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Valproic Acid Promotes Apoptosis and Cisplatin Sensitivity Through Downregulation of H19 Noncoding RNA in Ovarian A2780 Cells

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Abstract Cisplatin resistance is one of the main limitations in the treatment of ovarian cancer, which is partly mediated by long noncoding RNAs (lncRNAs). H19 is a lncRNA involving in cisplatin resistance in cancers. Valproic acid (VPA) is a commonly used drug for clinical treatment of seizure disorders. In addition, this drug may display its effects through regulation of noncoding RNAs controlling gene expression. The aim of the present study was the investigation of VPA treatment effect on H19 expression in ovarian cancer cells and also the relation of the H19 levels with apoptosis and cisplatin resistance. Briefly, treatment with VPA not only led to significant increase in apoptosis rate, but also increased the cisplatin sensitivity of A2780/CP cells. We found that following VPA treatment, the expression of H19 and EZH2 decreased, but the expression of p21 and PTEN increased significantly. To investigate the involvement of H19 in VPA-induced apoptosis and cisplatin sensitivity, H19 was inhibited by a specific siRNA. Our results demonstrate that H19 knockdown by siRNA induced apoptosis and sensitized the A2780/CP cells to cisplatin-induced cytotoxicity. These data indicated that VPA negatively regulates the expression of H19 in ovarian cancer cells, which subsequently leads to apoptosis induction, cell proliferation inhibition, and overwhelming to cisplatin resistance. The implication of H19→EZH2→p21/PTEN pathway by VPA treatment suggests

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that we could repurpose an old drug, valproic acid, as an effective drug for treatment of ovarian cancer in the future.

Keywords Cisplatin · EZH2 · H19 · Ovarian cancer · Valproic acid

Introduction

Ovarian cancer is the most lethal female reproductive system cancer, which accounts for about 3% of all cancers in women [1]. Platinum-based chemotherapy using cisplatin is considered as a primary treatment for advanced ovarian cancer; however, the high incidence of chemoresistance in cancer cells is a main clinical hurdle to achieve a successful therapy [2, 3]. Cisplatin resistance in ovarian cancer is a complicate process regulated by different molecular mechanisms [4]. Therefore, the identification of new therapeutic agents to overcome cisplatin resistance is important.

An increasing number of studies have reported that long noncoding RNAs (lncRNAs) play important roles in cancer chemoresistance [5]. H19 is a well-known lncRNA possessing critical functions in cancer development, progression, and metastasis. Although H19 was initially tumor suppressor [6], but its overexpression in several cancers supports its oncogenic role [6]. More recently, it has been shown that H19 is linked with doxorubicin resistance in breast and liver cancers. Moreover, H19 upregulation is associated with cisplatin resistance in ovarian cancer [7, 8].

H19 indirectly regulates several genes through interaction with chromatin-modifying complexes. For instance, H19 with EZH2 (an active component of polycomb repressive complex 2) suppresses the expression of a select group of genes [9]. A recent study has unveiled the regulatory function of H19 on EZH2 in nasopharyngeal carcinoma [10]. Moreover, EZH2 overexpression is associated with development and progression of tumors as well as inhibition of apoptosis and development of drug-resistance in most of cancers. EZH2 also regulates these effects through silencing of tumor suppressors and activation of cell proliferation-related genes [11]. For example, EZH2-mediated cell cycle transition from G1- to S-phase is associated with decrease of p21 expression [12]. Moreover, EZH2 binding to the promoter region of PTEN leads to silencing and activation of AKT [13]. These data collectively introduce H19 and EZH2 as two potential therapeutic targets in cancers to overwhelm drug resistance, induce apoptosis, and control cell proliferation.

Anticancer effects of a number of histone deacetylase inhibitors, such as valproic acid (VPA), have been approved in culture and animal studies. VPA is a branched short-chain fatty acid, which is commonly used for treatment of seizure disorders [14]. Previous study showed that VPA enhances the sensitivity of cancer cells to chemotherapeutic agents [15]. A most recent published report indicated that the clinical consensus of VPA can be mediated by alteration of noncoding RNA expression pattern [16]. This led us to investigate whether VPA treatment of ovarian cancer cells affects H19-expression, which in turn might influence the level of H19 target genes involved in apoptosis and cisplatin resistance.

Materials and Methods

Cell Lines and Cell Culture

Human epithelial ovarian cancer cells (A2780 S and A2780/CP) were purchased from the Pasteur Institute of Iran (NCBI, C461, and C454). The cells were cultured in RPMI-1640

medium supplemented with 10% FBS, penicillin (100 IU/mL), and streptomycin (100 μg/mL) at 37 °C under 5% CO_2 atmosphere.

