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hERG1 is involved in the pathophysiological process and inhibited by berberine in SKOV3 cells

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Abstract. The human ether-a-go-go-related potassium channel 1 (hERG1) is a functional component of the voltage-gated Kv11.1 potassium channel, which is commonly described as a crucial factor in the tumorigenesis of a variety of tumors. Ovarian cancer is one of the most severe types of cancer, with an extremely poor prognosis. Advances have been made in recent years; however, drug resistance and tumor recurrence remain critical issues underlying satisfactory treatment outcomes. Therefore, more effective antitumor agents with low levels of drug resistance for ovarian cancer treatment are urgently required in clinical practice. In the present study, hERG1 mRNA expression in ovarian tumor tissues and cell lines were measured by reverse transcription-quantitative polymerase chain reaction. Immunohistochemistry and western blotting were used to assess the expression levels of hERG1 protein. Cell proliferation, migration and invasion were assessed by Cell Counting Kit-8 assay and Transwell assay. A tumor xenograft assay was used to determine the growth of tumors *in vivo*. It was demonstrated that the expression levels of hERG1 were significantly elevated in ovarian cancer tissues and expressed in ovarian cancer cell lines, particularly in SKOV3 cells. Abnormal hERG1 expression was significantly associated with the proliferation, migration and invasion abilities of ovarian cancer. In addition, berberine (BBR) may be used as a potential drug in the treatment of ovarian cancer, possibly due to its inhibitory effects on the hERG1 channels. In conclusion, the present study demonstrated that hERG1 may be a potential therapeutic target in the treatment of ovarian cancer and provided novel insights into the mechanism underlying the antitumor effects of BBR in ovarian cancer.

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Key words: ovarian cancer, berberine, human ether-a-go-go-related potassium channel 1, proliferation, invasion, migration

Introduction

Ovarian cancer is one of the most common types of gynecological malignancies worldwide with an extremely low survival rate of 5 years (1,2). Patients diagnosed at the early stages of ovarian cancer respond to platinum-based combinatorial chemotherapies; however, relapse and drug resistance are prevalent, leading to treatment failure and poor prognosis (3). At present, due to the lack of reliable approaches for early diagnosis, ovarian cancer is generally diagnosed at an advanced stage when surgical resection or chemotherapy are generally ineffective (4). Therefore, molecular alterations in tumors, particularly those involved in cell proliferation, apoptosis and invasion signaling pathways, are being investigated for potential developments in early diagnosis and targeted therapy.

Potassium channels are the most widely distributed type of ion channel and are associated with numerous biological processes in almost all living organisms (5,6). Potassium channels are commonly expressed in excitable cells and are often aberrantly expressed in neoplastic cell lines and primary types of human cancer (7,8). This contributes to the regulation of several factors associated with neoplastic progression, including cell proliferation, apoptosis, invasion and metastasis (9-11). The inhibition of potassium channels has been demonstrated to exert antineoplastic activities in various types of tumors *in vitro* and *in vivo* (12,13). This evidence suggests that potassium channels may be considered as possible targets for antineoplastic therapy.

Human ether a-go-go related potassium channels (hERGs) are voltage-dependent potassium channels that serve important roles in the terminal repolarization on human ventricular myocytes (14). A number of studies over the past two decades have demonstrated that plasma membrane hERG1 is often aberrantly expressed in various types of cancer and serves essential roles in numerous crucial cellular events, including electrophysiological activity and signaling conduction (15,16). However, to the best of our knowledge, few investigations into the function of hERG1 in human ovarian cancer have been conducted (17,18)

and the underlying mechanisms regulating hERG1 expression in ovarian cancer progression remain unknown.

Previous studies have revealed that numerous traditional Chinese herbal medicines (19-21), including berberine (BBR), a clinically important natural isoquinoline alkaloid that was the subject of our previous study (22), exhibit significant antitumor activities and reverse the drug resistance of cancer cells. BBR has been reported to exert antitumor activity against various types of cancer, including gastric, oral, prostate and ovarian cancer (23-25). The molecular targets of BBR include protein kinase B, p53, mitogen-activated protein kinase, nuclear factor-κB and signal transducer and activator of transcription 3, which are pivotal factors in regulating cell growth, apoptosis, invasion and angiogenesis of cancer (26-28). However, since tumor progression is a complicated pathological process, other molecular targets may be involved in the antitumor effects exhibited by BBR. It is common that multi-target combination therapy for the treatment of tumors is administered as the main treatment approach to overcome drug resistance and improve the prognosis of patients with cancer (29). Therefore, investigations into the potential molecular mechanisms underlying the antitumor properties of BBR in ovarian cancer are necessary and valuable for the development of multi-targeted therapy.

The present study revealed that hERG1 was involved in the regulation of proliferation, migration and invasion of ovarian cancer cells. Treatment with BBR was observed to efficiently inhibit these biological activities *in vivo* and *in vitro*. The results of the present study provide novel insights into the molecular mechanism underlying the antitumor effects of BBR and may contribute to the development of novel therapeutic strategies for the treatment of ovarian cancer.

Materials and methods

Tissue samples. A total of 28 ovarian carcinoma tissue samples and matched adjacent non-tumorigenic tissues samples (2 cm from cancerous tissues) were obtained from patients who underwent surgical resection of the tumor at Harbin Medical University Cancer Hospital (Harbin, China) between May 2015 and March 2016. The average age of the patients was 48 years old. The samples were stored in liquid nitrogen until total RNA or protein were extracted. The present study was approved by the Ethical Committee of Harbin Medical University Cancer Hospital and written informed consent was obtained from all patients.

