SUZ12 Promotes Human Epithelial Ovarian Cancer by Suppressing Apoptosis via Silencing HRK

Hua Li, Qi Cai, Hong Wu, Vinod Vathipadickal, Zachary C. Dobbin, Tianyu Li, Xiang Hua, Charles N. Landen, Michael J. Birrer, Margarita Sánchez-Beato, and Rugang Zhang

Abstract

Epithelial ovarian cancer (EOC) ranks first as the cause of death for gynecological cancers in the United States. SUZ12 is a component of the polycomb repressive complex 2 (PRC2) and is essential for PRC2-mediated gene silencing by generating trimethylation on lysine 27 residue of histone H3 (H3K27Me3). The role of SUZ12 in EOC has never been investigated. Here, we show that SUZ12 is expressed at significantly higher levels in human EOC (n = 117) compared with either normal human ovarian surface epithelium (n = 35, P < 0.001) or fallopian tube epithelium (n = 15, P < 0.001). There is a positive correlation between expression of SUZ12 and EZH2 in human EOC (P < 0.001). In addition, expression of SUZ12 positively correlates with Ki67, a marker of cell proliferation (P < 0.001), and predicts shorter overall survival (P = 0.0078). Notably, knockdown of SUZ12 suppresses the growth of human EOC cells in vitro and in vivo in both orthotopic and subcutaneous xenograft EOC models. In addition, SUZ12 knockdown decreases the levels of H3K27Me3 and triggers apoptosis of human EOC cells. Mechanistically, we identified Harakiri (HRK), a proapoptotic gene, as a novel SUZ12 target gene, and showed that HRK upregulation mediates apoptosis induced by SUZ12 knockdown in human EOC cells. In summary, we show that SUZ12 promotes the proliferation of human EOC cells by inhibiting apoptosis and HRK is a novel SUZ12 target gene whose upregulation contributes to apoptosis induced by SUZ12 knockdown.

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Introduction

More than 85% of ovarian cancers are of epithelial origin (1). Epithelial ovarian cancers (EOC) are classified into distinct histologic types including serous, mucinous, endometrioid, and clear cell (1). The most common histology of EOC is serous (~60% of all cancers) and less common histologies include endometrioid, clear cell, and mucinous (2). Recently, an alternative classification has been proposed, in which EOC is broadly divided into 2 types (3). Type I EOC includes mucinous, low-grade serous, low-grade endometrioid, and clear cell carcinomas, and type II EOC includes high-grade serous and high-grade endometrioid carcinomas (3). EOC remains the most lethal gynecological malignancy in the United States (4). Thus, there is an urgent need to better understand the etiology of EOC to develop novel therapeutics for this disease.

SUZ12 is essential for polycomb repressive complex 2 (PRC2)–mediated gene silencing (5–8). In addition to SUZ12, PRC2 also contains the catalytic subunit EZH2 (9). PRC2 epigenetically silences gene transcription by generating trimethylation on lysine 27 residue of histone H3 (H3K27Me3). EZH2 lacks methyltransferase enzyme activity on its own, and has to complex with SUZ12 to attain histone methyltransferase activity (9, 10).

EZH2 is overexpressed in several types of cancers, including prostate and breast cancers (11–15). Its overexpression correlates with the aggressiveness and poor prognosis in breast and prostate cancers (11–13). Indeed, EZH2 is often overexpressed in human EOC and its knockdown triggers apoptosis of human EOC cells (16, 17). In contrast, the role SUZ12 in EOC remains poorly understood.

Here, we examined the expression of SUZ12 in human EOC specimens and discovered that SUZ12 is expressed at significantly higher levels in human EOCs compared with either normal human ovarian surface epithelium or fallopian tube epithelium. In addition, we showed that HRK upregulation positively correlates with expression of EZH2 and Ki67, a cell proliferation marker. Conversely, SUZ12 knockdown suppresses the growth of human EOC cells in vitro and in vivo in xenograft EOC models. Consistently,
SUZ12 knockdown induces apoptosis of human EOC cells. Mechanistically, we identified Harakiri (HRK), a proapoptotic gene, as a novel SUZ12 target gene whose upregulation contributes to apoptosis induced by SUZ12 knockdown in human EOC cells.

