Sinomenine hydrochloride exerts antitumor outcome in ovarian cancer cells by inhibition of long non-coding RNA HOST2 expression

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ABSTRACT
Background: Accumulating evidence displays that sinomenine hydrochloride (SH) are utilised to treat a variety of cancers. Nevertheless, the influences of SH on ovarian cancer stayblurry. We endeavoured to uncover the antitumor effects of SH on ovarian cancer and underlying mechanism(s).

Methods: Human ovarian epithelial cell line (HOEpiC), Caov3 and SKOV3 cells were administrated with SH and/or transfection with pc-long non-coding RNA (IncRNA) human ovarian cancer-specific transcript 2 (HOST2), then cell viability, cell cycle and apoptosis and the related-proteins were respectively inspected by MTT, flow cytometry, and Western blot. In addition, expression of HOST2 was investigated by real-time PCR.

Results: SH remarkably repressed cell viability, evoked apoptosis and induced cell cycle arrest in G0/G1. Moreover, SH statistically decreased HOST2 expression in Caov3 and SKOV3 cells. Overexpression of HOST2 significantly reversed the effects of SH on Caov3 cell viability, cell cycle and apoptosis. Clinical findings confirmed that HOST2 was profoundly higher expressed in ovarian cancer tissues and cells, and HOST2 predicted unfavourable prognosis of ovarian cancer individuals.

Conclusion: Our findings recommended that SH exerted the antitumor effect in ovarian cancer cells by hindering expression of HOST2.

INTRODUCTION
Ovarian cancer is a devastating gynecological malignant tumour, disturbing approximate 2–3% of women all over the world [1]. Even though substantial improvements have been undertaken for management approaches, ovarian cancer still characterises high mortality [2]. It has been reported that the 5-year survival rates are still below 45% due to the asymptomatic behaviour and the advanced stage at diagnosis [3]. Recently, about 295,414 new cases were identified and 184,799 deaths happened worldwide in 2018 [4]. Currently, the main treatments of ovarian cancer include cytoreductive operation, platinum-based chemotherapies, nanotechnology and immunological therapy [5,6]. Nevertheless, some severe side effects, such as acute and long-term toxicities, are frequent and diverse [7]. Consequently, further investigation or exploration is indispensable to develop novel approaches to lessening the burden of ovarian cancer.

Increasing evidence has suggested that Traditional Chinese Medicine (TCM) plays a decisive role in treatment of many human diseases including cancers for a long history. Sinomenine (SN) is a pure alkaloid which acquired from the roots and stems of Sinomenium actum Rehd.et Wils. [8]. Recently, more and more experts and scholars pour their attention into the therapeutic action of SN. They confirmed that SIN and its water-soluble form, sinomenine hydrochloride (SH), exert diverse functions in vitro or in vivo, such as anti-angiogenesis [9], anticonvulsant [10], anticarcinogen, anti-inflammation [11] and immunosuppression possessions [12]. A recent study demonstrated that SH increased sensitivity to ionising radiation in cervical cancer cells [13]. However, whether SIN displays the antitumor influences on ovarian cancer is still absence of adequate studies.

In recent times, long non-coding RNAs (lncRNAs) have been documented to activate or repress gene expression, playing critical roles in physiology and pathology of human diseases, including ovarian cancers [14,15]. Human ovarian cancer-specific transcripts (HOSTs) are rich in ovarian cancer, however, are infrequently expressed in normal or other malignancy tissues [16]. HOSTs have 5 five transcripts, namely HOST1, HOST2, HOST3, HOST4 and HOST5 [16]. Until now, HOST2 has been identified as novel member of lncRNAs family. It is 2.9 kb in length but does not have an apparent open reading frame (ORF) [16]. More interestingly, Gao et al. exposed that HOST2 endorses tumour cell proliferation, migration and invasion in epithelial ovarian cancer [17]. However, little information is available regarding the expression of HOST2 in ovarian cancer individuals and whether HOST2 can be utilised as a therapeutic target is covered.
Therefore, in this research, we attempted to explore whether SH demonstrates the antitumor outcome on ovarian cancer cells by assessing cell viability, cell cycle, and cell apoptosis. In addition, we enrolled ovarian cancer individuals, examined the expression of HOST2 and confirmed the connection of HOST2 and prognosis. Finally, we investigated whether SH exerts the antitumor effects by regulating the expression of HOST2. These discoveries might afford a new insight into clinical ovarian cancer medication with SH.

