Role of ADP Ribosylation Factor Guanylate Kinase 1 in the Malignant Biological Behavior of Gastric Cancer

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Abstract

Objectives: To study the effects of ADP ribosylation factor guanylate kinase 1 gene (*ASAP1*) on the biological behavior of malignant gastric cancer (GC), and explore its possible molecular mechanisms in tumorigenesis and tumor progression.

Methods: Quantitative PCR and western blotting (WB) were performed to measure *ASAP1* mRNA and protein expression in the GES-1 epithelial cell line, which is derived from normal human mucosa, and three GC cell lines. Molecular biology techniques such as lentivirus packaging, infection, and screening were used to obtain BGC823 GC cells overexpressing *ASAP1* and BGC823 and MKN45 cells with *ASAP1* knocked down. The Cell Counting Kit-8 assay, colony formation assay, flow cytometry using Annexin V/propidium iodide, Transwell migration and invasion assays, and scratch assay were used to assess the malignant biological behavior of GC cells with *ASAP1* overexpression and knockdown. WB was conducted to evaluate the effects of *ASAP1* expression on angiogenesis, as well as on the expression of matrix metalloproteinases (MMPs), apoptotic proteins, and epithelial-mesenchymal transition (EMT)-related proteins. Nude mice bearing transplanted tumors were evaluated to determine the effect of *ASAP1* knockdown on BGC823 GC cells.

Results: *ASAP1* expression in GC cells was greater than that in GES-1 normal gastric mucosal epithelial cells. *ASAP1* overexpression significantly enhanced the proliferation, invasion, and migration of GC cells and reduced apoptosis; whereas *ASAP1* knockdown significantly reduced the proliferation, invasion, and migration of GC cells and promoted apoptosis. In the *ASAP1*-knockdown group, expression of cleaved-caspase 3, cleaved-poly-ADP-ribose polymerase (PARP), and the epithelial marker E-cadherin increased significantly, whereas the expression of MMP2, MMP9, vascular endothelial growth factor A (VEGFA), and the mesenchymal markers N-cadherin, and vimentin decreased significantly ($P < 0.01$). Knockdown of *ASAP1* inhibited the growth of subcutaneously implanted tumors in nude mice.

Conclusions: *ASAP1* overexpression strongly promotes—whereas knockdown of *ASAP1* effectively weakens—the malignant biological behavior of GC cells, possibly by reducing VEGFA expression and thus reducing angiogenesis, upregulating the expression of cleaved-caspase 3 and cleaved-PARP, and reducing the activity of MMPs and EMT.

1 Introduction

Gastric cancer (GC) is one of the most common malignant tumors worldwide and the third leading cause of cancer-related deaths.¹ In 2018, more than 1 million new cases and approximately 783,000 deaths due to GC were reported globally.¹ Modern research on tumor molecular biology has proven that tumorigenesis and tumor progression are biological behaviors that involve multi-gene participation, multi-factor actions, multi-stage development, and extremely complex steps. Hence, it is highly relevant to identify highly specific and sensitive molecular biological markers, and to explore the underlying

mechanism of gastric tumorigenesis and tumor progression to improve the early detection rate of GC and establish new gene therapy targets, thereby strengthening prevention and treatment approaches for GC.²

