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RESEARCH ARTICLE

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Lidocine potentiates the cytotoxicity of 5-fluorouracil to choriocarcinoma cells by downregulating ABC transport proteins expression

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Abstract

Choriocarcinoma is a gestational trophoblastic cancer, which often occurs in the first 3 months of pregnancy. 5-Fluorouracil (5-Fu) is the widely used chemotherapeutic drug for choriocarcinoma but limited by drug resistance. Lidocaine, an aminamide-type anesthetic, shows potential anticancer and chemosensitization effects in recent years. Herein, we tested the possible chemosensitization activity of lidocaine on the cytotoxicity of 5-Fu in choriocarcinoma cells. Viabilities and apoptosis of choriocarcinoma JEG-3 and JAR cells after lidocaine and/or 5-Fu treatment were detected using Cell Counting Kit-8 assay, annexin V-FITC/PI (fluorescein isothiocyanate/propidium iodide) staining and Western blot analysis, respectively. Quantitative reverse transcription polymerase chain reaction was done to measure breast cancer resistance protein (ABCG2) messenger RNA level. Western blot analysis was carried out to detect ABCG2, P-glycoprotein (P-gp), MRP1, and MRP2 protein levels. pEX-ABCG2 was transfected to elevate ABCG2 level. Then, the influence of ABCG2 on lidocaine + 5-Fu-caused cell viability loss, apoptosis, and inactivation of PI3K/AKT pathway were analyzed. We found that lidocaine in low concentration had no significant cytotoxicity to JEG-3 and JAR cells, but stimulated cell apoptosis in high concentration. Moreover, lidocaine potentiated the cytotoxicity of 5-Fu to JEG-3 and JAR cells through decreasing viability and increasing apoptosis. Lidocaine treatment reduced the ABCG2, P-gp, MRP1, and MRP2 protein levels in cells. Overexpression of ABCG2 reversed the synergistic effects of lidocaine + 5-Fu on JEG-3 and JAR cell viability and apoptosis, as well as PI3K/AKT pathway. Our research verified that lidocaine potentiated the cytotoxicity of 5-Fu to choriocarcinoma cells by downregulating ATP-binding cassette (ABC) transport proteins expression.

KEYWORDS

5-fluorouracil, breast cancer resistance protein, choriocarcinoma, lidocaine, PI3K/AKT signaling pathway

1 | INTRODUCTION

Choriocarcinoma is a type of malignant gestational trophoblastic cancer, which originated from the villous trophoblastic hydatidiform mole and often happened in the first 3 months of pregnancy.[1,2] Despite the fact that choriocarcinoma has a high sensitivity to chemotherapy than other cancers, there are still lots of peoples who show inadequate reaction to conventional chemotherapy.[1] The occurrence of drug resistance is the main reason for the failure of chemotherapy.[3] More efficient therapeutic medicines and strategies are still needed.

5-Fluorouracil (5-Fu) is one of the most widely used chemotherapeutic drugs for choriocarcinoma.[4,5] As an analog of uracil, 5-Fu is converted to flurodeoxyuridine monophosphate (FdUMP) by a similar metabolic pathway of uracil in choriocarcinoma cells.[6] Then FdUMP inhibits choriocarcinoma cell DNA synthesis, causes cell injury and further induces cell apoptosis.[7] ATP-binding cassette (ABC) transport proteins, such as P-glycoprotein (P-gp), multidrug resistance protein (MRP) and breast cancer resistance protein (ABCG2), can transport chemotherapy drugs, including 5-Fu, out of tumor cells to decrease the cytotoxicity of drugs.[8,9] Therefore, combination therapy of multicomponents (rather than single-agent) is recommended for various cancer treatment, including choriocarcinoma therapy, in recent years, which can decrease the occurrence of drug resistance.[10,11] For example, Bailey et al[12] reported that ABCG2 had a high endogenous expression in choriocarcinoma JEG-3, JAR, and BeWo cells. Blazquez et al[13] proved that acetaminophen, the first-choice drug for pain relief during pregnancy, could reduce ABCG2 expression in choriocarcinoma JEG-3 cells. Wang et al[14] indicated that bortezomib aggravated the cytotoxicity of 5-Fu on choriocarcinoma cells.

As an aminamide-type anesthetic, lidocaine is widely used for regional anesthesia and pain relief.[15,16] Firstly, Johnson et al[17] pointed out that lidocaine resulted in local neurotoxicity by inducing mitochondrial dysfunction and activation of apoptosis pathways. Increasing number of reports in recent years revealed that lidocaine displayed anticancer activity on breast cancer, gastric cancer and colon cancer.[18-20] Moreover, Li et al proved that lidocaine sensitized the cytotoxicity of cisplatin to breast cancer cells via RARβ2 and RASSF1A,[15] which implied that lidocaine might also exert chemosensitization effect on choriocarcinoma. To date, no any literature is available regarding the chemo-sensitization activity of lidocaine on 5-Fu on choriocarcinoma.

Therefore, in this study, we tested the possible chemosensitization roles of lidocaine in the cytotoxicity of 5-Fu in choriocarcinoma cells. The findings of our research may provide a new combination of therapeutic strategy for choriocarcinoma therapy.

