or loss of function EZH2 mutations have been discovered in distinct cancer types. Notably, EZH2 is not only a critical epigenetic repressor through histone methylation, but also an activator of gene expression through different pathways [26].It is also clinically relevant in epigenetic cancer therapy and therefore many small molecule inhibitors have been developed that can specifically suppress the enzymatic activity of EZH2[27-29]. Notably, a phase ½ clinical trial of EPZ-6438 in patients with advanced solid tumors was launched.

Urological cancers of the prostate, bladder and the kidney are among the 10 most frequent cancers in Chinese men [30]. Prostate cancer is a major health concern in the older male populations all over the world and is the sixth most common cause of cancer related deaths in the world [31]. Androgen receptor (AR) plays a critical role in the development of prostate cancer and androgen deprivation therapy (ADT) is the first line therapy for newly diagnosed prostate cancer patients [32]. Nevertheless, most patients progress to castration-resistant prostate cancer (CRPC) and even metastatic prostate cancer [33].

Bladder cancer incidence is 3 times higher among males than females [34]. Nearly 386000 newly diagnosed cases and about 150000 deaths are reported annually worldwide [31]. Also, 75% to 80% of new patients are diagnosed with superficial non-muscle invasive bladder cancer (NMIBC) [35] Renal cell carcinoma is the eighth most common cancer in the United States [36] and its incidence is steadily rising in most areas of the world[37]. Total or partial nephrectomy is the optimal primary treatment. Nevertheless, renal cell carcinoma recurs in 20-40% of patients after resection, which is associated with tumor stage and grade [38].

In this review, we highlight the transcriptional function of EZH2 in cancer and the current insights into

the role of EZH2 in prostate, bladder and kidney cancer. Finally, we will review the development, translation and early clinical findings of therapeutics targeting EZH2 in cancer.

THE FUNCTIONAL ROLE OF EZH2

Human EZH2 gene is located on the long arm of chromosome 7 at 7q35 and encodes a 746 amino acid protein that is part of the PRC2 complex, which also includes SUZ12, EED, RbAp46 and RbAp48 as shown in Figure 1a [30]. PRC2 is a methyltransferase that methylates lysine 27 of histone H3 (H3K27me3) [39]. Many studies have implicated EZH2 as a key player in tumorigenesis. The role of EZH2 in cancer was first observed when it was identified as one of the top upregulated genes in aggressive prostate cancer [10]. Since then, similar findings have been reported in other human cancers including breast cancer, bladder cancer, renal cell carcinoma, etc [40-42]. In most cases, high EZH2 expression is associated with metastasis and advanced disease in each of these cancer types. Collectively, the biological function of EZH2 includes canonical H3K27 methylation, transactivation of gene expression and methylation of non-histone targets.

Canonical H3K27 methylation

As a core subunit of PRC2, EZH2 methylates H3K27me3 that leads to transcriptional silencing. The SET domain is the catalytic subunit of EZH2 [43]. For epigenetic silencing, EZH2 complexes with EED and SUZ12, which are the two other subunits of the PRC2 complex. Given its role as a transcriptional repressor (Figure 1b), substantial efforts have been dedicated to understand the mechanism by which EZH2 drives tumor

