Published in final edited form as:

Mol Cancer Ther. 2018 March ; 17(3): 591–602. doi:10.1158/1535-7163.MCT-17-0437.

Histone Methyltransferase EZH2: A therapeutic target for ovarian cancer

Bayley A. Jones¹, Sooryanarayana Varambally^{2,*}, and Rebecca C. Arend^{3,*}

¹University of Alabama at Birmingham School of Medicine, Birmingham, AL, United States

²Department of Pathology, University of Alabama at Birmingham, Birmingham, AL, United States

³Department of Obstetrics and Gynecology, University of Alabama at Birmingham, Birmingham, AL, United States

Abstract

Ovarian cancer is the fifth leading cause of cancer-related deaths in females in the United States. It is anticipated that there will be about 22,440 new cases and 14,080 deaths due to ovarian cancer in 2017. Most patients present with advanced stage disease, revealing the urgent need for new therapeutic strategies targeting pathways of tumorigenesis and chemotherapy resistance. While multiple genomic changes contribute to the progression of this aggressive disease, it has become increasingly evident that epigenetic events play a pivotal role in ovarian cancer development. One of the well-studied epigenetic modifiers, the histone methyltransferase EZH2, is a member of polycomb repressive complex 2 (PRC2) and is commonly involved in transcriptional repression. EZH2 is the enzymatic catalytic subunit of the PRC2 complex that can alter gene expression by trimethylating lysine 27 on histone 3 (H3K27). In ovarian cancer, EZH2 is commonly overexpressed, and therefore potentially serves as an effective therapeutic target. Multiple small molecule inhibitors are being developed to target EZH2, which are now in clinical trials. Thus, in this review, we highlight the progress made in EZH2 related research in ovarian cancer and discuss the potential utility of targeting EZH2 with available small molecule inhibitors for ovarian cancer.

Keywords

EZH2; ovarian cancer; epigenetics; histone methyltransferase

Introduction

As the fifth leading cause of cancer-related deaths in females in the United States, ovarian cancer will present with 22,440 new cases and will be the cause of 14,080 deaths in 2017 (1). Many patients present with advanced stage disease, contributing to the high death rate. Treating these patients generally consists of surgical resection followed by platinum/taxane-

To whom correspondence should be addressed: Rebecca C. Arend, M.D., University of Alabama at Birmingham, Department of Obstetrics and Gynecology, Division of Gynecologic Oncology, Address: 176F Rm 10250; 619 19th St South; Birmingham, AL 35249-7333, Phone: 205-934-4986, Fax: 205-975-6174.

*Senior author

Conflicts of Interest: The authors declare no potential conflicts of interest.

based chemotherapy (2). However, a significant challenge faced in treating these patients is the high rate of recurrence associated with platinum-resistance. Patients with platinum-resistant ovarian cancer carry a poorer prognosis with less than 20% of them responding to subsequent therapies (3).

Epithelial ovarian carcinomas (EOCs) account for 90% of ovarian cancers. These tumors are classified based on tumor cell type, such as endometrioid, clear cell, mucinous or serous ovarian cancer. It is important to distinguish between the types of EOCs as it has become clear that the subtypes differ with respect to risk factors, precursor lesions, patterns of metastasis, molecular events during oncogenesis, response to chemotherapy and outcome. In ovarian serous carcinomas, it is also important to distinguish between low-grade and high-grade tumors, as they represent distinct tumors rather than a spectrum of the same tumor type, and their histological and molecular features are entirely different. Therefore, standard chemotherapy treatment based on subtype rather than grade and stage may be the more powerful therapeutic approach (4). Both the poor prognosis and the molecular heterogeneity in EOC reveals the critical need for the development of new therapies in the treatment of ovarian cancer.

The regulation of covalent histone modifications at enhancers and promoters has become a popular research interest due to the important role these modifications have on altering gene expression and modulating cell fate specification. Polycomb repressive complex 2 (PRC2) is a transcriptional repressive complex that consists of three critical components: enhancer of zeste 2 (EZH2), embryonic ectoderm development (EED) and suppressor of zeste 12 (SUZ12). The catalytic subunit, EZH2, can trimethylate lysine 27 on histone 3 (H3K27) to promote transcriptional silencing by facilitating chromatin compaction. Alterations of this complex have been identified in many types of malignancies (5). Table 1 includes some of the cancers that have been found to have EZH2 dysregulation (6–21).

EZH2 mutations have been shown to display both oncogenic and tumor-suppressive behavior and gain-of-function and loss-of-function behavior. These opposing roles can be explained by the conserved molecular function of PRC2 in maintaining gene silencing. Studies have shown that the main function of PRC2 is in maintaining transcriptional silence rather than initiating it. Therefore, the function of PRC2 is not to determine which genes should be repressed, but rather to maintain the already established silent state of a gene, which is critical for stabilizing cell identity (6). Hematologic malignancies often carry gain-of-function and loss-of-function mutations of EZH2, whereas solid tumors, including ovarian cancer, often display EZH2 overexpression (5,7,8). The context-dependent role of EZH2 is not strictly segregated based on tumor type, thereby suggesting that dysregulation might depend on the identity of the cell of origin and on the early transformation events, such as tumorigenic alterations of other genes (6). The frequency of EZH2 dysregulation in malignancies highlights the involvement of EZH2 in tumor progression and the possible benefits of targeting it in the treatment of ovarian cancer.

EZH2 has been shown to play a critical role in cancer cell proliferation, invasion, tumor metastasis, angiogenesis and chemotherapy resistance (Figure 1). EZH2 also suppresses differentiation by repressing lineage-specifying factors (9). It has been shown to be

expressed in higher levels in cancer stem cell populations in human breast xenografts and primary breast tumor cells, and it is critical for the maintenance of these stem cell populations (10). Similarly, chronic myelogenous leukemia (CML) leukemic stem cells (LSCs) are dependent on EZH2, which is overexpressed in these cells. Genetic inactivation of EZH2 in a mouse CML model blocks leukemia initiation and development, and EZH2 inactivation in existing disease induces disease regression and improves survival (11,12). These findings suggest that EZH2 dysregulation can facilitate the initiation and progression of cancer by blocking differentiation and maintaining malignant stem cell populations. Since EZH2 suppresses transcriptional programs that underlie alternate fates, the consequences of altering EZH2 expression are likely to be highly cell type specific (5).

In this review, we highlight what is currently known about the role of EZH2 in various cancers and specifically its role in ovarian cancer. We discuss its interactions with microRNAs, long non-coding RNAs, ARID1A and the mammalian target of rapamycin (mTOR) pathway, as well as its potential implications in immunotherapy. We also highlight its role in the transformation of ovarian stem cells to malignant cells. Finally, we review current EZH2 inhibitors and suggest that targeting EZH2, particularly in combination with other molecular therapeutics, may prove to be a therapeutic strategy that has potential for success in the new age of personalized medicine.

EZH2 in ovarian cancer

EZH2 upregulation has been widely established in ovarian cancer. EZH2 expression promotes cell proliferation and invasion, inhibits apoptosis and enhances angiogenesis in epithelial ovarian cancers (EOCs) (13,14). Here, we will review the mechanisms by which EZH2 contributes to tumorigenesis in ovarian cancer.

In ovarian cancer, overexpression of EZH2 correlates with a high proliferative index and tumor grade. Knockdown of EZH2 inhibits growth of EOCs (serous, endometrioid, mucinous and clear cell types) *in vitro* and *in vivo*, and it induces apoptosis and suppresses invasion of EOC cells (13). ARHI is a tumor suppressor gene that acts as a negative regulator of EOC growth and progression. In EOC cell lines SKOV3 and OVCAR3, EZH2 trimethylation of ARHI lead to repression of ARHI. Inhibition of EZH2 with DZNep released this repression, leading to inhibition of EOC cell survival through restoring the expression of ARHI (15). Therefore, inhibition of EZH2 with subsequent release of repression of ARHI may be an additional treatment strategy in EOC.

NF-YA is a key regulator of EZH2 expression in EOC, and it is required for cell proliferation (16). In addition, lysine-specific demethylase 2B (KDM2B) may play an important role in ovarian cancer proliferation and invasion by increasing EZH2 expression. Using tissue samples from all subtypes of ovarian cancer, Kuang et al. found that EZH2 expression was positively correlated to KDM2B and that expression of EZH2 and KDM2B was significantly associated with tumor histological type, tumor FIGO stage and lymph node metastasis. Furthermore, they showed that knockdown of KDM2B decreases EZH2 expression, and as a result of KDM2B silencing, ovarian cancer cells had reduced proliferation and migration. Similar results were found *in vivo* (17).

One mechanism by which EZH2 may promote tumor proliferation is by regulating the cyclin dependent kinase (CDK) inhibitor, p57 (18). p57 regulates transcription, apoptosis, differentiation, development and migration (19). Guo et al. investigated the role of EZH2 and p57 in ovarian cancer using 55 tissue samples from epithelial tumors (serous, mucinous, clear cell and undifferentiated) and eight non-epithelial tumor tissue samples (immature teratoma, granulosa cell tumors, dysgerminomas, blastoma and metastatic cancer to the ovary). They found that 85.55% of epithelial ovarian carcinomas displayed EZH2 upregulation compared to only 37.5% for non-epithelial cancers. EZH2 upregulation was significantly correlated with poor cellular differentiation, advanced stage and lymph node metastasis. Well-differentiated tumors displayed positive EZH2 expression in 33.36% (n=11) of patients, while moderately and poorly differentiated tumors were positive for EZH2 expression in 82.61% (n=23) and 93.1% (n=29) of cases respectively. With regard to FIGO stage, 95.24% (n=21) of stage III/IV tumors had positive EZH2 expression, which was much higher than stage I/II tumors (71.43%, n=42). Looking at the same 63 patients, 22 patients had lymph node metastasis, and tumor specimens from all of these patients were positive for EZH2 expression (100%). This was much higher than tumors from patients without lymph node metastasis in which only 68.29% (n=41) of specimens were positive for EZH2. In the same study, there was an inverse correlation between EZH2 expression and p57 mRNA levels. *In vitro* loss of EZH2 increased p57 expression and suppressed cancer cell proliferation and migration in ovarian adenocarcinoma cell lines A2780 and SKOV3, while *in vivo* loss of EZH2 suppressed ovarian tumor formation (18). This study indicates that EZH2 acts as an oncogene in the tumorigenesis of ovarian cancer by targeting p57, and it highlights the potential benefit of targeting EZH2 in the treatment of ovarian cancer.