2 | MATERIALS AND METHODS

2.1 | Cell culture

Human choriocarcinoma-derived cell lines, JEG-3 and JAR, and human normal trophoblast cell line HTR8/SVneo (Stem Cell Bank, Chinese Academy of Science, Shanghai, China) were growth at 37°C with 5% CO_2 in Minimum Essential Media (MEM) medium (Gibco, Life Technologies, Carlsbad, CA) and Roswell Park Memorial Institute (RPMI)-1640 medium (Sigma-Aldrich, St Louis, MO) respectively containing with 1.5 g/L $NaHCO_3$ (Sigma-Aldrich), 0.11 g/L sodium pyruvate (Sigma-Aldrich), 100 U/mL benzyl penicillin-100 μg/mL streptomycin solutions (Sigma-Aldrich), and 10% fetal bovine serum (FBS; HyClone, Logan, UT).

2.2 | Preparation of lidocaine and 5-Fu

Lidocaine was obtained from Selleck Chemicals (Houston, TX) and 5-Fu was purchased from Sigma-Aldrich. Ultrapure water was used to dissolve lidocaine powder to 10 mM. Dimethyl sulfoxide (DMSO) was used to dissolve 5-Fu powder to 100 mM. Lidocaine and 5-Fu were filtered by 0.22 μM percolator and stored in −20°C until use.

2.3 | Detection of cell viability

Viability of JEG-3, JAR, and HTR8/SVneo cells was tested using Cell Counting Kit-8 (CCK-8) assay. Briefly, JEG-3, JAR or HTR8/SVneo cells were seeded into 96-well plates with 1×10^4 cells per well. Followed by different treatment and/or transfection, the CCK-8 solution (10 μL) was supplemented into the culture medium. Then, the cell plate was cultivated at 37°C for 1 hour. Subsequently, the absorbance of each well was measured using Microplate Reader (Molecular Device, Sunnyvale, CA) at 450 nm. Cell viability (%) was calculated as the percentage of control.

2.4 | Measurement of cell apoptosis

Annexin V-FITC/PI staining (Vazyme Biotech Co, Piscataway, NJ) was carried out to quantify the rate of apoptotic cells. Briefly, JEG-3, JAR or HTR8/SVneo cells were seeded into six-well plates with 1×10^5 cells per well. Followed by different treatment and/or transfection, the cells were collected, respectively, and cleaned with phosphate-buffered saline. Then, 100 μL cells were mixed with 100 μL kit solution at 37°C for 20 minutes. After that, cell samples were subjected to flow cytometer

analysis using Guava easyCyte 8HT (Millipore, Bedford, MA). Cell apoptosis was quantified by FCS Express software (De Novo Software, Los Angeles, CA).

2.5 | Quantitative reverse-transcription polymerase chain reaction

The ABCG2 mRNA expression in JEG-3 and JAR cells were determined using Quantitative reverse-transcription polymerase chain reaction (qRT-PCR). Total RNAs were isolated using TRIzol Plus RNA Purfication kit (Invitrogen, Carlsbad, CA). qRT-PCR was performed using SuperScript First-Stand Synthesis SuperMix for qRT-PCR (Invitrogen). The conditions of PCR program were set at 30 minutes at 58°C for reverse transcription, 2 minutes at 94°C for denaturation, 36 cycles of amplification for 45 seconds at 92°C, 60 seconds at 60°C and 60 seconds at 72°C. Sequences of primers were as follows: ABCG2 forward primer: 5′-ATGATGTTGTGATGGGCACTC-3′; reverse primer: 5′-CAGACCTAACTCTTGAATGACCCT-3′; GAPDH forward primer: 5′-ACCAC AGTCCATGCCATCAC-3′; reverse primer: 5′-TCCACCACCCTGTTGCTGTA-′3. Data were quantified using $2^{-\Delta\Delta C_t}$ method.[21]

2.6 | Cell transfection

The full-length ABCG2 sequence was constructed into pEX-2 plasmid (GenePharma, Shanghai, China), which was referred as pEX-ABCG2. Empty pEX-2 plasmid was utilized as negative control, which was referred as pEX. pEX-ABCG2 or pEX was transfected into JEG-3 and JAR cells using Lipofectamine 3000 reagent (Life Technologies Corporation, Carlsbad, CA). MEM medium added 0.5 mg/mL G418 (Sigma-Aldrich) was used to select transfected cells. qRT-PCR and Western blot analysis were performed to verify transfection efficiency.

2.7 | Western blot analysis

Total proteins used for Western blot analysis were isolated from JEG-3 and JAR cells using radioimmunoprecipitation assay buffer (RIPA) lysis and extraction buffer adding protease inhibitor. Proteins were separated using Bio-Rad Bis-Tris Gel system (Bio-Rad Laboratories, Hercules, CA) and transferred onto nitrocellulose membranes (0.22 μM, Millipore). Following primary antibodies were used in this study: Anti-Bcl-2 antibody (#15071), anti-Bax antibody (#2774), anti-Caspase -3 antibody (#9662), anti-caspase-9 antibody (#9508), anti-ABCG2 antibody (#4477), anti-P-gp antibody (#12683), anti-MRP1 antibody (#72202), anti-MRP2 antibody (#4446), anti-p-PI3K antibody (#4228), anti-PI3K antibody (#4292), anti-p-AKT antibody (#4060), anti-AKT antibody (#9272) and anti-GAPDH antibody (#5174; Cell Signaling Technology, Beverly, MA). After that, the membranes carried blots and antibodies were incubated with anti-mouse/rabbit IgG (H + L) Dylight^{M} 680 Conjugate (#5470, #5257; Cell Signaling Technology) for 1 hour at room temperature. The protein signals were recorded using Odyssey System (LI-COR Biosciences, Bad Homburg, Germany).

