Histone Methyltransferase EZH2: A Therapeutic Target for Ovarian Cancer

Bayley A. Jones1, Sooryanarayana Varambally2, and Rebecca C. Arend3

Abstract

Ovarian cancer is the fifth leading cause of cancer-related deaths in females in the United States. There were an estimated 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 tumorogenesis 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.

Introduction

As the fifth leading cause of cancer-related deaths in females in the United States, there were an estimated 22,440 new cases of ovarian cancer and 14,080 deaths due to ovarian cancer 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-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 histologic 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 reveal 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.

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EZH2 has been shown to play a critical role in cancer cell proliferation, invasion, tumor metastasis, angiogenesis, and chemotherapy resistance (Fig. 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 (LSC) 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 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. Because 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 (miRNA), long noncoding 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; refs. 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 leads 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

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<td>EZH2 overexpression</td>
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<td>EZH2 mutation (loss of function)</td>
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release of repression of ARH1 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 and colleagues found that EZH2 expression was positively correlated to KDM2B and that expression of EZH2 and KDM2B was significantly associated with tumor histologic 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 and colleagues 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 nonepithelial tumor tissue samples (immature teratoma, granulosa cell tumors, dysgerminomas, blastoma, and metastatic tumor to the ovary). They found that 85.55% of epithelial ovarian carcinomas displayed EZH2 upregulation compared with only 37.5% for nonepithelial 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 oncopgene 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 and colleagues 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 and colleagues 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-ß1. In their study, they observed downregulated E-cadherin and upregulated TGF-ß1 in EOCs, including serous, mucinous, clear cell, endometrioid, and undifferentiated types. However, only EZH2 and TGF-ß1 showed a positive correlation, suggesting that EZH2 regulates cell invasion via the regulation of TGF-ß1 expression (21). TGF-ß1 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; refs. 22–24). Despite the potential benefits of reduced TGF-ß1 expression by EZH2 inhibition, adverse consequences of EZH2 inhibition in EMT have been documented. Cardenas and colleagues proposed that EZH2 inhibition may drive EMT by removing repression of TGF-ß-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 protumorigenic events causing a more aggressive cancer phenotype, thus highlighting the need for further investigation into the role of EZH2 in the induction of EMT (25).

Tissue inhibitor of metalloproteinase 2 (TIMP2) is an endogenous regulator of matrix metalloproteinases (MMP). 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 and colleagues 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 proangiogenic and antiangiogenic pathways (28). VEGF is one of the most potent proangiogenic stimulators that affects endothelial proliferation and mobility and vascular permeability (29). Lu and colleagues 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, it is not surprising that EZH2 inhibitors are being pursued in clinical trials for EOC. As a summary, EZH2 is an important target in ovarian cancer and may be responsible for many aspects of ovarian cancer progression.
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 1 year, compared with a 2-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-resistant 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 and colleagues 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 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 and colleagues suggested that a potential mechanism by which EZH2 inhibits 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 and colleagues looked at the interaction of EZH2 with several differentially expressed genes (DEG) 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 miRNAs
miRNAs are small, endogenous noncoding RNAs molecules that are capable of modulating the posttranscriptional 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). miR-101, miR-298, and the enzyme Dicer, a key protein required for miRNA 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 miR-101. Downregulation of miR-101 results in overexpression of EZH2, thereby resulting in cancer progression (40–42). Liu and colleagues showed that there was a significant negative correlation between miR-101 and EZH2 mRNA in epithelial ovarian cancers (using serous, mucinous, and endometrioid tissue samples), with miR-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 miR-101 overexpression resulted in decreased proliferation and migration (18). This suggests that miR-101 may play an important role in ovarian cancer by regulating EZH2. Using these data, Liu and colleagues looked at the role of miR-101 and found miR-101 levels to be reduced in cisplatin-resistant cells (A2780/DDP and SKOV3/DDP). Furthermore, upregulation of miR-101 was able to sensitize cisplatin-resistant cell lines to treatment with cisplatin (43).

miR-298 has also been found to inhibit malignant EOC phenotypes by regulation of EZH2. Zhou and colleagues showed that EZH2 expression was significantly upregulated while miR-298 was significantly downregulated in human serous and nonserous EOC tissues. These findings were significantly associated with high clinical stage and pathologic grade, demonstrating the roles of EZH2 and miR-298 in the regulation of malignant phenotypes of EOC cells and the aggressive progression of EOC cancers. Ectopic expression of miR-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 miR-298. Their findings support the idea that the miR-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). Downregulation of miR-298 and concomitant upregulation of EZH2 can result in aggressive tumor phenotype in ovarian cancer.

EZH2 indirectly interacts with miRNA 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 and colleagues 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 noncoding RNAs
Long noncoding RNAs (lncRNA) 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 and colleagues showed that IncRNA 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 with 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; ref. 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 occurrence of certain gene loci as well as the methylation by EZH2. Ozes and colleagues showed that HOTAIR is overexpressed in platinum-resistant EOC cells (A2780_CR5). A positive feedback loop of a proposed NF-κB–HOTAIR axis may result cellular senescence and platinum resistance in EOC and it may do so by functional overlap with EZH2 (50). Ozes and colleagues further investigated the interaction of HOTAIR and EZH2 and showed that blocking their interaction using peptide nucleic acids (PNA) represses tumor growth and invasiveness of A2780_CR5 in vitro, reduces cell proliferation, and decreases NF-κB 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 GS126, respectively (51).