MTT Assay

Cell viability was evaluated by MTT assay. A2780 S and A2780/CP cells were seeded in a 96-well plate at a density of 4×10^3 cells/well. After 24 h, the culture medium was discarded and the cells were exposed to medium containing cisplatin (0.1 to 5.75 μg/mL) or VPA (5 to 1250 μg/mL) or their combination for 48 h. For control, some wells were cultured in medium without the addition of cisplatin and/or VPA. After discarding the media and washing with PBS, 100 μL of phenol red free-RPMI-1640 was mixed with 10 μL of MTT solution (5 mg/mL, Sigma-Aldrich) added to each well and incubated for another 4 h. The media were removed and 150 μL/well of DMSO was added to dissolve the formazan crystal and the plates were rapidly shaken for at least10 min. The absorbance was measured at 570 nm wavelength by an ELISA reader (Bio-Rad). The percentage of cell viability at each dose of drugs was calculated as A570 (drug-treated cells)/A570 (untreated cells) [17, 18]. The results of combined treatment of cisplatin and VPA were analyzed according to Zheng-Jun Jin method [19]. This method provides a q value, obtaining by the following formula: $q = EA + B/[(EA + EB) - (EA \times EB)]$, where EA + B, EA and EB indicate the average effects of combined treatment, cisplatin and VPA, respectively. Then $q \leq 0.85$ represents antagonism, $0.85 \leq q < 1.15$ represents additive, and $q \geq 1.15$ represents synergism. All assays were performed 3–4 times and the results were given as means ± SD.

Flow Cytometry

The Annexin-V/FITC apoptosis detection kit was used to detect the apoptotic rate, according to the manufacturer's instructions (cat. no. 556547, BD Biosciences). Briefly, 1×10^5 A2780 cells/well were plated on 6-well plates and then treated with cisplatin (1.4 μg/mL for A2780 S and 2.8 μg/mL for A2780/CP), VPA (300 μg/mL for A2780 S and 100 μg/mL for A2780/CP), and their combination (the concentrations of 1.4 μg/mL cisplatin and 300 μg/mL VPA for A2780 S cells and 2.8 μg/mL cisplatin and 100 μg/mL VPA for A2780/CP cells) for 48 h. After that, the cells were harvested, centrifuged, and washed twice with PBS and stained with Annexin-V/FITC and Propidium iodide (PI) for 15 min at room temperature in the dark. The percentage of apoptotic cells was determined using a flow cytometer (BD Biosciences).

RNA Extraction, cDNA Synthesis, and RT-PCR

The A2780 cells at the density of 1×10^5 cells/well were seeded in 6-well plates for 24 h and cisplatin (1.4 μg/mL for A2780 S and 2.8 μg/mL for A2780/CP), VPA (300 μg/mL for A2780 S and 100 μg/mL for A2780/CP), or their combination (the concentrations of 1.4 μg/mL cisplatin and 300 μg/mL VPA for A2780 S cells and 2.8 μg/mL cisplatin and 100 μg/mL VPA for A2780/CP cells) were added into medium and incubated for 48 h. Total RNA was isolated from the ovarian cancer cells using Trizol and quantified at 260 nm by a NanoDrop spectrometer (Thermo Scientific). For reverse transcription, 1 μg of total RNA was converted to cDNA using a PrimeScript™ RT Reagent Kit (cat. no. RR037A, Takara) according to the manufacturer's instruction. The thermal program was set as 95 °C for 30 min, 85 °C for 5 s, and 4 °C for 10 min. Real-time PCR was conducted using a SYBR®Green one-step RNA

PCR kit (cat. no. RR066A,Takara) in a Rotor-Gene 6000 instrument (Corbett Life Science) as follows: 95 °C for 10 min, 30 cycles of 95 °C for 15 s, 60 °C for 30 s, and 72 °C for 30 s. GAPDH was used as a reference gene. The results were analyzed using $2^{-\Delta\Delta Ct}$ method. The PCR primers were designed by Oligo 7 software (Molecular Biology Insights Inc.) and the sequences are shown in Table 1.

Western Blotting

A2780 cells were treated with cisplatin, VPA, or their combination (the concentrations were mentioned in the previous section) for 48 h. The expression of p21, PTEN, and EZH2 proteins was validated by Western blotting. Briefly, total proteins were extracted from cultured cells using cold RIPA buffer. The proteins were separated on 10% SDS–polyacrylamide gels, transferred onto PVDF membranes and blocked with 5% skim milk. Then, membranes were incubated with primary antibody at the dilution of 1:5000 for p21 (cat. no. ab96137) and 1:10,000 for EZH2 (cat. no. ab191080) and PTEN (cat. no. ab109454) overnight and secondary antibody at the dilution of 1:5000 (cat. no. ab6721) for 1 h. Chemiluminescence substrate (Sigma-Aldrich) was used to detect the desired bands. β-actin (cat. no. ab119716) was used as a control. All antibodies were purchased from Abcam.

SiRNA Transfection

A2780/CP cells seeded in 6-well plates were allowed to grow to 70–80% confluent within 2–3 days. Cells were transfected with H19 siRNA (si-H19) or scrambled (control) (Life Technologies) using lipofectamine 2000 (Invitrogen) according to manufacturers' guidelines. After 24 h, the media were replaced with RPMI-1640 containing 10% FBS. The cells were collected for further analyses 2 days later.