ADP ribosylation factor guanylate kinase 1 gene (*ASAP1*), which consists of SRC homology 3 (SH3), ankyrin repeat (ANK), and pleckstrin homology (PH) domains, is located on chromosome 8q24. It contains six domains, including an N-terminal bar, PH, Arf-GAP, ANK, proline-rich, and C-terminal SH3 domains (proline-rich).³ In addition, *ASAP1* also contains a proline helix structure consisting of tandem repeating E/DLPPKP.⁴ As a member of the Arf-GAP family, *ASAP1* hydrolyzes GTP to regulate actin reorganization and cytoskeletal dynamics to control cell movement, as well as to participate in and regulate the formation of focal adhesion, the invasion of pseudopodia, and plasma membrane folds.⁵⁻⁹ *ASAP1* also binds to Cbl-interacting protein of 85 kDa, CD2-associated protein, cortactin, CRK-like proto-oncogene adaptor protein, and Src to impart corresponding biological effects that regulate cell invasion. Studies have shown that *ASAP1* is closely related to the malignant biological behavior of breast, colorectal, and ovarian cancers, among others. Junnila *et al.* (2010) used affinity capture-based transcript analysis (TRAC assay) to perform expression analysis of 11 genes such as *ASAP1* in GC and non-malignant GC tissues, and showed that *ASAP1* was differentially expressed between them.¹⁰ Müller *et al.*⁵ showed that *ASAP1* is significantly upregulated in GC tissues. Our previous studies indicated that *ASAP1* was highly expressed in human GC tissues, and *ASAP1* expression levels was negatively associated with disease-free survival time and overall survival time.¹¹ Hence, further research on the anti-GC effect of *ASAP1* is of great significance.

2 Materials And Methods

2.1 Cell culture

GC cell lines, including BGC823, MGC803 and MKN45, and the GES-1 normal human epithelial cell line were purchased from ShangHai Zishi Biotechnology Co., Ltd. (Shanghai, China). All cells were grown in RPMI-1640 medium, supplemented with 10% fetal bovine serum (FBS) (Gibco, Gaithersburg, MD, USA) and 100 U/mL penicillin/streptomycin. The cells were maintained in a humidified incubator with 5% CO₂ atmosphere at 37°C.

2.2 Quantitative PCR

Total RNA from cells was isolated using TRIzol (Invitrogen, Carlsbad, CA, USA). Then the cDNA was synthesized using the PrimeScript RT Reagent Kit (TaKaRa, Shiga, Japan). Quantitative PCR (qPCR) was performed using the SYBR Premix Ex Taq™ II (TaKaRa, Shiga, Japan) according to the manufacturer's instructions. GAPDH was used to normalize the *ASAP1* expression and the $2^{-\Delta\Delta C_t}$ method was used to calculate the gene expression levels. *ASAP1* and GAPDH primers were synthesized by Shanghai Biotechnology Co., Ltd. (Shanghai, China). The following primer pairs were used in this study: 5'-GAGAGGTATGAAGGTTATTCA-3' (forward) and 5'-ATCACCACCTTACGAGCCACC-3' (reverse) for *ASAP1*

and 5'-GCGGGGCTCCAGAACATCAT-3' (forward) and 5'-CCAGCCCCAGCGTCAAGGTG-3' (reverse) for GAPDH.

2.3 Transfection

Two small interfering RNAs (siRNAs) targeting ASAP1 and negative control (siNC) were synthesized by Kilton Biotechnology Co., Ltd (Shanghai, China). The overexpression plasmid, pcDNA3.1-ASAP1 and an empty plasmid were also constructed by Kilton Biotechnology Co., Ltd. Finally, the cells were transfected using Lipofectamine 2000 (Invitrogen, USA) according to the manufacturer's instructions. The following siRNA sequences were used in Transfection: 5'-CCAGGGAUUUACUUGCACUUU-3' (sense) and 5'-AGUGCAAGUAAAUCCCUUGGUU-3' (antisense) for siASAP1-1, and 5'-CAGUACUUUUGUGUAGUACAA-3' (sense) and 5'-UUGUACUACACAAAAGUACUG-3' (antisense) for siNC.

2.4 Cell Counting Kit-8 assay

Cell Counting Kit-8 (CCK-8) (Beyotime Biotechnology, Beijing, China) was used to measure the cell proliferation. Briefly, 5×10^3 cells transfected were seeded into 96-well plates and cultured at 37°C for 0, 12, 24, and 48 h. Then 10 mL CCK-8 solution was added to each well and incubated for 1 h. A microplate reader (Molecular Devices, Sunnyvale, CA, USA) was used to measure the optical density at 450 nm.