Materials and Methods

Cell culture
Human EOC cell lines SKOV3, PEO1, and OVCAR10 were cultured according to American Type Culture Collection and as we have previously described (16, 18). The cell line identification was confirmed by DNA Diagnostic Center (www.dnacenter.com).

FACS, immunofluorescence staining, and immunoblot analysis

Fluorescence-activated cell sorting (FACS) and indirect immunofluorescence staining were conducted as described previously (19). The following antibodies were used for immunofluorescence: rabbit anti-H3K27Me3 (Cell Signaling, 1:1,000), and rabbit anti-H3K9Me2 (Abcam, 1:500). The antibodies used for immunoblot were from indicated suppliers: rabbit anti-H3K27Me3 (Cell signaling, 1:1,000), rabbit anti-H3K9Me3 (Abcam, 1:2,000), mouse antihistone H3 (Millipore, 1:10,000), mouse anti–glyceraldehyde-3-phosphate dehydrogenase (GAPDH; Millipore, 1:10,000), rabbit anti-PARP p85 fragment (Promega, 1:1,000), rabbit anti-phosphate dehydrogenase (GAPDH; Millipore, 1:10,000), rabbit anti-H3K9Me2 (Abcam, 1:400) for 1 hour, detecting the biotinylated goat anti-rabbit antibody (220A 1:40) at 4°C, followed by biotinylated goat anti-mouse IgG (DAKO, 1:400) for 1 hour, detecting the antibody complexes with the labeled streptavidin–biotin system, and visualizing them with the chromogen 3,30-diaminobenzidine. Sections were lightly counterstained with hematoxylin. In addition, anti-EZH2 (Millipore; 1:100) and anti-Ki67 (Dako, 1:100) antibodies were used on consecutive sections as we have previously described (20).

siRNA, shRNA, lentivirus packaging, and infection

The sense sequences of 2 individual short hairpin RNA (shRNA) to the human SUZ12 gene (shSUZ12) are: 5'-GCTTATCATGTGAGTTGTAATG 3' and 5'-CTCATAGCACCAATAAGCGTGC-3', respectively. Lentivirus packaging was achieved using virapower system (Invitrogen) according to manufacturer’s instruction. PE01 and SKOV3 at 40% to 50% confluence were infected with lentivirus expressing shSUZ12 or vector control. The infected cells were selected with 1 µg/mL (for PE01) or 3 µg/mL (for SKOV3) of puromycin, respectively. shHRK was purchased from Dharmacon (Cat: L-008216-00-0005) and transfection was conducted following the manufacturer’s instruction. A siRNA to luciferase (siGL2) was used as a negative control.

Inducible expression of shRNA resistant SUZ12

To generate shRNA resistant SUZ12 expression construct that do not affect the protein sequence, but resistant to the shSUZ12 #1, 3 rounds of mutagenesis were carried out to mutate every third base of the coding region in SUZ12 open reading frame (ORF) targeted by shSUZ12 #1 using Quickchange II XL Site-Directed Mutagenesis kit (Stratagene, Cat. No: 200521). Mutagenic primers are as the following: Round 1: forward: 5’-GTCAGCTCATTTGCAGAATTCA-CTTCACCGGTTTCCTCCAC-3’ and reverse: 5’-GTGGAAGAAACCCGCTGAATGTGAGTGGACAAATGAGCTGAC-3’T; Round 2 forward: 5’-CTCATACCTCA-CGGGCTTTTCCAAAATGATAAGC-3’ and reverse: 5’-GCTTATCATTTGTGAAAAGCCGT-GAATGTGAG-3’T; and Round 3 forward: 5’-GTCAGCTCATTTGCAGAATTCA-CTTCACCGGTTTCCTCCAC-3’ and reverse: 5’-GGAAAAGCCGTGAATGTGAG-3’T. The shRNA resistant SUZ12 was then subcloned into an inducible retroviral vector pRetroX-Tight-Pur (Retro-X Tet-On, Invitrogen) and the inducible SUZ12 SKOV3 cell line was generated following manufacturer’s instruction. Twenty-four hours after infection with shSUZ12 #1 virus, shRNA resistant SUZ12 was induced by DOX (Clontech, 500 ng/mL) following manufacturer’s instruction.

