Arsenic sulfide inhibits the progression of gastric cancer through regulating the circRNA_ASAP2/Wnt/β-catenin pathway

Jing Hu[a], Bin Hu[a], Li Deng[a], Lin Cheng[a], Qunhong Fan[a] and Caibao Lu[b]

In our paper, the effects of As_4S_4 treatments on the growth and migration of gastric cancer (GC) cells were explored, and the potential underlying molecular mechanisms were also identified. Cell viability was evaluated by cell counting kit 8 assay. The expression of Ki-67 was examined using immunofluorescence staining. Cell apoptosis was assessed by flow cytometry. The migratory and invasion abilities of cells were determined using Transwell assay. The mRNA and protein levels of related gene were examined by RT-qPCR and western blotting, respectively. CircRNAs chip was performed to identify the differentiated expression of circRNAs in GC cells following the treatment with As_4S_4. Our results revealed that the proliferation, migration and invasion of GC cells were remarkably suppressed by the treatment with As_4S_4, while cell apoptosis was promoted. Furthermore, circRNA_ASAP2 was a novel target of As_4S_4 in GC, and it is involved in As_4S_4-modulated biological behavior alterations in GC cells. In addition, the activities of the Wnt/β-catenin signaling in GC cells were affected by the overexpression circRNA_ASAP2 and the treatment with As_4S_4. Moreover, the behavior changes in GC cells caused by the knockdown of circRNA_ASAP2 were reversed by the treatment with Wnt agonist SKL2001. In summary, As_4S_4 could function as an antitumor agent in GC through regulating the circRNA_ASAP2/Wnt/β-catenin pathway, which in turn influences the growth and metastasis of GC cells. *Anti-Cancer Drugs* 33: e711–e719 Copyright © 2021 The Author(s). Published by Wolters Kluwer Health, Inc.

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Keywords: arsenic sulfide, circRNA_ASAP2, gastric cancer, Wnt/β-catenin

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Introduction

Gastric cancer (GC) is one of the most aggressive tumors and the second leading cause of cancer-related mortality. In recent years, the global occurrence of GC has been increasing [1,2]. The molecular mechanisms underlying involved in the tumor progression of GC are complex, including the networking of different pathways. Therefore, the pathogenesis of GC is still not completely understood. Furthermore, the prognosis and survival of GC patients is poor [1–4]. Although recent development has been made on the treatments of GC using endoscopic and surgical resection, the therapeutic outcomes of GC patients are still unfavorable [4,5]. A lot of factors are associated with the prognosis of GC patients, including the incidence of metastasis and the staging at diagnosis, and the overall mortality of GC patients at late stages is quite high [1–5]. Therefore, it is urgent to explore the molecular mechanisms involved in the development of GC. More importantly, the treatment plans of GC patients could be optimized in future clinical practice.

Arsenic sulfide (As_4S_4) is a key ingredient of realgar, a type of traditional Chinese medicine which has been used for the treatment of leukemia [6,7]. More recently, the antitumor activity of arsenic has been well studied, and arsenic and its combinations have been used for the treatment of cancer [6,7]. Previous studies have revealed that As_4S_4 is able to inhibit the growth and migration of tumor cells in numerous types of cancer including colon and GC [8–11]. Furthermore, As_4S_4 could promote apoptosis and suppress the migration/invasion of GC cells both in vitro and in vivo [9,10]. In addition, As_4S_4 may inhibit the progression of GC through regulating its downstream miRNAs such as miR-4665-3p [11]. Moreover, As_4S_4 induced the apoptosis of GC cells via inhibiting NFATc3 [12,13]. However, the detailed molecular mechanisms underlying As_4S_4-modulated GC cell growth and migration remain largely unknown and require further investigation.

CircRNAs are a group of noncoding RNAs with a continuous loop [14,15]. As they are lack free 5'- and 3'- overhangs, circRNAs are resistant to the degradation by exonuclease [14,15]. CircRNAs exert their regulatory function as 'sponges' of miRNAs, and previous studies have elucidated the biological roles of circRNAs and the molecular mechanisms [16]. CircRNAs are key regulators of gene expression,

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but the detailed functions of most circRNAs are unclear [14–16]. Recently, the involvement of circRNAs during the onset and progression of cancer has been revealed [17,18]. CircRNAs are novel biomarkers with diagnostic and therapeutic values for patients with cancer [17–21]. Moreover, impaired levels of circRNAs have been detected in different types of cancer, which are associated with the growth and migration of tumor cells [22–25]. Furthermore, a recent study has indicated that circRNA_ASAP2 is involved in the development of GC [26]. However, the detailed functions and downstream signaling of circRNAs in the pathogenesis of GC are still unknown.

Wnt/β-catenin signaling is highly conserved, and it takes part in the regulation of biological processes such as cell proliferation, differentiation, apoptosis, migration and invasion [27]. Aberrant activity of the Wnt/β-catenin pathway could induce the renewal of cancer stem cells and promote the proliferation/differentiation of tumor cells, consequently leading to tumor progression [28]. Dysregulation of Wnt/β-catenin has been observed in numerous types of cancer including GC, and targeted therapies against Wnt/β-catenin signaling have been developed in cancer treatment [29–32]. Furthermore, previous reports have revealed the regulatory effects of arsenic on the Wnt/β-catenin pathway in cancer [33].