2.5 Clone formation assay

The clone formation assay was used to assess cloning ability. Cells were seeded in 6-well plates (1000 cells/well) and cultured at 37°C for 14 days. Then the supernatants were discarded, and the cells were fixed in methanol and stained with crystal violet (0.1%; Sigma-Aldrich, St. Louis, MO, USA). The number of colonies was determined by light microscopy.

2.6 Cell apoptosis assay

The Annexin V-FITC apoptosis detection kit (BD Biosciences, Franklin Lakes, NJ, USA) was used to detect cell apoptosis. In brief, harvested cells were sequentially stained with Annexin V-FITC for 15 min and propidium iodide (PI) for 5 min in the dark. Then, a flow cytometer (BD Biosciences) was used to analyze the stained cells. The data were analyzed by FlowJo software.

2.7 Transwell invasion assay

We evaluated cell invasion ability by the Transwell invasion assay (BD Biosciences) in 24-well plates. The upper chambers were precoated with 60 µL of 1:3 diluted Matrigel (BD Biosciences). About 6×10^4 cells were seeded into the upper chambers in 200 µL serum-free medium. The lower chambers were filled with 600 µL complete medium containing 10% FBS. Following incubation for 24 h at 37°C with 5% CO₂, we removed the cells on the upper membranes with a cotton wool, fixed them in 4% paraformaldehyde, and stained them with 0.1% crystal violet. Lastly, we counted the invading cells in five randomly selected fields by a microscope. Each experiment was performed in triplicate for at least three times.

2.8 Wound healing assay

We seeded 1×10^6 cells/well into 6-well plates and cultured them for 12 h in serum-free medium. A perpendicular scratch wound was created by using a 10 μ L pipette tip. We obtained images at 0 and 48 h to observe wound healing with an inverted microscope (100 $\times$ magnification; Olympus Corporation, Tokyo, Japan). The distance between the two edges of a wound was calculated by ImageJ software. Healing rate = (0h average gap-24h average gap)/0h average gap.

2.9 Western blot analysis

Western blot was carried out according to the manufacturer's protocol. Briefly, proteins were detected with primary antibodies specifically recognizing ASAP1(1:500; Ab125729; Abcam), MMP2(1:1000; Ab97779; Abcam), MMP9(1:1000; Ab137867; Abcam), VEGFA(1:1000; Ab214424; Abcam), Cleaved-Caspases3(1:500; Ab2302; Abcam), Cleaved-PARP(1:1000; Ab32064; Abcam), E-cadherin (1:500; Ab15148, Abcam), N-cadherin (1:5000; Ab76011, Abcam), Vimentin(1:2000; Ab137321, Abcam) and GAPDH(1:1000; #5174; CST). Total protein was collected from cells using RIPA buffer, separated by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis, and electro-transferred onto PVDF membranes. After blocking in 5% non-fat milk for 2 h at 37°C, the membranes were incubated with primary antibodies overnight at 4°C, and probed with secondary antibodies for 2 h at 37°C. Finally, the protein bands were detected by an ECL kit according to the manufacturer instructions. Each band was quantified using ImageJ software.

2.10 Animal experiments

Five-week-old male nude mice (Shanghai, China) were randomly divided into three groups (control, siNC, and siASAP1 groups). The animal experiments were carried out in accordance with protocols approved by the Research Animal Resource Center of Fujian Medical University (Fujian, China), and were approved by the independent Ethics Committee of Fujian Medical University. The same number of BGC823 cells (4×10^6) transfected with siNC or siASAP1 were injected into the right flank of mice. Tumor volume was monitored on the 8th day after inoculation and was measured every 3 days (volume = $\pi/6 \times \text{length} \times \text{width}^2$). All mice were euthanized by cervical dislocation 20 days after injection, mice were euthanized, and the tumors were resected and weighed.

2.11 Statistical analysis.

GraphPad Prism software (version 6.0; San Diego, CA, USA) was used for the statistical analyses. All data from three independent experiments presented as the mean $\pm$ standard deviation. The Student's *t*-test was used to assess the comparisons between two groups. Multigroup comparisons were assessed by analysis of variance. $P < 0.05$ was considered statistically significant.