Immunohistochemical assay. All tissues were initially fixed in 10% formalin for 48 h at 4˚C and embedded in paraffin. Section of 5 μm thickness were pre-incubated with 5% goat serum (cat. no. C0265; Beyotime Institute of Biotechnology, Shanghai, China) as a blocking buffer for 2 h at room temperature, and then incubated with primary hERG1 antibody (1:200; cat. no. sc-377388; Santa Cruz Biotechnology, Inc., Dallas, TX, USA) overnight at 4˚C; After washing three times with PBS at room temperature, the sections were incubated with peroxidase-labeled anti-mouse antibody (1:50; cat. no. A0216; Beyotime Institute of Biotechnology) for 30 min at room temperature. The peroxidase activity was detected with diaminobenzidine (DAB; Beyotime Institute of Biotechnology). The stained sections were analyzed using a fluorescence Olympus BX51 microscope (magnification, x200; Olympus Corporation, Tokyo, Japan). The immunohistochemical staining results were scored as the sum of intensity and percentage scores, according to the staining intensity (0=negative; 1=weak/trace; 2=moderate; and 3=strong) and the percentage of positive cells (0, ≤10; 1, 11-25; 2, 26-50; 3, 51-75; and 4, 76-100%). This grading produced a final score of 0-12, and samples were separated into groups based on low (0-4) or high (6-12) scores.

Reagents. BBR and DMSO were purchased from Sigma-Aldrich; Merck KGaA (Darmstadt, Germany). BBR was dissolved in 10% DMSO and 90% deionized water. The drug solution was diluted to concentrations of 5, 10, 30, 50 and 100 μM in RPMI-1640 culture medium (Beyotime Institute of Biotechnology) prior to use at room temperature. For cellular experiments, cells were incubated with different concentrations of BBR (5, 10, 30, 50 or 100 μM) for 24, 48 and 72 h at 37˚C, respectively. Cells in the blank control group were treated with 1% DMSO.

Cell culture and transfection. OVCAR-3, SKOV3, HO-8910, A2780 and ES-2 ovarian carcinoma cell lines were obtained from the Cancer Institute of Harbin Medical University. OVCAR-3 and SKOV3 cells were maintained in RPMI-1640 medium (Beyotime Institute of Biotechnology) supplemented with 12% (v/v) FBS (Thermo Fisher Scientific, Inc., Waltham, MA, USA). HO-8910, A2780 and ES-2 ovarian carcinoma cells were maintained in DMEM (Beyotime Institute of Biotechnology) supplemented with 12% (v/v) FBS (Thermo Fisher Scientific, Inc.). All cell lines were cultured at 37˚C with 5% CO_2 in a humidified incubator.

Short hairpin (sh)RNA-hERG1 plasmid (4 μg) and shRNA-control (scrambled short hairpin RNA sequence) plasmids (4 μg) (Shanghai GeneChem Co., Ltd., Shanghai, China), were used to transfect SKOV3 cells. All transfections were performed using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol. Cells were harvested at 24 h after transfection.

Cell proliferation assay. Cell proliferation was determined by Cell Counting kit-8 (CCK8) assay. Briefly, SKOV3 cells were seeded in 96-well plates at $1x10^5$ cells/well and maintained for 24 h at 37˚C to allow cell adhesion. Subsequently, the cells in various groups (5, 10, 30, 50 or 100 μM berberine for 24, 48 or 72 h) were incubated with 10 μl CCK8 for 2 h and the absorbance was measured at 450 nm using a microplate reader (Olympus Corporation). The proliferation of treated cells was assessed by comparison with the blank control group. Each experiment was performed in triplicate.

Total RNA extraction and reverse transcription-quantitative polymerase chain reaction (RT-qPCR). OVCAR-3, SKOV3, HO-8910, A2780 and ES-2 ovarian carcinoma cells and tissue specimens were homogenized in TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc.) to isolate total RNA, according to the manufacturer's protocols. The concentration of RNA was detected with a NanoDrop spectrophotometer (NanoDrop Technologies; Thermo Fisher Scientific, Inc.). The total RNA of each sample (1 μg) was reverse transcribed using random primers and a PrimeScript 1st Strand cDNA Synthesis kit (Takara Biotechnology Co., Ltd., Dalian, China), according to the

manufacturer's protocols. qPCR was performed using a SYBR green fluorescent dye (PCR master mix; Applied Biosystems; Thermo Fisher Scientific, Inc.) with the following thermocycling conditions: Pre-denaturation at 95˚C for 45 sec, followed by 40 cycles of denaturation at 95˚C for 15 sec, and annealing and extension at 55˚C for 30 sec. The relative hERG1 expression levels were calculated based on the $2^{-\Delta\Delta Cq}$ method and normalized to GAPDH level in each sample (30). Each experiment was performed in triplicate. The primer sequences for qPCR were as follows: hERG1 forward, 5'-CAGCGGCTGTACTCGGGCACAG-3' and reverse, 5'-CAGAAGTGGTCGGAGAACTC-3'; and GAPDH forward, 5'-GTCAACGGATTTGGTCGTATTG-3' and reverse, 5'-AGTGATGGCATGGACTGTGG-3'.