EZH2 upregulation also promotes invasion and metastasis in ovarian cancer (13). The initial stages of tumor invasion are characterized by the disruption of cell-cell adhesion, and thereby reduced expression of the tumor suppressor gene E-cadherin. Cao et al. showed that increased levels of EZH2 in aggressive tumor cells of epithelial origin silence E-cadherin expression by trimethylation of histone H3K27 at the promoter site (20). Rao et al. demonstrated that cell invasion and/or metastasis pathways may be tumor type specific. They showed that high EZH2 levels could facilitate an increased malignant phenotype of the tumor and that cell invasion and/or metastasis in ovarian carcinoma may be supported by EZH2 regulation of TGF- β 1. In their study, they observed downregulated E-cadherin and upregulated TGF- β 1 in EOCs, including serous, mucinous, clear cell, endometrioid and undifferentiated types. However, only EZH2 and TGF- β 1 showed a positive correlation, suggesting that EZH2 regulates cell invasion via the regulation of TGF- β 1 expression (21). TGF- β 1 has been shown to promote invasive behavior in human ovarian cancer cells by elevating matrix metalloproteinase secretion and increasing metastatic potential by inducing epithelial-mesenchymal transition (EMT) (22–24). Despite the potential benefits of reduced TGF- β 1 expression by EZH2 inhibition, adverse consequences of EZH2 inhibition in EMT have been documented. Cardenas et al. proposed that EZH2 inhibition may drive EMT by removing repression of TGF- β -stimulated ZEB2, a late stage inducer of EMT. Mesenchymal characteristics of ovarian cancer cells were enhanced by both EZH2 knockdown and by enzymatic inhibitors. Therefore, EZH2 inhibition may lead to pro-tumorigenic events

causing a more aggressive cancer phenotype, thus highlighting the need for further investigation into the role of (25).

Tissue inhibitor of metalloproteinase 2 (TIMP2) is an endogenous regulator of matrix metalloproteinases (MMPs). MMPs facilitate tumor cell invasion by degrading extracellular matrix (ECM) components, resulting in a mechanism of metastasis initiation. One mechanism by which they may promote ovarian cancer invasion and migration is by EZH2 inhibition of TIMP2. Yi et al. found EZH2 to be inversely correlated to TIMP2 expression in serous and endometrioid ovarian carcinoma. Using EOC cell lines (A2780, CAOV3, C13*, ES2, HO8910, OV2008 and SKOV3), they showed that EZH2 inhibits TIMP2 expression via DNA hypermethylation and trimethylation of histone H3 lysine 27 (H3K27me3), thereby inducing chromatin compaction and transcriptional silencing. As a result of this inhibition, MMP2 and MMP9 were activated. In subsequent *in vivo* experiments, EZH2 was found to promote ovarian cancer metastasis by repression of TIMP2. Their findings suggest an additional mechanism by which EZH2 may contribute to the progression of ovarian cancer (26).

Tumor angiogenesis is necessary for tumor metastasis and for cancer cell proliferation in distant organs (14,27). EZH2 plays a critical role in angiogenesis by interacting with both pro-angiogenic and anti-angiogenic pathways (28). VEGF is one of the most potent pro-angiogenic stimulators that affects endothelial proliferation and mobility and vascular permeability (29). Lu et al. used genomic profiling of endothelial cells from ovarian HGSC and normal ovary to show that EZH2 expression is significantly increased in tumor-associated endothelial cells (30). Based on this finding, investigations into the role of EZH2 in promoting angiogenesis revealed that EZH2 interacts with the VEGF pathway in EOC cell lines HeyA8 and SKOV3ip1 (14). VEGF increased EZH2 expression in ovarian tumor vasculature. In turn, EZH2 inactivated the antiangiogenic factor, vasohibin 1 (VASH1), by methylation. When VASH1 is activated, it inhibits endothelial-cell migration, proliferation and tube formation, and it limits tumor-associated angiogenesis and increases vessel maturity. Therefore, inhibition of VASH1 by EZH2 promoted tumor angiogenesis. EZH2 gene silencing was able to restore increased VASH1 levels in tumor endothelial cells (14). Given the critical role that EZH2 plays in tumor angiogenesis in EOC, it is clear that targeting EZH2 may prove beneficial not only at the tumor cellular level but also at the level of neovascularization, which plays an important role in metastasis and tumor growth in ovarian cancer.

Apart from ovarian cancer proliferation, invasion, metastasis and angiogenesis, it is important to consider the role of EZH2 in cancer treatment resistance. Patients with platinum-resistant ovarian cancer typically have a low response rate to subsequent chemotherapy treatments and a median survival of less than one year, compared to a two year median survival for patient with platinum-sensitive recurrent ovarian cancer (31). This highlights the importance of investigating the resistance pathways to first-line ovarian cancer treatment. Studies have shown that the overexpression of EZH2 contributes to acquired cisplatin resistance in ovarian cancer cells, while downregulation of EZH2 is found in cisplatin-sensitive cells (32,33). EZH2 has been shown to epigenetically silence tumor suppressor genes, such as ARNTL and hMLH1, which may contribute to chemoresistance in

these tumors (34,35). Hu et al. showed that EZH2 expression was elevated in drug-resistant ovarian cancer cells (cisplatin-resistant cell line A2780/DDP). They also demonstrated that loss of EZH2 resensitizes ovarian cancer cells to cisplatin and that EZH2 knockdown enhances sensitivity to cisplatin *in vivo*. In *in vitro* experiments, EZH2 downregulation suppressed cell viability and proliferation in cisplatin-resistant cancer cells, and knockdown of EZH2 in ovarian cancer cells induced G2/M cell cycle arrest (32).

Yang et al. suggested that a potential mechanism by which EZH2 inhibition reverses cisplatin resistance in ovarian cancer is by inhibiting autophagy. Using SKOV3/DDP ovarian cancer cells, they showed that inhibition of EZH2 in these cells did not induce apoptosis but rather reduced autophagy. They discovered that inhibition of EZH2 with subsequent downregulation of H3K27me3 expression led to activation of the INK/ARF/Rb pathway with upregulation of p14, p16, p53 and pR protein expression. These cells demonstrated increased volume, flat granules and β -galactosidase staining, all of which are characteristics of cellular senescence. In addition, they found that cisplatin becomes effective at lower doses with partial EZH2 silencing in these cells (36). Wang et al. looked at the interaction of EZH2 with several differentially expressed genes (DEGs) in cisplatin-resistant human ovarian cancer cells (CP70 cell line). They found that cell cycle, DNA replication and pyrimidine metabolism were significantly enriched in the DEGs that directly interacted with EZH2 (33). Another mechanism by which EZH2 confers resistance is by physical alteration of DNA. Loss of H3K27 methylation in ovarian cancer cells was able to reverse chemotherapy resistance, likely by altering gene expression and changing chromatin structure, which thereby increased the susceptibility to damage by DNA-targeting agents (37).

EZH2 and microRNAs

MicroRNAs (miRNAs) are small, endogenous non-coding RNAs molecules that are capable of modulating the post-transcriptional regulation of numerous cellular genes (38). Levels of PRC2 proteins, including EZH2, have been shown to be regulated by miRNAs. In turn, EZH2 itself regulates many miRNAs, some of which act as tumor suppressors by attenuating growth, invasiveness and self-renewal of cancer cells (39). miRNA-101, miRNA-298 and the enzyme Dicer, a key protein required for mRNA processing, interact with EZH2 in ovarian cancer, and their interactions are thought to contribute to chemotherapy resistance. EZH2 has been identified as a target of miRNA-101 (miR-101). Downregulation of miRNA-101 results in overexpression of EZH2, thereby resulting in cancer progression (40–42). Liu et al. showed that there was a significant negative correlation between miRNA-101 and EZH2 mRNA in epithelial ovarian cancers (using serous, mucinous and endometrioid tissue samples), with miRNA-101 being underexpressed in EOC tissues (43). EZH2 inhibition reduced proliferation and migration in ovarian cancer cells (A2780 and SKOV3) and suppressed tumor formation *in vivo*, while miRNA-101 overexpression resulted in decreased proliferation and migration (18). This suggests that miRNA-101 may play an important role in ovarian cancer by regulating EZH2. Using this data, Liu et al. looked at the role of miRNA-101 and found miRNA-101 levels to be reduced in cisplatin-resistant cells (A2780/DDP and SKOV3/DDP). Furthermore, upregulation of miRNA-101 was able to sensitize cisplatin-resistant cell lines to treatment with cisplatin (43).

miRNA-298 has also been found to inhibit malignant EOC phenotypes by regulation of EZH2. Zhou et al. showed that EZH2 expression was significantly upregulated while miRNA-298 was significantly downregulated in human serous and non-serous EOC tissues. These findings were significantly associated with high clinical stage and pathologic grade, demonstrating the roles of EZH2 and miRNA-298 in the regulation of malignant phenotypes of EOC cells and the aggressive progression of EOC cancers. Ectopic expression of miRNA-298 was shown to efficiently inhibit cell migration and invasion *in vitro* (SKOV3 and OVCAR3 cell lines); however, EZH2 overexpression could restore the migration and invasion capabilities that were suppressed by miRNA-298. Their findings support the idea that the miRNA-298-EZH2 axis may play an important role in the progression of EOC and may serve as a potential therapeutic target for the treatment of EOC (44). Down regulation of miR-298 and concomitant upregulation of EZH2 can result in aggressive tumor phenotype in ovarian cancer.

EZH2 indirectly interacts with microRNAs via regulation of Dicer, an enzyme involved in regulating the maturation of miRNAs in the cytoplasm from miRNA precursors (38). Dicer is decreased in 60% of epithelial ovarian cancers, and its downregulation is associated with poor clinical outcomes (45). Kuang et al. showed that Dicer downregulation promotes cell proliferation, migration and cell cycle progression in ovarian cancer cell lines (A2780 and SKOV3). They demonstrated that Dicer expression is significantly decreased in cisplatin-resistant ovarian cancer cell line A2780/DDP and that knockdown of Dicer in parental cell lines decreased the sensitivity of the cells to cisplatin treatment. In the same study, EZH2 inhibition increased Dicer expression *in vitro*, suggesting that EZH2 regulation of Dicer may also contribute to drug resistance in ovarian cancer (38). The role of DICER in high-grade serous ovarian cancer was demonstrated *in vivo* using a *DICER-PTEN* double knockout mouse model. The mice developed aggressive tumors that metastasized throughout the abdominal cavity, leading to ascites and ultimately a 100% mortality rate by 13 months (46).