Statistical Analysis

All statistical tests were implemented by GraphPad Prism statistical software, version 5.01 (GraphPad, USA). Each experiment was performed at least in triplicate. All data were presented as mean ± standard deviation (SD). Real-time PCR data analysis was performed using the ΔΔCT method in Microsoft office excel 2007 software, and final data were normalized by GAPDH expression level as an endogenous control. The gene expression data

Table 1 Real-time PCR primers used in this study

Gene	Sequence (5′ to 3′)	PCR size (bp)	Accession no.
H19	TGCTGCACTTTACAACCACTG ATGGTGTCTTTGATGTTGGGC	182	NM001293171
p21	GGAGACTCTCAGGGTCGAAA GGATTAGGGCTTCCTCTTGG	96	NM001291549
EZH2	TTGTTGGCGGAAGCGTGTAAAATC TCCCTAGTCCCGCGCAATGAGC	207	XM011515901
PTEN	ACACGACGGGAAGACAAGTT CTGGTCCTGGTATGAAGAATG	157	NM000314
GAPDH	ACGGATTTGGTCGTATTGGG TGATTTTGGAGGGATCTCGC	231	NM001289746

obtained by real-time PCR was statistically analyzed. One-way ANOVA test was also applied for statistical analysis of apoptotic values obtained by flow cytometry. $p < 0.05$ value was considered the level of statistical significance.

Results

Cisplatin and VPA Combined Treatment Reduces Cell Viability

A2780 cells were exposed to increasing concentrations of cisplatin (0.1 to 5.75 μg/mL) for 48 h. As shown in Fig. 1a, b, the IC_{50} of cisplatin to be 1.4 μg/mL for A2780 S and 2.8 μg/mL for A2780/CP cells. VPA effect on cell viability was also assessed, and the results showed a dose-dependent decrease in cell viability suggested that A2780/CP cells were more sensitive to VPA treatment ($IC_{50} = 400$ μg/mL) than A2780 S cells ($IC_{50} = 650$ μg/mL).

The effect of cisplatin plus VPA on cell viability was investigated by exposure of A2780 cells to different concentrations VPA (5–400 μg/mL) and/or sub-cytotoxic cisplatin (0.1–

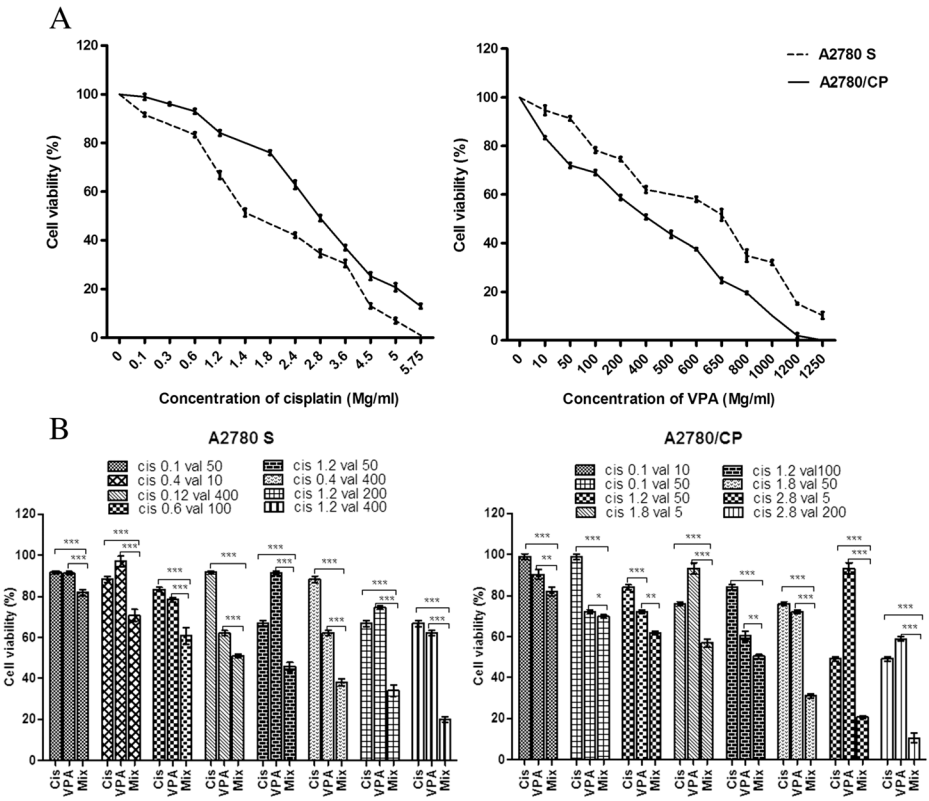

Fig. 1 Combination therapy of cisplatin and VPA inhibited ovarian cancer cell growth. A2780 cells were treated with the different concentrations of VPA, cisplatin (**a**), and their combination (**b**); the cell viability was determined using MTT. Combination treatment showed a more lethal effect on A2780 cells compared to the each agent alone. Data represent the mean and the standard deviation from three independent experiments (*$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$)

Table 2 IR (%) of cisplatin (μg/mL) combined with VPA (μg/mL) in A2780 S cells

VPA	IR	0.1	*q*	0.4	*q*	1.2	*q*
0	–	8.44 + 0.8	–	16.63 + 1.0	–	38.22 + 1.52	–
10	5.31 + 0.8	14.7 + 0.29	1.10[a]	29.24 + 0.8	1.39[b]	46.29 + 1.98	1.12[b]
50	8.7 + 0.89	18.24 + 0.13	1.112[a]	36.54 + 1.2	1.53[b]	54.23 + 0.96	1.24[b]
200	25.36 + 0.88	35.25 + 1.02	1.113[a]	44.2 + 0.85	1.16[b]	74.98 + 0.54	1.39[b]
400	37.94 + 1/36	46.15 + 0.87	1.06[a]	61.94 + 1.23	1.29[b]	82.94 + 0.2	1.34[b]