Human ovarian tissue microarrays and specimens

Tissue microarrays, including core samples from 117 primary human EOCs, 35 cases of normal ovary tissues and 15 cases of fallopian tube tissues were obtained from FCCC Biosample Repository Core Facility. Use of these human specimens was approved by the Institutional Review Board.

Immunohistochemical staining and scoring

The expression of SUZ12 was detected using avidin–biotin–peroxidase methods and as previously described (18). Briefly, tissue sections were subjected to antigen retrieval by steaming in 0.01 mol/L of sodium citrate buffer (pH 6.0) for 30 minutes. After quenching endogenous peroxidase activity with 3% hydrogen peroxide and blocking nonspecific protein binding with 1% bovine serum albumin, sections were incubated overnight with primary monoclonal SUZ12 antibody (220A 1:40) at 4°C, followed by biotinylated goat anti-mouse IgG (DAKO, 1:400) for 1 hour, detecting the antibody complexes with the labeled streptavidin–biotin system, and visualizing them with the chromogen 3,30-diaminobenzidine. Sections were lightly counterstained with hematoxylin. In addition, anti-EZH2 (Millipore; 1:100) and anti-Ki67 (Dako, 1:100) antibodies were used on consecutive sections as we have previously described (16).

RNA isolation, qRT-PCR, and PCR array

RNA was isolated using Trizol (Invitrogen) according to manufacturer’s instruction. For quantitative real-time PCR (qRT-PCR), Trizol-isolated RNA was further purified using an RNEasy kit (Qiagen) following manufacturer’s instruction. The primers for HRK genes used for qRT-PCR are: forward: 5’-GCAAACAGGTGTGGAAAAACCCCT-3’ and reverse: 5’-ATTGGGGTGTCTGTGGAGCC-3’. Expression of the housekeeping gene β-2-microglobulin was used to normalize mRNA expression. Apoptosis genes PCR array was purchased from SABiosciences (Cat: PAHS-012A) and the analysis was conducted following the manufacturer’s instruction. The RT-PCR data was analyzed by using RT2 Profiler PCR Array Data Analysis version 3.4. For survival analysis, RNA was isolated and amplified from
SUZ12 Promotes EOC via Silencing HRK

We sought to determine the expression of SUZ12 protein in human EOCs by immunohistochemistry (IHC). The specificity of the anti-SUZ12 antibody was confirmed in our study. A single band at the predicted molecular weight (≈83 kDa) was detected in human SKOV3 cells and was absent after expression of a shRNA to the human SUZ12 gene (shSUZ12) that effectively knocked down SUZ12 mRNA expression (Supplementary Fig. S1A and data not shown). In addition, SUZ12 staining was lost when primary anti-SUZ12 antibody was replaced with an isotype-matched IgG control (Supplementary Fig. S1B).

We next examined SUZ12 expression in 117 cases of human EOCs and 35 cases of normal human ovaries by IHC. The specificity of the anti-SUZ12 antibody was confirmed in our study. A single band at the predicted molecular weight (≈83 kDa) was detected in human SKOV3 cells and was absent after expression of a shRNA to the human SUZ12 gene (shSUZ12) that effectively knocked down SUZ12 mRNA expression (Supplementary Fig. S1A and data not shown). In addition, SUZ12 staining was lost when primary anti-SUZ12 antibody was replaced with an isotype-matched IgG control (Supplementary Fig. S1B).