2.8 | Statistical analysis

Results were showed as mean ± standard deviation (SD) from at least three repeated experiments. Statistical analyses were carried out with the help of Graphad 6.0 statistical software (Graphad Software, San Diego, CA). Comparisons between different groups were done using one-way analysis of variance (ANOVA). $P < 0.05$ was considered as significant.

3 | RESULTS

3.1 | Cytotoxicity of lidocaine

First of all, the effects of lidocaine on JEG-3, JAR and HTR8/SVneo cell viability and apoptosis were tested. As presented in Figure 1A, 10 or 100 μM lidocaine incubation both had no obvious influences on JEG-3 cell viability, but 1000 μM lidocaine incubation lowered JEG-3 cell viability to 87.64% ± 5.42%. Figure 1B showed that the percentages of apoptotic JEG-3 cells had no obvious changes after 10 or 100 μM lidocaine treatment but remarkably increased to 7.89% ± 2.14% after 1000 μM lidocaine incubation ($P < 0.05$). Western blot analysis assay displayed that bcl-2-like protein 4 (Bax), cleaved-caspase-3, and cleaved-caspase-9 levels were all upregulated after 1000 μM lidocaine incubation (Figure 1C). Moreover, analogous outcomes were found in JAR cells, which showed that 10 or 100 μM lidocaine incubation had no obvious influences on JAR cells, while 1000 μM lidocaine incubation decreased JAR cell viability to 89.20% ± 3.38% (Figure 1D). The rates of apoptotic cells had no obvious changes after 10 or 100 μM lidocaine incubation but notably increased to 7.89% ± 2.14% after 1000 μM lidocaine incubation (Figure 1E, $P < 0.05$). Besides, the results of Figure 1F,G illustrated that 10 to 1000 μM lidocaine incubation all had no remarkably influence on HTR8/SVneo cell viability and apoptosis. These above results indicated that lidocaine in low concentration had no significant cytotoxicity to choriocarcinoma cells but