Antagonistic, additive, or synergistic effects were indicated when $q < 0.85$, $0.85 \le q \le 1.15$, or $q > 1.15$, respectively

IR inhibitive rate of cell growth

[a] Representative of additive effect

[b] Representative of synergistic effect

2.8 μg/mL), and the results showed more significant reduction of cell viability in combination therapy than monotherapy. This result suggested the additive and synergistic effects of cisplatin and VPA in combination therapy. Co-treatment resulted in decrease of mean IC_{50} values of cisplatin and VPA to 0.12 and 400 μg/mL for A2780 S cells and 1.2 and 100 μg/mL for A2780/CP cells, respectively (Fig. 1a, b). The synergistic effect in combination treatment group occurred until sub-toxic dose of cisplatin reached to 0.4 and 1.2 μg/mL in A2780 S and A2780/CP cells, respectively (Tables 2 and 3).

Cisplatin and VPA Promote Apoptosis

Apoptosis rate induced by VPA and/or cisplatin was determined by Annexin-V/PI staining. As shown in Fig. 2, treatment with VPA or cisplatin markedly increased the number of apoptotic cells. After treatment with cisplatin, VPA, and both components, the early apoptosis rate was 28.34, 6.44, and 17.93% in A2780 S cells. The ratio of late apoptotic cells was significantly increased in the co-treated A2780 S cells (38.82%) compared with cisplatin (14.88%) or VPA (7.36%) treatment alone ($p < 0.001$). The percentage of Annexin V^+/PI^- cells (early apoptosis) after treatment with cisplatin, VPA, and their combination was 31.2, 3.43, and 5.01% in A2780/CP, respectively. In addition, VPA efficiently increased cisplatin-induced late apoptosis in A2780/CP cells (23.59% for cisplatin, 20.85% for VPA, and 80.33% for cisplatin and VPA treated cells) ($p < 0.001$).

Table 3 IR (%) of cisplatin (μg/mL) combined with VPA (μg/mL) in A2780/CP cells

VPA	IR	0.1	*q*	1.2	*q*	1.8	*q*
0	–	1.04 + 0.24	–	15.86 + 0.14	–	24.1 + 1.0	–
5	8.7 + 0.54	10.7 + 0.29	1.11[a]	30.24 + 0.8	1.3[b]	44.29 + 1.98	1.47[b]
10	16.58 + 0.42	17.87 + 0.13	1.025[a]	36.54 + 1.2	1.24[b]	55.77 + 0.96	1.41[b]
50	28.1 + 1	30.25 + 0.89	1.05[a]	38.2 + 0.85	1.17[b]	68.98 + 1.14	1.51[b]
200	41.2 + 1	48.15 + 0.24	1.14[a]	65.24 + 0.87	1.3[b]	78.24 + 0.2	1.42[b]

Antagonistic, additive, or synergistic effects were indicated when $q < 0.85$, $0.85 \le q \le 1.15$, or $q > 1.15$, respectively