EZH2 and long non-coding RNAs

Long non-coding RNAs (lncRNAs) have been linked to EZH2 in ovarian cancer. lncRNAs are involved in a number of important biologic processes, such as shaping chromosome conformation and allosterically regulating enzymatic activity. Patterns of expression mediate cell state, differentiation, development and disease. The overexpression, deficiency or mutations in these genes have been found in multiple types of cancers (47). Using 64 tissue samples of serous, mucinous and endometrioid tumors, Qiu et al. showed that lncRNA HOX transcript antisense RNA (HOTAIR) expression is correlated with the prognosis of patients with EOC. The mean overall survival (OS) for patients with tumors with low HOTAIR expression was 64.41 months compared to 34.53 months for high HOTAIR expression. In addition, HOTAIR expression was correlated with EOC metastasis. They found that silencing HOTAIR impairs EOC cell migration and invasion *in vitro* (SKOV3.ip1, HO8910-PM and HEY-A8 cells) and inhibits EOC metastasis *in vivo* (using injection of HOTAIR-knockdown HEY-A8 cells into mice) (48). The HOTAIR has been shown to interact with PRC2 via EZH2 (49). The interaction of HOTAIR and PRC2 appears to be required for the occupancy of certain gene loci as well as the methylation by EZH2. Ozes et al. showed that HOTAIR is overexpressed in platinum-resistant EOC cells (A2780_CR5). A positive

feedback loop of a proposed NF- κ B-HOTAIR axis may result cellular senescence and platinum resistance in EOC and it may do so by functional overlap with EZH2 (50). Ozes et al. further investigated the interaction of HOTAIR and EZH2 and showed that blocking their interaction using peptide nucleic acids (PNAs) resensitizes resistant A2780_CR5 EOC cells to platinum, reduces cell invasion and decreases NF- κ B transcriptional activity. These findings were demonstrated *in vivo* in mice injected with A2780_CR5 cells. Increased chemotherapy sensitivity and reduced cell survival were also found with independent blocking of HOTAIR and EZH2 with siRNA HOTAIR and EZH2 inhibitor GSK126, respectively (51).

Despite these data, a recent study by Portoso et al. used MDA-MB-231 breast cancer cells to show that HOTAIR can exert repressive effects on chromatin independent of PRC2. Silencing of the luciferase reporter in these cells required continuous presence of *MS2-HOTAIR* but not H3K27me3. They suggested that recruitment of PRC2 may be a downstream consequence of gene silencing and may serve functions other than chromatin targeting (52). This study highlights the need for further investigations into the role of lncRNAs in ovarian cancer, such as HOTAIR, to determine their potential function in molecular therapeutics for ovarian cancer.

Ovarian cancer stem cells and EZH2

EZH2 has been shown to play a role in the maintenance of ovarian stem cell-like cells (OCSCs), or side populations (SP), in ovarian tumors. These ovarian SP cells are thought to play an important role in tumor formation, tumor growth and resistance to therapy as a result of their low degree of differentiation, their ability to self-renew and their highly invasive nature (53,54). Rizzo et al. found that the proportion of SP in ovarian cancer ascites increased following chemotherapy. EZH2 expression was increased in SP cells from ovarian cancer ascites compared to non-SP cells. Using the IGROV1 ovarian cancer cell line, SP cells persisted after treatment with chemotherapy, suggesting that these cells are important for tumor resistance and that platinum treatment may select for SP cell populations. Following knockdown of EZH2, there was a reduction in the SP in IGROV1, PEO14 and PEO23 ovarian cancer cell lines. These cells displayed less stem cell-like behavior, such as loss of anchorage-independent growth and reduced formation of large spherules of more than thirty-two cells. The same results were seen in cells with knockdown of both EZH2 and EZH1. Rizzo et al. supported these findings with studies *in vivo*. Following siRNA knockdown of EZH2 and EZH1, IGROV1 and PEO23 cells were injected into a NOD/SCID mouse model. They found a reduction in tumor volume in the EZH2+EZH1 knockdowns compared to the controls (54).

EZH2 may regulate ovarian stem cell-like cells by controlling the levels of ALDH1A1 expression. ALDH1A1 has been reported to be a marker of cancer stem cells in certain types of cancers, including ovarian and breast cancer. ALDH1A1 has found to be downregulated more than 2-fold in more than 96% of EOC cases identified in the Cancer Genomic Atlas ovarian database. Using normal human ovarian surface epithelial (HOSE) cells and EOC cells, Li et al. showed an upregulation of EZH2 and a downregulation of ALDH1A1 in EOC cells compared to HOSE cells. Thus, they showed that EZH2 expression negatively

correlated to ALDH1A1 expression in EOC cells. They confirmed this interaction by showing that the presence of H3K27Me3 at the 9q21.13 chromosomal location of the ALDH1A1 target gene was dependent on EZH2. EZH2 knockdown in SKOV3 ovarian cancer cells resulted in up to 22-fold upregulation of ALDH1A1 (55).

Liu et al. suggested that human ovarian SP proliferation may be regulated via EZH2 signaling through the pRb-E2F pathway. They used CD44+/CD117+ ovarian cancer stem cells isolated from six tumor samples, including two serous, one mucinous, one clear cell, one endometrioid and one mixed or undifferentiated sample. They demonstrated the interaction between EZH2 and this pathway using EZH2-targeted miRNA-98. Most OCSCs transfected with miRNA-98 were arrested in the G₀/G₁ phase of the cell cycle and the percentage of cells in the G₂/M phase was decreased. This suggests that EZH2 knockdown affects cell cycle regulation via miRNA-98. In addition, they demonstrated a significant decrease in the levels of *Hdac1*, *E2f1*, *Ccne*, *Cdk2* and *pRb* mRNAs in OCSCs transfected with miRNA-98. These mRNAs are all part of the pRB-E2F signaling pathway, suggesting that EZH2 regulates this pathway. *In vivo* studies using BALB/c nude mice showed that xenografts formed by miRNA-98 transfected OCSCs were smaller and had reduced proliferation capacity than those formed by mutant miRNA-98 transfected OCSCs (53). Taken together, their *in vitro* and *in vivo* studies offer a possible mechanism by which EZH2 promotes tumorigenesis by regulation of OCSCs, thereby supporting the use of targeting EZH2 as a therapeutic approach.

Recent studies have suggested that many cases of HGSC arise from the fallopian tube. Kim et al. used a *Dicer-Pten* mouse model to show that primary fallopian tube tumors spread to the ovary and then aggressively metastasize throughout the abdominal cavity. These cancers clinically resembled human serous cancers and highly expressed genes that are known to be upregulated in human serous ovarian cancer, such as secreted phosphoprotein 1 (*spp1*) and CA125 (*Muc16*). In addition, ovariectomized mice still developed high-grade serous ovarian cancers, while early removal of fallopian tube prevented cancer formation. This finding further suggests a fallopian tube origin for serous ovarian cancer. Interestingly, the primary carcinomas in these mice were first observed in the stroma of the fallopian tube, suggesting that these cancers may have a mesenchymal origin (46).

Additional studies have identified a pre-metastatic intramucosal neoplasm that is found almost exclusively in the fallopian tube, called serous intraepithelial neoplasm (STIC). STICs are considered the earliest morphological manifestation of serous carcinoma from the fallopian tube and arise from non-ciliated secretory cells of the endosalpinx (56). Yamamoto et al. showed that fallopian tube stem cells (FTSCs) can develop into HGSC in mouse xenografts and suggest that transformed FTSCs are the *in vitro* correlate to STIC. They generated clones of FTSCs that contained many small, undifferentiated and highly proliferative cells. They then induced immortalization or transformation of these FTSCs (FTSCⁱ) by introducing SV40/hTERT or SV40/hTERT/c-MYC by retroviral infection. The transformed FTSCs were injected into immunodeficient mice. Xenografts from the tumors that developed demonstrated immunological and pathological markers of human HGSC, such as gain of p53, WT1, EZH2 and MUC4 expression. In addition, 2395 genes that were altered in HGSC tumor samples were altered in a similar manner in the FTSCⁱ xenografts.

Protein expression of EZH2 was upregulated in precancerous lesions of HGSC, STIC, invasive serous cancer and FTSC^t tumor xenografts. Consequently, the expression of many downstream targets of EZH2 was downregulated compared to normal fallopian tube epithelium (57). Their data highlights the need for further investigation into the role of EZH2 in the transformation of FTSCs to HGSC and the impact that EZH2 inhibition would have on this process.

ARID1A and EZH2 in OCCC and endometriod carcinoma

The SWI/SNF chromatin-remodeling complex modulates transcription and is essential for differentiation, proliferation and DNA repair. Loss of SWI/SNF function has been associated with malignant transformation, as several components of the complex have been shown to act as tumor suppressors (58). ARID1A is one of the accessory subunits of the SWI/SNF chromatin-remodeling complex that is believed to confer specificity in the regulation of gene expression, particularly those involved in the repression of key cell cycle regulators (58). For example, ARID1A acts as a tumor suppressor by regulating p53-controlled genes in a p53-dependent fashion, thereby regulating tumor growth (59). Mutations in ARID1A lead to genomic instability and consequently may lead to the uncontrolled proliferation seen in malignant transformation (58,60). It should be noted that ARID1A deletion alone has been shown to be insufficient for ovarian endometriod tumor initiation and that additional genetic modifications, such as alterations in the PI3K/Akt pathway, may be necessary to drive tumorigenesis *in vivo* (61). The antagonistic roles played by EZH2 and ARID1A in the regulation of EZH2/ARID1A target genes makes targeting EZH2 a potential therapeutic strategy in these cancers. In fact, EZH2 inhibition has been shown to be synthetically lethal in ARID1A-mutated cells by selectively promoting apoptosis in these cells (62).

ARID1A mutations are commonly seen in many types of cancer, including EOC. Wiegand et al. found ARID1A mutations in 46% of ovarian clear cell carcinoma (OCCC) and in 30% of ovarian endometriod carcinoma. None of the 76 high-grade serous ovarian carcinomas in their study had mutated ARID1A (60). Another study by Jones et al. found 57% of OCCC to have mutated ARID1A (63). ARID1A knockdown in ovarian epithelial cell lines (IOSE-80PC and OSE4) with ARID1A shRNA enhanced cellular proliferation *in vitro*. OSE4 cells with ARID1A shRNA were injected into mice and displayed increased tumorigenicity compared to mice injected with control shRNA (59). Loss of ARID1A in OCCC tumors correlates with chemoresistance and shorter overall survival of patients treated with platinum-based chemotherapy, making loss of ARID1A a negative prognostic factor (64). Therefore, targeting EZH2 in OCCCs and endometriod carcinomas that display loss of ARID1A may prove to be a promising therapeutic strategy to improve outcomes in these patients.