In this paper, we aimed to explore the effects of As_4S_4 treatments on tumor progression of GC, and the biological behavior changes such as proliferation, apoptosis, migration and invasion in GC cells treated with As_4S_4 were examined in vitro. Additionally, the novel targets of As_4S_4 were also identified. Our results revealed that circRNA_ASAP2 and the Wnt/β-catenin signaling were involved in As_4S_4-modulated biological behavior alterations in GC cells. Taken all together, As_4S_4 could exert antitumor roles on GC by regulating the circRNA_ASAP2/Wnt/β-catenin pathway, subsequently affecting the growth and metastasis of GC cells. More importantly, our findings may provide guidance for the treatment of GC patients in future clinic.

Materials and methods

Cell culture and reagent

Two human GC cell lines (AGS and HCG-27) and normal gastric epithelial cells (GES-1) were purchased from Beijing ComWin Biotech co. Ltd. (Beijing, China). HCG-27 and GES-1 cells were cultured using RPMI 1640 medium, and AGS cells were maintained in DMEM/F12 medium (1:1). All culture media were supplemented with 10% FBS, 100 μg/ml streptomycin and 100 U/ml penicillin (all purchased from GE Healthcare Life Science). Cells were cultured in a humid incubator at 37°C supplied with 5% CO_2. The solution of mined natural realgar with high purity was prepared using PBS (GE Healthcare Life Science) and sterilized using a filter. The concentration of As in PBS solution was evaluated using inductively coupled plasma atomic emission spectrometry, and the experiments were performed at the Instrumental Analysis Centre in Shanghai Jiao Tong University (Shanghai, China). The concentration of As_4S_4 stock solution was 211.39 μM, and the solution was kept at 4°C until further use. According to the blood arsenic levels in patients treated with As_4S_4, stock solution was diluted to prepare working solutions ranged from 0.125 to 8 μM using culture media. In further function studies, cells were treated with 1 μM/2 μM As_4S_4 or 40 μM Wnt Agonist II (SKL2001; Calbiochem, UK) for 48 h as indicated.

Cell transfection

To generate the knockdown model of circRNA_ASAP2, shRNAs of circRNA_ASAP2 (sh-circRNA_ASAP2) and the negative control (sh-NC) were purchased (Genepharm Co. Ltd., Shanghai, China). Then, shRNA segments were annealed and cloned in pU6-Luc-Puro lentivirus vector (Genepharm Co. Ltd.). In order to produce GC cells overexpressing circRNA_ASAP2, wildtype (pc-circRNA_ASAP2) or mutant (pc-NC) fragments were amplified using PCR and subsequently inserted into PLCDH-cir vector (Invitrogen; Thermo Fisher Scientific, Waltham, USA). The up- or downregulation of circRNA_ASAP2 was confirmed using reverse transcription-quantitative PCR (RT-qPCR). All the transfections were carried out using Lipofectamine 3000 (Invitrogen; Thermo Fisher Scientific). Following 24 h, culture supernatants were aspirated, and complete culture media were added for further culture.

RT-qPCR

RNAs were isolated with the TRIzol reagent (Invitrogen; Thermo Fisher Scientific). Extracted RNAs were reverse transcribed using a PrimeScript RT kit (TaKaRa Biotechnology Co., Ltd., China). Subsequently, qPCR was conducted using SYBR Green PCR Master Mix (TaKaRa Biotechnology Co., Ltd.). PCR reactions were performed on an ABI system (ABI 7500; Thermo Fisher Scientific). GAPDH was used as control. Forward and reverse primer pairs used for qPCR were: circRNA_ASAP2, 5′-CCTGACCTGCATCGAGTGTT-3′ and 5′-GTAAGTTCTGTCATCAGCAGCTC-3′; DVL2, 5′-CATGAGCAATGACGATGCTGTG-3′ and 5′-AGCTG GATCAATTGGCTGTATGG-3′; GSK-3β, 5′-GAGC CACTGATTACACGTCCAG-3′ and 5′-CCAACTGAT CCACACCACTGTC-3′; β-catenin, 5′-CACAAGCA GAGTGCTGAAGGTG-3′ and 5′-GATTCCTGAGAG TCCAAAGACAG-3′; CyclinD1, 5′-CATCTACACCG ACAACTCCATC-3′ and 5′-TCTGGCATTTTGG AGAGGAAG-3′; GAPDH, 5′-GTCTCCTCTGACT TCAACAGCG-3′ and 5′-ACCACCCTGTTGCTGT AGCCAA-3′. The program used for qPCR was as followed: 95°C for 5 min, 45 cycles of 95°C for 15 s, 60°C for 20 s and 72°C for 10 s, followed by final extension at 72°C for 2 mins.