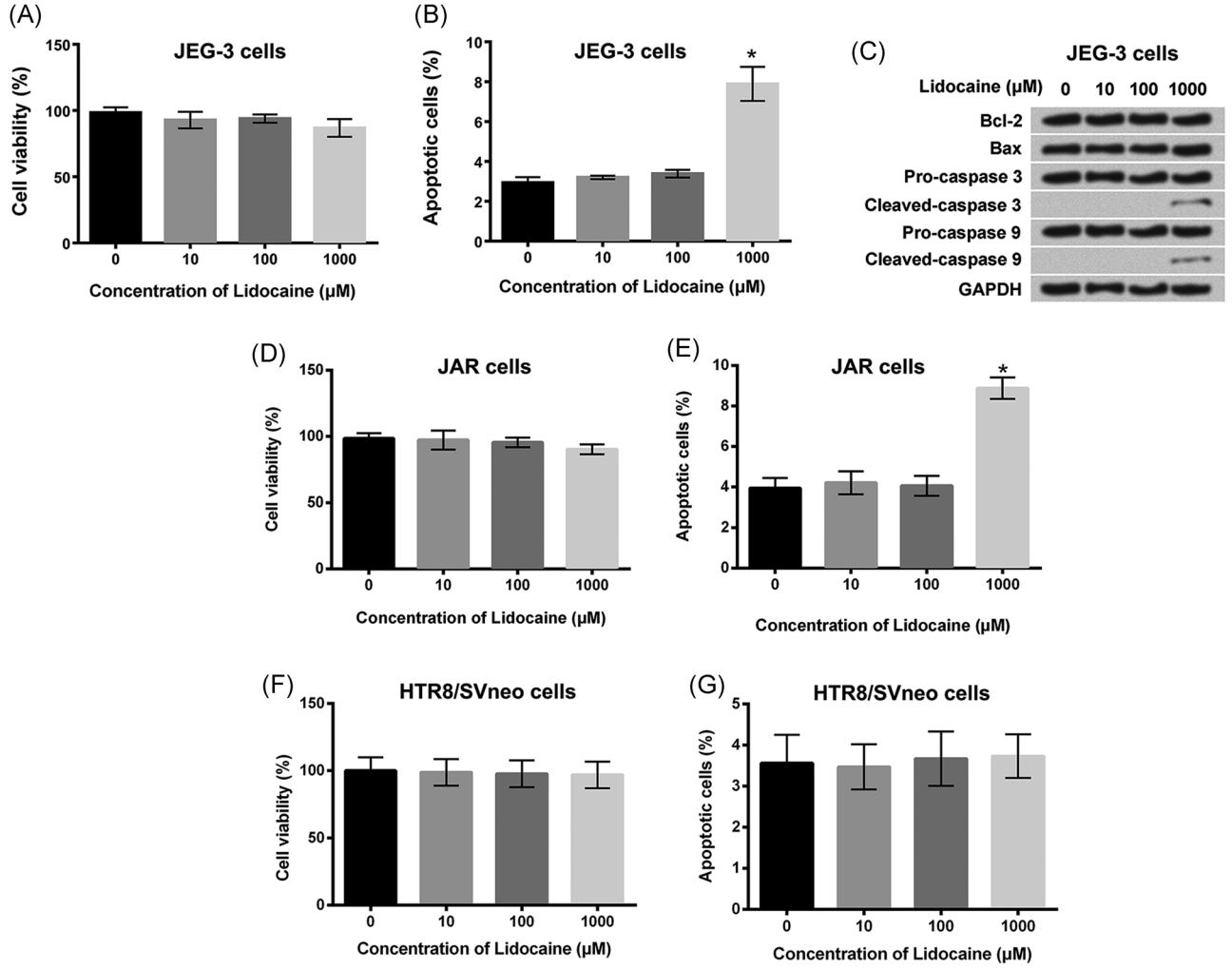

FIGURE 1 Cytotoxicity of lidocaine. A, D, and F, Cell Counting Kit-8 assay was carried out to test cell viability after 0, 10, 100, or 1000 μM lidocaine treatment. B, E, and G, The rates of apoptotic cells were measured after 0, 10, 100, or 1000 μM lidocaine treatment using Annexin V-FITC/PI staining. C, Western blot analysis was done to analyze Bcl-2, Bax, caspase-3, and caspase-9 levels in JEG-3 cells after 0, 10, 100, or 1000 μM lidocaine treatment. *$P < 0.05$ vs 0 μM lidocaine treatment group. Bax, bcl-2-like protein 4; Bcl-2, B-cell lymphoma 2; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; FITC, fluorescein isothiocyanate; PI, propidium iodide

induced choriocarcinoma cell viability inhibition and apoptosis in high concentration.

3.2 | Cytotoxicity of lidocaine combined with 5-Fu

Then, the effects of single 5-Fu incubation or 5-Fu + lidocaine incubation on JEG-3 and JAR cell viability and apoptosis were determined. Figure 2A displayed that 5-Fu incubation inhibited JEG-3 cell viability in a concentration-dependent pathway. The rate of JEG-3 cell viability reduced to 62.85% ± 2.46% after 100 μM 5-Fu incubation ($P < 0.05$). Moreover, after 100 μM 5-Fu stimulation, the percentage of apoptotic JEG-3 cells was dramatically elevated (Figure 2B, $P < 0.01$). The Bax, cleaved-caspase-3 and cleaved-caspase-9 levels in JEG-3 cells were all upregulated after 10 or 100 μM 5-Fu stimulation, while the Bcl-2 level was downregulated after 100 μM 5-Fu treatment (Figure 2C) In addition, Figure 2D presented that the JEG-3 viability after cotreatment with low doses (1, 10, or 100 μM) of lidocaine and 5-Fu were remarkably decreased ($P < 0.05$ or $P < 0.01$). Figure 2E indicated that the apoptosis of JEG-3 cells were noticeably increased after 5-Fu cotreatment with lidocaine ($P < 0.05$ or $P < 0.01$). Analogous outcomes were found in JAR cells (Figure 2F-J, $P < 0.05$ or $P < 0.01$). These outcomes evidenced that lidocaine could enhance the cytotoxicity of 5-Fu to choriocarcinoma