3 Results

3.1 ASAP1 expression is upregulated in GC cells

In this study, we used qPCR and Western blot assays to determine the expression of ASAP1 in three GC cell lines (BGC823, MKN45 and MGC803) and GES-1 normal gastric epithelial cell. We found that the expression of ASAP1 was higher in the GC lines (especially BGC823 and MKN45 cell lines) than in GES-1 cells at both the mRNA and protein levels (Fig. 1). Therefore, BGC823 and MKN45 cells were selected for knockdown and BGC823 was selected for overexpression.

3.2 Silencing and overexpression of ASAP1 in GC cells

To silence or overexpress ASAP1, we used specific siRNA to transfect BGC823 and MKN45 cells, and a plasmid overexpressing ASAP1 (oeASAP1) was transfected into BGC823 cells. As demonstrated by qPCR and Western blot, ASAP1 expression was successfully suppressed in BGC823 and MKN45, and upregulated in BGC823 cells (Fig. 2a, b and c).

3.3 Silencing of ASAP1 inhibits cell proliferation and induces the apoptosis of GC cells

To elucidate the role of ASAP1 in GC cell proliferation and apoptosis in vitro, CCK-8 assay, colony formation assays and flow cytometry test were performed. The results from the CCK-8 and colony formation assays showed that ASAP1 silencing decreased the growth of BGC823 and MKN45 cells, whereas ASAP1 overexpression increased the proliferation of BGC823 cells (Fig. 3a and b). Flow cytometry test showed that silencing of ASAP1 could significantly enhanced apoptotic rate in BGC823 and MN45 cells, whereas overexpress ASAP1 had the opposite effects in BGC823 cells (Fig. 3c). These data demonstrate that ASAP1 plays a negative role in GC cell proliferation.

3.4 ASAP1 knockdown suppresses GC cell invasion and migration

To investigate ASAP1's role in GC cell invasion and migration, we performed transwell invasion assays and wound-healing assays using BGC823 and MKN45 cells. Knockdown of ASAP1 in BGC823 and MKN45 cells significantly impaired cell invasion and migration abilities, respectively. In contrast, when ASAP1 was upregulated in BGC823 cells, the cell invasion and migration abilities were both improved (Fig. 4a and b). The above-mentioned results demonstrate that ASAP1 knockdown can serve a suppressive role in GC cell migration and invasion.

3.5 Knockdown of ASAP1 changes the proteins levels related to malignant behaviors of GC cells

VEGFA plays an important role in the transport of nutrients for tumors before angiogenesis. MMPs are a class of proteolytic enzymes that are closely related to the invasion and metastasis of malignant tumors. Cysteiny aspartate specific proteinases (caspases) are a group of proteases related to apoptosis and cytokine maturation.

Western blot was performed to investigate the effect of ASAP1 silence on the malignant behavior of tumor cells in protein level of VEGFA, Cleaved-Caspase 3, Cleaved-PARP, MMP2 and MMP9. These related protein levels changed along with the knockdown of ASAP1 (Fig. 5a, b and c), which further indicated that ASAP1 knockdown might impaired the malignant biological behaviors GC cells.

To further investigate the mechanism related to ASAP1 silence inhibiting malignant phenotype of GC, we detected the protein level of EMT markers. Western blot results showed that ASAP1 downregulation markedly suppressed the expression of N-cadherin and Vimentin and significantly increased the expression of E-cadherin (Fig. 5d).

These data uncovered that knockdown of ASAP1 may suppress malignant behaviors of GC cells through reducing VEGFA, upregulation the expression of cleaved-caspase 3 and cleaved-PARP MMPs expression and reducing the activity of MMPs and EMT.