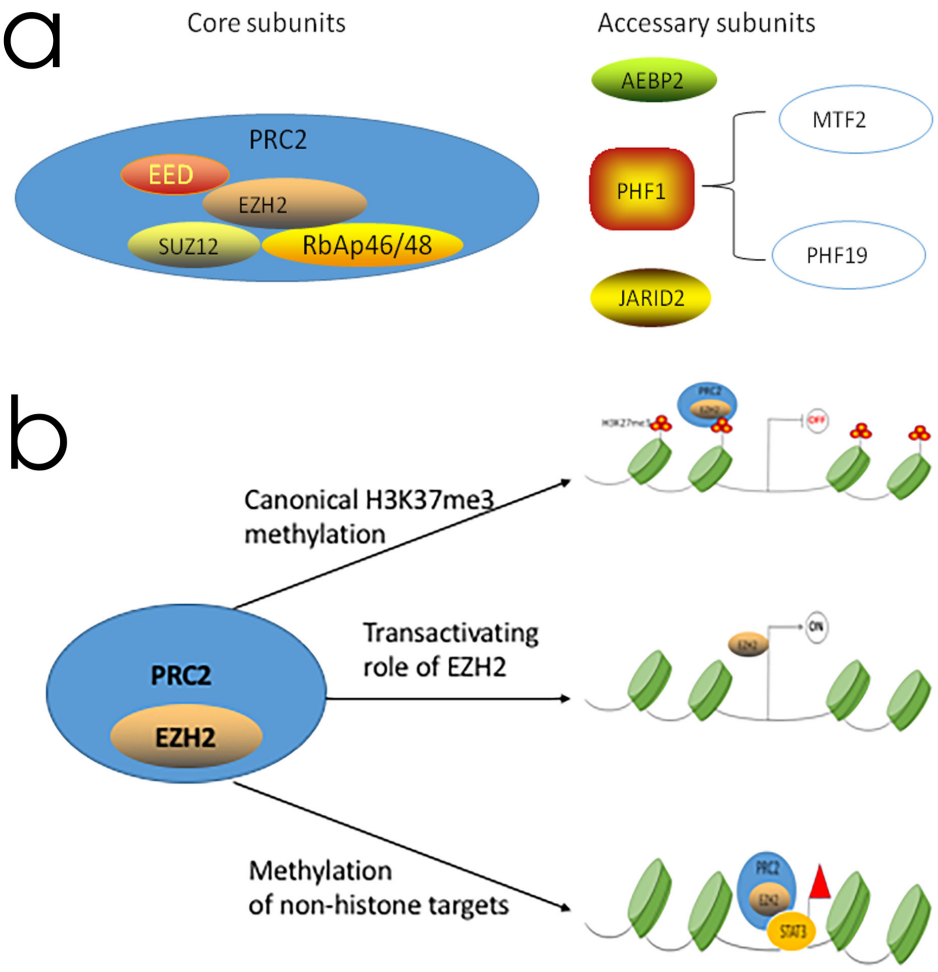

Figure 1: The PRC2 complex structure and the function of EZH2 in transcriptional regulation. a. The PRC2 complex consist of four core subunits, namely, EZH2, EED, SUZ12 and RbAp46/48 and additional proteins like AEBP2, PHF1, and JARID2. **b**. The functional role of EZH2: as a subunit of PRC2, EZH2 methylates H3K27 which contributing transcriptional silencing, EZH2 also have a PRC2 independent role in transcriptional activation and can methylate a number of non-histone protein substrates. OFF and ON refer to transcriptional silencing and activation, respectively.

Table 1: List of downstream targets of EZH2 in urological cancer

Cancer type	Target genes	Function	Contribution to tumorigenesis	Reference
Prostate	MSMB	Inhibits MMP secretion	Proliferation and invasion	83
	DAB2IP	Inhibition of NF-kB/Ras pathway	Transformation, proliferation and invasion	75, 76
	E-cadherin	maintain epithelial cellular adhesion	invasion	92
	ADRB2	B-adrenergic signaling	Transformation and invasion	77
	SLIT2	Chemorepellent protein	Proliferation and invasion	79
	TIMP2/3	ECM degradation	Invasion	82
	PCAT-1	Transcriptional repressor lincRNA	Proliferation	84
	RKIP	Inhibition of Raf and NF-kB pathways	Invasion	85
Bladder	APAF-1	apoptosis promoting factor	Proliferation and invasion	91
	E-cadherin	maintain epithelial cellular adhesion	Invasion	92
Kidney	E-cadherin	maintain epithelial cellular adhesion	Invasion	53

development.