Western blot analysis. Briefly, total protein was extracted from tissues or OVCAR-3, SKOV3, HO-8910, A2780 and ES-2 ovarian carcinoma cell lines with RIPA buffer (Beyotime Institute of Biotechnology) and the protein concentrations were determined using a bicinchoninic acid protein assay (Pierce; Thermo Fisher Scientific, Inc.). Subsequently, proteins (50 μg/lane) were separated by SDS-PAGE (8% gel) and transferred onto a nitrocellulose membrane (Bio-Rad Laboratories, Inc., Hercules, CA, USA). To prevent non-specific binding, the membrane was blocked with 5% skimmed milk in PBS with Tween-20 (0.25%) for 2 h at room temperature. Subsequently, the membrane was incubated with hERG1 antibody (1:1,000; cat. no. sc-377388; Santa Cruz Biotechnology, Inc.) or β-actin antibody (1:1,000; cat. no. ab8227; Abcam, Cambridge, UK) in PBS at 4˚C overnight. Subsequently, the membrane was rinsed with PBST three times, which was followed by incubation with corresponding IRDye® 800CW fluorescent secondary antibodies (1:8,000; cat. nos. anti-rabbit 926-32211 and anti-mouse 926-32210; LI-COR Biosciences, Lincoln, NE, USA) for 1 h at room temperature. Finally, the protein bands were visualized using an Odyssey infrared imaging system (LI-COR Biosciences) at 800 nm.

Transwell assays. SKOV3 cells in serum-free RPMI-1640 medium (200 μl containing $5x10^5$ cells for BBR group, 200 μl containing $2.5x10^5$ cells for other groups) were added to the upper Transwell chambers (pore size, 8 μm; Corning Inc., Corning, NY, USA). The chambers were coated with Matrigel (BD Biosciences, Franklin Lakes, NJ, USA) for the invasion assays but not the migration assays. The bottom chamber was filled with 700 μl RPMI-1640 medium containing 15% FBS. The cells were cultured in an incubator at 37˚C with 5% CO_2 for 24 h, then the cells were fixed in 4% paraformaldehyde for 15 min. Subsequently, the cells were washed with PBS and stained with 0.1% crystal violet for 10 min at room temperature. For analysis, five fields were randomly selected and the number of stained cells was counted under a light microscope at magnification of x200 (Nikon Corporation, Tokyo, Japan). Data are expressed as the mean number of cells per insert.

In vivo tumor xenograft study. Five-week-old BALB/c-nu/nu nude mice were purchased from the Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). The experimental protocols were approved by the Ethics Committee of Harbin Medical University (Harbin, China). A total of 72 mice (36 females, 36 males) weighing 20-25 g each, were maintained under a 12-h light/dark cycle at 20.0-26.1˚C and 35-70% humidity. Food and water were freely accessible to mice. SKOV3 cells ($2x10^6$ per mouse) were subcutaneously injected into the flank of each mouse. Following the outgrowth of palpable tumors, the mice were randomly divided into four groups (n=3 for each group) and fed by oral gavage with saline or BBR (20 mg/kg), twice per week. BBR was dissolved in carboxymethylcellulose sodium for use via oral gavage. Tumors volumes were calculated using the following formula: Volume=L x $(W)^2/2$, where L is the longest diameter and W is the shorter diameter. At the end of the present study, mice were euthanized and tumor tissues were harvested for further research.

Table I. Associations between hERG1 expression and clinicopathological features of patients with ovarian cancer.

Variables	hERG1 expression, n		P-value
	Low	High	
Age, years			0.6193
≤50	7	8	
>50	8	5	
Tumor diameter, cm			0.8232
<8	3	8	
≥8	4	13	
Ascite			0.7051
Negative	6	7	
Positive	9	6	
Lymph node metastasis			0.0044[a]
Negative	9	5	
Positive	1	13	
Distant metastasis			0.0204[b]
Negative	7	3	
Positive	4	14	
Differentiation			0.7483
Good	3	4	
Moderate	5	3	
Poor	7	6	
Tumor location			0.694
Bilateral	8	10	
Unilateral	6	4	

Data were analyzed using a χ^2 test. hERG1, human ether-a-go-go-related potassium channel 1. [a]P<0.01, [b]P<0.05.

Statistical analysis. Data are presented as the mean ± standard error of the mean of triplicate experiments and were analyzed with SPSS version 13.0 software (SPSS, Inc., Chicago, IL, USA). The χ^2 test was used to analyze the association of hERG1 expression with clinicopathologic features of ovarian cancer. Unpaired Student's t-test and one-way analysis of variance with a Student-Newman-Keuls post hoc test were used to determine the significance of differences between two groups and three or more groups, respectively. P<0.05 was considered to indicate a statistically significant difference.