Immunofluorescence analysis of Ki-67

Fixation of cells was carried out using ice-cold acetone (SigmaAldrich, Darmstadt, Germany), and cells were

incubated at room temperature for 20 mins. Subsequently, cells were washed in PBS three times and blocked with FBS for 30 mins. Then, cells were incubated with primary antibody against Ki-67 (1:100; cat. no. 9129; Cell Signaling Technology) at 4°C overnight. The following day, cells were further incubated using a secondary antibody conjugated with Alexa-Fluor 568 (1:2000, Molecular Probes, Eugene, Oregon, USA) in dark at room temperature for 1 h. The cells incubated with secondary antibody alone were used as negative control. Cell nuclei were counterstained with DAPI solution (Vector Laboratories, Peterborough, UK). Then, cells were washed and mounted to a glass slide using Mowiol solution containing 10% Mowiol D488 (Calbiochem). The intensity of staining was checked under Leica DMLB microscope. Images were captured with CCD camera (Cool-SNAP-Pro; Media Cybernetics, Rockville, Maryland, USA) and analyzed using Image-Pro Plus (version 6.0; Media Cybernetics).

Western blot analysis

Total protein was isolated using RIPA buffer (Beyotime Institute of Biotechnology, Shanghai, China). The concentration of extracted protein samples was determined using a BCA assay kit (Beyotime Institute of Biotechnology). Equal amounts of samples (40 μg) were used for SDS-PAGE (10% gel), and separated samples were further transferred on nitrocellulose membranes (Millipore, Billerica, Massachusetts, USA). Then, block of membranes was performed using PBS solution containing 5% skimmed milk, and samples were kept on a shaker for one hr. Membranes were incubated using primary antibodies against DVL*2* (1:1000; cat. no. 32165; Cell Signaling), p-GSK-3β (1:500; cat. no. 9336S; Cell Signaling), cyclinD1 (1:2000; cat. no. 2978T; Cell Signaling), β-catenin (1:2000; cat. no. 9562S; Cell Signaling) or β-actin (1:500; cat. no. 4970S; Cell Signaling) in cold room with shaking overnight. Next day, membranes were rinsed and incubated with secondary antibodies conjugated with HRP: anti-rabbit IgG (1:10 000; cat. no. 7074; Cell Signaling) or anti-mouse (1:10 000; cat. no. 7076; Cell Signaling) for 1 h. Protein bands were visualized using an ECL kit (Pierce Biotechnology; Thermo Fisher Scientific). Blots were quantified using densitometry with Image J (NIH, Bethesda, Maryland, USA).

Cell counting kit 8 assay

To evaluate the IC_{50} value of As_4S_4, the viability of GC cells was examined using a cell counting kit 8 (CCK8) assay after treatment. Briefly, cells were seeded onto 96-well plates at the density of 1×10^4 cells per well and incubated overnight. Next day, cells were treated with As_4S_4 at serial concentrations (ranged from 0.125 to 8 μM) for 24, 48 or 72 h, respectively. Otherwise in function study, cells were placed onto 96 wells, and the proliferative activity of cells was determined on days 1, 2, 3 and 4. In each assay, 10 μL of CCK8 solution (Dojindo Molecular Technologies, Inc., Kumamoto, Japan) was added to each cell. Subsequently, cells were incubated for an additional 2 h, and the absorbance (wavelength =450 nm) was evaluated and recorded on microplate reader. IC_{50} was calculated using SPSS (version 25.0; SPSS, Inc., Chicago, Illinois, USA).

Migration and invasion assay

The migratory and invasive abilities of cells were determined by a Transwell assay. In migration assay, a total of 1×10^4 cells were diluted in serum-free media and inoculated onto the upper chamber of Transwell (pore size =8 μm; BD Biosciences, Franklin Lakes, USA). In invasion assay, the upper chamber was precoated with Matrigel (Sigma-Aldrich, St. Louis, USA). Briefly, 500 μl of complete culture media was added into the lower chamber, and cells were incubated for two days. Nonmigratory/-invasive cells were then removed with a cotton bud. Then, migrated/invaded cells were fixed using ice-cold methanol at room temperature for 10 min, followed by the staining with 0.5% crystal violet. Stained cells were subsequently counted in ten random fields using an inverted light microscope with magnification at ×200 (Olympus Corporation, Japan).

Evaluation of cell apoptosis

To determine the cell apoptotic rate, cells were seeded onto six wells plate at the density of 5×10^4/well and then centrifugated. Cell pellets were rinsed and fixed using ice-cold 70% ethanol for 30 mins. Then, cells were centrifugated and re-suspended in PBS. The Annexin V-fluorescein isothiocyanate (FITC)/propidium iodide (PI) apoptosis kit (BD Biosciences, Franklin Lakes, New Jersey, USA) was employed to assess cell apoptotic rate. Cells were incubated using 10 μL Annexin V-FITC as well as 5 μL PI solution in cold room for 30 min in dark, then cell apoptotic rate was evaluated with a flow cytometer (BD Biosciences). The results were analyzed using Flowjo (version 10.6, Flowjo LLC, Ashland, Oregon, USA).

CircRNAs chip

Total RNA was extracted with TRIzol (Invitrogen; Thermo Fisher Scientific) and RNeasy kit (Qiagen, Germany). For circRNA array, fluorescence-labeled targets were generated. Human circRNAs array (v*2*; CapitalBio Technology) was performed with ~15 000 genes mounted onto the chip, and target sequences of circRNAs were obtained from Circbase. Labeled targets were then hybridized with the samples, which were then scanned using an Agilent Microarray Scanner (Agilent Technologies, Santa Clara, California, USA). Data were normalized according to the Quantile algorithm. Experiments were performed according to the protocols provided by Agilent Technologies Inc. Shanghai Corporation. Fold change >*2* was used for the analysis of differentially expressed circRNAs. The five most

up- or downregulated circRNAs were presented. Has_circ_0006089 was used for further experiments.