EZH2 and cancer initiation

EZH2 is essential for self renewal in stem cells [44]. Analogous to its role in normal stem cells, EZH2 suppresses differentiation via canonical H3K27 methylation to repress lineage specifying factors [45, 46]. EZH2 is essential survival and proliferation of breast tumor initiating cells [44]. High EZH2 levels have been observed in cancer stem cell (CSC) populations isolated from primary breast cancer cells compared to normal breast cell lines [44]. Further, EZH2 activates RAF1-β-catenin signaling pathway that promotes expansion of breast tumor initiating cells. Therefore, it is hypothesized that EZH2 promotes cancer initiation by blocking differentiation [47]. However, EZH2 is also essential for differentiation programs of several distinct cancer types [48]. Therefore, the primary role of EZH2 is envisaged to include suppression of lineage specifying transcription programs in CSC and its effects on stemness and differentiation are probably secondary consequences.

EZH2 and tumor metastasis

EZH2 mediates silencing of FOXC1 and DNA damage repair pathways thereby driving oncogenesis [44, 49, 50]. Moreover, EZH2 promotes epithelial-mesenchymal transition (EMT) by epigenetically suppressing E-cadherin (also known as CDH1) via

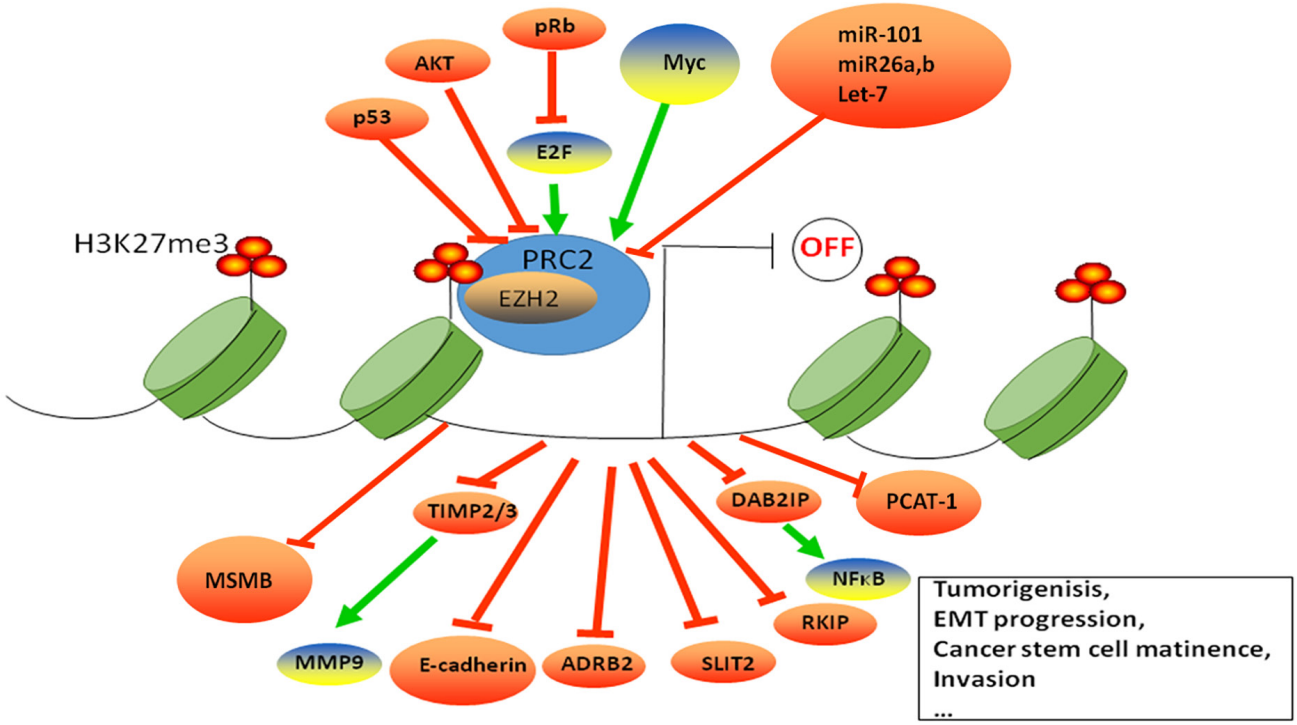

Figure 2: EZH2 regulation and function in prostate cancer. EZH2 is the enzymatic subunit of PRC2, which catalyzes H3K23me3. EZH2 induces transcriptional silencing of tumor suppressor genes, which subsequently cause tumor initiation, growth and progression. EZH2 is regulated by E2F, p53, MYC, AKT, miR101, miR26a, miR26b and Let-7.

Table 2: List of upstream targeting EZH2 in urological cancer

Cancer type	Upstream modulator	Reference
prostate	AKT, p53, E2F, Myc, miR-101,miR-26a/b, Let-7	66,77,78,86
bladder	Myc, E2F, miR-144, miR-101, miR-26a	81,82,84,87
kidney	YB-1, LncRNA MALAT1	97,98

canonical H3K27me3 modification of its promoter [50-53], facilitated by MEK/ERK signaling [54]. Further, EZH2 interacts with HDAC1/HDAC2 and Snail to form a co-repressor complex that contributes to E-cadherin promoter repression [55]. EZH2 is also required to recruit Snail-Ring1A/B complex to the E-cadherin promoter [56]. Consequently, E-cadherin inhibition is correlated with advanced stage cancer with poor clinical outcomes [56].

EZH2 and tumor progression