Materials and methods

Collection of tissues in individuals with ovarian cancer

We collected the ovarian cancer tissues and neighbouring non-tumour tissues from 66 individuals who underwent ovarian cancer operation at the Department of Gynaecology in Jining No.1 People’s Hospital from February 2014 to February 2019. Ovarian cancer was diagnosed by histological examination. None received specific therapy, such as chemotherapy or radiotherapy, prior to the surgery. The collected tissues were instant frozen for further valuation. Moreover, individuals with ovarian cancer were followed up for up to 36 months. Our research was allowed by the Ethics Committee of Jining No.1 People’s Hospital. Written informed consents were obtained by all the members.

Cell culture

A human ovarian epithelial cell line (HOEpiC) and 5 ovarian cancer cell lines (A2780, Caov3, Hey, OVCAR3, and SKOV3) were gotten from Chinese Type Culture Collection (Chinese Academy of Sciences, Beijing, China). These cells were preserved in RPMI 1640 medium (Life Sciences), complemented with 10% foetal bovine serum (FBS, Life Sciences), 1% penicillin and streptomycin (Sigma, MO, USA) at 37°C in a moistened air atmosphere comprising 5% CO2.

Cell treatment and transfection

For cell treatment, different concentrations of SH (CAS number 6080-33-7, Sigma) (0.25, 0.5, 0.75 and 1 μm/ml) were administrated to HOEpiC, Caov3 and SKOV3 cells for 24 h. The concentrations were referred a previous study [18]. The chemical structure of SH was shown in Figure 1. The molecular formula of SH is C19H23NO4·HCl, and the molecular weight of SH is 365.85.

Real-time quantitative PCR (RT-qPCR)

Entire RNA was extracted from both ovarian cancer tissues or different ovarian cell lines using TRIzol reagent (Invitrogen). First-strand cDNA was created using an oligo (dT) primer and Superscript III Reverse Transcriptase (Life Technologies). PCR was accomplished based on the subsequent procedure: 1 min at 94°C; 40 cycles of 10 s at 98°C, 30 s at 60°C, and 30 s at 72°C, followed by a final extension of 5 min at 72°C. The comparative RNA level was presented using the 2^DDCT manner. β-actin was used as the internal reference.

Western blot analyses

Total proteins were acquired from the cell lines using RIPA lysis buffer (Beyotime, China) with protease inhibitor cocktail
CDK4 and CDK6 in both Caov3 and SKOV3 cells. SH meaningfully reduced all the protein levels of CyclinD1, CDK4 and CDK6. The quantitative results demonstrated that expression of cell cycle-related proteins comprising CyclinD1, CDK4 and CDK6 (all \( p < .05 \)) in a dose-dependent manner in both Caov3 and SKOV3 cells. Moreover, we observed that SH noticeably diminished the level of Bcl-2, but upraised the levels of Bax and cleaved-caspase 3 in Caov3 (\( p < .01 \) or \( p < .001 \), Figure 5(D–E)). Similarly, we observed that SH noticeably diminished the percentage of cells in G0/G1 phase (\( p < .05 \) or \( p < .001 \), Figure 5(F)). These outcomes suggested that SH evoked apoptosis in Caov3 and SKOV3 cells.

**SH decreases the expression of HOST2**

In consideration of the functional parts of HOST2 in ovarian cancer tissues and cell lines, we subsequently discovered whether SH stimulation can affect HOST2 expression in Caov3 and SKOV3 cells. Results in Figure 4(A,B) revealed that SH administration conspicuously lessened HOST2 expression in both Caov3 and SKOV3 cells, as compared to the control groups (\( p < .05 \) or \( p < .01 \) or \( p < .001 \)) in a dose-dependent way. These involved results illustrated that SH could decrease HOST2 expression in Caov3 and SKOV3 cells.