Statistical analyses

All the data were presented as means ± standard error of mean. Data were analyzed with SPSS (version 25.0; SPSS, Inc., USA). The significance of differences between/among experimental groups was determined using the Student's *t* test or one-way ANOVA, and post hoc Tukey test was performed following ANOVA. The statistically significant difference was indicated as $P < 0.05$.

Results

Treatment with As_4S_4 was able to inhibit the growth and migration of GC cells

In order to investigate the biological behavior alteration of GC cells following the treatment with As_4S_4, AGS and HGC-27 cells were treated with As_4S_4 at serial concentrations (0.125–8 μM). The results indicated that as the concentration of As_4S_4 increased, the viability of GC cells was reduced in a dose-dependent manner (Fig. 1a,b). IC_{50} of As_4S_4 in both cell lines fell within the range of 1–2 μM, thus GC cells were treated with As_4S_4 at concentrations of 1 or 2 μM in further function studies. Moreover, to confirm the findings of the CCK8 assay, immunofluorescence staining of proliferation-associated marker Ki-67 was performed on treated cells. Our results revealed that the staining intensities of Ki-67 were remarkably decreased in GC cells treated with 1 μM and 2 μM As_4S_4 compared to nontreated control (Fig. 1c–f).

In addition, the apoptotic rates of GC cells were notably increased after the treatment with 1 and 2 μM As_4S_4 (Fig. 2a–d). Furthermore, the results of Transwell assay suggested that the migration and invasion were significantly reduced in GC cells treated with 1 and 2 μM As_4S_4 (Fig. 2e–h) Taken all together, the growth and migration of GC cells were remarkably suppressed by the treatment with As_4S_4.

CircRNA_ASAP2 was involved in As_4S_4-modulated biological behavior changes in GC cells

To further explore the molecular mechanisms of As_4S_4-regulated signaling in GC, circRNAs chip was performed on AGS cells treated with 2 μM As_4S_4 for 48 h. The data revealed the differentiated expression of circRNAs in GC cells treated with As_4S_4 compared to the nontreated control. The five most up- or downregulated circRNAs were presented in Fig. 3a. As a previous study has indicated that circRNA_ASAP2 (has_circ_0006089) was associated with the progression of GC [26], further experiments were conducted to investigate the involvement of circRNA_ASAP2 in As4S4-modulated biological behavior alterations in GC cells. Moreover, the expression of circRNA_ASAP2 was significantly decreased in GC cells treated with 1 and 2 μM As_4S_4 (Fig. 3b). In addition, reduced levels of circRNA_ASAP2 were detected in GC cells compared to GES-1 cells (Fig. 3c). Furthermore, GC cell models overexpressing circRNA_ASAP2 were generated through the transfection with pc-circRNA_ASAP2. The transfection efficiencies were confirmed by RT-qPCR; however, the overexpression of circRNA_ASAP2 was suppressed by the treatment with 2 μM As_4S_4 (Fig. 3d). Moreover, the CCK8 assay suggested that the proliferative ability of GC cells was enhanced by pc-circRNA_ASAP2, which was reversed by the treatment with 2 μM As_4S_4 (Fig. 3e,f). In addition, flow cytometry indicated that the apoptotic rate of GC cells was reduced by the transfection with pc-circRNA_ASAP2, but these effects were rescued by the treatment with 2 μM As_4S_4 (Fig. 3g–j). Additionally, Transwell assay revealed that the migrative and invasive activities of AGS and HGC-27 cells were remarkably promoted by the transfection with pc-circRNA_ASAP2; however, these influences were abrogated by the treatment with 2 μM As_4S_4 (Fig. 3k–n). All in all, these findings suggested that circRNA_ASAP2 was involved in As_4S_4-regulated downstream signaling in GC cells.