IR inhibitive rate of cell growth

[a] Representative of additive effect

[b] Representative of synergistic effect

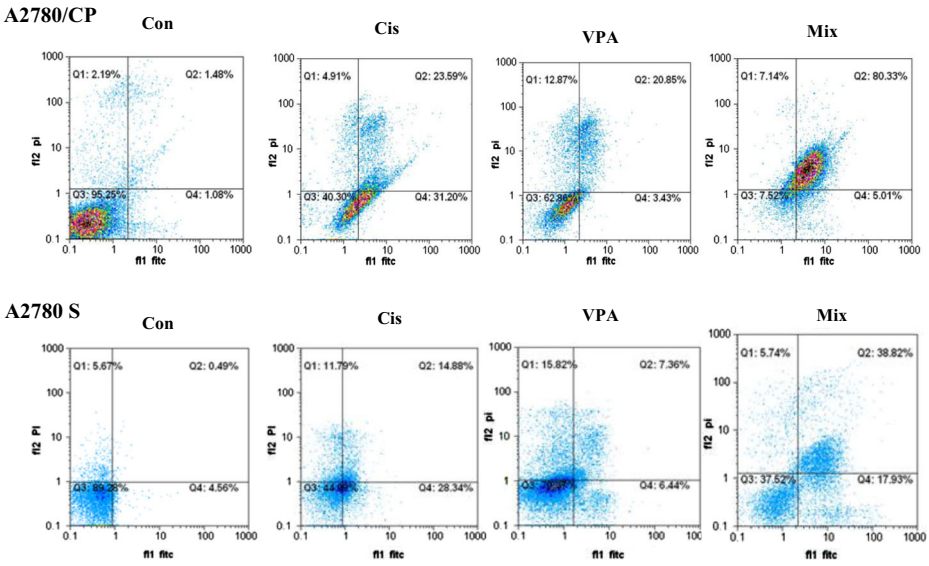

Fig. 2 VPA promoted cisplatin-mediated apoptosis in ovarian cancer cells. A2780 cells were treated with cisplatin (1.4 μg/mL for A2780 S and 2.8 μg/mL for A2780/CP) and/or VPA (300 μg/mL for A2780 S and 100 μg/mL for A2780/CP), and apoptosis rate was measured by Annexin-V/PI double staining. Annexin V^+/PI^- cells (the lower right quadrant, Q3) and Annexin V^+/PI^+ cells (the upper right quadrant, Q2) were considered to be in the early and late stages of apoptosis, respectively. Annexin V^-/PI^+ cells (the upper left quadrant, Q1) were necrotic. The total percentage of apoptotic cells (Q2 + Q3.) in the combination treatment was 85.34% for A2780/CP and 56.75% for A2780 S cells, which was much greater than cisplatin (54.79% for A2780/CP cell and 43.22% for A2780 S cell) or VPA (23.59% for A2780 CP cell and 13.79% for A2780 S cell) treatment alone (***$p < 0.001$). In the co-treatment group, the number of late apoptotic cells increased dramatically as compared with other groups (****$p < 0.001$)

Alteration of Proliferation and Apoptosis Gene Expression Due to Cisplatin and VPA

Since H19 lncRNA is related to cisplatin resistance in cancers, the effect of VPA on expression levels of H19 and its target genes were examined. As shown in Fig. 3a, H19 level significantly increased in A2780/CP cells as compared with the parental A2780 cell ($p < 0.01$). Real-time PCR confirmed that VPA resulted in downregulation of H19 and EZH2 in A2780 cells ($p < 0.001$ and $p < 0.05$, Fig. 3a). Interestingly, more decrease in expression level of H19 was observed in the combination therapy compared to applying each drug separately. Concordantly, EZH2 expression level decreased further in the cells treated with the both drugs ($p < 0.01$, Fig. 3a). However, a significant increase in mRNA level of p21 and PTEN was observed in the cells treated with VPA. Combination treatment had a much greater effect on the expression of p21 and PTEN and increased them respectively to 270.59-fold ($p < 0.0001$) and 8.69-fold ($p < 0.05$) in A2780/CP cells.

Cisplatin and VPA Enhance Tumor Suppressor Protein Expression

We investigated the expression levels of p21, PTEN, and EZH2 using Western blotting. As shown in Fig. 3b, cisplatin and VPA separately had significant effects on the enhancement of p21 and PTEN in both cell lines. Interestingly, more expression level of these proteins was observed when the mixture of cisplatin and VPA was used. Furthermore, cisplatin treatment increased the levels of EZH2. Conversely, VPA or the combination of VPA and cisplatin reduced EZH2 expression (Fig. 3b).