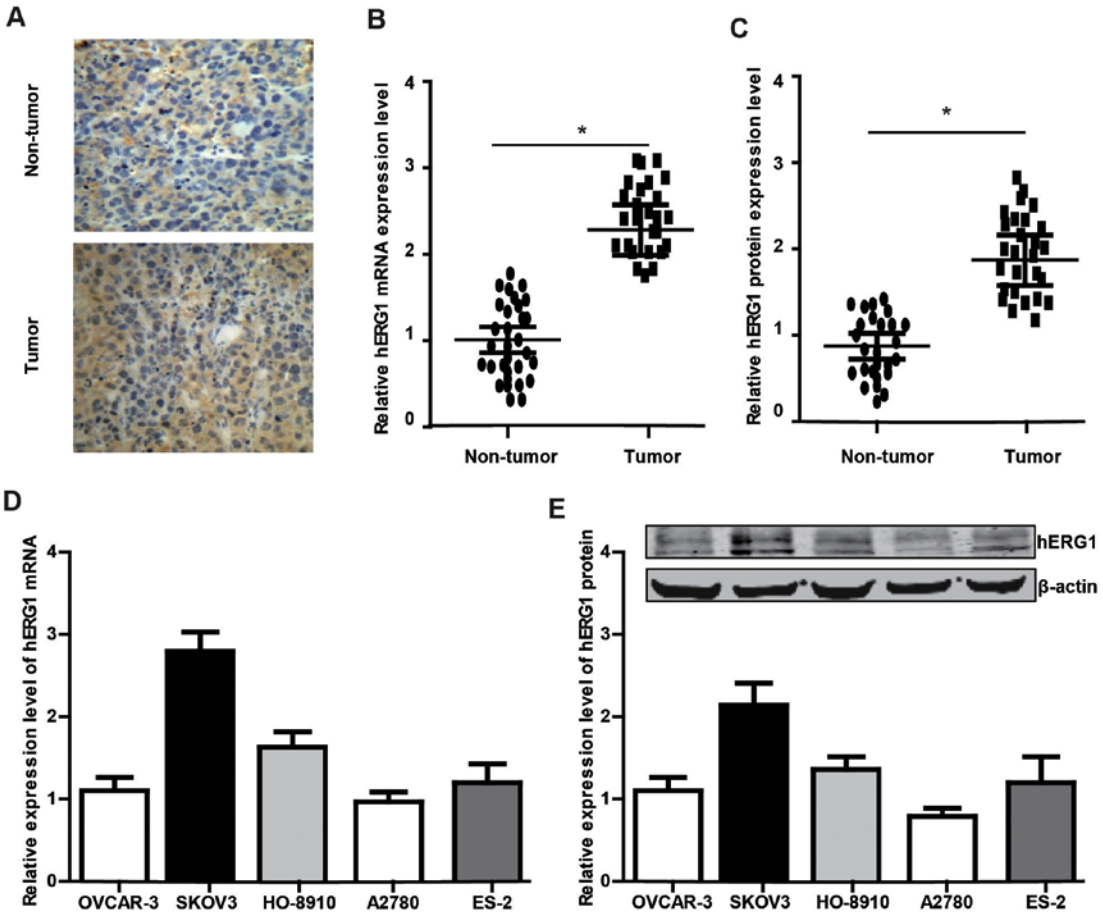

Figure 1. Expression patterns of hERG1 in ovarian cancer tissues and cell lines. (A) Expression levels of hERG1 analyzed by immunohistochemistry (magnification, x400). (B) Reverse transcription-quantitative polymerase chain reaction and (C) western blot analysis of hERG1 expression in ovarian cancer and matched adjacent non-tumor tissues (n=28). (D) Relative mRNA and (E) protein expression levels of hERG1 in various ovarian cancer cell lines (n=3). *P<0.05. hERG1, human ether-a-go-go-related potassium channel 1.

Results

hERG1 is upregulated in human ovarian cancer. In order to determine the role of hERG1 in ovarian cancer development, the mRNA and protein expression levels of hERG1 were detected by RT-qPCR and western blotting. A total of 28 tumor tissues and matched non-tumor tissues were obtained from patients with ovarian cancer with a median age of 48 years in the present study. The association of hERG1 expression with clinicopathologic features of ovarian cancer is presented in Table I. These results demonstrate that hERG1 expression level is significantly associated with ovarian cancer distant metastasis and lymph node metastasis. The immunohistochemical assay results revealed that the protein expression levels of hERG1 were markedly higher in ovarian cancer tissues compared with non-tumor tissues (Fig. 1A). As presented in Fig. 1B and C, the mRNA and protein expression levels of hERG1 were significantly upregulated in tumor tissues compared with non-tumor tissues, as expected. Furthermore, the expression level of hERG1 in OVCAR-3, SKOV3, HO-8910, A2780 and ES-2 ovarian cancer cell lines was investigated. The results (Fig. 1D and E) revealed that hERG1 was generally expressed in these ovarian cancer cell lines and highly expressed in SKOV3, in particular. Therefore, in the present study, SKOV3 cells were used for further analysis.

BBR inhibits cell proliferation and hERG1 in SKOV3 cells. Subsequently, the effects of BBR on SKOV3 cells were investigated using a CCK8 assay. SKOV3 cells were treated for 24, 48 and 72 h with BBR at concentrations ranging between 5 and 100 μM, and the results were compared with the control group treated with DMSO. The CCK8 assay results demonstrated that BBR inhibited the proliferation of SKOV3 cells in a time- and dose-dependent manner (Fig. 2A). The proliferation of cells treated for 48 h decreased more significantly compared with cells treated for 24 h ($P<0.05$); however, no difference was observed between cells in the 48 and 72 h groups. Simultaneously, BBR significantly reduced the mRNA and protein expression levels of hERG1 in a dose-dependent manner (Fig. 2B-D), which suggests that hERG1 may serve a role in the proliferation of SKOV3 cells. Expression levels of hERG1 decreased significantly in response to 10 μM BBR and the IC_{50} of BBR was 9.8 μM at 48 h. Therefore, in subsequent experiments BBR was administered to SKOV3 cells for 48 h at a concentration of 10 μM.