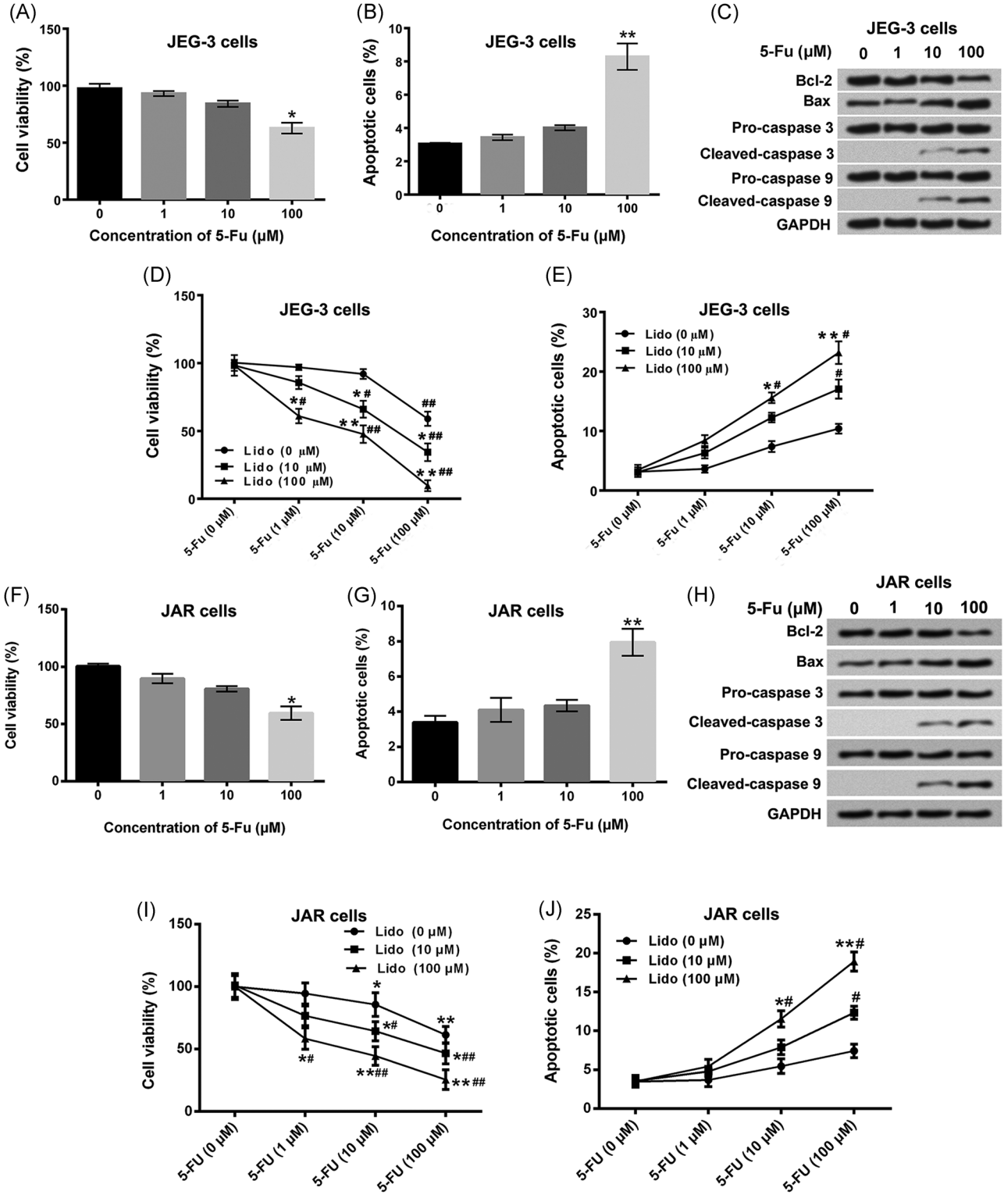

FIGURE 2 Cytotoxicity of lidocaine combined with 5-Fu. A and F, Viabilities of JEG-3 and JAR cells after 0, 1, 10, or 100 μM 5-Fu stimulation were detected. B and G, The rates of apoptotic JEG-3 and JAR cells after 0, 1, 10, or 100 μM 5-Fu treatment were assessed. C and H, The Bcl-2, Bax, caspase-3 and caspase-9 levels in JEG-3 and JAR cells after 0, 1, 10, or 100 μM 5-Fu treatment were evaluated. D and I, Viabilities of JEG-3 and JAR cells after cotreatment lidocaine (0, 10, or 100 μM) with 5-Fu (0, 1, 10, or 100 μM) were determined. E and J, The rates of apoptotic JEG-3 and JAR cells after cotreatment lidocaine (0, 10, or 100 μM) with 5-Fu (0, 1, 10, or 100 μM) were assessed. $^{*}P < 0.05$ or $^{**}P < 0.01$ vs 0 μM lidocaine treatment group; $^{\#}P < 0.05$ or $^{\#\#}P < 0.01$ vs 0 μM 5-Fu treatment group. 5-Fu, 5-fluorouracil; Bax, bcl-2-like protein 4; Bcl-2, B-cell lymphoma 2; GAPDH, glyceraldehyde-3-phosphate dehydrogenase

cells by decreasing cell viability and increasing cell apoptosis.

3.3 | Effect of lidocaine on the expression level of ABC transport proteins in choriocarcinoma cells

Subsequently, the ABCG2 mRNA and protein levels in JEG-3 and JAR cells after 10 or 100 μM lidocaine treatment were determined. As shown in Figure 3A, the ABCG2 mRNA levels in JEG-3 and JAR cells were markedly decreased after 10 or 100 μM lidocaine incubation ($P < 0.05$ or $P < 0.01$). Moreover, the ABCG2 protein levels in JEG-3 and JAR cells were also downregulated after 10 or 100 μM lidocaine treatment (Figure 3B). Furthermore, the results of Figure 3C presented that 10 μM 5-Fu treatment had no significant effects on ABCG2, P-gp, MRP1, and MRP2 expression levels in JEG-3 and JAR cells; while 10 μM lidocaine and 10 μM 5-Fu cotreatment suppressed the ABCG2, P-gp, MRP1, and MRP2 expression levels in JEG-3 and JAR cells. In summary, these above findings indicated that lidocaine enhanced the cytotoxicity of 5-Fu to choriocarcinoma cells might be via downregulating the expression of ABC transport proteins in choriocarcinoma cells.