Results

SUZ12 is expressed at significantly higher levels in human EOCs compared with either normal human ovarian surface epithelium or fallopian tube epithelium

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Statistical analysis

Quantitative data were expressed as mean ± SD, unless otherwise stated. ANOVA with Fisher’s Least Significant Difference was used to identify significant differences in multiple comparisons. The χ² test was used to analyze the relationship between categorical variables. Overall survival was defined as the time elapsed from the date of diagnosis and the date of death from ovarian cancer, otherwise were considered as censored observations. Kaplan–Meier survival plots were generated and comparisons made using the log-rank statistic. For all statistical analyses, the level of significance was set at 0.05.
We scored expression of SUZ12 as high (H score ≥ 50) or low (H score < 50) based on a histologic score as previously described (25, 26). SUZ12 was scored as high in 76.9% (90/117) of human EOCs. In contrast, SUZ12 was scored high in 20% (7/35) and 13.3% (2/15) of normal human ovarian surface epithelium and fallopian tube epithelium, respectively (Table 1). Statistical analysis revealed that SUZ12 was expressed at significantly higher levels in human EOCs compared with either normal human ovarian surface epithelium (P < 0.001) or fallopian tube epithelium (P < 0.001; Table 1). Consistently, SUZ12 was expressed at higher levels in human EOC cell lines compared with normal human ovarian surface epithelial cells (Supplementary Fig. S1D). On the basis of these studies, we conclude that SUZ12 is expressed at significantly higher level in human EOCs compared with either normal human ovarian surface epithelium or fallopian tube epithelium.

SUZ12 expression positively correlates with expression of EZH2 and Ki67, and predicts shorter overall survival

Following this observation, we sought to determine the correlation between expression of SUZ12 and EZH2, or Ki67, a marker of cell proliferation. We examined the expression of EZH2 and Ki67 by IHC in the same set of human EOC specimens (Fig. 1B and Table 1). There was a significant, positive correlation between expression of SUZ12 and EZH2 (P < 0.001) or Ki67 (P < 0.001; Table 1). We next examined the correlation between SUZ12 expression and clinical and pathological features of human EOCs. There is a trend toward significance between expression of SUZ12 and tumor stage (P = 0.122; Table 1). Notably, the majority of the examined EOC cases are type II high-grade serous subtypes that are typically of stage III/IV (Table 1). Furthermore, we assessed the correlation between SUZ12 expression and prognosis of EOC patients (n = 50), for which long-term follow-up data were available. There was a significant correlation between expression of SUZ12 and overall survival in EOC patients (P = 0.0078; Fig. 1C). On the basis of these results, we conclude that SUZ12 expression positively correlates with expression of EZH2 and Ki67, and predicts poor overall survival in EOC patients.

SUZ12 knockdown suppresses the growth of human EOC cells in vitro and in vivo

As SUZ12 expression correlates with expression of a cell proliferation marker Ki67, we sought to determine the effects of SUZ12 knockdown on proliferation of human EOC cells. Toward this goal, we used 2 individual shRNAs to the human SUZ12 gene (shSUZ12). The knockdown efficacy of shSUZ12 in SKOV3 human cells was confirmed by immunoblot (Fig. 2A). Notably, the level of H3K27Me3 was significantly reduced in shSUZ12 expressing SKOV3 cells as determined by both immunoblot and immunofluorescence staining (Fig. 2A and B). In contrast, shSUZ12 has no effects on the level of trimethylated lysine 9 histone H3 (H3K9Me3) that is generated by the histone methyltransferases such as Suv39H1, Suv39H2, and SETDB1 (Fig. 2A and B; ref. 27). Together, these data support the premise that SUZ12 is necessary for H3K27Me3 epigenetic modification in human EOC cells.

Compared with controls, SUZ12 knockdown significantly inhibited both anchorage-dependent and independent
growth in soft agar in SKOV3 cells ($P < 0.001$; ref. Fig. 2C and D). In addition, SUZ12 knockdown also decreased the expression levels of H3K27Me3 and suppressed both anchorage-dependent and independent growth in PEO1 cells (Supplementary Fig. S2), suggesting that the observed growth inhibitory effects are not cell line specific.