Knockdown of hERG1 in SKOV3 cells. To investigate the effects of hERG1 on tumor biological activities, expression levels of hERG1 in SKOV3 cells were downregulated using a shRNA-hERG1 plasmid. As presented in Fig. 3, the mRNA and protein expression levels of hERG1 in SKOV3 cells transfected

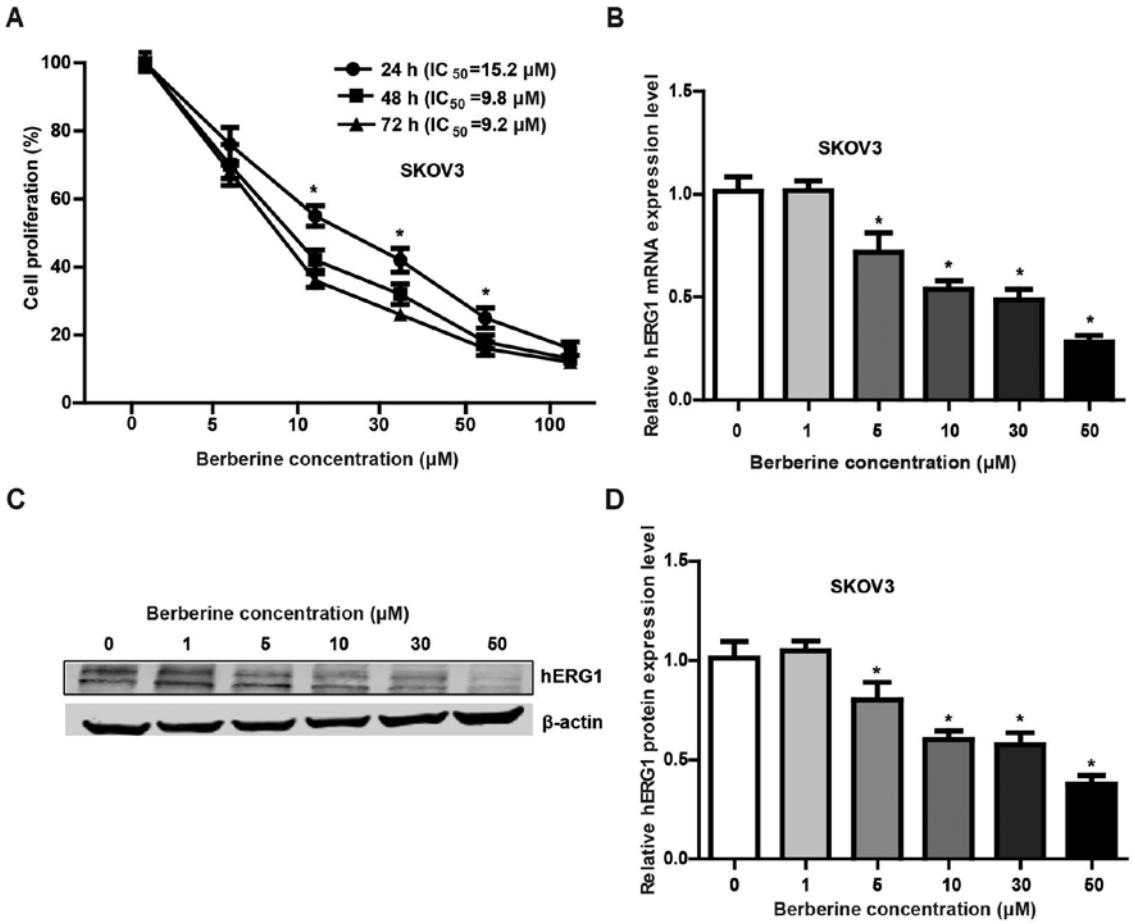

Figure 2. BBR inhibits cell proliferation and hERG1expression in SKOV3 cells. (A) BBR (10, 30, 50 and 100 μM) inhibited the growth of SKOV3 cells in a time- and dose-dependent manner. Proliferation of cells treated with indicated doses of BBR for 24, 48 and 72 h was assessed using a Cell Counting kit-8 assay. *P<0.05 vs. 0 μM. (B) BBR inhibited the expression of hERG1 mRNA in a dose-dependent manner at 48 h. *P<0.05 vs. 0 μM. (C) BBR inhibited the expression of hERG1 protein in a dose-dependent manner at 48 h. (D) Quantification of hERG1 protein expression levels. (n=3). *P<0.05 vs. 0 μM. BBR, berberine; hERG1, human ether-a-go-go-related potassium channel 1.