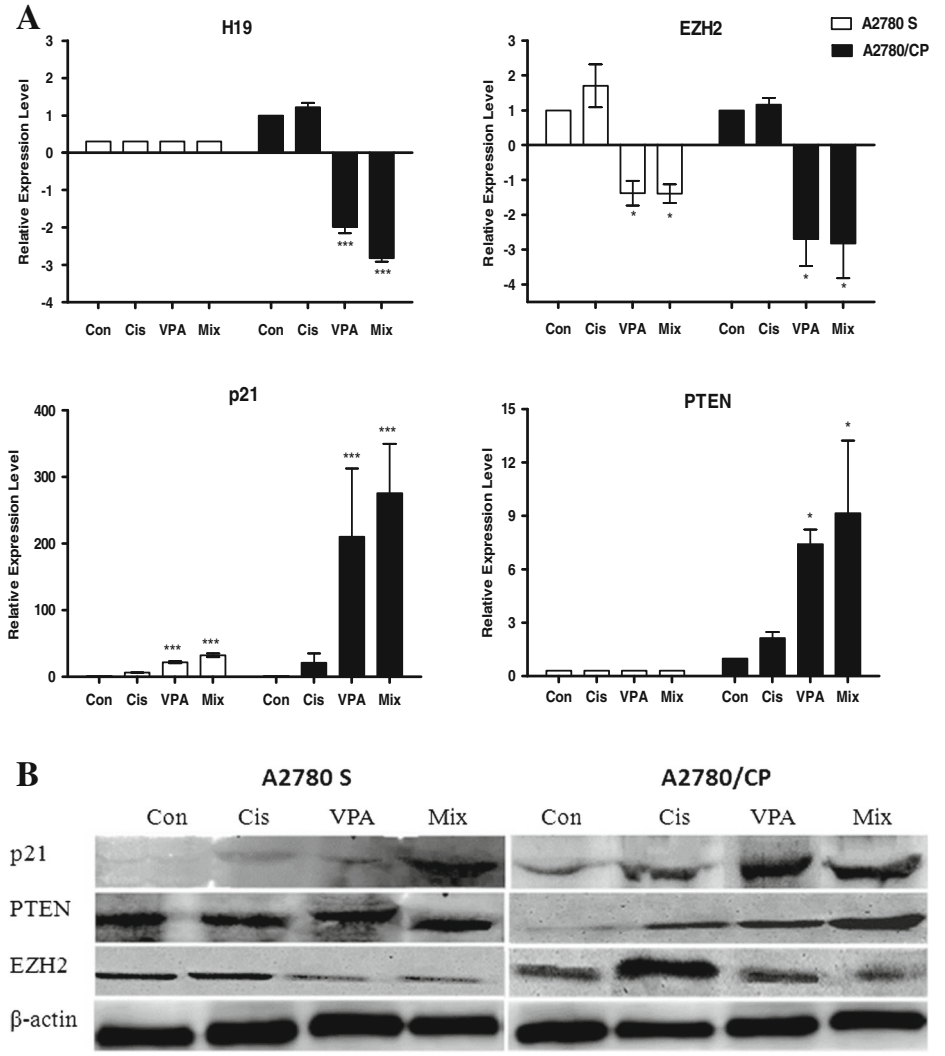

Fig. 3 VPA in combination with cisplatin regulates the expression of H19, EZH2, p21, and PTEN. **a** Real-time-PCR showed that VPA alone or in combination with cisplatin decreases the expression of H19 and EZH2, which are normally upregulated in cisplatin resistance cells. Significant upregulation of p21 and PTEN genes were observed in A2780 cells following combination treatment compared to untreated cells. Data represent the mean and the standard deviation of three independent experiments. $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$ versus untreated cells (ANOVA). **b** A2780 cells were cultured with cisplatin and/or VPA and subjected to Western blotting. Combination treatment more strongly upregulated p21 and PTEN expression compared to control group. β-actin was used as the loading control

Blocking H19 Promotes Apoptosis, Cell Mortality, and Drug Sensitivity

To investigate whether the VPA effects on induction of apoptosis and reduction of cisplatin resistance is mediated through blocking H19, the impact of H19 knockdown on A2780 cells was studied. Lipofectamine-mediated transfection of si-H19 into A2780 cells induced apoptosis up to 14.3%. Apoptosis upregulated to 79.99% in

H19-knockdown cells when cisplatin was added to the culture (Fig. 4a). Monotherapy using cisplatin induced apoptosis to 54.79% (Fig. 2). A2780/CP cells were treated with increasing concentrations of cisplatin after being transfected with si-H19 or scrambled oligonucleotide. Compared with the negative control, H19 downregulation markedly increased the sensitivity to cisplatin in A2780/CP cells in a dose-dependent