The loss of ARID1A protein expression is thought to be a molecular event that occurs very early in the development of the majority of OCCC and endometriod carcinomas arising from endometriomas. Ayan et al. analyzed ARID1A expression in 47 ovarian endometriotic cysts, which contained OCCC (24 cases), endometriod carcinoma (20 cases) and mixed clear cell and endometriod carcinoma (3 cases). ARID1A loss was detected in 31 (66%) of the 47 carcinomas and the normal-appearing endometriotic cyst epithelium immediately adjacent to

the carcinoma. (65). Transformation from endometriosis to ovarian cancer is rare and the mechanism is unclear. The microenvironment around the endometrium likely has many free radicals. Repeated damage and repair in these areas along with abnormalities in chromatin remodeling due to loss of ARID1A may contribute to malignant transformation (66).

It is critical to note the enzymatic and non-enzymatic function of EZH2 in ARID1A-mutated ovarian cancer. Targeting only the catalytic subunit of EZH2 in ARID1A-mutated cancers may be insufficient in suppressing the oncogenic activity of EZH2. Kim et al. demonstrated this by highlighting the dependence of ARID1A-mutant cancers on EZH2 in OCCC and endometrial adenocarcinoma cell lines. The ARID1A-mutant cancer cells were sensitive to EZH2 knockdown but showed varying responses to EZH2 enzymatic inhibitors GSK126, GSK343 and JQEZ5 (67,68)(67,68)(67,68). Investigation into these discrepancies revealed that dependence on EZH2 in ARID1A-mutant cancers is likely to be dictated by more than the enzymatic function of EZH2 and that EZH2's non-enzymatic function may lead to the stabilization of the PRC2 complex. Thus, both enzymatic and non-enzymatic functions of EZH2 should be taken into consideration with regard to future EZH2 inhibitors in order to elicit the best responses in ARID1A-mutant ovarian cancer (69).

PI3K/Akt/mTOR pathway and EZH2

The PI3K/Akt/mTOR intracellular signaling pathway is one of the most highly mutated systems in human cancers, including ovarian cancer. It is involved in regulating many cellular functions, including cell metabolism, cell growth, cell migration, cell cycle entry and cell survival (70). The mTOR pathway is thought to play a critical role in tumor growth, tumorigenesis and pathological angiogenesis (71,72).

The interaction of EZH2 and the PI3K/Akt/mTOR pathway has been shown in ARID1A-mutant cancer. Guan et al. showed that ARID1A deletion alone was insufficient for ovarian endometriod tumor initiation and that additional genetic modifications, such as PTEN deletion, were necessary to drive tumorigenesis *in vivo* (61). Bitler et al. showed that inhibition of EZH2 resulted in increased expression of EZH2-targeted *PIK3IP1*, which is an inhibitor of the PI3K/Akt/mTOR pathway. They demonstrated that inhibiting EZH2 triggered apoptosis in ARID1A-mutated OCCC cells and regression of ARID1A-mutated tumors *in vivo*. EZH2 inhibition was selective against ARID1A-mutated OVCSE xenografts in reducing tumor growth compared to controls, suggesting that the response to the EZH2 inhibitor was dependent on having an ARID1A mutation. They showed that the synthetic lethality of EZH2 inhibition in ARID1A-mutated cells was due to increased expression of the EZH2-target gene *PIK3IP1*, an inhibitor of the PI3K/Akt pathway. Therefore, increased PI3K activity led to increased sensitivity to EZH2 inhibition. This data reaffirms the interaction of ARID1A mutations and PI3K/Akt pathways in OCCC and suggests that therapeutic targeting of EZH2 in ARID1A-mutated OCCC is a possible treatment strategy in these patients (73).

Similar findings in other cancers show that the communication between EZH2 and the PI3K/Akt/mTOR pathway are not dependent on ARID1A mutations, prompting the need for further investigation into additional interactions of EZH2 with this pathway in OCCC and well as other types of ovarian cancer. (74). Furthermore, direct interactions between mTOR

and EZH2 may also contribute to the pathogenesis of ovarian cancer. Such direct interactions were verified in colorectal cancer where EZH2 was seen to epigenetically repress multiple negative regulators of mTOR, such as the *TSC2* gene, leading to activation of mTOR and subsequent mTOR-related events. (75).

Based upon the data supporting the roles of mTOR and its cofactors and EZH2 in the carcinogenesis of ovarian cancer, further investigations into how the dysregulation of EZH2 affects the PI3K/Akt/mTOR pathway may lead to a better understanding of how these two pathways could be targeted for therapy. Furthermore, additional investigations into how the interactions of EZH2 and the PI3K/Akt/mTOR pathway differ among the various types of ovarian cancer may lead to better optimization of targeted therapeutics.

Immunotherapy and EZH2

In addition to the role EZH2 expression plays in cancer progression as described above, it also plays a significant role in T-cell immunity in the tumor microenvironment. A study by Peng et al. used HGSC tissue samples and a syngeneic mouse ovarian cancer model (ID8 cells) to demonstrate that targeting EZH2 (and other epigenetic modulators) in cancer cells led to the expression of immune-protective signature genes. EZH2 inhibition increased effector T cell infiltration, slowed down tumor progression, and improved the therapeutic efficacy of the PD-L1 (B7-H1) checkpoint blockade (76).

In the same study, Peng et al. showed that the histone methyltransferase activity of EZH2 mediated the repression of T helper 1 (Th1)-type chemokines CXCL9 and CXCL10 in primary ovarian cancer cells generated from fresh ovarian cancer tissues and/or ascites fluid from patients with HGSC (76). These chemokines play important roles as T-cell chemoattractants to the tumor environment. Therefore, EZH2 inhibition increases the trafficking of CD8+ T cells into the tumor, promoting anti-tumor function (77). EZH2 expression leads to tumor immune-evasion both by the repression of chemokines and the inhibition of tumor-infiltrating CD8+ T cells. In their ID8 ovarian cancer mouse model, EZH2 inhibitors elicited potent tumor immunity and blocked cancer progression by increasing tumor infiltration of T cells and Th1-type chemokine expression through epigenetic reprogramming. Based on the data from these studies, targeting EZH2 for epigenetic reprogramming may condition tumors and improve cancer therapy, thereby improving patient outcomes (76).

Although EZH2 expression in the tumor microenvironment has an inhibitory effect on T cell infiltration, it is critical for survival, proliferation and activity of effector T cells (77). Zhao et al. showed that EZH2+ T cells are functional effector T cells in the tumor microenvironment that mediate potent antitumor immunity in human cancer and are associated with long-term survival in ovarian cancer patients. In fact, they suggest that the percentage of EZH2+ CD8+ T cells precisely predicts patient outcomes and long-term survival in patients with ovarian cancer. They also showed that EZH2 regulates T cell polyfunctionality by EZH2-associated H3K27me3, with polyfunctionality meaning the expression of multiple chemokines. EZH2 controls effector T cell survival by regulating Bcl-2 expression. It does so by suppressing Notch repressors and promoting Notch activation, leading to anti-apoptotic Bcl-2 activation. A loss of polyfunctional effector T

cells contributes to the cancer microenvironment; therefore, EZH2's control of effector T cell polyfunctionality enhances survival. Zhao et al. also found that T cell EZH2 was controlled by glycolytic metabolism in the tumor microenvironment in ovarian cancer by maintaining high expression of miRNA-101 and miRNA-26a. Cancer restricts T cell EZH2 expression by limiting aerobic glycolysis, thereby weakening T cell-mediated antitumor immunity (78).

With regard to clinical applications of EZH2 inhibition, Zhao et al. acknowledged that the potential effects that systemic epigenetic manipulation may have on T cell effector function must be taken into consideration. They suggest that a tumor-specific targeting approach of EZH2 may be a good option in clinical exploration of epigenetic therapy (78). However, a study by Zingg et al. showed that in melanoma systemic inhibition did not compromise CD8⁺ T cell function. Therefore, they proposed that the discrepancy between the role of EZH2 in cancer immune evasion and in anti-tumor immune response might be related to the specific tumor model or immunotherapies used (79). This study in melanoma highlights the need for further investigation into the role of EZH2 in the immune response in ovarian cancer, since the use of EZH2 inhibitors with different immunotherapies and/or *in vivo* models may not result in loss of immune cell functionality. Therefore, EZH2 inhibition combined with immunotherapy may prove to be a successful therapeutic strategy that does not compromise immune cell function.

EZH2 in non-epithelial ovarian cancers

Although the vast majority of research concerning the role of EZH2 in ovarian cancer has been studied in epithelial ovarian cancers, some recent studies are beginning to investigate its role in non-epithelial cancers, including granulosa cell tumors (GCTs) and small cell carcinoma of the ovary, hypercalcemic type (SCCOHT). Xu et al. showed that overexpression of EZH2 with subsequent hypermethylation of CDH13, DKK3 and FOXL2 gene promoters contributed to the development of GCTs. Using 30 GCT tissue samples and 30 control samples, they showed that the methylation of these gene promoters was significantly higher in GCT tissues than the controls. They also found EZH2 protein expression in 11 of 30 GCT tissue samples but no EZH2 protein expression in the controls (80).

SCCOHT is a rare cancer, typically affecting young women. Despite surgical debulking and adjuvant chemotherapy, recurrence is rapid and the prognosis is poor. Wang et al. demonstrated that EZH2 may serve as a potential target for this deadly disease, particularly through its interaction with *SMARCA4*, which is a gene found to be mutated in over 90% of SCCOHT cases. SMARCA4 is an ATPase of the SWI/SNF chromatin-remodeling complex, which regulates the expression of genes involved in cell cycle, differentiation and chromosome organization. Wang et al. suggested that loss of SMARCA4 creates a dependency of SCCOHT tumors on EZH2 by epigenetic rewiring. They found EZH2 overexpression in SCCOHT tumor samples and in SCCOHT cell lines (BIN67, SCCOHT-1 and COV434). Furthermore, they showed that the EZH2 inhibitor, EPZ-6438, suppressed tumor growth in the SCCOHT-1 and BIN67 mouse models and induced cell cycle arrest and apoptosis in all three SCCOHT cell lines (81).

A study by Chan-Penebre et al. showed similar results with *in vitro* and *in vivo* studies, with SMARCA2- and SMARCA4-deficient SCCOHT exhibiting dependence on PRC2. They found BIN67, COV434, TOV112D and OVK18 cell lines to be preferentially sensitive to EZH2 inhibition with tazemetostat when compared to other ovarian cancer cell lines. Similar results were seen *in vivo* using BIN67, COV434 and TOV112D xenograft studies. They confirmed EZH2 dependency in SCCOHT with a CRISPR pooled screen. Chan-Penebre also conducted a Phase 1 study of EZH2 inhibition in patients with SMARCA4-deficient SCCOH. Two patients were treated with the EZH2 inhibitor tazemetostat. One showed stable disease while the other showed partial response after eight weeks of treatment. These results highlight the notable clinical benefit of using EZH2 inhibitors in SCCOH patients (82).