3.6 ASAP1 silencing represses GC subcutaneously xenografted growth *in vivo*

To investigate the function of ASAP1 *in vivo*, a subcutaneous xenograft model was established. Eight days after subcutaneous cancer cell inoculation, all nude mice transfected with cells developed tumors. However, siASAP1 cells had greatly reduced tumor volumes and weights compared with control and siNC cells (Fig. 6).

4 Discussion

GC is currently one of the major malignancies affecting humans. The abnormal expression of tumor-related genes is considered one of the main mechanisms of tumorigenesis and tumor progression in GC. Targeted therapy for cancer driver genes is a novel path for the prevention and treatment of GC.

As a multifunctional scaffold protein, *ASAP1* regulates cell movement, participating in and regulating focal adhesions, invading pseudopodia, and fold formation in the plasma membrane.^{6,12-14} With rapid improvements in molecular biotechnology, *ASAP1* is expected to become a novel tumor diagnostic marker. Biological therapy targeting *ASAP1* may have an enormous impact on the development of antitumor drugs. Hence, it is important to study the influence and mechanism of *ASAP1* on malignant biological behavior of GC.

The results of this study showed that the mRNA and protein expression levels of *ASAP1* in GES-1 epithelial cells derived from normal human gastric mucosa were significantly lower than that in the three GC cell lines, namely, BGC823, MGC803, and MKN45 (all $P < 0.05$), suggesting that *ASAP1* may be closely related to gastric tumorigenesis and tumor progression, which deserves further investigation.

Activation of oncogenes or inactivation of tumor suppressor genes leads to abnormal cell proliferation, differentiation, and apoptosis, which should be key to tumorigenesis and tumor progression. In 2009,

Sabe *et al.*¹⁵ pioneered the use of RNA interference (RNAi) and showed that siRNA-mediated *ASAP1* silencing effectively inhibited the aggressive activity of breast cancer. In 2017, Yang *et al.*¹⁶ showed that knockdown of *ASAP1-IT1* effectively inhibited bladder cancer stem cells, thereby inhibiting the recurrence, metastasis, and chemotherapy resistance of bladder cancer. In 2018, Zhang *et al.*¹⁷ showed that interference of *ASAP1-IT1* inhibited the proliferation, migration, and metastasis of non-small cell lung cancer cells. Guo *et al.*¹⁸ showed that knocking out *ASAP1-IT1* significantly reduced the number of cholangiocarcinoma colonies. Liu *et al.*¹⁹ showed that the application of RNAi technology downregulated *ASAP1* expression, in turn, inhibiting REF52 cell spread and migration. In 2018, Zhang *et al.*²⁰ showed that *ASAP1* overexpression promoted the migration and invasion of ovarian cancer cells, enhanced cancer cell proliferation and survival, and inhibited the apoptosis of ovarian cancer cells. In this study, knockdown of *ASAP1* significantly reduced proliferation and colony formation, increased the number of apoptotic cells, and decreased the wound healing ability of BGC823 and MKN45 cells, whereas *ASAP1* overexpression had opposite effects, indicating that knocking down *ASAP1* inhibits the development of gastric tumors.

The malignant biological behavior of tumor cells is closely related to angiogenic factors. VEGFA is one of the most important positive regulators of angiogenesis. It provides nutrition for the proliferation and growth of tumor cells and the establishment of vascular network by increasing the permeability of blood vessels. VEGFA plays an important role in the transport of nutrients for tumors before angiogenesis. In 2011, Hashimoto *et al.*²¹ showed that RNAi of *ASAP1* significantly inhibited VEGFA-induced lumen formation, possibly affecting the angiogenesis of breast cancer via the GEP100-Arf6-*ASAP1*-cortacin pathway. This study showed that knocking down *ASAP1* in BGC823 GC cells significantly reduced VEGFA protein expression, which subsequently blocked relevant signal transduction pathways, thereby suppressing tumor angiogenesis, proliferation, and growth, and even the invasion and metastasis of malignant GC.