There is increasing evidence that EZH2 promotes angiogenesis in clear renal cell carcinoma, inflammatory breast cancer, nasopharyngeal carcinoma (NPC) and glioblastomas (GBM) [13, 40, 57]. High expression levels of EZH2 and VEGF correlate with TMN stage and distant metastasis in advanced clear renal cell carcinoma [13]. In nasopharyngeal carcinoma (NPC), elevated EZH2 levels were associated with an aggressive phenotype with poor prognosis and enhanced microvessel density [18]. EZH2 also promotes angiogenesis by inhibiting miR-1/Endothelin-1, which is an autocrine regulator of endothelial cells during neovascularization. Conversely, EZH2 represses angiogenesis during hypoxia and ischemia through its hypoxia response element (HRE) [58]. In endothelial cells, hypoxia results in EZH2 overexpression that regulates two pro-angiogenic genes, eNOS and BDNF, by augmenting the abundance of H3K27me3 at their promoters. However, from a therapeutic perspective, it is not clear if the EZH2 targets are essential for all cancer types.

Transactivating role of EZH2

Several studies have also identified a PRC2-independent role of transcriptional activation for EZH2 (Figure 1b) [59-62]. In a castration-resistant prostate cancer model, EZH2 acted as a co-activator for critical transcription factors including the androgen receptor (AR) that was independent of its transcriptional repressor function [63]. Further, EZH2 physically bridged the estrogen receptor (ER) and components of Wnt signaling to induce the gene expression in breast cancer cells [59]. EZH2 also activated NF-κB targets of NOTCH1 in breast cancer cells [61, 62].

Methylation of non-histone targets

Another PRC2-independent role of EZH2 is methylation of non-histone targets (Figure 1b). In a castration-resistant prostate cancer model, EZH2 methylated AR and modulated AR recruitment to its target sites [63]. Further, EZH2 promoted tumorigenicity of glioblastoma stem-like cells by methylating STAT3 [64]. EZH2 also methylates non-histone substrates that are recognized by the ubiquitination machinery for degradation [65].

Therefore, the biological functions of EZH2 include epigenetic repression through histone methylation as well as transcriptional activation of genes by modulating activity of various transcription factors and other associated proteins. However, the functional significance of the non-canonical functions of EZH2 to tumorigenesis is unclear at the present.