Future direction of targeting EZH2 in ovarian cancer

Recent advances in understanding the role of EZH2 in cancer have led to the development of different inhibitors, many of which are being studied in clinical trials. A list of current inhibitors and their mechanisms of action are found in Table 2 (67,68,83–94). One inhibitor, tazemetostat, developed by Epizyme, Inc., has recently been granted fast track designation by the FDA for treatment of patients with relapsed or refractory lymphoma, thus highlighting its promising therapeutic potential (95). The SCCOHT study has been the only clinical study using an EZH2 inhibitor specifically in ovarian cancer. Although a number of ongoing clinical trials are currently using EZH2 inhibitors, none are investigating the use of EZH2 inhibitors specifically in ovarian cancer subjects.

In the new era of personalized medicine, using EZH2 inhibitors to specifically target ovarian tumors with EZH2 upregulation could elicit better responses than what is seen with current therapies and ultimately improve outcomes in these patients. Many studies have shown the benefits of using comprehensive genomic profiling (CGP) in ovarian cancer (96). A study done at University of Texas MD Anderson Cancer Center in Houston, Texas, treated 188 patients with advanced malignancy, 18% of whom were diagnosed with ovarian cancer, showed that matching therapies to molecular aberrations led to higher rates of stable disease, longer time-to-treatment failure and overall survival. These outcomes were compared to patients with unmatched therapy (97). In ovarian cancer, genomic technologies could help provide opportunities for the identification of novel biomarkers and drivers of ovarian tumorigenesis and chemotherapy resistance. We need more clinical trials to validate potential biomarkers and their implications for targeted therapy (98). Therapies targeting molecular aberrations, such as EZH2, could significantly enhance ovarian patient outcomes with further studies (99).

EZH2 inhibition alone may not be effective; therefore, it is important to consider its therapeutic value when used in combination with other drugs, such as those targeting the SWI/SNF chromatin-remodeling complex, the mTOR/Akt pathway and immunotherapy. The increase in the use of CGP to determine molecular aberrations, such as dysregulation of EZH2, highlights the importance of having more targeted agents available that could be used based on the results. Both EZH2 inhibitors and the reintroduction of tumor suppressor microRNA-101 are potential therapies (40,42). A model in Figure 1 depicts the mode of

regulation and action of EZH2 in ovarian cancers. Targeting EZH2 with small molecule inhibitors can re-activate the tumor suppressors repressed by EZH2.

EZH2 dysregulation plays a profound role in the development, progression, and therapeutic resistance of many types of cancer, specifically ovarian cancer. Further investigation into the use of EZH2 inhibitors, as a single agent or in combined therapy, could provide a significant therapeutic advance in the treatment of ovarian cancer.

Acknowledgments

R.C.A. is supported by ACS-IRG Junior Faculty Development Grant, ABOG/AAOGF Scholarship Award, Foundation for Women's Cancer and Norma Livingston Foundation. S.V. is supported by National Institutes of Health R01CA154980.

References

1. Siegel RL, Miller KD, Jemal A. Cancer Statistics, 2017. *CA Cancer J Clin.* 2017; 67(1):7–30. DOI: 10.3322/caac.21387 [PubMed: 28055103]
2. Cannistra SA. Cancer of the ovary. *N Engl J Med.* 2004; 351(24):2519–29. DOI: 10.1056/NEJMr041842 [PubMed: 15590954]
3. Slaughter K, Holman LL, Thomas EL, Gunderson CC, Lauer JK, Ding K, et al. Primary and acquired platinum-resistance among women with high grade serous ovarian cancer. *Gynecol Oncol.* 2016; 142(2):225–30. DOI: 10.1016/j.ygyno.2016.05.020 [PubMed: 27208536]
4. Gilks CB, Prat J. Ovarian carcinoma pathology and genetics: recent advances. *Hum Pathol.* 2009; 40(9):1213–23. DOI: 10.1016/j.humpath.2009.04.017 [PubMed: 19552940]
5. Kim KH, Roberts CW. Targeting EZH2 in cancer. *Nat Med.* 2016; 22(2):128–34. DOI: 10.1038/nm.4036 [PubMed: 26845405]
6. Comet I, Riising EM, Leblanc B, Helin K. Maintaining cell identity: PRC2-mediated regulation of transcription and cancer. *Nat Rev Cancer.* 2016; 16(12):803–10. DOI: 10.1038/nrc.2016.83 [PubMed: 27658528]
7. Di Croce L, Helin K. Transcriptional regulation by Polycomb group proteins. *Nat Struct Mol Biol.* 2013; 20(10):1147–55. DOI: 10.1038/nsmb.2669 [PubMed: 24096405]
8. Volkel P, Dupret B, Le Bourhis X, Angrand PO. Diverse involvement of EZH2 in cancer epigenetics. *Am J Transl Res.* 2015; 7(2):175–93. [PubMed: 25901190]
9. Ezhkova E, Pasolli HA, Parker JS, Stokes N, Su IH, Hannon G, et al. Ezh2 orchestrates gene expression for the stepwise differentiation of tissue-specific stem cells. *Cell.* 2009; 136(6):1122–35. DOI: 10.1016/j.cell.2008.12.043 [PubMed: 19303854]
10. Chang CJ, Yang JY, Xia W, Chen CT, Xie X, Chao CH, et al. EZH2 promotes expansion of breast tumor initiating cells through activation of RAF1-beta-catenin signaling. *Cancer Cell.* 2011; 19(1):86–100. DOI: 10.1016/j.ccr.2010.10.035 [PubMed: 21215703]
11. Scott MT, Korfi K, Saffrey P, Hopcroft LE, Kinstrie R, Pellicano F, et al. Epigenetic Reprogramming Sensitizes CML Stem Cells to Combined EZH2 and Tyrosine Kinase Inhibition. *Cancer Discov.* 2016; 6(11):1248–57. DOI: 10.1158/2159-8290.CD-16-0263 [PubMed: 27630125]
12. Xie H, Peng C, Huang J, Li BE, Kim W, Smith EC, et al. Chronic Myelogenous Leukemia-Initiating Cells Require Polycomb Group Protein EZH2. *Cancer Discov.* 2016; 6(11):1237–47. DOI: 10.1158/2159-8290.CD-15-1439 [PubMed: 27630126]
13. Li H, Cai Q, Godwin AK, Zhang R. Enhancer of zeste homolog 2 promotes the proliferation and invasion of epithelial ovarian cancer cells. *Mol Cancer Res.* 2010; 8(12):1610–8. DOI: 10.1158/1541-7786.MCR-10-0398 [PubMed: 21115743]
14. Lu C, Han HD, Mangala LS, Ali-Fehmi R, Newton CS, Ozbun L, et al. Regulation of tumor angiogenesis by EZH2. *Cancer Cell.* 2010; 18(2):185–97. DOI: 10.1016/j.ccr.2010.06.016 [PubMed: 20708159]

15. Fu Y, Chen J, Pang B, Li C, Zhao J, Shen K. EZH2-induced H3K27me3 is associated with epigenetic repression of the ARHI tumor-suppressor gene in ovarian cancer. *Cell Biochem Biophys*. 2015; 71(1):105–12. DOI: 10.1007/s12013-014-0168-1 [PubMed: 25077680]
16. Garipov A, Li H, Bitler BG, Thapa RJ, Balachandran S, Zhang R. NF-YA underlies EZH2 upregulation and is essential for proliferation of human epithelial ovarian cancer cells. *Mol Cancer Res*. 2013; 11(4):360–9. DOI: 10.1158/1541-7786.MCR-12-0661 [PubMed: 23360797]
17. Kuang Y, Lu F, Guo J, Xu H, Wang Q, Xu C, et al. Histone demethylase KDM2B upregulates histone methyltransferase EZH2 expression and contributes to the progression of ovarian cancer in vitro and in vivo. *Onco Targets Ther*. 2017; 10:3131–44. DOI: 10.2147/OTT.S134784 [PubMed: 28706445]
18. Guo J, Cai J, Yu L, Tang H, Chen C, Wang Z. EZH2 regulates expression of p57 and contributes to progression of ovarian cancer in vitro and in vivo. *Cancer Sci*. 2011; 102(3):530–9. DOI: 10.1111/j.1349-7006.2010.01836.x [PubMed: 21205084]
19. Guo H, Tian T, Nan K, Wang W. p57: A multifunctional protein in cancer (Review). *Int J Oncol*. 2010; 36(6):1321–9. [PubMed: 20428755]
20. Cao Q, Yu J, Dhanasekaran SM, Kim JH, Mani RS, Tomlins SA, et al. Repression of E-cadherin by the polycomb group protein EZH2 in cancer. *Oncogene*. 2008; 27(58):7274–84. DOI: 10.1038/onc.2008.333 [PubMed: 18806826]
21. Rao ZY, Cai MY, Yang GF, He LR, Mai SJ, Hua WF, et al. EZH2 supports ovarian carcinoma cell invasion and/or metastasis via regulation of TGF-beta1 and is a predictor of outcome in ovarian carcinoma patients. *Carcinogenesis*. 2010; 31(9):1576–83. DOI: 10.1093/carcin/bgq150 [PubMed: 20668008]
22. Lin SW, Lee MT, Ke FC, Lee PP, Huang CJ, Ip MM, et al. TGFbeta1 stimulates the secretion of matrix metalloproteinase 2 (MMP2) and the invasive behavior in human ovarian cancer cells, which is suppressed by MMP inhibitor BB3103. *Clin Exp Metastasis*. 2000; 18(6):493–9. [PubMed: 11592306]
23. Thiery JP. Epithelial-mesenchymal transitions in tumour progression. *Nat Rev Cancer*. 2002; 2(6):442–54. DOI: 10.1038/nrc822 [PubMed: 12189386]
24. Do TV, Kubba LA, Du H, Sturgis CD, Woodruff TK. Transforming growth factor-beta1, transforming growth factor-beta2, and transforming growth factor-beta3 enhance ovarian cancer metastatic potential by inducing a Smad3-dependent epithelial-to-mesenchymal transition. *Mol Cancer Res*. 2008; 6(5):695–705. DOI: 10.1158/1541-7786.MCR-07-0294 [PubMed: 18505915]
25. Cardenas H, Zhao J, Vieth E, Nephew KP, Matei D. EZH2 inhibition promotes epithelial-to-mesenchymal transition in ovarian cancer cells. *Oncotarget*. 2016; 7(51):84453–67. DOI: 10.18632/oncotarget.11497 [PubMed: 27563817]
26. Yi X, Guo J, Guo J, Sun S, Yang P, Wang J, et al. EZH2-mediated epigenetic silencing of TIMP2 promotes ovarian cancer migration and invasion. *Sci Rep*. 2017; 7(1):3568. doi: 10.1038/s41598-017-03362-z [PubMed: 28620234]
27. Crea F, Fornaro L, Bocci G, Sun L, Farrar WL, Falcone A, et al. EZH2 inhibition: targeting the crossroad of tumor invasion and angiogenesis. *Cancer Metastasis Rev*. 2012; 31(3–4):753–61. DOI: 10.1007/s10555-012-9387-3 [PubMed: 22711031]
28. Thanappapras D, Hu W, Sood AK, Coleman RL. Moving beyond VEGF for anti-angiogenesis strategies in gynecologic cancer. *Curr Pharm Des*. 2012; 18(19):2713–9. [PubMed: 22390757]
29. Su JL, Yang PC, Shih JY, Yang CY, Wei LH, Hsieh CY, et al. The VEGF-C/Flt-4 axis promotes invasion and metastasis of cancer cells. *Cancer Cell*. 2006; 9(3):209–23. DOI: 10.1016/j.ccr.2006.02.018 [PubMed: 16530705]
30. Lu C, Bonome T, Li Y, Kamat AA, Han LY, Schmandt R, et al. Gene alterations identified by expression profiling in tumor-associated endothelial cells from invasive ovarian carcinoma. *Cancer Res*. 2007; 67(4):1757–68. DOI: 10.1158/0008-5472.CAN-06-3700 [PubMed: 17308118]
31. Davis A, Tinker AV, Friedlander M. “Platinum resistant” ovarian cancer: what is it, who to treat and how to measure benefit? *Gynecol Oncol*. 2014; 133(3):624–31. DOI: 10.1016/j.ygyno.2014.02.038 [PubMed: 24607285]