We next sought to determine the effects of SUZ12 knockdown on the growth of SKOV3 cells in vivo in immunocompromised mice. Toward this goal, a luciferase gene was retrovirally transduced into control or shSUZ12-expressing SKOV3 cells to monitor the cell growth in vivo via noninvasive imaging as previously described (18). These cells were injected unilaterally into the bursa sac covering the ovary in female immunocompromised mice ($n = 9$ for each of the groups). Tumor growth was monitored by measuring luciferase activity every 2 weeks starting at week 2 after injection, and the tumor growth was followed for a total of 8 weeks. Indeed, SUZ12 knockdown significantly suppressed the orthotopically xenografted SKOV3 human EOC cells compared with controls (Fig. 3A and B). In addition, we also examined the growth of control and shSUZ12-expressing SKOV3 cells in a s.c. xenograft model in immunocompromised mice. The same growth inhibitory effects of shSUZ12 were also observed in the s.c. xenograft EOC model (Fig. 3C and D). SUZ12 knockdown in the surgically dissected xenografted tumors was confirmed by IHC staining (Fig. 3E and F).

### Table 1. SUZ12 expression in human EOCs and its correlation with clinicopathological variables or expression of EZH2 and Ki67

<table>
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<th>SUZ12 protein expression</th>
<th>Low (n)</th>
<th>High (n)</th>
<th>Total (n)</th>
<th>High (%)</th>
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<td>18</td>
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<td>70</td>
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$^a$Compared with epithelial ovarian cancer, $P < 0.001$.

$^b$Compared with serous epithelial ovarian cancer, $P < 0.001$.

$^c$Spearman’s Rank Order Correlation also showed SUZ12 positively correlated with Ki67 ($P < 0.001$), and $r_s$ is 0.465.

$^d$Spearman’s Rank Order Correlation also showed SUZ12 positively correlated with EZH2 ($P < 0.001$), and $r_s$ is 0.515.
3E). On the basis of these results, we conclude that SUZ12 knockdown suppresses the growth of human EOC cells in vivo in both orthotopic and s.c. xenograft EOC models.

SUZ12 knockdown triggers apoptosis of human EOC cells

Next, we sought to determine the cellular mechanism by which SUZ12 knockdown inhibits the growth of human EOC cells. DNA content analysis determined by FACS showed that there was an increase in the percentage of the sub-G1 phase in SUZ12 knockdown cells compared with controls (Fig. 4A and B), suggesting that these cells may undergo apoptosis. Indeed, markers of apoptosis were significantly induced in SUZ12 knockdown cells compared with controls. Those markers include increased percentage of Annexin V positively stained cells as measured by Guava Nexin assay and upregulation of cleaved Lamin A, PARP p85, and caspase 3 as determined by immunoblot (Fig. 4C–E). The degree of apoptosis induced by shSUZ12 correlated with the level of SUZ12 knockdown by 2 individual shSUZ12 (Fig. 4), suggesting that the observed apoptosis in SUZ12 knockdown EOC cells was not because of off-target effects.

To further limit the potential off-target effect, we conducted the rescue experiments. Toward this goal, we generated a shSUZ12 #1 resistant HA-tagged SUZ12-expressing construct that expresses the wild-type protein. Expression of shSUZ12 efficiently knocked down the expression of endogenous SUZ12 mRNA as reflected by semiquantitative RT-PCR using primers targeting the 3' end untranslated region (UTR; Fig. 5A). The expression of shSUZ12 #1 resistant HA-tagged SUZ12 was confirmed at both the mRNA level by semiquantitative RT-PCR using primers targeting the ORF and at the protein level by immunoblot using an anti-HA antibody (Fig. 5A and B). Indeed, expression of HA-tagged shSUZ12-resistant wild-type SUZ12 protein partially rescued the decrease of H3K27Me3 level and suppressed the expression of markers of apoptosis such as expression of cleaved Lamin A, PARP p85, and caspase 3 in SUZ12 knockdown SKOV3 cells (Fig. 5B). Notably, the ectopic HA-tagged shSUZ12 #1 resistant wild-type SUZ12 protein is expressed at a level lower than the endogenous SUZ12 level observed in SKOV3 cells (Fig. 5A and B), suggesting that the observed effects are not because of supraphysiologic levels of ectopic SUZ12 expression.