DYSREGULATION AND FUNCTIONAL ROLES OF EZH2 IN UROLOGY CANCER

EZH2 and prostate cancer

Varambally *et al* first demonstrated a positive association between EZH2 protein levels and prostate cancer aggressiveness [10]. Since then, many studies have highlighted the association between EZH2 expression and prostate cancer development [66-68]. Notably, EZH2 overexpression is not only associated with metastasis, but also with higher risk of recurrence after radical prostatectomy [10]. Hence, EZH2 is considered a potential diagnostic and prognostic biomarker in prostate cancer (Figure 2).

EZH2 is regulated transcriptionally, post-transcriptionally, and translationally (Figure 2). It also integrates and modulates many signaling pathways (Table 2). The E2F transcriptional factors bind to EZH2 and EED promoters and regulate their expression during E2F mediated cell proliferation via EZH2 [69]. In contrast, activated p53 suppresses EZH2 gene expression by repressing the EZH2 promoter via p21 that inactivates pRB/E2F transcriptionally [70]. Further, SKP2-TRAF6 pathway tightly regulates EZH2 expression by ubiquitination [71]. A recent study showed that a transcriptional repressor, ZFN217 interacted with EZH2

to enhance H3K23me3 levels of FPN promoter to promote prostate cancer growth [72]. The splicing factor SF3B3 stimulates inclusion of exon 14 of EZH2 that promotes proliferation [73]. The lncRNA MALAT1 interacts with the N-terminal of EZH2 to enhance migration and invasion in castration-resistant prostate cancer [74].

As a histone trimethyltransferase, EZH2 represses transcription of a number of tumorigenesis and metastasis suppressor genes thereby regulating prostate cancer development. A list of direct targets of EZH2 is shown in Table 1, which indicates that EZH2 is a bonafide oncogene. Enhanced EZH2 expression suppresses DABI2P expression that is part of the Ras-NFkB signaling pathway resulting in initiation and metastasis of prostatic tumors [75, 76] . Moreover, EZH2 represses the expression of adrenergic receptor beta 2 (ADRB2), which is a critical mediator of β-adrenergic signaling that ultimately leads to cell transformation and invasion [77]. E-cadherin is another EZH2 target that mediates epithelial to mesenchymal transition [50]. Also, PSP94, SLIT2 and CDKN2A are downstream targets of EZH2 in prostate cancer that mediates tumorigenesis and metastasis. PSP94 is a suppressor of tumor growth and metastasis; SLIT2 inhibits prostate cancer cell proliferation and invasion; and CDKN2A is a critical tumor suppressor gene [78-80]. Moreover, a direct relationship between EZH2 and TIMP2/3-tissue inhibitors of metalloproteinase-results in enhanced proteolytic activity of MMP-9 in prostate cancer cells [81]. The lncRNA, DANCER represses TIMP2/3 expression by mediating the binding of EZH2 on their promoters thereby promoting prostate cancer invasiveness [82]. High expression of the EZH2 gene is also associated with low MSMB levels in metastasizing prostate cancer [83]. Meanwhile, EZH2 can repress long non-coding RNA, PCAT-1, which is a prostate specific regulator of cell proliferation [84]. In addition, Raf-1 kinase inhibitor protein (RKIP), a tumor and metastasis suppressor is repressed by EZH2. Lack of RKIP disrupts major cellular signaling pathways like Raf-1/MEK/ERK, NFkβ, and GPCR resulting in prostate cancer development and metastasis [85]. Furthermore, RNNX1, a direct target of AR is repressed by H2K27me3 and is negatively regulated by EZH2 [86]. Notably, miR-26a and miR-138a block the G1/S-phase transition in prostate cancer, independent of EZH2, via a concerted inhibition of crucial cell cycle regulators [87].