32. Hu S, Yu L, Li Z, Shen Y, Wang J, Cai J, et al. Overexpression of EZH2 contributes to acquired cisplatin resistance in ovarian cancer cells in vitro and in vivo. *Cancer Biol Ther.* 2010; 10(8):788–95. [PubMed: 20686362]
33. Wang H, Yu Y, Chen C, Wang Q, Huang T, Hong F, et al. Involvement of enhancer of zeste homolog 2 in cisplatin-resistance in ovarian cancer cells by interacting with several genes. *Mol Med Rep.* 2015; 12(2):2503–10. DOI: 10.3892/mmr.2015.3745 [PubMed: 25955318]
34. Yeh CM, Shay J, Zeng TC, Chou JL, Huang TH, Lai HC, et al. Epigenetic silencing of ARNTL, a circadian gene and potential tumor suppressor in ovarian cancer. *Int J Oncol.* 2014; 45(5):2101–7. DOI: 10.3892/ijo.2014.2627 [PubMed: 25175925]
35. Wang J, Yu L, Cai J, Jia J, Gao Y, Liang M, et al. The role of EZH2 and DNA methylation in hMLH1 silencing in epithelial ovarian cancer. *Biochem Biophys Res Commun.* 2013; 433(4):470–6. DOI: 10.1016/j.bbrc.2013.03.037 [PubMed: 23523787]
36. Sun Y, Jin L, Liu JH, Sui YX, Han LL, Shen XL. Interfering EZH2 Expression Reverses the Cisplatin Resistance in Human Ovarian Cancer by Inhibiting Autophagy. *Cancer Biother Radiopharm.* 2016; 31(7):246–52. DOI: 10.1089/cbr.2016.2034 [PubMed: 27610467]
37. Abbosh PH, Montgomery JS, Starkey JA, Novotny M, Zuhowski EG, Egorin MJ, et al. Dominant-negative histone H3 lysine 27 mutant derepresses silenced tumor suppressor genes and reverses the drug-resistant phenotype in cancer cells. *Cancer Res.* 2006; 66(11):5582–91. DOI: 10.1158/0008-5472.CAN-05-3575 [PubMed: 16740693]
38. Kuang Y, Cai J, Li D, Han Q, Cao J, Wang Z. Repression of Dicer is associated with invasive phenotype and chemoresistance in ovarian cancer. *Oncol Lett.* 2013; 5(4):1149–54. DOI: 10.3892/ol.2013.1158 [PubMed: 23599754]
39. Cao Q, Mani RS, Ateeq B, Dhanasekaran SM, Asangani IA, Prensner JR, et al. Coordinated regulation of polycomb group complexes through microRNAs in cancer. *Cancer Cell.* 2011; 20(2):187–99. DOI: 10.1016/j.ccr.2011.06.016 [PubMed: 21840484]
40. Varambally S, Cao Q, Mani RS, Shankar S, Wang X, Ateeq B, et al. Genomic loss of microRNA-101 leads to overexpression of histone methyltransferase EZH2 in cancer. *Science.* 2008; 322(5908):1695–9. DOI: 10.1126/science.1165395 [PubMed: 19008416]
41. Cao P, Deng Z, Wan M, Huang W, Cramer SD, Xu J, et al. MicroRNA-101 negatively regulates Ezh2 and its expression is modulated by androgen receptor and HIF-1 α /HIF-1 β . *Mol Cancer.* 2010; 9:108.doi: 10.1186/1476-4598-9-108 [PubMed: 20478051]
42. Friedman JM, Liang G, Liu CC, Wolff EM, Tsai YC, Ye W, et al. The putative tumor suppressor microRNA-101 modulates the cancer epigenome by repressing the polycomb group protein EZH2. *Cancer Res.* 2009; 69(6):2623–9. DOI: 10.1158/0008-5472.CAN-08-3114 [PubMed: 19258506]
43. Liu L, Guo J, Yu L, Cai J, Gui T, Tang H, et al. miR-101 regulates expression of EZH2 and contributes to progression of and cisplatin resistance in epithelial ovarian cancer. *Tumour Biol.* 2014; 35(12):12619–26. DOI: 10.1007/s13277-014-2585-6 [PubMed: 25260883]
44. Zhou F, Chen J, Wang H. MicroRNA-298 inhibits malignant phenotypes of epithelial ovarian cancer by regulating the expression of EZH2. *Oncol Lett.* 2016; 12(5):3926–32. DOI: 10.3892/ol.2016.5204 [PubMed: 27895750]
45. Merritt WM, Lin YG, Han LY, Kamat AA, Spannuth WA, Schmandt R, et al. Dicer, Drosha, and outcomes in patients with ovarian cancer. *N Engl J Med.* 2008; 359(25):2641–50. DOI: 10.1056/NEJMoa0803785 [PubMed: 19092150]
46. Kim J, Coffey DM, Creighton CJ, Yu Z, Hawkins SM, Matzuk MM. High-grade serous ovarian cancer arises from fallopian tube in a mouse model. *Proc Natl Acad Sci U S A.* 2012; 109(10):3921–6. DOI: 10.1073/pnas.1117135109 [PubMed: 22331912]
47. Quinn JJ, Chang HY. Unique features of long non-coding RNA biogenesis and function. *Nat Rev Genet.* 2016; 17(1):47–62. DOI: 10.1038/nrg.2015.10 [PubMed: 26666209]
48. Qiu JJ, Lin YY, Ye LC, Ding JX, Feng WW, Jin HY, et al. Overexpression of long non-coding RNA HOTAIR predicts poor patient prognosis and promotes tumor metastasis in epithelial ovarian cancer. *Gynecol Oncol.* 2014; 134(1):121–8. DOI: 10.1016/j.ygyno.2014.03.556 [PubMed: 24662839]

49. Rinn JL, Kertesz M, Wang JK, Squazzo SL, Xu X, Brugmann SA, et al. Functional demarcation of active and silent chromatin domains in human HOX loci by noncoding RNAs. *Cell*. 2007; 129(7): 1311–23. DOI: 10.1016/j.cell.2007.05.022 [PubMed: 17604720]
50. Ozes AR, Miller DF, Ozes ON, Fang F, Liu Y, Matei D, et al. NF-kappaB-HOTAIR axis links DNA damage response, chemoresistance and cellular senescence in ovarian cancer. *Oncogene*. 2016; 35(41):5350–61. DOI: 10.1038/onc.2016.75 [PubMed: 27041570]
51. Ozes AR, Wang Y, Zong X, Fang F, Pilrose J, Nephew KP. Therapeutic targeting using tumor specific peptides inhibits long non-coding RNA HOTAIR activity in ovarian and breast cancer. *Sci Rep*. 2017; 7(1):894.doi: 10.1038/s41598-017-00966-3 [PubMed: 28420874]
52. Portoso M, Ragazzini R, Brencic Z, Moiani A, Michaud A, Vassilev I, et al. PRC2 is dispensable for HOTAIR-mediated transcriptional repression. *EMBO J*. 2017; 36(8):981–94. DOI: 10.15252/embj.201695335 [PubMed: 28167697]
53. Liu T, Hou L, Huang Y. EZH2-specific microRNA-98 inhibits human ovarian cancer stem cell proliferation via regulating the pRb-E2F pathway. *Tumour Biol*. 2014; 35(7):7239–47. DOI: 10.1007/s13277-014-1950-9 [PubMed: 24771265]
54. Rizzo S, Hersey JM, Mellor P, Dai W, Santos-Silva A, Liber D, et al. Ovarian cancer stem cell-like side populations are enriched following chemotherapy and overexpress EZH2. *Mol Cancer Ther*. 2011; 10(2):325–35. DOI: 10.1158/1535-7163.MCT-10-0788 [PubMed: 21216927]
55. Li H, Bitler BG, Vathipadiekal V, Maradeo ME, Slifker M, Creasy CL, et al. ALDH1A1 is a novel EZH2 target gene in epithelial ovarian cancer identified by genome-wide approaches. *Cancer Prev Res (Phila)*. 2012; 5(3):484–91. DOI: 10.1158/1940-6207.CAPR-11-0414 [PubMed: 22144423]
56. Carlson JW, Miron A, Jarboe EA, Parast MM, Hirsch MS, Lee Y, et al. Serous tubal intraepithelial carcinoma: its potential role in primary peritoneal serous carcinoma and serous cancer prevention. *J Clin Oncol*. 2008; 26(25):4160–5. DOI: 10.1200/JCO.2008.16.4814 [PubMed: 18757330]
57. Yamamoto Y, Ning G, Howitt BE, Mehra K, Wu L, Wang X, et al. In vitro and in vivo correlates of physiological and neoplastic human Fallopian tube stem cells. *J Pathol*. 2016; 238(4):519–30. DOI: 10.1002/path.4649 [PubMed: 26415052]
58. Reisman D, Glaros S, Thompson EA. The SWI/SNF complex and cancer. *Oncogene*. 2009; 28(14): 1653–68. DOI: 10.1038/onc.2009.4 [PubMed: 19234488]
59. Guan B, Wang TL, Shih Ie M. ARID1A, a factor that promotes formation of SWI/SNF-mediated chromatin remodeling, is a tumor suppressor in gynecologic cancers. *Cancer Res*. 2011; 71(21): 6718–27. DOI: 10.1158/0008-5472.CAN-11-1562 [PubMed: 21900401]
60. Wiegand KC, Shah SP, Al-Agha OM, Zhao Y, Tse K, Zeng T, et al. ARID1A mutations in endometriosis-associated ovarian carcinomas. *N Engl J Med*. 2010; 363(16):1532–43. DOI: 10.1056/NEJMoa1008433 [PubMed: 20942669]
61. Guan B, Rahmanto YS, Wu RC, Wang Y, Wang Z, Wang TL, et al. Roles of deletion of Arid1a, a tumor suppressor, in mouse ovarian tumorigenesis. *J Natl Cancer Inst*. 2014; 106(7)doi: 10.1093/jnci/dju146
62. Bitler BG, Fatkhutdinov N, Zhang R. Potential therapeutic targets in ARID1A-mutated cancers. *Expert Opin Ther Targets*. 2015; 19(11):1419–22. DOI: 10.1517/14728222.2015.1062879 [PubMed: 26125128]
63. Jones S, Wang TL, Shih Ie M, Mao TL, Nakayama K, Roden R, et al. Frequent mutations of chromatin remodeling gene ARID1A in ovarian clear cell carcinoma. *Science*. 2010; 330(6001): 228–31. DOI: 10.1126/science.1196333 [PubMed: 20826764]
64. Katagiri A, Nakayama K, Rahman MT, Rahman M, Katagiri H, Nakayama N, et al. Loss of ARID1A expression is related to shorter progression-free survival and chemoresistance in ovarian clear cell carcinoma. *Mod Pathol*. 2012; 25(2):282–8. DOI: 10.1038/modpathol.2011.161 [PubMed: 22101352]
65. Ayhan A, Mao TL, Seckin T, Wu CH, Guan B, Ogawa H, et al. Loss of ARID1A expression is an early molecular event in tumor progression from ovarian endometriotic cyst to clear cell and endometrioid carcinoma. *Int J Gynecol Cancer*. 2012; 22(8):1310–5. DOI: 10.1097/IGC.0b013e31826b5dcc [PubMed: 22976498]