The malignant biological behavior of tumor cells is closely related to programmed cell death. Cysteiny aspartate specific proteinases (caspases) are a group of proteases related to apoptosis and cytokine maturation. Caspase 3 is an important protease and an execution factor of apoptosis. It often exists in the form of inactive zymogen. The activation of caspase 3 marks an irreversible process of apoptosis.²² Cleaved-caspase 3 is the active fragment of activated caspase 3. Song *et al.*²³ showed that the activation of caspase 3 induces apoptosis in GC cells. Related studies have shown that the rate of positive expression of cleaved-caspase 3 in cervical cancer tissues is low and is negatively correlated with the differentiation of cervical cancer tissues. Patients with high cleaved-caspase 3 expression levels have a better prognosis.^{24–26} PARP is a deoxyribonucleic acid repair enzyme that maintains the stability of genetic materials and is activated during DNA damage and repair, thereby regulating apoptosis. After cleavage by activated caspase 3, PARP is transformed into cleaved-PARP that in turn trigger apoptosis.²⁷ Wang *et al.*²⁸ showed that compared with healthy gastric tissues, PARP expression is upregulated in GC tissues. This study shows that cleaved-caspase 3 and cleaved-PARP protein expression were downregulated in BGC823 *ASAP1* knockdown GC cells, suggesting that knocking down *ASAP1* promotes

the expression of cleaved-caspase 3 and cleaved-PARP proteins, further confirming that *ASAP1* regulates apoptosis of GC cells.

The invasion and metastasis of tumor cells are continuous processes that involve the interaction between cancer cells and host cells. MMPs are a class of proteolytic enzymes that are closely related to the invasion and metastasis of malignant tumors. Among these, MMP2 and MMP9 both induce and enhance the formation of blood vessels and invade blood vessels and lymphatic vessels through degradation, destruction, and infiltration, eventually leading to tumor invasion and metastasis.²⁹ Downregulation of MMP2 and MMP9 inhibits the invasion and metastasis of tumor cells.^{30–31} In this study, knockdown of *ASAP1* significantly downregulated the expression of MMP2 and MMP9 proteins and inhibited the invasion and migration of GC cells.

Invasion and metastasis of tumor cells often require a specific cell phenotype. The EMT is a rapid, reversible, temporary process that occurs under certain specific physiological and pathological conditions when epithelial cells lose their normal polarity and tight connection between cells and convert from epithelial phenotype to mesenchymal phenotype. It mainly manifests as downregulation of the epithelial marker E-cadherin, upregulation of the mesenchymal marker N-cadherin, and the transformation of keratin into Vimentin as the core of cytoskeleton.³² Among these, E-cadherin and N-cadherin are key components of the epithelial monolayer cell connection.³³ Vimentin is the main component of the intermediate silk protein family that maintains cell integrity and provides stress resistance and promotes tumor growth and invasion.^{34–35} In 2017, Li *et al.*³⁶ blocked *ASAP1* expression by RNAi, which reversed the EMT process of breast cancer cells. In 2018, Zhang *et al.*²⁰ showed that *ASAP1* overexpression enhanced tumor cell motility and invasiveness of ovarian cancer cells. In this study, knockdown of *ASAP1* inhibited the phenotype loss of GC epithelial cells and reduced the appearance of mesenchymal cell phenotypes, which was manifested by the upregulation of E-cadherin and downregulation of N-cadherin and Vimentin, thereby confirming that knockdown of *ASAP1* effectively reverses the EMT process and inhibits invasion and metastasis of malignant tumors.

This study also used nude mouse xenograft models to evaluate the effects of knocking down *ASAP1* in BGC823 gastric tumorigenesis *in vivo*, and to verify the relationship between *ASAP1* and GC. The animal experiments showed that the growth rate of the subcutaneously transplanted tumors with *ASAP1* knockdown was significantly slower and tumor weight was also significantly reduced, confirming that the proliferation and growth of *ASAP1*-knockdown BGC823 GC cells were significantly inhibited.