HRK is a SUZ12 target gene that contributes to apoptosis induced by SUZ12 knockdown

Next, we sought to determine the molecular mechanism by which SUZ12 knockdown induces apoptosis of human EOC cells. Toward this goal, we sought to identify the direct SUZ12 target genes that are implicated in promoting apoptosis observed in SUZ12 knockdown EOC cells. We compared the expression of apoptosis-regulating genes
between control and SUZ12 knockdown SKOV3 cells using a qRT-PCR array that consists of 84 apoptosis-regulating genes (Supplementary Table S1 and Fig. S3). We hypothesized that apoptosis-promoting genes are epigenetically silenced by SUZ12 in EOC cells via H3K27Me3 and SUZ12 knockdown induces apoptosis of EOC cells by upregulating the expression of these genes. Thus, we cross-examined the apoptosis-regulating qRT-PCR array data in SKOV3 cells with a H3K27Me3 ChIP followed by next generation sequencing (ChIP-seq) dataset in SKOV3 cells (28). This analysis revealed 2 H3K27Me3 direct target genes that are upregulated more than 2-fold in SUZ12 knockdown SKOV3 cells, namely, HRK and TNFRSF9.

BH3-only Bcl-2 family member HRK is a proapoptotic gene (29). Notably, HRK has been implicated in regulating apoptosis of human ovarian cancer cells and murine ovarian reserve (30, 31). Thus, we tested the role of HRK in mediating apoptosis induced by SUZ12 knockdown. We confirmed the upregulation of HRK in SUZ12 knockdown SKOV3 cells by qRT-PCR (Fig. 6A). Significantly, we showed that SUZ12 directly binds to the HRK genomic locus by ChIP analysis (Fig. 6B). Likewise, we showed that H3K27Me3 epigenetic marker is present in the HRK genomic locus (Fig. 6B). Importantly, knockdown of SUZ12 severely diminished the association of SUZ12 and H3K27Me3 with the HRK genomic locus (Fig. 6B). This result suggests that the presence of H3K27Me3 in HRK genomic locus is dependent upon SUZ12 and the association of SUZ12 to the HRK genomic locus is specific. It has previously been shown that DNA methylation contributes to HRK downregulation in certain types of cancers such as prostate (32). Thus, we treated EOC cells with 5-azacytidine (5-AzaC), a DNA demethylation drug (33), and examined the expression of HRK in these cells. 5-AzaC failed to upregulate HRK expression in EOC cells (Supplementary Fig. S4). This result suggests that HRK expression is not regulated by DNA methylation in human EOC cells. Consistent with our findings, based on the newly released the Cancer Genome Atlas (TCGA) ovarian cancer database.
HRK gene promoter methylation is very rare in human ovarian cancer (<1% cases show >50% of promoter methylation in human HRK gene promoter; http://cancergenome.nih.gov/; ref. 34).

Next, we sought to determine whether apoptosis induced by SUZ12 knockdown is mediated, in part, by upregulation of HRK. Toward this goal, we knocked down the HRK expression in SUZ12 knockdown cells. HRK knockdown was confirmed by qRT-PCR (Supplementary Fig. S5). Indeed, HRK knockdown notably suppressed the expression of markers of apoptosis such as the percentage of sub-G1 phase cells and expression of cleaved lamin A, caspase 3, and PARP p85, in SUZ12 knockdown cells (Fig. 6C and D and Supplementary Fig. S6). Similar results were also obtained in OVCAR10 human EOC cell line (Supplementary Fig. S7), showing that the observed effects are not cell line specific. On the basis of these results, we conclude that HRK is a novel SUZ12 target gene whose upregulation contributes to apoptosis induced by SUZ12 knockdown in human EOC cells.

Discussion

Herein, we showed that SUZ12 is expressed at significantly higher levels in human EOCs compared with either normal human ovarian surface epithelium or fallopian tube epithelium. SUZ12 is located at 17q21 and gene amplification has been shown to contribute to SUZ12 upregulation in a number of cancer types (20). However, based on the newly released TCGA ovarian cancer database (34), SUZ12 gene amplification is very rare in human ovarian cancer (<1% cases show >3 copy of SUZ12 gene; http://cancergenome.nih.gov/), suggesting that gene amplification is not a major mechanism that underlies SUZ12 upregulation in human EOCs. Recently, microRNA has also been
implicated in regulating the expression of SUZ12 (35). Nevertheless, future studies are warranted to elucidate the mechanisms by which SUZ12 is upregulated in human EOCs.