66. Takeda T, Banno K, Okawa R, Yanokura M, Iijima M, Irie-Kunitomi H, et al. ARID1A gene mutation in ovarian and endometrial cancers (Review). *Oncol Rep.* 2016; 35(2):607–13. DOI: 10.3892/or.2015.4421 [PubMed: 26572704]
67. McCabe MT, Ott HM, Ganji G, Korenchuk S, Thompson C, Van Aller GS, et al. EZH2 inhibition as a therapeutic strategy for lymphoma with EZH2-activating mutations. *Nature.* 2012; 492(7427): 108–12. DOI: 10.1038/nature11606 [PubMed: 23051747]
68. Verma SK, Tian X, LaFrance LV, Duquenne C, Suarez DP, Newlander KA, et al. Identification of Potent, Selective, Cell-Active Inhibitors of the Histone Lysine Methyltransferase EZH2. *ACS Med Chem Lett.* 2012; 3(12):1091–6. DOI: 10.1021/ml3003346 [PubMed: 24900432]
69. Kim KH, Kim W, Howard TP, Vazquez F, Tsherniak A, Wu JN, et al. SWI/SNF-mutant cancers depend on catalytic and non-catalytic activity of EZH2. *Nat Med.* 2015; 21(12):1491–6. DOI: 10.1038/nm.3968 [PubMed: 26552009]
70. Cantley LC. The phosphoinositide 3-kinase pathway. *Science.* 2002; 296(5573):1655–7. DOI: 10.1126/science.296.5573.1655 [PubMed: 12040186]
71. Guertin DA, Sabatini DM. Defining the role of mTOR in cancer. *Cancer Cell.* 2007; 12(1):9–22. DOI: 10.1016/j.ccr.2007.05.008 [PubMed: 17613433]
72. Engelman JA, Luo J, Cantley LC. The evolution of phosphatidylinositol 3-kinases as regulators of growth and metabolism. *Nat Rev Genet.* 2006; 7(8):606–19. DOI: 10.1038/nrg1879 [PubMed: 16847462]
73. Bitler BG, Aird KM, Garipov A, Li H, Amatangelo M, Kossenkova AV, et al. Synthetic lethality by targeting EZH2 methyltransferase activity in ARID1A-mutated cancers. *Nat Med.* 2015; 21(3): 231–8. DOI: 10.1038/nm.3799 [PubMed: 25686104]
74. Dai YJ, Wang YY, Huang JY, Xia L, Shi XD, Xu J, et al. Conditional knockin of Dnmt3a R878H initiates acute myeloid leukemia with mTOR pathway involvement. *Proc Natl Acad Sci U S A.* 2017; 114(20):5237–42. DOI: 10.1073/pnas.1703476114 [PubMed: 28461508]
75. Wei FZ, Cao Z, Wang X, Wang H, Cai MY, Li T, et al. Epigenetic regulation of autophagy by the methyltransferase EZH2 through an MTOR-dependent pathway. *Autophagy.* 2015; 11(12):2309–22. DOI: 10.1080/15548627.2015.1117734 [PubMed: 26735435]
76. Peng D, Kryczek I, Nagarsheth N, Zhao L, Wei S, Wang W, et al. Epigenetic silencing of TH1-type chemokines shapes tumour immunity and immunotherapy. *Nature.* 2015; 527(7577):249–53. DOI: 10.1038/nature15520 [PubMed: 26503055]
77. Karantanos T, Chistofides A, Barhdan K, Li L, Boussiotis VA. Regulation of T Cell Differentiation and Function by EZH2. *Front Immunol.* 2016; 7:172.doi: 10.3389/fimmu.2016.00172 [PubMed: 27199994]
78. Zhao E, Maj T, Kryczek I, Li W, Wu K, Zhao L, et al. Cancer mediates effector T cell dysfunction by targeting microRNAs and EZH2 via glycolysis restriction. *Nat Immunol.* 2016; 17(1):95–103. DOI: 10.1038/ni.3313 [PubMed: 26523864]
79. Zingg D, Arenas-Ramirez N, Sahin D, Rosalia RA, Antunes AT, Haeusel J, et al. The Histone Methyltransferase Ezh2 Controls Mechanisms of Adaptive Resistance to Tumor Immunotherapy. *Cell Rep.* 2017; 20(4):854–67. DOI: 10.1016/j.celrep.2017.07.007 [PubMed: 28746871]
80. Xu Y, Li X, Wang H, Xie P, Yan X, Bai Y, et al. Hypermethylation of CDH13, DKK3 and FOXL2 promoters and the expression of EZH2 in ovary granulosa cell tumors. *Mol Med Rep.* 2016; 14(3): 2739–45. DOI: 10.3892/mmr.2016.5521 [PubMed: 27431680]
81. Wang Y, Chen SY, Karnezis AN, Colborne S, Santos ND, Lang JD, et al. The histone methyltransferase EZH2 is a therapeutic target in small cell carcinoma of the ovary, hypercalcaemic type. *J Pathol.* 2017; 242(3):371–83. DOI: 10.1002/path.4912 [PubMed: 28444909]
82. Chan-Penebre E, Armstrong K, Drew A, Grassian AR, Feldman I, Knutson SK, et al. Selective Killing of SMARCA2- and SMARCA4-deficient Small Cell Carcinoma of the Ovary, Hypercalcemic Type Cells by Inhibition of EZH2: In Vitro and In Vivo Preclinical Models. *Mol Cancer Ther.* 2017; 16(5):850–60. DOI: 10.1158/1535-7163.MCT-16-0678 [PubMed: 28292935]
83. Miranda TB, Cortez CC, Yoo CB, Liang G, Abe M, Kelly TK, et al. DZNep is a global histone methylation inhibitor that reactivates developmental genes not silenced by DNA methylation. *Mol Cancer Ther.* 2009; 8(6):1579–88. DOI: 10.1158/1535-7163.MCT-09-0013 [PubMed: 19509260]