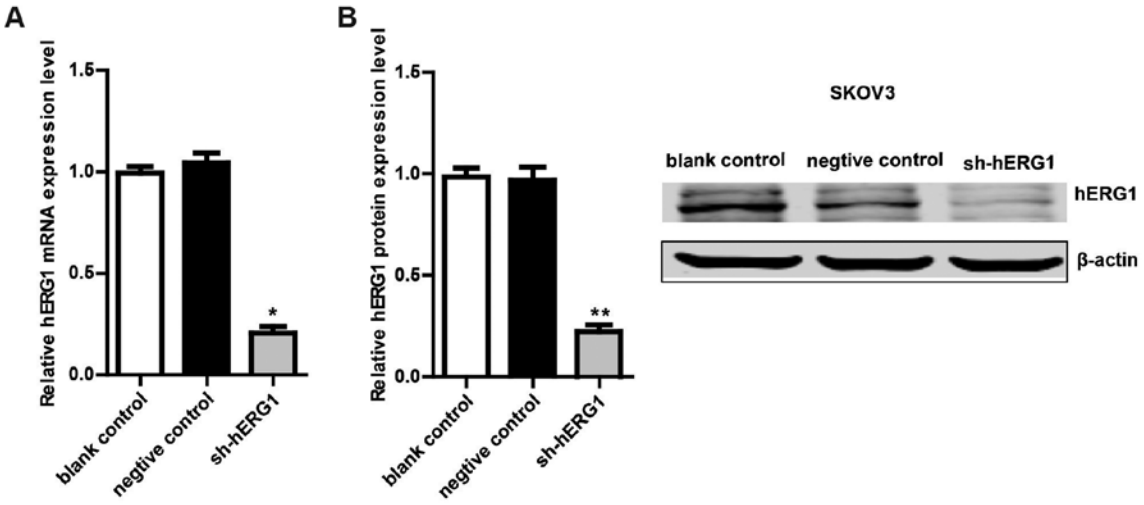

Figure 3. Knockdown of hERG1 in SKOV3 cells. (A) Knockdown of hERG1 by transfection with sh-hERG plasmid significantly reduced the mRNA expression levels of hERG1 in SKOV3 cells compared with the control group. (B) Knockdown of hERG1 gene by sh-hERG plasmid significantly reduced the protein expression of hERG1 compared with the control group in SKOV3 cells (n=3). *P<0.05, **P<0.01 vs. blank control. hERG1, human ether-a-go-go-related potassium channel 1; sh, short hairpin RNA.

with the shRNA-hERG1 plasmid were significantly decreased compared with in the control groups.

hERG1 is involved in the pathophysiological process of SKOV3 cells. The effects of hERG1 on the proliferation