Despite these data, a recent study by Portoso and colleagues 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 highlighted the need for further investigations into the role of IncRNAs 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 (OCSC), 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 and colleagues 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 with 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 spheres of more than 32 cells. The same results were seen in cells with knockdown of both EZH2 and EZH1. Rizzo and colleagues 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 with 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 and colleagues showed an upregulation of EZH2 and a downregulation of ALDH1A1 in EOC cells compared with 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 and colleagues 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 endometriod, and one mixed or undifferentiated cell. They demonstrated the interaction between EZH2 and this pathway using EZH2-targeted miR-98. Most OCSCs transfected with miR-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 miR-98. In addition, they demonstrated a significant decrease in the levels of Hidac1, E2f1, Ccne, Cdk2, and pRb mRNAs in OCSCs transfected with miR-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 miR-98-transfected OCSCs were smaller and had reduced proliferation capacity than those formed by mutant miR-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 and colleagues 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 Muc16. In addition, ovariectomized mice still developed high-grade serous 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 premetastatic 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 nonciliated secretory cells of the endosalpinx (56). Yamamoto and colleagues showed that fallopian tube stem cells (FTSC) 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 (FTSC\(^{C}\)) 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 immunologic 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).

**ARID1A and EZH2 in OCCC and endometrioid 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 endometrioid 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 make 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. Wieand and colleagues found ARID1A mutations in 46% of ovarian clear cell carcinoma (OCCC) and in 30% of ovarian endometrioid carcinoma. None of the 76 high-grade serous ovarian carcinomas in their study had mutated ARID1A (60). Another study by Jones and colleagues 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 with mice injected with control shRNA (59).

**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 to be necessary for the development of ovarian endometrioid tumor initiation and that additional genetic modifications, such as PTEN deletion, were necessary to drive tumorigenesis in vivo (61). Bitler and colleagues showed that inhibition of EZH2 resulted in decreased expression of EZH2-targeted PI3K/P, 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 OVISE xenografts in reducing tumor growth compared with 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. These data reaffirm the interaction of ARID1A mutations and PI3K/Akt pathways in OCCC and suggest 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 is 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 led to the broader 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 and colleagues used HGSC tissue samples and a syngeneic mouse ovarian cancer model (1D8 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 and colleagues 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 antitumor 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 1D8 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 and colleagues 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 antipoptotic 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 and colleagues also found that T cell EZH2 was controlled by glycolytic metabolism in the tumor microenvironment in ovarian cancer by maintaining high expression of miR-101 and miR-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 and colleagues 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 and colleagues 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 antitumor 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, because 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 nonepithelial 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 nonepithelial cancers, including granulosa cell tumors (GCT) and small cell carcinoma of the ovary, hypercalcemic type (SCCOHT). Xu and colleagues 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...
Table 2. Current EZH2 inhibitors and their mechanism of action

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mechanism</th>
<th>Ref.</th>
<th>Chemical structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>DZNep</td>
<td>SAH hydrolase inhibitor of methyltransferase activity</td>
<td>(83)</td>
<td><img src="image1" alt="Chemical structure" /></td>
</tr>
<tr>
<td>EPZ005687</td>
<td>SAM-competitive inhibitor of PRC2</td>
<td>(84)</td>
<td><img src="image2" alt="Chemical structure" /></td>
</tr>
<tr>
<td>GSK126</td>
<td>SAM-competitive inhibitor of PRC2</td>
<td>(67)</td>
<td><img src="image3" alt="Chemical structure" /></td>
</tr>
<tr>
<td>UNC1999</td>
<td>SAM-competitive inhibitor of PRC2</td>
<td>(85)</td>
<td><img src="image4" alt="Chemical structure" /></td>
</tr>
<tr>
<td>EPZ-6438</td>
<td>SAM-competitive inhibitor of PRC2</td>
<td>(86)</td>
<td><img src="image5" alt="Chemical structure" /></td>
</tr>
<tr>
<td>EI1</td>
<td>SAM-competitive inhibitor of PRC2</td>
<td>(87)</td>
<td><img src="image6" alt="Chemical structure" /></td>
</tr>
<tr>
<td>GSK343</td>
<td>SAM-competitive inhibitor of PRC2</td>
<td>(68)</td>
<td><img src="image7" alt="Chemical structure" /></td>
</tr>
<tr>
<td>GSK926</td>
<td>SAM-competitive inhibitor of PRC2</td>
<td>(68)</td>
<td><img src="image8" alt="Chemical structure" /></td>
</tr>
<tr>
<td>EPZ011989</td>
<td>SAM-competitive inhibitor of PRC2</td>
<td>(88)</td>
<td><img src="image9" alt="Chemical structure" /></td>
</tr>
<tr>
<td>ZLD10A</td>
<td>SAM-competitive inhibitor of PRC2</td>
<td>(89)</td>
<td><img src="image10" alt="Chemical structure" /></td>
</tr>
<tr>
<td>CPI-1205</td>
<td>SAM-competitive inhibitor of PRC2</td>
<td>(90)</td>
<td><img src="image11" alt="Chemical structure" /></td>
</tr>
<tr>
<td>CPI-360</td>
<td>SAM-competitive inhibitor of PRC2</td>
<td>(91)</td>
<td><img src="image12" alt="Chemical structure" /></td>
</tr>
</tbody>
</table>

(Continued on the following page)
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 with 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 miR-101 are potential therapies (40, 42). A model in Fig. 1 depicts the mode of regulation and action of EZH2 in ovarian cancers. Targeting EZH2 with small-molecule inhibitors can reactivate the tumor suppressors repressed by EZH2.

EZH2 dysregulation plays a profound role in the development, progression, and therapeutic resistance of many types of cancer,

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 and colleagues 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 and colleagues 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 and colleagues 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 compared with 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 I 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
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.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References


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