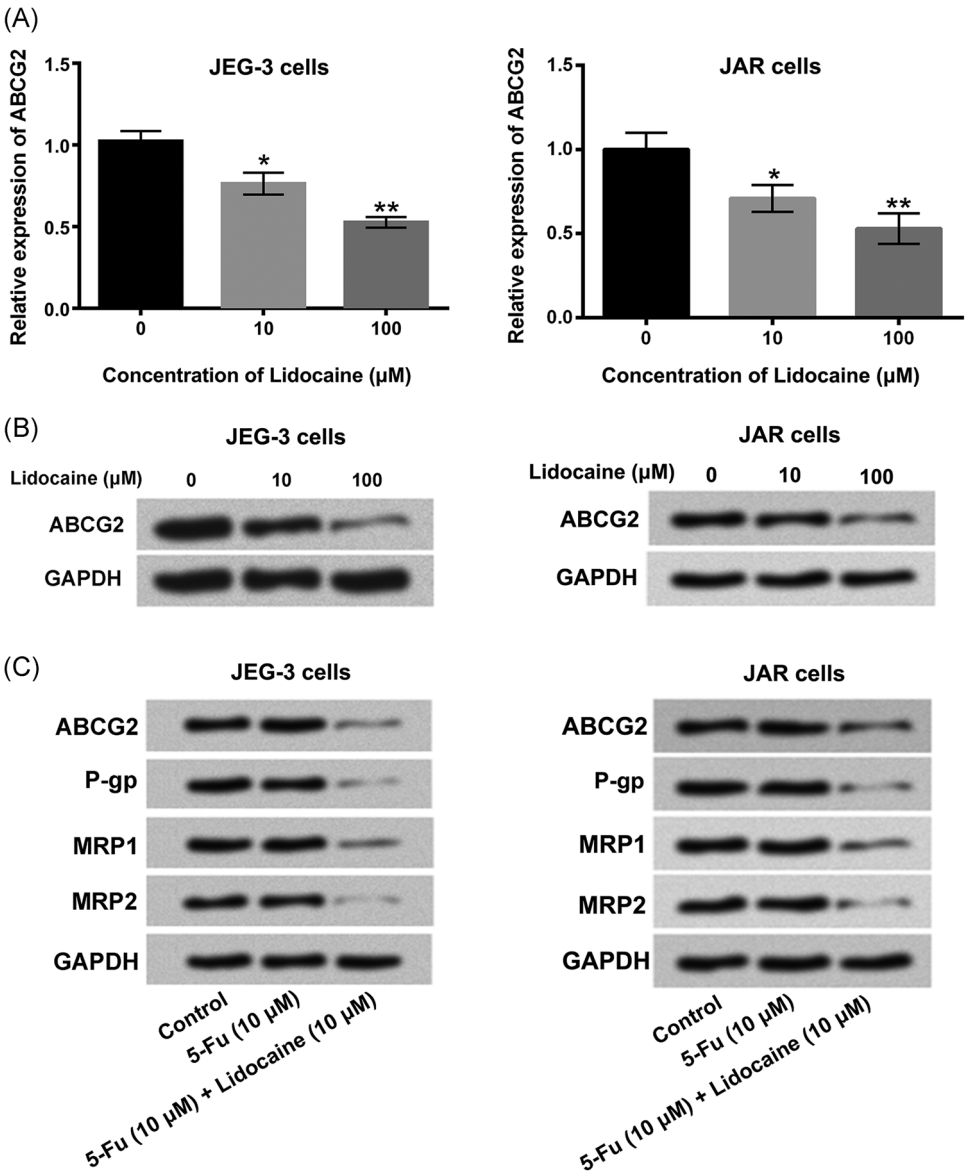

FIGURE 3 Effects of lidocaine on ABC transport proteins expression in choriocarcinoma cells. A, The ABCG2 messenger RNA levels in JEG-3 and JAR cells followed by 0, 10, or 100 μM lidocaine treatment were detected. B, The ABCG2 protein level was determined. C, After 10 μM 5-Fu and/or 10 μM lidocaine treatment, the ABCG2, P-gp, MRP1, and MRP2 levels in JEG-3 and JAR cells were assessed. *$P < 0.05$ or **$P < 0.01$ vs 0 μM lidocaine treatment group. 5-Fu, 5-fluorouracil; ABC, ATP-binding cassette; ABCG2, breast cancer resistance protein; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; P-gp, P-glycoprotein