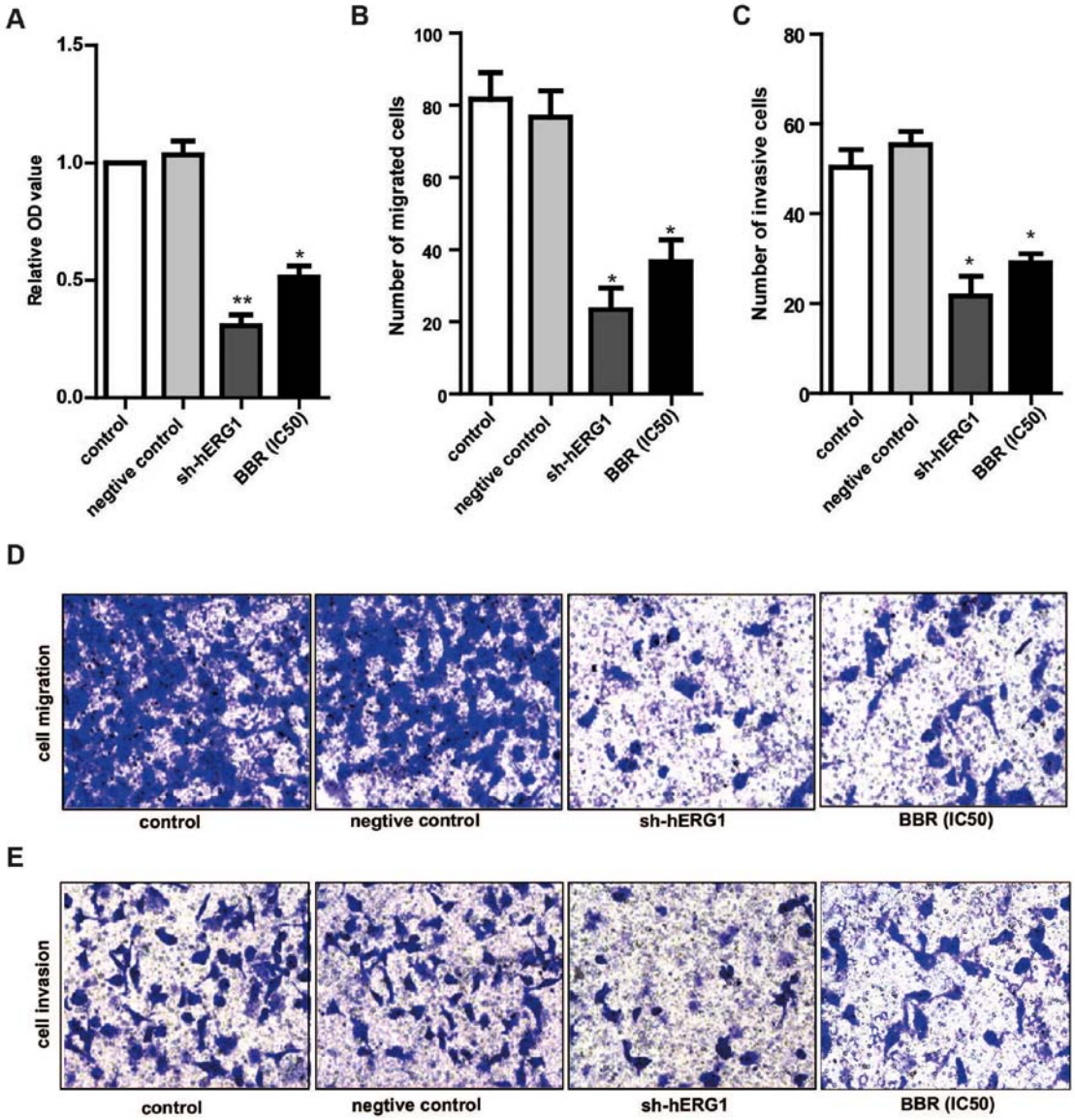

Figure 4. hERG1 is involved in the pathophysiological processes of SKOV3 cells. (A) Knockdown of hERG1 significantly reduced the cell proliferation activity in SKOV3 cells compared with in control cells. BBR at a concentration of 10 μM was used as a positive control. Quantification of the (B) migration and (C) invasion abilities of SKOV3 cells in different treatment groups. Images (magnification, x200) represent the (D) migrating cells and (E) invading cells in the Transwell and Matrigel invasion assays, respectively. n=3. *P<0.05, **P<0.01 vs. control. BBR, berberine; hERG1, human ether-a-go-go-related potassium channel 1; IC_{50}, half-maximal inhibitory concentration; sh, short hairpin RNA; OD, optical density.

of SKOV3 cells were detected *in vitro* using a CCK8 assay (Fig. 4A). The results demonstrated that knockdown of hERG1 in SKOV3 cells significantly reduced the cell proliferation activity compared with the control cells. BBR (10 μM) was considered as a positive control group. As expected, knockdown of hERG1 exhibited similar cytotoxic effects as BBR treatment. These results suggest that hERG1 may serve a role in cell proliferation.

Furthermore, to determine whether hERG1 is involved in the process of cell migration and invasion, which represent the tumor migration and invasive abilities, Transwell assays were performed following the transfection of SKOV3 cells with sh-hERG1 or scramble control. As presented in Fig. 4B-E, knockdown of hERG1 significantly reduced the migration and invasion abilities of SKOV3 cells. BBR (10 μM) was used as a positive control. The results of the present study indicate that hERG1 may be involved in the proliferation, migration and invasion processes of SKOV3 cells, and that BBR may be a potential therapeutic drug in the treatment of ovarian cancer.

Knockdown of hERG1 inhibits ovarian tumor growth in vivo. To assess whether hERG1 is involved in ovarian cancer growth *in vivo*, nude mice were inoculated with SKOV3 cells, which were previously transfected with sh-hERG1 or shRNA-control plasmids. Following the outgrowth of palpable tumors, nude mice were randomly divided into four groups (n=3 for each group) and fed via oral gavage with saline or BBR (20 mg/kg) twice per week. As presented in Fig. 5, the size and weight of ovarian cancer mass significantly decreased following the administration of BBR by oral gavage compared with the control group. Similarly, knockdown of hERG1 also significantly decreased tumor growth of xenografts compared with