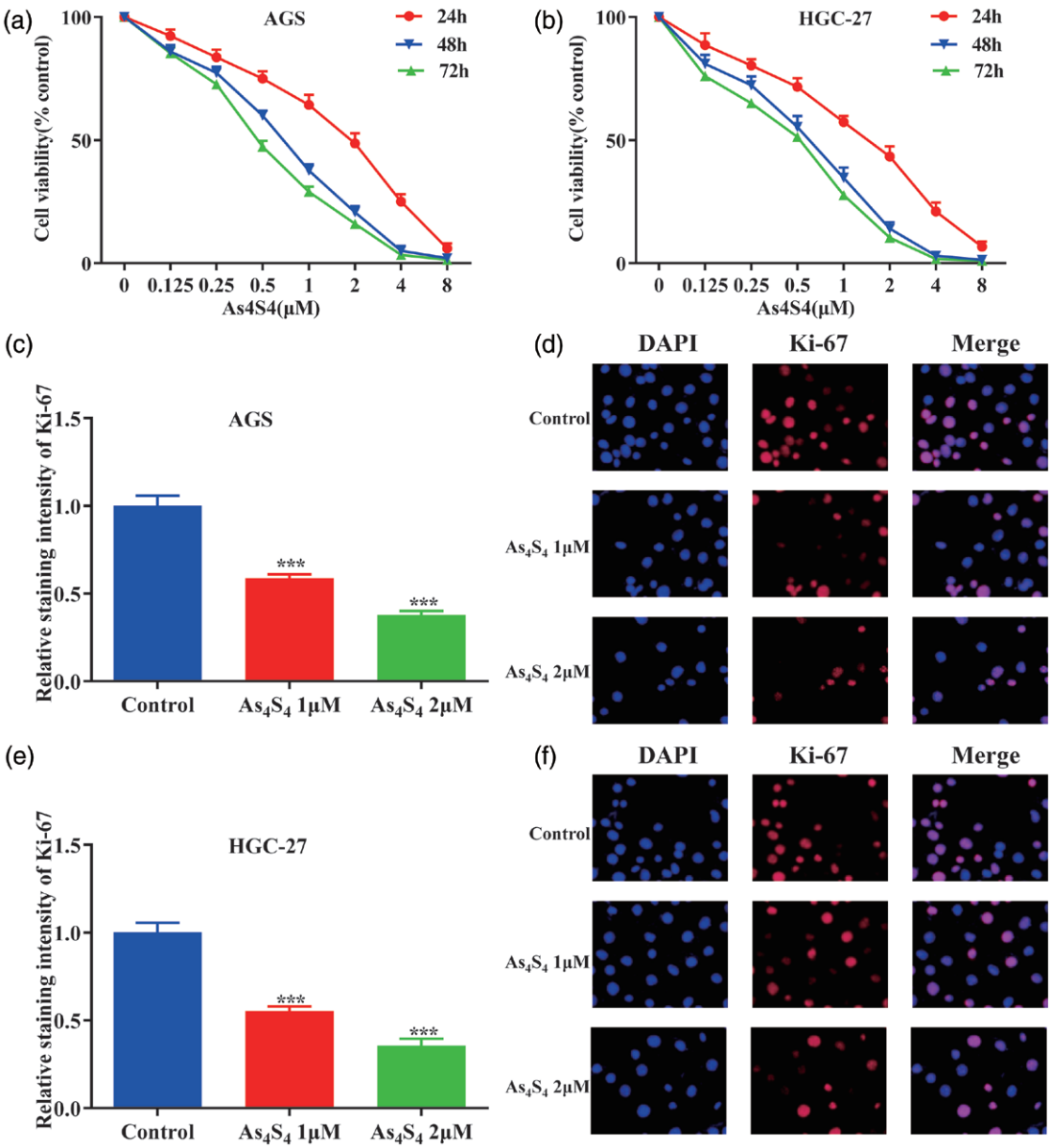
The Wnt/β-catenin pathway could be novel target of As4S4/circRNA_ASAP2-regulated behavior alterations in GC cells

As previous studies have revealed the effects of arsenic on the Wnt/β-catenin signaling [33], the expression levels of Wnt/β-catenin-associated molecules were examined in treated GC cells. Our results indicated that the mRNA levels of Wnt/β-catenin-related genes including DVL2, GSK-3β, β-catenin and CyclinD1 were increased in GC cells transfected with pc-circRNA_ASAP2, which was abolished by the treatment with 2 μM As_4S_4 (Fig. 4a,b). In consistence with these findings, the protein levels of DVL2, p-GSK-3β, β-catenin and CyclinD1 were also elevated after the transfection with pc-circRNA_ASAP2, and these effects were abrogated in GC cells treated with 2 μM As_4S_4 (Fig. 4c,d). Moreover, the proliferative activity of GC cells was inhibited by the transfection with sh-circRNA_ASAP2, which was rescued by activation of Wnt signaling through the treatment with SKL2001 (Fig. 4e,f). In addition, the apoptotic rate of GC cells was increased by sh-circRNA_ASAP2, but these effects were reversed by Wnt agonist SKL2001 (Fig. 4g,h). Furthermore, the migration and invasion of GC cells were remarkably suppressed after the transfection with sh-circRNA_ASAP2, which was abolished by the treatment with SKL2001 (Fig. 4i,j). All in all, these data revealed the involvement of the Wnt/β-catenin signaling in $As4S_4$/circRNA_ASAP2-modulated behavior changes of GC cells.

Discussion

As_4S_4 is extracted from realgar and has been used for the treatment of leukemia [6,7]. Recent studies have revealed the potential antitumor roles of arsenic, which is considered a promising therapy for cancer treatment