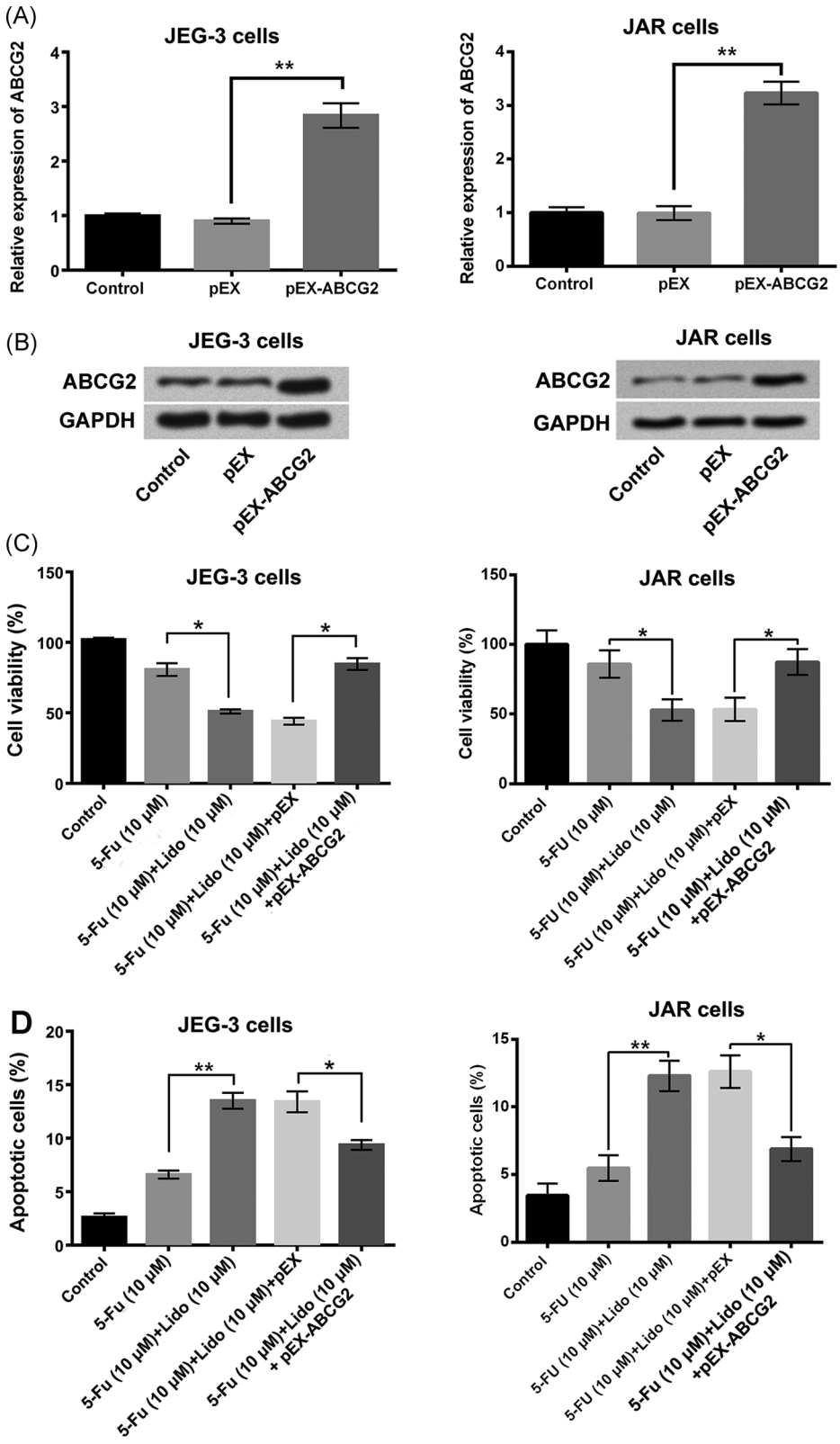

FIGURE 4 Roles of ABCG2 in the cytotoxicity of 5-Fu + lidocaine on choriocarcinoma cells. A and B, JEG-3 and JAR cells were transfected with pEX or pEX-ABCG2, the ABCG2 mRNA and protein levels were determined. C and D, Viabilities of JEG-3 and JAR cells after 5-Fu (10 μM) and/or lidocaine (10 μM) treatment and/or pEX-ABCG2 transfection were measured. D, The rates of apoptotic JEG-3 and JAR cells after 5-Fu (10 μM) and/or lidocaine (10 μM) treatment and/or pEX-ABCG2 transfection were assessed $^{*}P < 0.05$; $^{**}P < 0.01$. 5-Fu, 5-fluorouracil; ABC, ATP-binding cassette; ABCG2, breast cancer resistance protein; GAPDH, glyceraldehyde-3-phosphate dehydrogenase

3.4 | Roles of ABCG2 in the cytotoxicity of 5-Fu + lidocaine on choriocarcinoma cells

To further explore the effects of ABCG2 on the cytotoxicity of 5-Fu + lidocaine on choriocarcinoma cells, pEX-ABCG2 was transfected into JEG-3 and JAR cells. Figure 4A,B displayed that the ABCG2 mRNA and protein levels in JEG-3 and JAR cells were both increased after pEX-ABCG2 transfection ($P < 0.01$ of mRNA level). Figure 4C presented that compare to 5-Fu (10 μM) + lidocaine (10 μM) + pEX treatment group, the viabilities of JEG-3 and JAR cells were remarkably increased in 5-Fu (10 μM) + lidocaine (10 μM) + pEX-ABCG2 treatment group ($P < 0.05$). Furthermore, Figure 4D pointed out that pEX-ABCG2 also mitigated the effects of 5-Fu + lidocaine on JEG-3 and JAR cell apoptosis ($P < 0.05$). These above outcomes evidenced that ABCG2 unleashed essential activity in the effects of 5-Fu + lidocaine on choriocarcinoma cell viability inhibition and apoptosis, and further implied that lidocaine potentiated the cytotoxicity of 5-Fu to JEG-3 cells by downregulating ABC transport proteins, including ABCG2.

3.5 | The activation of PI3K/AKT signaling pathway after 5-Fu and/or lidocaine treatment in choriocarcinoma cells

Finally, the PI3K/AKT pathway in JEG-3 and JAR cells after 5-Fu and/or lidocaine treatment was assessed. The phospho-phosphoinositide 3-kinases (p-PI3K) and phospho-protein kinase B (p-AKT) expression levels in JEG-3 and JAR cells were both decreased after single 10 μM 5-Fu treatment and further reduced after 5-Fu (10 μM) + lidocaine (10 μM) treatment (Figure 5). However, 5-Fu + lidocaine treatment-induced expression decreases of p-PI3K and p-AKT were both reversed by pEX-ABCG2 transfection. These results suggested that lidocaine enhanced the inhibitory effects of 5-Fu on PI3K/AKT pathway in JEG-3 cells also through lowering ABCG2.

4 | DISCUSSION

Combination therapy is an efficient therapeutic strategy for cancer treatment.[11,22] Herein, we found that high concentration of lidocaine caused choriocarcinoma cell apoptosis s through lowering Bax, cleaved-caspase-3 and cleaved-caspase-9. Moreover, the low concentration of lidocaine significantly potentiated the cytotoxicity of 5-Fu to choriocarcinoma cells via decreasing cell viability and enhancing cell apoptosis. Lidocaine reduced the expression of ABC transport proteins in choriocarcinoma cells. Overexpression of ABCG2 reversed the lidocaine + 5-Fu-induced choriocarcinoma cell viability inhibition, apoptosis and inactivation of PI3K/AKT pathway.

5-Fu exerted its cytotoxicity by inhibiting DNA synthesis and enhancing cell apoptosis.[7,23] Lidocaine had been reported to exert anticancer and chemosensitization effects on cancer therapy.[14,18,20] Considering that lidocaine has few side effects on patient's health, looking for possible antitumor influences of lidocaine will not only be helpful for exploring the other beneficial activity of lidocaine, but provide possible chemosensitization drug for cancer treatment. Study on multiple actions of