We showed that SUZ12 expression positively correlates with expression of EZH2 in human EOCs (Fig. 1B and Table 1). However, we cannot rule out the possibility that SUZ12 may also have additional EZH2-independent functions in EOC cells. Interestingly, it has previously been reported that H3K27Me3 levels do not correlate with the EZH2 levels in human EOC specimens (36). It is possible that additional factors such as H3K27Me3 demethylase JMJD3 and UTX may also regulate H3K27Me3 levels in EOC cells (37). In addition, SUZ12 expression correlates with expression of a cell proliferation marker, Ki67 (Fig. 1B and Table 1). EZH2 has been shown as a poor prognosis marker in a number of cancer types (38). Similarly, we found that a higher level of SUZ12 expression positively correlates with a poor overall survival in human EOC patients. Collectively, PRC2 could serve as independent prognosis biomarkers for EOCs.

The proapoptotic HRK gene is located at 12q13.1 and is transcriptionally regulated in several cancer cell types (29, 39, 40). Interestingly, we discovered that HRK is a novel target of SUZ12-mediated gene silencing via H3K27Me3, and showed that its upregulation contributes to apoptosis induced by SUZ12 knockdown in human EOC cells (Fig. 6). Notably, SUZ12 knockdown severely diminished the H3K27Me3 level (Fig. 2A). Thus, inhibition of SUZ12 by shRNA might affect pathways in addition to HRK in these cells. Consistently, it has been shown that SUZ12 also regulates the expression of genes implicated in cell-cycle progression and DNA damage and repair (20). In the future, it will be interesting to investigate additional SUZ12 target genes that contribute to growth inhibition observed in SUZ12 knockdown EOC cells.

We showed that knockdown of SUZ12 is sufficient to decrease the levels of H3K27Me3 in human EOC cells (Fig. 2). This is consistent with the premise that SUZ12 is required for PRC2 complex–mediated gene silencing via H3K27Me3 epigenetic modification. Indeed, SUZ12 expression positively correlates with the expression of EZH2 (Fig. 1B and Table 1). These results suggest that the interaction between SUZ12 and EZH2 may be an alternative target for inactivating PRC2 in addition to the methyltransferase activity of EZH2. Consistently with this idea,
phosphorylation of EZH2 by CDK1 kinase at Thr487 is thought to decrease H3K27Me3 levels by disrupting the interaction between SUZ12 and EZH2 (41).

In summary, the data reported here show that SUZ12 is often overexpressed in human EOCs and its expression positively correlates with EZH2, a high proliferation index, and a poor overall survival in EOC patients. Knockdown of SUZ12 suppresses the growth of human EOC cells in vitro and in vivo in both orthotopic and s.c xenograft EOC models. Indeed, knockdown of SUZ12 triggers apoptosis of human EOC cells. Furthermore, these observed effects correlate with a decrease in H3K27Me3 levels, suggesting that SUZ12 functions via PRC2 to suppress apoptosis of human EOC cells. Mechanistically, we identified the pro-apoptotic HRK gene as a novel SUZ12 target gene whose upregulation contributes to apoptosis induced by SUZ12 knockdown in human EOC cells.

Disclosure of Potential Conflict of Interest
No potential conflicts of interest were disclosed.

References


Authors’ Contributions
Conception and design: H. Li, R. Zhang
Development of methodology: H. Li, Q. Cai, X. Hua, C.N. Landen
Acquisition of data (provided animals, collected and managed patients, provided facilities, etc.): Q. Cai, H. Wu, V. Vathipadiekal, Z.C. Dobbin, X. Hua, C.N. Landen, M.J. Birrer
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): H. Li, Q. Cai, H. Wu, V. Vathipadiekal, T. Li, C.N. Landen, M.J. Birrer
Writing, review, and/or revision of the manuscript: H. Li, M.J. Birrer, R. Zhang
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): X. Hua, C.N. Landen
Study supervision: R. Zhang
Contribute Fundamental materials for research: M. Sanchez-Beato

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