Fig. 1

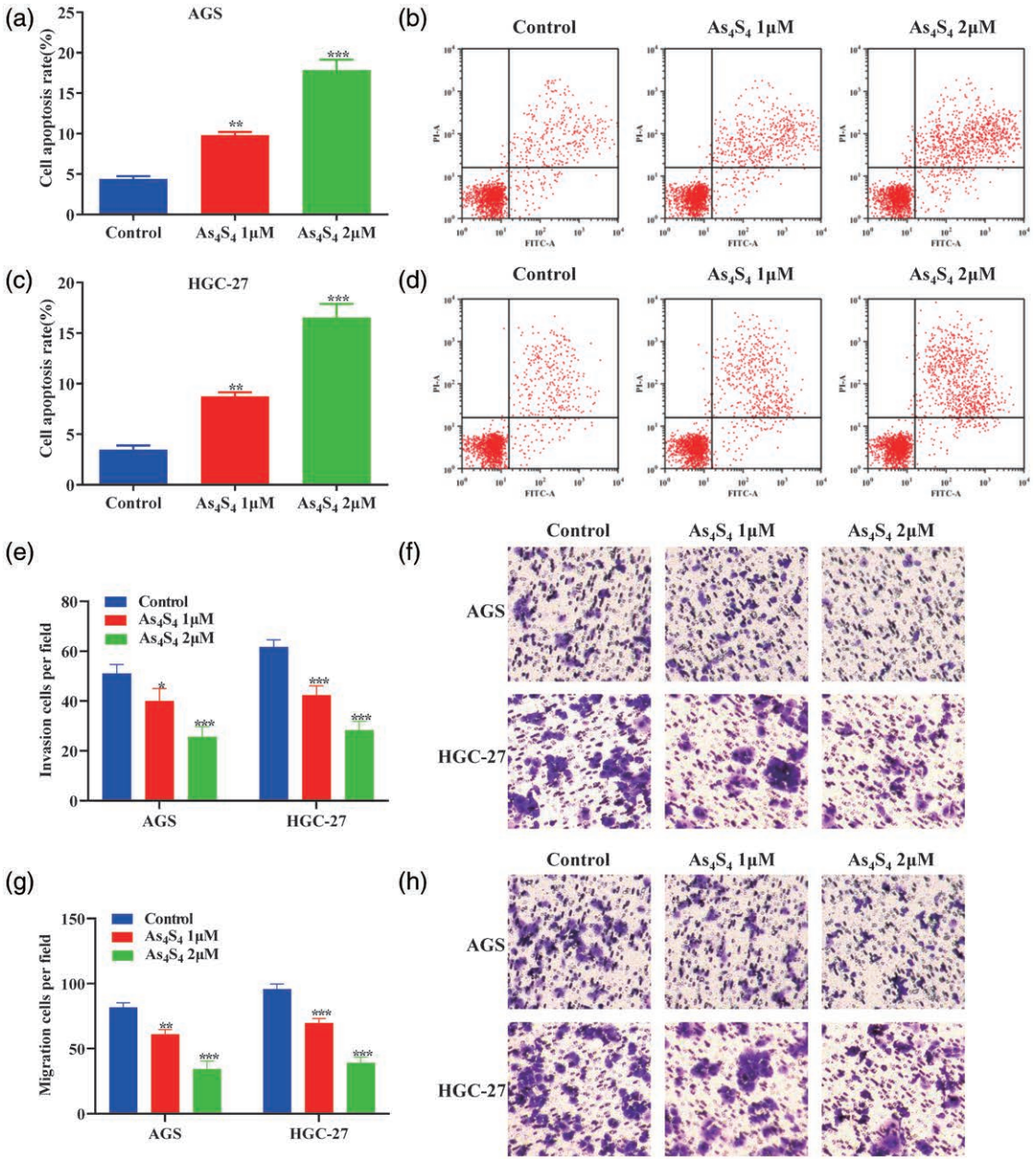


The growth and metastasis of GC cells were suppressed by As_4S_4. (a,b) AGS and HGC-27 cells were treated with As_4S_4 ranged from 0.125 to 8 μM. GC cell viability was remarkably decreased in cells treated with As_4S_4 for 48 and 72 h. (c–f) The expression levels of proliferation-related gene Ki-67 were notably reduced after the treatment with 1 and 2 μM As_4S_4. $^{***}P < 0.001$. GC, gastric cancer.

[8,9]. Furthermore, previous reports have suggested that As_4S_4 could suppress tumor progression in various types of cancer including GC [8–11]. However, the exact effects of As_4S_4 treatment on GC are still unclear. CircRNAs are novel noncoding RNAs, which are considered key regulators of numerous biological processes [14–16]. Moreover, dysregulation of certain circRNAs could be associated with the development of tumor [17–21], but the detailed functions of most circRNAs remain largely unknown. In this study, the effects of As_4S_4 treatment on the biological behaviors of GC cells were investigated in vitro. More importantly, the putative downstream molecules in As_4S_4-modulated signaling in GC were identified.

Our results revealed that the proliferation, migration and invasion of AGS and HGC-27 cells were remarkably suppressed by the treatment with As_4S_4; however, cell apoptosis was notably promoted. These findings suggested that the growth and migration of GC cells could be inhibited by As_4S_4. In consistence with our data, previous