EZH2 and bladder cancer

Many studies have indicated that there are multiple modes of regulating EZH2 that act in concert. EZH2 can be transcriptionally induced by E2F family transcription factors [69, 88]. Further, it can be regulated post-transcriptionally by the interaction with many microRNAs and long non-coding RNAs [89]. Moreover, its protein level can be modulated by ubiquitination linked degradation through PI3K-Akt phosphorylation [90]. EZH2 transcriptional activity correlates with methylation of the APAF-1 gene, which is associated with superficial transitional cell carcinoma of the bladder [91]. Further, EZH2 mediates transcriptional silencing of the tumor suppressor gene, E-cadherin [50, 92]. In addition, the pRB-E2F pathway tightly regulates EZH2 expression that promotes bladder cancer development [93]. Further, BDR4 regulates EZH2 transcription by upregulating c-Myc, thereby suggesting a novel therapeutic target in bladder cancer [94].

Several miRNAs are involved in EZH2 regulation. The microRNAs are small non-coding transcripts, 20-22 nucleotides long that participate in many fundamental biological processes including development, apoptosis, differentiation and proliferation [95]. Some like miR-101, miR-144 directly regulate EZH2 post-transcriptionally [96, 97]. In mouse fibroblasts, histone demethylase KDM2B induces expression of miR-101 that targets EZH2 [98]. A similar NDY1/KDM2B-miR101-EZH2 axis was identified in bladder cancer [99]. Meanwhile, miR144-EZH2 axis promotes bladder cell proliferation by regulating the Wnt signaling pathway [96]. Conversely, EZH2 also regulates a wide variety of miRNAs like the miR200 family and miR143 through epigenetic repression. These miRNAs regulate tumor suppressors thereby modulating tumor growth, maintain cancer stem phenotype and cancer cell invasiveness.

Several lncRNAs interact with PRC2 and facilitate access to the promoter of some target genes. LncRNAs act as scaffolds for chromatin modifying factors that alter histone markers thereby modifying gene expression [100]. The lncRNA UNMIBC physically associates with EZH2 and is associated with recurrence of primary invasive bladder cancer [101]. Further, lncRNA H19 is an enhancer that promotes bladder cancer metastasis by inhibiting E-cadherin expression through epigenetic silencing [92]. Also, the lncRNA UBC1 is physically associated with the PRC2 complex and frequently upregulated in bladder cancer [102]

EZH2 and renal cell carcinoma

Many studies have demonstrated that EZH2 plays crucial roles in the initiation, growth and progression of renal cell carcinoma (RCC) [103-106]. Wagener *et al* suggested that EZH2 is an independent prognostic marker indicating poor cancer specific survival (CSS) in RCC [107] . Hinz *et al* demonstrated that high EZH2 levels indicated less aggressive tumor phenotypes with a favorable prognosis in RCC [12].

Many factors regulate EZH2 in regard to RCC. YB1 regulates EZH2 post-transcriptionally [108]. Long non-coding RNAs, such as HOTAIR and MALAT1, promote

aggressive renal cell carcinoma by associating with EZH2 [109, 110]. MiR101 suppresses EZH2 that results in decreased renal cancer cell proliferation [111]. MiR138 induces RCC senescence by targeting EZH2 [112]. Du *et al* showed that CDH5, a chromatin remodeling factor, suppressed the expression of EZH2 [113].

Meanwhile, EZH2 enhanced proliferation and invasion of the renal cell carcinoma cell line ACHN via Wnt / β-catenin pathway [114]. Also, EZH2 positively correlated with VEGF expression [13]. Additionally, high EZH2 expression repressed E-cadherin and was associated with advanced disease state and poor survival of RCC patients [53].

EZH2 AS A THERAPEUTIC TARGET

As discussed in previous sections, EZH2 has a critical role in tumor initiation, growth and progression in urological cancers. Further, downregulation of EZH2 demonstrates potential benefits for suppressing the urological cancers [115-120]. Therefore, there is great interest and effort to develop EZH2 specific inhibitors and multiple phase I trials are underway to analyze potential clinical benefits.