**SH restraints cell growth, cell cycle arrest and apoptosis via inhibition of HOST2 in SKOV3 cells**

To further inquiry the influences of HOST2 in ovarian cell growth, cell cycle arrest and apoptosis, HOST2 was overexpressed in SKOV3 cells. As expected, the enhancement of HOST2 was observed in SKOV3 cells (\( p < .001 \), Figure 5(A)), implying high transfection efficiency. Thereafter, after transfection with pc-HOST2, we administrated with SH and assessed the impacts of SH on cell viability, cell cycle and cell apoptosis, as well as related-proteins. Interestingly, we observed that overexpression of HOST2 statistically reversed the inhibition of cell viability induced by SH (\( p < .05 \), Figure 5(B)). In addition, the data presented that overexpression of HOST2 markedly decreased the percentages of cells in G0/G1 phase (Figure 5(C)). The cell cycle-related proteins also demonstrated that HOST2 overexpression significantly elevated the expression of CyclinD1, CDK4 and CDK6 (all \( p < .05 \), Figure 5(D–E)). Simultaneously, overexpression of HOST2 statistically overturned the enhancement of cell apoptosis induced by SH (\( p < .01 \), Figure 5(F)). The results of apoptosis-related proteins confirmed the results, namely, overexpression of HOST2 notably elevated the expression of Bcl-2, while markedly decreased the levels of Bax and cleaved-caspase 3 induced in SKOV3 cells (\( p < .05 \) or \( p < .01 \), Figure 5(G–H)). All above-mentioned discoveries recommended that SIN restrained cell growth, cell cycle arrest and apoptosis via inhibition of HOST2.

**HOST2 is unregulated in ovarian cancer tissues and cell lines and predicts unfavourable prognosis**

The expression of HOST2 was distinguished by qPCR in 66 ovarian cancer individuals and adjacent non-tumour cancer tissues. As exposed in Figure 6(A), the RNA expression of HOST2 was highly expressed in ovarian tumour tissues, but was lowly expressed in non-cancer tissues, with a noteworthy
Figure 2. Sinomenine hydrochloride (SH) reserves cell viability and induces cell arrest in G0/G1 phase in Caov3 and SKOV3 cells. HOEpiC, Caov3 and SKOV3 cells were exposed to varied concentrations of SH (0, 2.5, 5, 7.5 and 10 μM/ml) for 24 h. A, SH had no impact on cell viability of HOEpiC cells; B and C, SH administration presented a significant inhibition of cell viability in Caov3 and SKOV3 cells in a dose-dependent manner; D and E, SH statistically raised the cells in G0/G1 phase but dismissed the percentage of cells in S phase in Caov3 and SKOV3 cells; F, SH meaningfully reduced the protein levels of CyclinD1, cyclin-dependent kinase (CDK4) and CDK6 in Caov3 cells; G, representative pictures of cell cycle-related proteins in Caov3 cells; H, SH meaningfully reduced all the protein levels of CyclinD1, CDK4 and CDK6 in SKOV3 cells; I, representative pictures of cell cycle-related proteins in SKOV3 cells. *p < .05, **p < .01, ***p < .001 compared to the corresponding controls.

Figure 3. Sinomenine hydrochloride (SH) induces apoptosis in Caov3 and SKOV3 cells. Caov3 and SKOV3 cells were exposed to SH (7.5 μM/ml) for 24 h. A, SH markedly unregulated the percentage of apoptotic rates in Caov3 cells; B, SH obviously diminished the level of Bcl-2, but upraised the levels of Bax and cleaved-caspase 3 in Caov3 cells; C, representative pictures of cell apoptosis-related proteins in Caov3 cells; D, SH decisively increased the percentage of apoptotic rates in SKOV3 cells; E, SH clearly weakened the level of Bcl-2, but elevated the levels of Bax and cleaved-caspase 3 in SKOV3 cells; F, representative pictures of cell apoptosis-related proteins in SKOV3 cells. **p < .01, ***p < .001 compared to the corresponding controls.
difference \( (p < .01) \). In addition, we explored the connection between HOST2 expression and ovarian cancer individuals prognosis. Of the 66 participants, 36 individuals were categorised as high expression group, while 30 individuals were categorised as low expression group. The outcomes of follow up presented that the higher the expression of HOST2, the worse and the unfavourable prognosis \( (p = .0146) \) (Figure 6(B)). These above statistics specified that HOST2 was related...
to the poor prognosis of ovarian cancer individuals. Furthermore, we assessed the expression of HOST2 in different ovarian cancer cell lines. As shown in Figure 6(C), the data demonstrated that compared to the HOEpiC cells, HOST2 was enhanced in different ovarian cancer cell lines ($p < 0.05$ or $p < 0.01$) compared to the corresponding controls.