Fig. 2

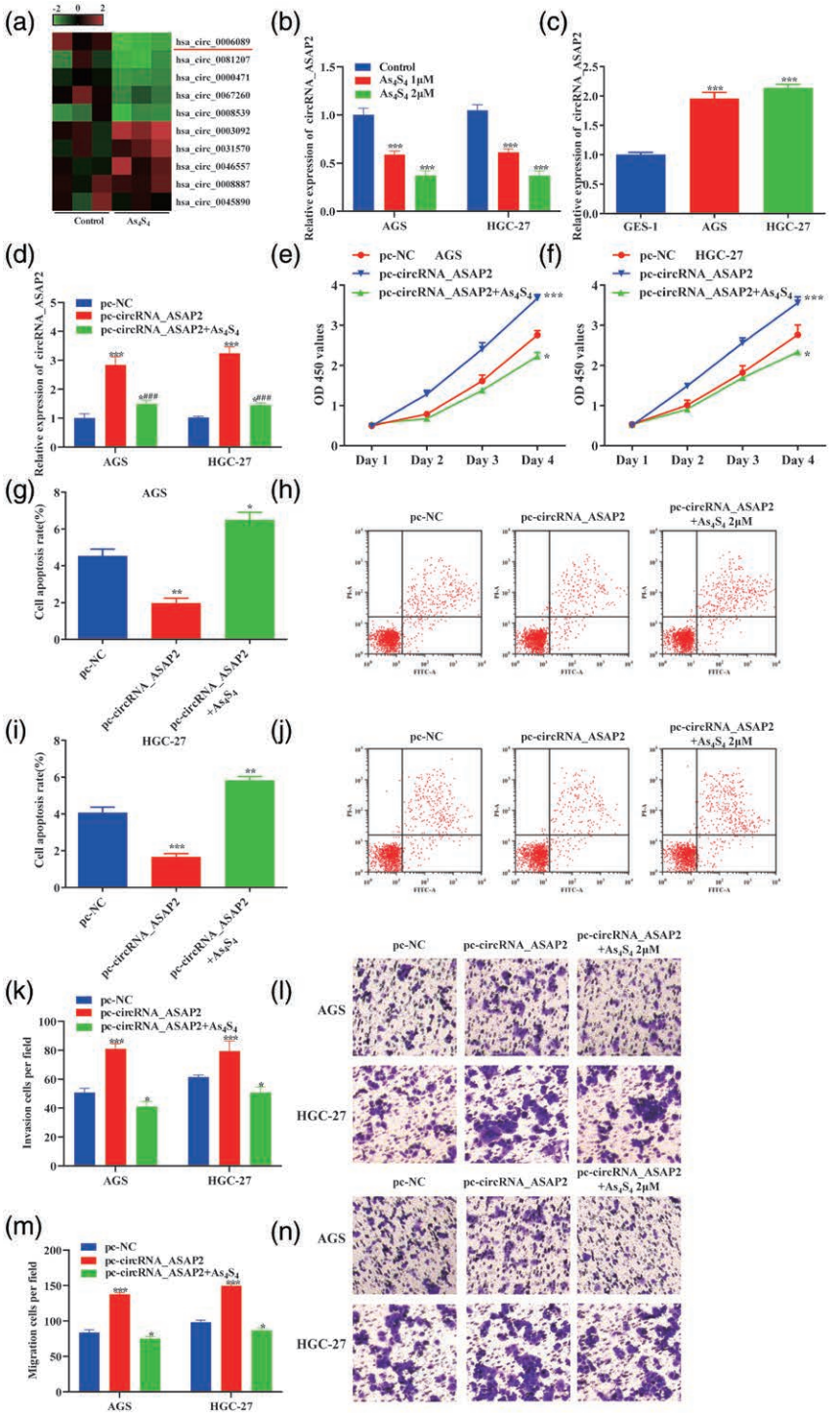


Treatment with As_4S_4 promoted the apoptosis of GC cells, whereas cell migration and invasion were inhibited. (a–d) The apoptotic rates of GC cells were notably increased after the treatment with 1 and 2 μM As_4S_4. (e–h) The migratory and invasive activities of GC cells were decreased by the treatment with As_4S_4. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. GC, gastric cancer; NC, negative control.

studies have also revealed that As_4S_4 treatment could suppress tumor progression in GC through various signaling pathways [8–11]. In our study, circRNA_ASAP2 was identified as the novel target of As_4S_4 in GC, and it was involved in As_4S_4-regulated biological behavior changes in GC cells. First, the overexpression of circRNA_ASAP2 in GC cells transfected with pc-circRNA_ASAP2 was suppressed by the treatment with As_4S_4. Second, the proliferation, migration and invasion were significantly promoted in GC cells after the transfection with pc-circRNA_ASAP2, but these effects were abolished by As_4S_4 treatment. Similarly, a recent study has also revealed the involvement of circRNA_ASAP2 in the regulation of tumor growth and metastasis in GC [26].