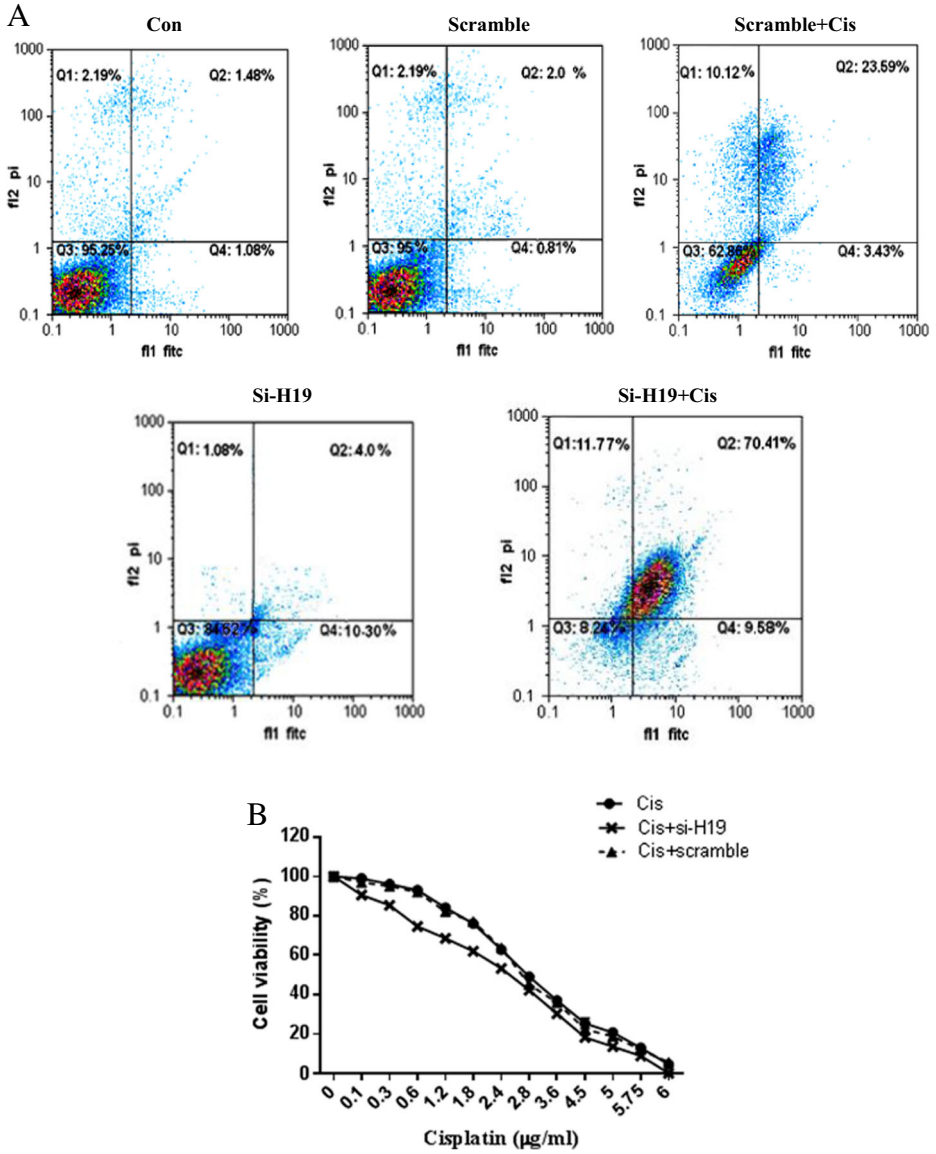

Fig. 4 H19 knockdown increased apoptosis, cell death, and cisplatin sensitivity. **a** Si-H19 transfected A2780/CP cells were harvested, and apoptosis was measured by Annexin-V/PI staining. H19 knockdown notably increased rate of apoptotic cells in cisplatin-treated samples ($p < 0.0001$, ANOVA). **b** Cisplatin sensitivity in H19-suppressed cells. Si-H19 transfection into A2780/CP cells showed a significant reduction of cell viability due to cisplatin in compare to untransfected and scramble groups ($p < 0.05$, ANOVA)

manner. IC_{50} was evaluated as 2.43 μg/mL for H19 downregulated group and 2.8 μg/mL for the control. These results suggested that inhibition of H19 makes the A2780/CP cells sensitive to cisplatin ($p < 0.05$; Fig. 4b).

H19 Inhibition Increases Apoptosis by Influencing EZH2, p21, and PTEN

H19 promotes cisplatin resistance via several potential target genes, such as EZH2. We evaluated whether growth inhibition and increased rate of apoptosis upon H19 depletion in A2780/CP cells was associated with EZH2 and its target genes, p21 and PTEN. Western blot results in line with real-time PCR data showed that p21 and PTEN protein levels increased in si-H19 transfected cells. In contrast, EZH2 protein decreased (Fig. 5b).

Discussion

LncRNAs are essential molecules regulating biological processes and diseases, in particular, cancers. Accumulating evidences have demonstrated that changes in specific lncRNAs levels cause development of cisplatin resistance in cancers [5, 20]. Among these, H19 was identified as one of the most important lncRNAs involved in proliferation, invasion, metastasis, and chemoresistance of tumors [7–10].

Oncogenic properties of H19 have been reported in recent years. Yang et al. in 2012 reported that H19 decreases apoptosis in gastric cancer through targeting p53 [21]. H19 overexpression negatively regulates EZH2 and E-cadherin and conversely increases cell invasion in nasopharyngeal carcinoma [10]. Li et al. in 2014 reported that H19 upregulation resulted in metastasis and tumorigenesis in gastric cancer [22]. Upregulated H19 alters responses to conventional chemotherapy. Association of H19 with cell proliferation and temozolomide resistance in glioma cells has been demonstrated [23]. In addition, H19 causes acquisition of doxorubicin resistance through targeting MDR1 gene in hepatocellular carcinoma and cisplatin resistance in A549 cells [7, 24]. Enhancing the sensitivity of lung adenocarcinoma cells to cisplatin by downregulation of H19 might be associated with induction of G0/G1 cell-cycle arrest and apoptosis [24]. Zheng et al. in 2016 indicated that H19 suppression induced sensitivity to the antitumor effect of cisplatin in human ovarian cancer cells [2]. These studies confirm that H19 can be a novel target to overcome drug resistance in cancers. In consistent with these, our results indicated higher expression of H19 in the cisplatin-resistant ovarian cancer cells (A2780/CP) than that of the cisplatin-sensitive cells. Moreover, treatment with cisplatin increased the expression of H19 significantly showing the involvement of H19 in response to cisplatin. Collectively, these data emphasize on the oncogenic function of H19 and also its critical role in cisplatin resistance in ovarian cancer.

The use of known drugs, such as VPA, is a strategy to overcome drug resistance in cancers. VPA has been found to improve the effect of chemotherapeutic agents. VPA has synergistic cytotoxicity with cisplatin on larynx, HNSCC, ovarian and melanoma cancer cells in vitro [15, 25–27]. In consistent with these results, our results demonstrated that the combination of VPA with cisplatin enhanced cisplatin-mediated cytotoxicity, apoptosis, and ovarian cancer cell sensitivity to cisplatin.

Although there are several reports confirming the regulatory effects of VPA on microRNAs [16, 28, 29], a recently published study found out that H19 expression can be suppressed by