84. Knutson SK, Wigle TJ, Warholc NM, Sneeringer CJ, Allain CJ, Klaus CR, et al. A selective inhibitor of EZH2 blocks H3K27 methylation and kills mutant lymphoma cells. *Nat Chem Biol.* 2012; 8(11):890–6. DOI: 10.1038/nchembio.1084 [PubMed: 23023262]
85. Katona BW, Liu Y, Ma A, Jin J, Hua X. EZH2 inhibition enhances the efficacy of an EGFR inhibitor in suppressing colon cancer cells. *Cancer Biol Ther.* 2014; 15(12):1677–87. DOI: 10.4161/15384047.2014.972776 [PubMed: 25535899]
86. Kaniskan HU, Jin J. Chemical probes of histone lysine methyltransferases. *ACS Chem Biol.* 2015; 10(1):40–50. DOI: 10.1021/cb500785t [PubMed: 25423077]
87. Qi W, Chan H, Teng L, Li L, Chuai S, Zhang R, et al. Selective inhibition of Ezh2 by a small molecule inhibitor blocks tumor cells proliferation. *Proc Natl Acad Sci U S A.* 2012; 109(52):21360–5. DOI: 10.1073/pnas.1210371110 [PubMed: 23236167]
88. Campbell JE, Kuntz KW, Knutson SK, Warholc NM, Keilhack H, Wigle TJ, et al. EPZ011989, A Potent, Orally-Available EZH2 Inhibitor with Robust in Vivo Activity. *ACS Med Chem Lett.* 2015; 6(5):491–5. DOI: 10.1021/acsmedchemlett.5b00037 [PubMed: 26005520]
89. Song X, Zhang L, Gao T, Ye T, Zhu Y, Lei Q, et al. Selective inhibition of EZH2 by ZLD10A blocks H3K27 methylation and kills mutant lymphoma cells proliferation. *Biomed Pharmacother.* 2016; 81:288–94. DOI: 10.1016/j.biopha.2016.04.019 [PubMed: 27261606]
90. Vaswani RG, Gehling VS, Dakin LA, Cook AS, Nasveschuk CG, Duplessis M, et al. Identification of (R)-N-((4-Methoxy-6-methyl-2-oxo-1,2-dihydropyridin-3-yl)methyl)-2-methyl-1-(1-(2,2,2-trifluoroethyl)piperidin-4-yl)ethyl)-1H-indole-3-carboxamide (CPI-1205), a Potent and Selective Inhibitor of Histone Methyltransferase EZH2, Suitable for Phase I Clinical Trials for B-Cell Lymphomas. *J Med Chem.* 2016; 59(21):9928–41. DOI: 10.1021/acs.jmedchem.6b01315 [PubMed: 27739677]
91. Bradley WD, Arora S, Busby J, Balasubramanian S, Gehling VS, Nasveschuk CG, et al. EZH2 inhibitor efficacy in non-Hodgkin's lymphoma does not require suppression of H3K27 monomethylation. *Chem Biol.* 2014; 21(11):1463–75. DOI: 10.1016/j.chembiol.2014.09.017 [PubMed: 25457180]
92. He Y, Selvaraju S, Curtin ML, Jakob CG, Zhu H, Comess KM, et al. The EED protein-protein interaction inhibitor A-395 inactivates the PRC2 complex. *Nat Chem Biol.* 2017; 13(4):389–95. DOI: 10.1038/nchembio.2306 [PubMed: 28135237]
93. Qi W, Zhao K, Gu J, Huang Y, Wang Y, Zhang H, et al. An allosteric PRC2 inhibitor targeting the H3K27me3 binding pocket of EED. *Nat Chem Biol.* 2017; 13(4):381–8. DOI: 10.1038/nchembio.2304 [PubMed: 28135235]
94. Lingel A, Sendzik M, Huang Y, Shultz MD, Cantwell J, Dillon MP, et al. Structure-Guided Design of EED Binders Allosterically Inhibiting the Epigenetic Polycomb Repressive Complex 2 (PRC2) Methyltransferase. *J Med Chem.* 2017; 60(1):415–27. DOI: 10.1021/acs.jmedchem.6b01473 [PubMed: 27992714]
95. Epizyme, I. Epizyme Announces Tazemetostat Fast Track Designation for Follicular Lymphoma and Plenary Session on Phase 2 NHL Data at ICML. 2017. <<https://globenewswire.com/news-release/2017/04/25/970819/0/en/Epizyme-Announces-Tazemetostat-Fast-Track-Designation-for-Follicular-Lymphoma-and-Plenary-Session-on-Phase-2-NHL-Data-at-ICML.html>>
96. Petrillo M, Nero C, Amadio G, Gallo D, Fagotti A, Scambia G. Targeting the hallmarks of ovarian cancer: The big picture. *Gynecol Oncol.* 2016; 142(1):176–83. DOI: 10.1016/j.ygyno.2016.03.037 [PubMed: 27058837]
97. Wheler JJ, Janku F, Naing A, Li Y, Stephen B, Zinner R, et al. Cancer Therapy Directed by Comprehensive Genomic Profiling: A Single Center Study. *Cancer Res.* 2016; 76(13):3690–701. DOI: 10.1158/0008-5472.CAN-15-3043 [PubMed: 27197177]
98. Krzystyniak J, Ceppi L, Dizon DS, Birrer MJ. Epithelial ovarian cancer: the molecular genetics of epithelial ovarian cancer. *Ann Oncol.* 2016; 27(Suppl 1):i4–i10. DOI: 10.1093/annonc/mdw083 [PubMed: 27141069]
99. Ramalingam P. Morphologic, Immunophenotypic, and Molecular Features of Epithelial Ovarian Cancer. *Oncology (Williston Park).* 2016; 30(2):166–76. [PubMed: 26892153]
100. Kleer CG, Cao Q, Varambally S, Shen R, Ota I, Tomlins SA, et al. EZH2 is a marker of aggressive breast cancer and promotes neoplastic transformation of breast epithelial cells. *Proc*

- Natl Acad Sci U S A. 2003; 100(20):11606–11. DOI: 10.1073/pnas.1933744100 [PubMed: 14500907]
101. Varambally S, Dhanasekaran SM, Zhou M, Barrette TR, Kumar-Sinha C, Sanda MG, et al. The polycomb group protein EZH2 is involved in progression of prostate cancer. *Nature*. 2002; 419(6907):624–9. DOI: 10.1038/nature01075 [PubMed: 12374981]
 102. Xu K, Wu ZJ, Groner AC, He HH, Cai C, Lis RT, et al. EZH2 oncogenic activity in castration-resistant prostate cancer cells is Polycomb-independent. *Science*. 2012; 338(6113):1465–9. DOI: 10.1126/science.1227604 [PubMed: 23239736]
 103. Zhou J, Roh JW, Bandyopadhyay S, Chen Z, Munkarah AR, Hussein Y, et al. Overexpression of enhancer of zeste homolog 2 (EZH2) and focal adhesion kinase (FAK) in high grade endometrial carcinoma. *Gynecol Oncol*. 2013; 128(2):344–8. DOI: 10.1016/j.ygyno.2012.07.128 [PubMed: 22871469]
 104. Jia N, Li Q, Tao X, Wang J, Hua K, Feng W. Enhancer of zeste homolog 2 is involved in the proliferation of endometrial carcinoma. *Oncol Lett*. 2014; 8(5):2049–54. DOI: 10.3892/ol.2014.2437 [PubMed: 25295088]
 105. Bachmann IM, Halvorsen OJ, Collett K, Stefansson IM, Straume O, Haukaas SA, et al. EZH2 expression is associated with high proliferation rate and aggressive tumor subgroups in cutaneous melanoma and cancers of the endometrium, prostate, and breast. *J Clin Oncol*. 2006; 24(2):268–73. DOI: 10.1200/JCO.2005.01.5180 [PubMed: 16330673]
 106. Zingg D, Debbache J, Schaefer SM, Tuncer E, Frommel SC, Cheng P, et al. The epigenetic modifier EZH2 controls melanoma growth and metastasis through silencing of distinct tumor suppressors. *Nat Commun*. 2015; 6:6051. doi: 10.1038/ncomms7051 [PubMed: 25609585]
 107. Tang SH, Huang HS, Wu HU, Tsai YT, Chuang MJ, Yu CP, et al. Pharmacologic down-regulation of EZH2 suppresses bladder cancer in vitro and in vivo. *Oncotarget*. 2014; 5(21):10342–55. DOI: 10.18632/oncotarget.1867 [PubMed: 25431950]
 108. Zhang H, Qi J, Reyes JM, Li L, Rao PK, Li F, et al. Oncogenic Deregulation of EZH2 as an Opportunity for Targeted Therapy in Lung Cancer. *Cancer Discov*. 2016; 6(9):1006–21. DOI: 10.1158/2159-8290.CD-16-0164 [PubMed: 27312177]
 109. Poirier JT, Gardner EE, Connis N, Moreira AL, de Stanchina E, Hann CL, et al. DNA methylation in small cell lung cancer defines distinct disease subtypes and correlates with high expression of EZH2. *Oncogene*. 2015; 34(48):5869–78. DOI: 10.1038/onc.2015.38 [PubMed: 25746006]
 110. Kondo Y, Shen L, Suzuki S, Kurokawa T, Masuko K, Tanaka Y, et al. Alterations of DNA methylation and histone modifications contribute to gene silencing in hepatocellular carcinomas. *Hepatol Res*. 2007; 37(11):974–83. DOI: 10.1111/j.1872-034X.2007.00141.x [PubMed: 17584191]
 111. Morin RD, Johnson NA, Severson TM, Mungall AJ, An J, Goya R, et al. Somatic mutations altering EZH2 (Tyr641) in follicular and diffuse large B-cell lymphomas of germinal-center origin. *Nat Genet*. 2010; 42(2):181–5. DOI: 10.1038/ng.518 [PubMed: 20081860]
 112. Ernst T, Chase AJ, Score J, Hidalgo-Curtis CE, Bryant C, Jones AV, et al. Inactivating mutations of the histone methyltransferase gene EZH2 in myeloid disorders. *Nat Genet*. 2010; 42(8):722–6. DOI: 10.1038/ng.621 [PubMed: 20601953]
 113. Ntziachristos P, Tsigos A, Van Vlierberghe P, Nedjic J, Trimarchi T, Flaherty MS, et al. Genetic inactivation of the polycomb repressive complex 2 in T cell acute lymphoblastic leukemia. *Nat Med*. 2012; 18(2):298–301. DOI: 10.1038/nm.2651 [PubMed: 22237151]

Figure 1. A model depicting mechanism of overexpression of EZH2 in ovarian cancer and its role in regulating gene expression

Targeting EZH2 can lead to re-activation of repressed tumor suppressor, thus blocking the EZH2 mediated cell proliferation, invasion, metastasis and radiation resistance.

Table 1

EZH2 status in various cancers

EZH2 Status	Associated cancer type	Reference(s)
EZH2 overexpression	Ovarian	(13,30)
	Breast	(100)
	Prostate	(101,102)
	Endometrial	(103,104)
	Melanoma	(105,106)
	Bladder	(107)
	Lung	(108,109)
	Liver	(110)
EZH2 mutation (gain of function)	Non-Hodgkin's lymphoma (Follicular lymphoma and diffuse large B cell lymphoma)	(111)
EZH2 mutation (loss of function)	Myelodysplastic syndrome (MDS), myeloproliferative neoplasms (MPN)	(112)
	T cell acute lymphoblastic leukemia	(113)

Table 2

Current EZH2 inhibitors and their mechanism of action

Compound	Mechanism	Ref.	Chemical structure
DZNep	SAH hydrolase inhibitor of methyltransferase activity	(83)
EPZ005687	SAM-competitive inhibitor of PRC2	(84)
GSK126	SAM-competitive inhibitor of PRC2	(67)
UNC1999	SAM-competitive inhibitor of PRC2	(85)

Compound	Mechanism	Ref.	Chemical structure
EPZ-6438	SAM-competitive inhibitor of PRC2	(86)
EI1	SAM-competitive inhibitor of PRC2	(87)
GSK343	SAM-competitive inhibitor of PRC2	(68)
GSK926	SAM-competitive inhibitor of PRC2	(68)

Compound	Mechanism	Ref.	Chemical structure
EPZ011989	SAM-competitive inhibitor of PRC2	(88)
ZLD10A	SAM-competitive inhibitor of PRC2	(89)
CPI-1205	SAM-competitive inhibitor of PRC2	(90)
CPI-360	SAM-competitive inhibitor of PRC2	(91)
CPI-169	SAM-competitive inhibitor of PRC2	(91)

Compound	Mechanism	Ref.	Chemical structure
A-395	EED inhibitor	(92)
EED226	EED inhibitor	(93)