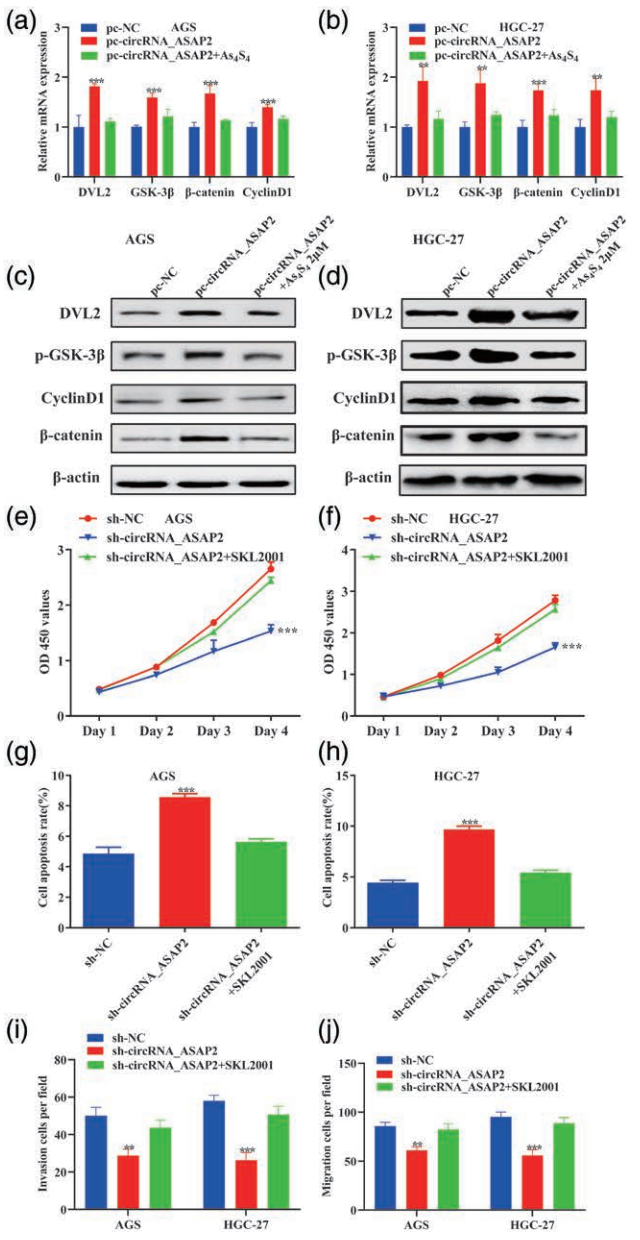
The Wnt/β-catenin could regulate numerous biological processes, and dysregulation of the Wnt/β-catenin pathway may promote tumor cell growth [27,28]. Moreover,

Fig. 3



The involvement of circRNA_ASAP2 in As_4S_4-regulated behavior changes of GC cells. (a) Differentially expressed circRNAs in GC cells treated with As_4S_4 were revealed by circRNAs chip. (b) The expression levels of circRNA_ASAP2 were notably downregulated in GC cells treated with 1 and 2μM As_4S_4. (c) Downregulation of circRNA_ASAP2 in GC cells compared to GES-1 cell line. (d) Overexpression of circRNA_ASAP2 in GC cells transfected with pc- circRNA_ASAP2 was suppressed by the treatment with As_4S_4. (e,f) The proliferation of GC cells was promoted by pc-circRNA_ASAP2, but these effects were abolished by the treatment with 2 μM As_4S_4. (g–j) The apoptosis of GC cells was inhibited by the transfection with pc-circRNA_ASAP2, which was reversed by As_4S_4 treatment. (k–n) The migration and invasion of GC cells were notably enhanced after the transfection with pc-circRNA_ASAP2, which was abrogated by the treatment with 2 μM As_4S_4. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{\#\#\#}P < .001$. GC, gastric cancer; NC, negative control.

Fig. 4



The Wnt/β-catenin signaling was involved in As4S$_4$/circRNA_ASAP2-regulated behavior alterations in GC cells. (a,b) The mRNA levels of Wnt/β-catenin-related genes were reduced in GC cells transfected with pc-circRNA_ASAP2, but these effects were reversed by the treatment with 2 μM As$_4$S$_4$. (c,d) Similarly, the protein levels of DVL2, p-GSK-3β, β-catenin and CyclinD1 were increased following the transfection with pc-circRNA_ASAP2, which was abolished by the treatment with As$_4$S$_4$ (e,f) The proliferation of GC cells was suppressed by sh-circRNA_ASAP2, and these effects were rescued by the treatment with SKL2001. (g,h) The apoptosis of GC cells was enhanced by the transfection with sh-circRNA_ASAP2, which was abrogated by Wnt agonist SKL2001. (i,j) The migratory and invasive activities of GC cells were significantly inhibited by sh-circRNA_ASAP2, but these influences were reversed by SKL2001 treatment. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. GC, gastric cancer; NC, negative control.

previous studies have that arsenic could affect tumor progression through regulating the Wnt/β-catenin pathway [33]. In our study, the results indicated that the Wnt/β-catenin pathway could be novel target of As$_4$S$_4$/circRNA_ASAP2-regulated behavior alterations in GC cells. The levels of the Wnt/β-catenin-related genes were increased in GC cells transfected with pc-circRNA_ASAP2, which was abrogated by the treatment with 2 μM As$_4$S$_4$. Furthermore, the biological behavior changes in GC cells caused by the knockdown of circRNA_ASAP2 were abolished by Wnt agonist SKL2001. In consistence with our findings, uncontrolled activity of the Wnt/β-catenin signaling has been detected in numerous types of cancer including GC, and targeted therapies against the Wnt/β-catenin pathway have been developed in the treatment of patients with GC [29–32].

In summary, As$_4$S$_4$ could function as an antitumor agent in GC by downregulating circRNA_ASAP2 and inactivating the Wnt/β-catenin pathway, which subsequently inhibits the growth and migration of GC cells. Therefore, As$_4$S$_4$/circRNA_ASAP2/Wnt/β-catenin could be involved in the regulation of tumor progression in GC, and this novel signaling could be a putative candidate for targeted therapy in GC. More importantly, these findings may provide guidance for the treatment of GC patients in clinical practice.

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Conflicts of interest

There are no conflicts of interest.

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