Discussion

In this research, we explored the therapeutic action of SH and its underlying mechanism(s) in ovarian cancer. We confirmed that SH had no cytotoxicity on normal HOEpiC at certain concentrations, but exhibited statistic repression of cell growth and increase of cell apoptosis in Caov3 and SKOV3 cells. In addition, the data showed that HOST2 was significantly increased in ovarian cancer tissues and cell lines, and higher HOST2 expression and worse survival rate. Interestingly, we identified that SH could also decrease the expression of HOST2 in a dose-dependent manner, and overexpression of HOST2 partly reversed the effects of SH on cell viability, cell cycle and cell apoptosis. In summary, HOST2 might be an effect target of SH on management of ovarian cancer.

SIN is a pure alkaloid taken out from *Sinomenium acutum* Rehd. et Wils [19]. It has been well acknowledged that SIN presents a variety of therapeutic actions, such as anti-inflammation and anti-immune. SH is a form of hydrochloride of SIN, has recently been identified to possess an anti-proliferative consequence on tumour cells [20–22]. Nonetheless, the exact mechanism is still blur. Herein, we probed the special possessions of SH on human ovarian cancer cells and inspected the potential fundamental mechanism(s). Uninhibited proliferation of tumour cells plays an indispensable role in progression and development of cancers [23], we attempted to inspect whether SH repressed ovarian cancer cell proliferation. Our findings established that SH inhibited Caov3 and SKOV3 cell viability in a dose-dependent way, however, SH had no cytotoxic outcome on HOEpiC. These results implied that SH could repress the ovarian cancer cell proliferative action.

To elucidate the fundamental mechanisms for the anti-proliferative consequence of SH, we examined the cell cycle and cell apoptosis of Caov3 and SKOV3 cells after administration with SH. It has been well-recognized that dysfunction of G1 phase progression are existed in most human malignant tumour cells. Our findings exhibited that SH management triggered a significant escalation in the percentage of cells in G0/G1 stage and a reduction in the proportion of cells in S stage. Mechanically, cell cycle changes are firmly delimited by a sequence of cell cycle supervisors. CyclinD1, an imperative and optimistic regulator, cooperates with CDK4 and CDK6 to produce holoenzymes and can next phosphorylate the tumour-suppressor protein Rb in G1 stage, playing critical roles in cell cycle transitions. Our data revealed that SH management lowered the expression of the cell cycle-positive supervisors, comprising CyclinD1, CDK4 and CDK6, which was in line with previous research [18,24,25]. In addition to cell proliferation, cell apoptosis is another one of the main targeted mechanism for cancer management [26]. Our results proved that SH prompted apoptosis in both Caov3 and SKOV3 cells. The molecular mechanism(s) were further explored by measuring the expression of anti-apoptotic protein Bcl-2 and pro-apoptotic protein Bax and caspase3. Administration of SH markedly decreased the levels of Bcl-2 but elevated the expression of Bax and caspase3, and our results were similar with Lu et al. [22], Jiang et al. [21], and Deng et al. [27].
HOST2 is a newly discovered IncRNA, and it was originally found in ovarian cancer [16] and exerted proliferative effects and migration facilitation in ovarian cancer [17]. However, accumulating evidence proposes that HOST2 also plays a part in other cancer cells, such as hepatocellular carcinoma [28], breast cancer [29,30], pancreatic cancer [31], HPV-positive cervical cancer [32] and gastric cancer [33]. Therefore, the functions of HOST2 in cancer cells deserve further research. Based on the above studies, we assumed that SH might display its functions by regulating HOST2. We evaluated the expression of HOST2 after administration with SH and interestingly, we found that SH could downregulate the expression of HOST2 in both Caov3 and SKOV3 cells in a dose-dependent way. This was the first research confirming the relationship between SH and HOST2. To confirm whether SH exerted its functions by downregulating the expression of HOST2, we overexpressed the HOST2 by transfection with pc-HOST2. As expected, the data demonstrated that after overexpression of HOST2, the effects of SH on cell viability, cell cycle and apoptosis were partly and significantly reversed. Furthermore, we investigated and verified the functions of HOST2 in clinical specimens. As shown in our data, HOST2 was highly expressed in cancer tissues compared to the non-tumour tissues, and HOST2 predicted unfavourable prognosis in ovarian cancer individuals. Our clinical research further confirmed the tumour-promoting action of HOST2 and it might be a potential therapeutic target in ovarian cancer. However, the internal research mechanism conserving the SH and HOST2 is also valuable to study more.

In summary, our study verified that SH exerted the antitumor outcome in ovarian cancer cells, and these effects might be achieved by hindering expression of HOST2.

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Disclosure statement
No potential conflict of interest was reported by the authors.

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