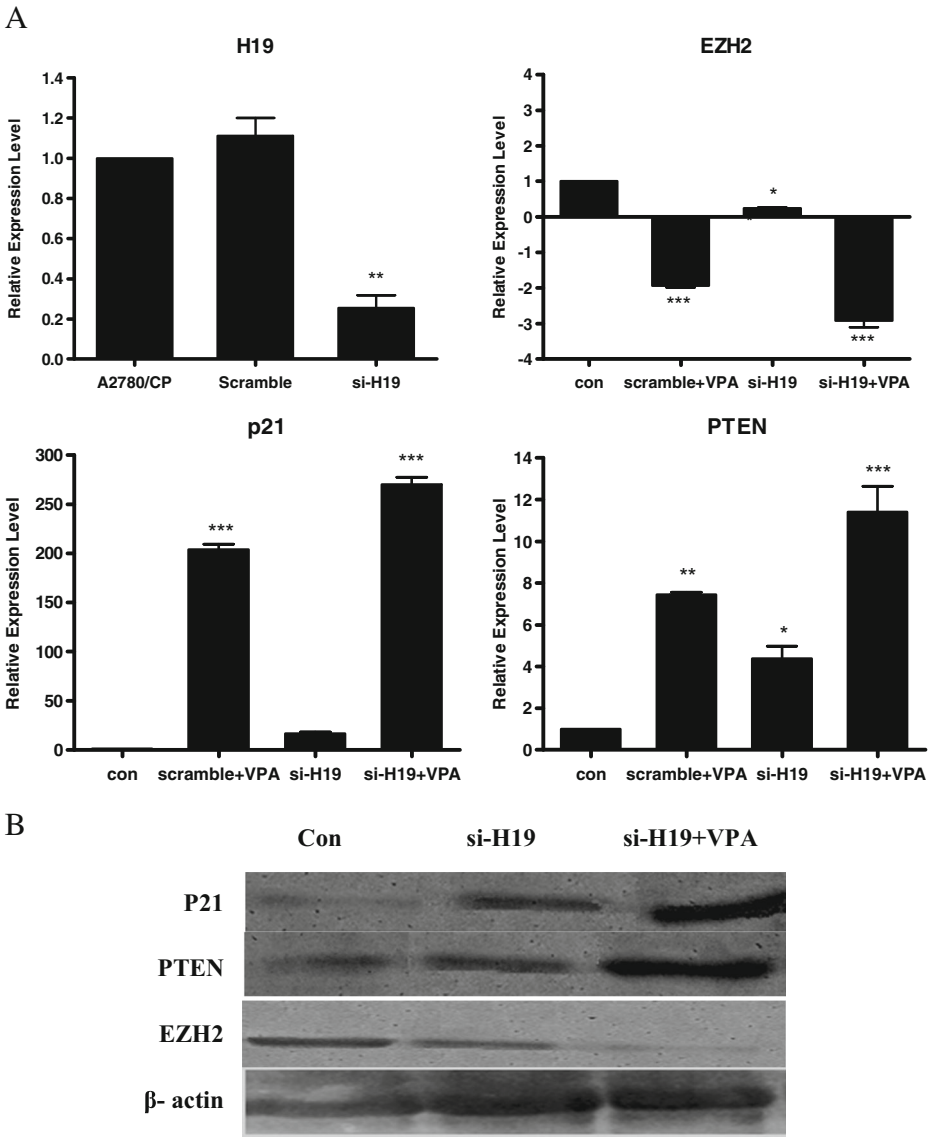

Fig. 5 H19 inhibition regulates EZH2, p21, and PTEN. Si-H19 transfected A2780/CP cells were treated with cisplatin. (**a**) Real-time PCR of H19 knockdown cells revealed a significant reduction of EZH2 mRNA, while p21 and PTEN transcripts were upregulated. **b** Western blot demonstrated that treatment of H19-suppressed A2780/CP cells with VPA significantly downregulated EZH2 and upregulated p21 and PTEN. Data represent the mean and the standard deviation of three independent experiments. $*p < 0.05$ and $***p < 0.001$ versus untreated cells (ANOVA)

VPA in A549 cells [30]. In line with this study, our results demonstrated that VPA treatment significantly decreased the expression of H19 in cisplatin-resistant ovarian cancer cell line. Moreover, VPA-mediated downregulation of H19 induces apoptosis in A2780/CP cells and more induction of apoptosis is achieved by mixture of VPA with cisplatin. This result

suggested the additive and synergistic effects of cisplatin and VPA in combination therapy. These are consistence with previous reports, which indicated that H19 downregulation promotes apoptosis in gastric and ovarian cancer cells [2, 20].

H19 positively regulates the expression of EZH2 [10]. EZH2 reduction induces apoptosis and sensitivity to cisplatin via upregulation of p21 in human nonsmall cell lung and gastric cancers [31]. Chen et al's study demonstrated that the increased level of EZH2 led to silencing of p53, p21, and PTEN, while EZH2 inhibition resulted in cell-cycle arrest and apoptosis induction in lymphoblastic leukemia [32].

In the present study, we provided a further evidence that H19 downregulation led to apoptosis and enhanced sensitivity to cisplatin in A2780 cells through downregulation of EZH2 and its target genes, p21 and PTEN. Taken together, our results confirmed that VPA contributes to increased cisplatin toxicity in A2780/CP cells through H19/EZH2/p21/PTEN pathway.

Conclusion

To the best of our knowledge, this is the first study indicating the relationship of H19 with VPA-induced apoptosis and overwhelming to cisplatin resistance in ovarian cancer cells. Our results in line with other studies emphasized on the pivotal function of H19 on cell proliferation, escape apoptosis, and chemoresistance. VPA epigenetically downregulate H19 and could drive the cells to apoptosis, growth inhibition, and cisplatin sensitivity. These results indicated that H19/EZH2/PTEN and p21 pathways might be used as novel therapeutic targets to overcome cisplatin resistance in ovarian cancer and purposes VPA as an effective drug to achieve this goal in the future.

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Compliance with Ethical Standards

Competing Interests The authors declare that they have no competing interests.

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