In conclusion, we show that *ASAP1* is highly expressed in GC cells. *ASAP1* overexpression significantly enhances the proliferation, reduces apoptosis, and improves the invasion and migration ability of GC cells. Knocking down *ASAP1* significantly reduces the proliferation, promotes the apoptosis, and weakens the invasion and migration of GC cells. Downregulation of VEGFA expression reduces angiogenesis, increases the expression of cleaved-caspase 3 and cleaved-PARP, and decreases the activity of MMPs and EMT in GC cells, thereby effectively suppressing the malignant biological behavior of GC cells. Future studies may present novel opportunities for the prevention and treatment of GC.

5 Declarations

ETHICS APPROVAL AND CONSENT TOPARTICIPATE

The animal experiments were carried out in accordance with protocols approved by the Research Animal Resource Center of Fujian Medical University (Fujian, China), and were approved by the independent Ethics Committee of Fujian Medical University.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA ANDMATERIALS

The data sets used in this study are available from the corresponding author on reasonable request.

COMPETING INTERESTS

The authors declare that they have no conflicting interests.

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AUTHORS' CONTRIBUTIONS

S.Y., X.Q.C., Q.L. and S.Y.Z. contributed to the conception of the manuscript. Q.L. and S.Y.Z. designed, performed the experiments and analyzed data. F.Y., R.F. and Q.X. partially performed some experiments and analyzed data. Q.L., S.Y.Z., X.Q.C. and S.Y. drafted and revised the manuscript. All authors read and approved the final manuscript.

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Figures

Figure 1

Expression levels of ASAP1 in normal GES-1 cell and three GC cell lines were determined by qRT-PCR and Western blot. (*P<0.05 vs. GES-1)

(a) BGC823

(b) MKN45

(c) BGC823

Figure 2

Silencing and overexpression of ASAP1 in GC cells. (* $P < 0.01$) (a) The efficiency of ASAP1 silencing in BGC823 was detected by qRT-PCR and Western blot. (b) The efficiency of ASAP1 silencing in MKN45 was detected by qRT-PCR and Western blot. (c) The efficiency of ASAP1 overexpression in BGC823 was detected by qRT-PCR and Western blot.

Figure 3

ASAP1 silencing inhibited, while overexpression promoted the cell progression of GC cells. (* $P < 0.01$) (a) The CCK-8 assay for the cell proliferation of BGC823 and MKN45 cells with ASAP1 silencing and BGC823 cells with ASAP1 overexpression. (b) The clone formation assay for the cell clonogenic capacity of BGC823 and MKN45 cells with ASAP1 silencing and BGC823 cells with ASAP1 overexpression. (c) The

flow cytometry assay for the cell apoptosis of BGC823 and MKN45 cells with ASAP1 silencing and BGC823 cells with ASAP1 overexpression.

Figure 4

ASAP1 knockdown suppressed, while overexpression promoted the invasion and migration of GC cells. (*P<0.01) (a) The Transwell assay for the invasion of BGC823 and MKN45 cells with ASAP1 silencing

and BGC823 cells with ASAP1 overexpression. (b) Wound healing assay for the migration of BGC823 and MKN45 cells with ASAP1 silencing and BGC823 cells with ASAP1 overexpression.

Figure 5

ASAP1 silencing inhibited the proteins levels related to malignant behaviors in BGC823 cells. (a) Western blot was used to assess the effect of ASAP1 silencing on VEGFA. (b) Western blot was carried out to evaluate the effect of ASAP1 silencing on the protein levels of cleaved caspase-3 and cleaved PARP in

BGC823 cells. (c) Western blot was performed to evaluate the effect of ASAP1 silencing on the protein levels of MMP2 and MMP9 in BGC823 cells.

Figure 6

ASAP1 silencing suppressed GC tumor growth in vivo. Nude mice were injected with BGC823 cells transfected with siNC or siASAP1. (a) image of tumor-bearing nude mice. (b) Tumor growth curves and (c) average tumor weights in si-ASAP1 group were greatly lower than those in control and siNC groups. (*P<0.01)