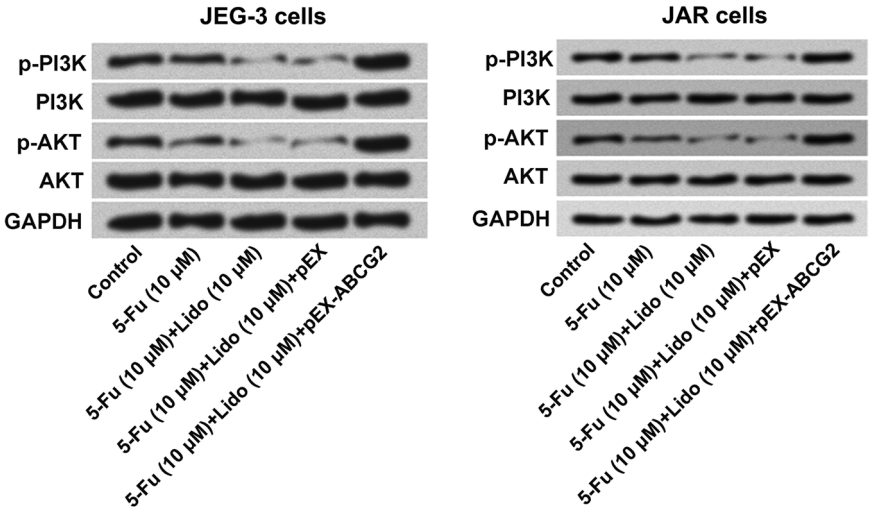

FIGURE 5 The activation of PI3K/AKT pathways in choriocarcinoma cells after 5-Fu + lidocaine treatment.
The p-PI3K, PI3K, p-AKT, and AKT levels in JEG-3 and JAR cells after 5-Fu (10 μM) and/or lidocaine (10 μM) treatment and/or pEX-ABCG2 transfection were analyzed. 5-Fu, 5-fluorouracil; ABCG2, breast cancer resistance protein; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; p-PI3K, phospho-phosphoinositide 3-kinases; p-AKT, phospho-protein kinase B

known medicines is very ponderable for medicinal development. In current experiments, we found that lidocaine treatment had no obvious influence on human normal trophoblast HTR/SVneo cells. In comparisons to either single treatment, the viabilities of JEG-3 and JAR cells were decreased after cotreatment with lidocaine and 5-Fu. Moreover, lidocaine and 5-Fu in combination exhibited superior proapoptosis effects on JEG-3 and JAR cells. These results suggested that lidocaine enhanced the cytotoxicity of 5-Fu to choriocarcinoma cells via synergistically suppressing cell viability and potentiating cell apoptosis. Considering that lidocaine had been demonstrated to enhance the cytotoxicity of cisplatin to breast cancer cells,[15] we could get a conclusion that lidocaine may be a wonderful chemo-sensitizer to improve the cytotoxicity of chemotherapy drugs to cancer cells.

ABC transport proteins can transport chemotherapy drugs, including 5-Fu, out of the tumor cells to reduce the cytotoxicity of drugs.[8] The previous study demonstrated that EJG-3 cells exposure to high concentration of 5-Fu for a long time will develop the drug resistance.[14] ABCG2 is a member of ABC transport proteins superfamily, which is associated with the drug resistance of many cancer cells.[24,25] Hu et al[26] proved that downregulation of ABCG2 potentiated the chemotherapy sensitivity of 5-Fu to human colon cancer cells. We discovered that lidocaine decreased the ABCG2 mRNA and protein levels in JEG-3 and JAR cells in dose-dependent manner. Moreover, lidocaine treatment suppressed the ABCG2, P-gp, MRP1, and MRP2 expression levelsin 5-Fu-treated JEG-3 and JAR cells. Overexpression of ABCG2 reversed the synergistic effects of lidocaine + 5-Fu on choriocarcinoma cell viability inhibition and cell apoptosis inducement, which suggested that ABC transport proteins were closely related to the influences of lidocaine + 5-Fu on choriocarcinoma cells and further illustrated that lidocaine potentiated the cytotoxicity of 5-Fu to choriocarcinoma cells by lowering ABC transport proteins.

To further understand the chemo-sensitization effects of lidocaine, we analyzed the PI3K/AKT pathway in JEG-3 and JAR cells after 5-Fu and/or lidocaine treatment. The activation of PI3K/AKT signaling pathway had been verified in numerous cancers, including choriocarcinoma, which was involved in the modulation of cell survival, proliferation, differentiation, and migration.[27,28] 5-Fu can inactivate PI3K/AKT signaling pathway in many cancer cells.[29] Zhang et al[30] proved that downregulation of USP22 potentiated the chemosensitivity of hepatocellular carcinoma cells to 5-Fu by inactivating AKT. In our research, Western blot analysis displayed that the p-PI3K and p-AKT levels in JEG-3 and JAR cells were both decreased after single 5-Fu treatment. Lidocaine potentiated the inhibitory effects of 5-Fu on p-PI3K and p-AKT. However, the inhibitory effect of lidocaine + 5-Fu on PI3K/AKT signaling pathway was reversed by ABCG2 overexpression, which further demonstrated that the critical regulatory roles of ABCG2 in lidocaine + 5-Fu combination therapy.

Taken together, our research affirmed that lidocaine showed chemosensitization activity to potentiated the cytotoxicity of 5-Fu to choriocarcinoma cells by means of lowering ABC transport proteins and inactivating PI3K/AKT pathway. We propose that lidocaine could be as a better combination drug of 5-Fu for choriocarcinoma therapy. However, the lidocaine concentration used for paracervical block in pregnant women is approximately 0.5-1 mg/kg.[31] In future, more in vivo studies are still needed to affirm the findings of our research and explore the suitable concentration of lidocaine used for choriocarcinoma therapy.

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

JW and XZ designed research; XZ and WP analyzed data; XZ and HL performed research; XZ drafted the manuscript; JW revised the manuscript and finally approved the article.

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