3-deazaadenosine (DZNep) has been widely used to inhibit EZH2. However, DZNep is not specific to EZH2[27]. It depletes PRC2 proteins and inhibits histone H3K27 methylation in various cancer types [121-126]. Among the drawbacks, DZNep has a very short plasma half-life and mediates non-specific inhibition of histone methylation and is toxic in animal models [127]. Therefore, currently efforts have been directed towards developing inhibitors that are potent and specific to EZH2 to reduce toxicity and improve antitumor activities. EPZ005687 is a potent inhibitor of EZH2 that demonstrates 500 fold greater selectivity compared to other human protein methyl transferases and 50 fold more selective than EZH1[28, 128]. GSK126 is another inhibitor with a 1000 fold more selective compared to 20 other human methyl transferases and 150 fold more selective over EZH1 [129]. And GSK343 is inhibitor with a 1000 fold over other human methyl transferases and 60 fold over EZH1[130]. EI1 is another EZH1 inhibitor that shows >10000-fold selectivity over other methyl transferases and 90 fold more selectivity over EZH1 [131]. In all these cases, there is increased expression of PRC2 targets. Notably, many of these compounds require frequent injection. Hence, UNC1999, the first orally available inhibitor with high *in vitro* potency for wild-type and mutant EZH2 as well as EZH1 is preferred [132]. Currently, another EZH2 inhibitor, EPZ-6438 has been developed that has better pharmacokinetic properties than EPZ005687 and better oral bioavailability [133]. In June 2013, a phase 1/2 clinical trial of EPZ7438 was launched in patients with either advanced solid tumor or B cell lymphomas (NCT01897571). In addition, a biologically active biphenolic compound, honokiol was isolated from *Magnolia officinalis* that inhibited human urinary bladder cancer (UBC) cell proliferation, migration and invasion by downregulating EZH2[117]. EZH2 can also be inhibited by disrupting PRC2 stability through the use of a peptide known as stabilized alpha-helix of EZH2(SAH-EZH2) that is derived from the domain of EZH2 that interacts with EED[134].

Meanwhile, reports of therapy resistance to EZH2 inhibitors have also been reported. In a cell line model of acquired resistance to EZH2 inhibitor EPZ-6438, two novel secondary mutations of EZH2 (Y111L and Y661D) were identified following prolonged exposure to EZH2 inhibitors that were associated with therapy resistance [135]. A combination of GSK126 and DZNep significantly increased cell death *in vitro* in murine and human prostate cancer cell lines [136]. Recent data also suggests that concomitant administration of small molecule inhibitors of EZH2 significantly increases the antitumor efficacy of conventional chemo-and radiotherapies in CRPC [115].

In summary, the development of EZH2 inhibitors for cancer therapy is in early stages and there have been reports of resistance that need to be addressed. Further studies are ongoing for potential combination therapy that includes use of EZH2 inhibitors.

CONCLUSIONS, QUESTIONS AND FUTURE DIRECTIONS

In conclusion, we have reviewed both clinical and basic studies that clearly indicate that EZH2 is an oncogene in urological cancers. Whole genome analysis has indicated that the downstream targets of EZH2 are cancer specific [137]. Since EZH2 has a dual role in epigenetic repression and signaling activation, it is interesting to investigate the consequence of gain of function EZH2 mutations towards cancer development in terms of PRC2 dependency. Compared to its epigenetic role, the signaling pathways involving EZH2 need to be further studied in detail.

EZH2 was originally discovered as a regulator of body patterning in fruit flies.[138] As of now, it is recognized as a critical driver of cancer initiation, growth and progression through transcriptional regulation of chromatin structure. Future investigations into the role of EZH2 in urological cancers would require application of advanced techniques including microarrays and RNAseq. Newer technological advances will potentially pave the way for novel EZH2 inhibitors for therapeutic use in near future.

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CONFLICTS OF INTERESTS

There is no conflict of interests declared in this review.

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