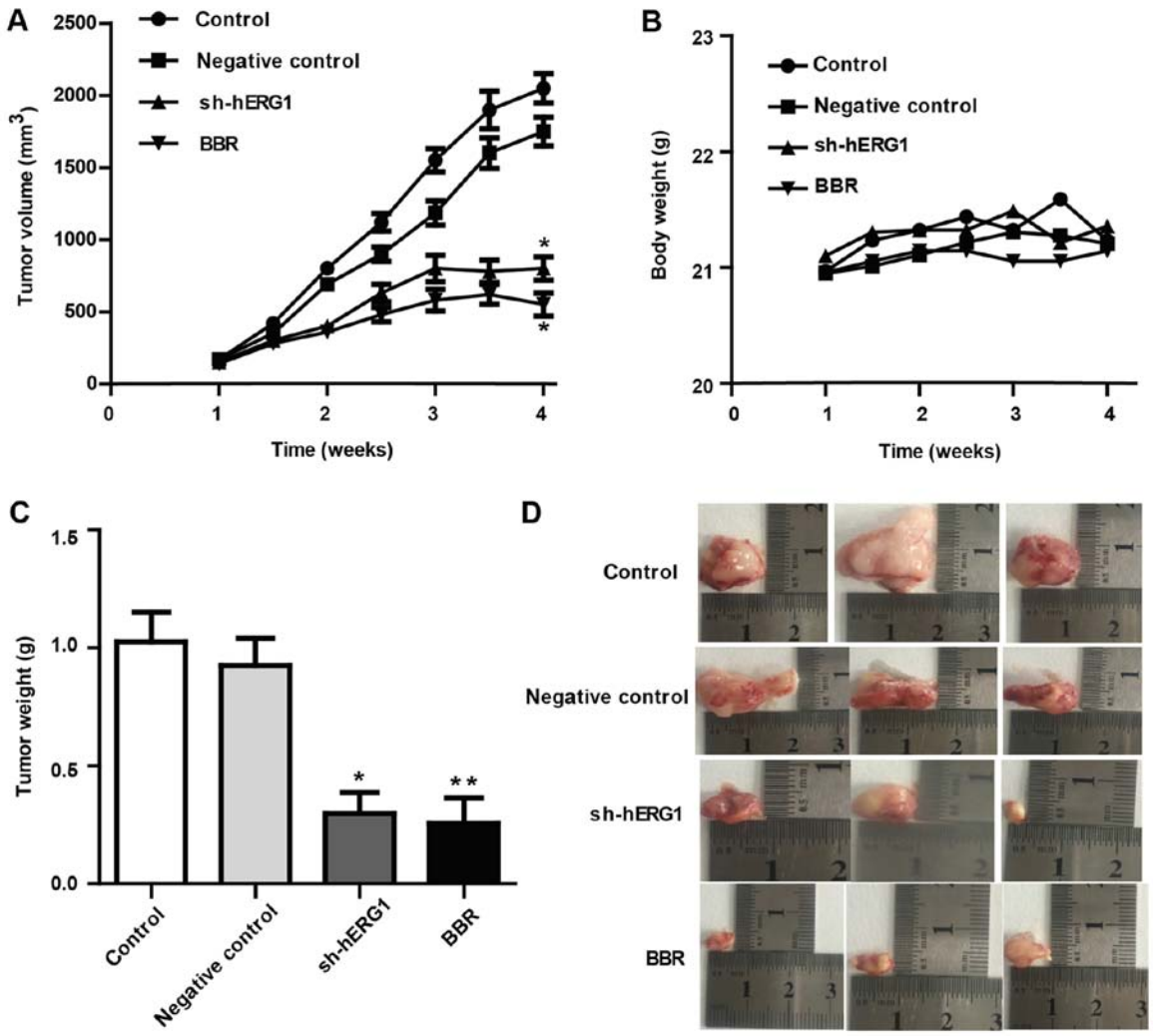

Figure 5. Knockdown of hERG1 inhibits ovarian tumor growth *in vivo*. (A) When tumors reached 100 mm^3 in ~1 week, BBR treatment was initiated. Mice in the control group were injected with saline. (B) Body weight of mice with xenografts. No notable difference was detected in the body weight of mice among the four groups. (C) Weight of tumor samples from nude mice. (D) Macroscopic appearance of the tumors at 4 weeks after drug administration. n=3. *P<0.05, **P<0.01 vs. control. BBR, berberine; hERG1, human ether-a-go-go-related potassium channel 1; sh, short hairpin RNA.

the control group. During the experiments, no notable weight loss was observed in mice of the different groups. These findings indicate that hERG1 may be involved in the pathogenesis of ovarian cancer and may be considered as a potential prognostic indicator in the treatment of ovarian cancer.

Discussion

Great efforts have been made in the treatment of ovarian cancer clinically; however, the prognosis of ovarian cancer remains poor due to drug resistance and tumor recurrence. In addition, tumor markers with appropriate specificity and sensitivity for the diagnosis and treatment of ovarian cancer remain to be identified (31,32). Therefore, it is urgent to determine novel molecular markers to improve early diagnosis and effective therapy of ovarian cancer.

Over the past decades, BBR has drawn extensive attention due to its antitumorigenic effects in various types of human cancer cells and animal models (33,34). Treatment with BBR has been reported to act on numerous targets and signaling pathways underlying pathophysiological process, including proliferation, apoptosis, angiogenesis, migration and invasion in a variety of cancer cells (35-37). In our previous study, BBR was observed to exert a strong inhibitory effect on hERG potassium channels (22,38,39), which are often aberrantly expressed in carcinoma. Therefore, the present study proposed that BBR may inhibit ovarian cancer by targeting hERG1.

In the present study, it was demonstrated that the potassium ion channel protein, hERG1, serves a pivotal role in the pathophysiological process of SKOV3 ovarian cancer cells and may be inhibited by BBR *in vitro* and *in vivo*. The results of the present study suggest that BBR exerts a strong cytotoxic effect against ovarian cancer cells. This was demonstrated by the time- and dose-dependent inhibition of the proliferation of human ovarian cancer SKOV3 cells. A similar cytotoxic effect was achieved by knockdown of hERG1, which strongly suggests that hERG1 is involved in the ovarian cancer cell proliferation process. Additionally, the migration and invasion abilities of ovarian cancer SKOV3 cells were investigated. The results of the present study revealed that knockdown of hERG1 markedly reduced the

migration and invasion abilities of ovarian cancer SKOV3 cells, which further suggests that hERG1 is an important regulator in the ovarian cancer phenotype. The results of the *in vivo* experimental results similarly supported the hypothesis of the present study, in which hERG1 was involved in the process of tumor growth and BBR could be a potential drug for the treatment of ovarian cancer.

During past decades, a number of studies have demonstrated the association between the activity of ion channels and the progression of different types of cancer, and considerable achievements have been made in understanding the role of ion channels in cancer (7,40,41). hERG1 has been reported to exhibit oncogenic properties, is often aberrantly expressed in cancer and has essential roles in numerous crucial cellular events (15,16). To the best of our knowledge, the present study revealed for the first time, that hERG1, as an important part of the potassium channel, may be the pivotal molecule to sense stimuli, transmit signals and eventually cause a series of signal pathway alterations within the cell.

In conclusion, hERG1 was demonstrated to be associated with the pathophysiological process of ovarian cancer SKOV3 cells, and the extract of a natural Chinese herbal medicine, BBR, may be of potential use as an anticancer agent for patients with ovarian cancer. The anticancer effects of BBR may be mediated by targeting the potassium hERG1 channel and eventually inducing a series of pathophysiological alterations in ovarian cancer cells. However, further studies are required to evaluate how BBR regulates the expression of hERG1 and the mechanism underlying the hERG1-mediated phenotypic alterations in ovarian cancer.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

DZ was responsible for the design of the study and writing of the article. KZ was a major contributor in data analysis and was responsible for some experimental operations. XF performed the western blotting experiments. JZ performed the cell culture, transfection and proliferation assays. XL performed RT-qPCR, Transwell assays and the *in vivo* tumor xenograft study. DY was responsible for the immunohistochemical assays, and the revision of the article. MD was responsible for the design of the study and revision of the article. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study has been approved by the Ethical Committee of the Harbin Medical University Cancer Hospital (Harbin, China) and written informed consent was obtained from all recruited patients.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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