Research article

Role of ADP ribosylation factor guanylate kinase 1 in the malignant biological behavior of gastric cancer

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A R T I C L E   I N F O

A B S T R A C T

Aim: This study aims to investigate the influence of *ASAP1* (ADP ribosylation factor guanylate kinase 1) on the malignant behavior of gastric cancer (GC) cells and to elucidate the potential molecular mechanisms involved in cancer development and progression.
Methods: We assessed the impact of *ASAP1* overexpression and knockdown on GC cell malignancy using CCK8, colony formation, flow cytometry (Annexin V/propidium iodide), Transwell migration, invasion, and scratch assays. Western blot analysis was used to assess the effects of *ASAP1* on angiogenesis, matrix metalloproteinases (MMPs), apoptotic proteins, epithelial-mesenchymal transition (EMT)-related proteins, as well as AKT and *p*-AKT. The influence of *ASAP1* knockdown was also evaluated in nude mice bearing BGC823 cell-derived tumors.
Results: Our findings revealed that *ASAP1* was significantly overexpressed in GC cells, enhancing their proliferation, invasion, and migration, while reducing apoptosis. Conversely, *ASAP1* knockdown reversed these effects, markedly increasing the expression of cleaved-caspase 3 (Casp3), PARP, and the epithelial marker E-cadherin, and significantly decreasing MMP2, MMP9, VEGFA, and mesenchymal markers such as N-cadherin and vimentin. Additionally, it reduced AKT, and *p*-AKT levels ($P < 0.01$). Tumor growth in nude mice was suppressed following *ASAP1* knockdown.
Conclusion: The overexpression of *ASAP1* significantly promotes malignant behaviors in GC cells, whereas its knockdown diminishes these effects. This modulation is potentially through the downregulation of VEGFA, leading to reduced angiogenesis, Cleaved-Casp3 and Cleaved-PARP overexpression, and a decrease in MMPs, EMT, AKT, and *p*-AKT activity.

1. Introduction

Gastric cancer (GC) represents a widespread malignancy globally and stands as the third leading cause of cancer-related mortality [1]. According to recent statistics in 2020, there were over one million newly reported cases of GC worldwide, resulting in an estimated 769,000 deaths attributed to the disease [1]. Contemporary investigations in tumor molecular biology have demonstrated that the onset and progression of tumors entail intricate biological processes, encompassing the involvement of multiple genes, factors, stages, and complex pathways. Consequently, there is a pressing need to pinpoint highly specific and sensitive molecular markers to delineate the underlying mechanism governing gastric tumorigenesis and tumor advancement. Such efforts are crucial for enhancing the early detection rates of GC and identifying novel targets for gene therapy, thus bolstering prevention and treatment strategies for GC [2].

ASAP1 (ADP ribosylation factor guanylate kinase 1 gene), comprising SRC homology 3 (SH3), ankyrin repeat (ANK), and pleckstrin homology (PH) domains, is situated on chromosome 8q24. It encompasses six domains, including an N-terminal bar, PH, Arf-GAP, ANK, proline-rich, and C-terminal SH3 [3]. In addition, *ASAP1* features a proline helix structure composed of tandem repeating E/DLPPKP sequences [4]. *ASAP1*, belonging to the Arf-GAP family, plays a role in GTP hydrolysis, regulating actin reorganization and cytoskeletal dynamics to control cell movement. It also participates in and regulates the formation of focal adhesion, pseudopodia invasion, and plasma membrane folding [4,5]. *ASAP1* interacts directly with actin filaments to modulate the actin cytoskeleton via its BAR-PH domain [6].

Moreover, *ASAP1* binds to various proteins such as Cbl-interacting protein of 85 kDa, CD2-related protein, cortactin, CRK-like proto-oncogene adaptor protein, and Src, influencing cell invasion. *ASAP1* is closely associated with the malignant behavior of breast, colorectal, and ovarian cancers [7–12]. Junnila et al. conducted expression analysis of ASAP1 and other genes in both GC and non-malignant GC tissues using affinity capture-based transcript analysis (TRAC assay), revealing differential expressed of ASAP1 between them [13]. Previous studies have shown that *ASAP1* is significantly overexpressed in GC tissues, and is negatively correlated with disease-free survival time and overall survival time [14,15]. Therefore, further investigation is warranted to explore the potential anti-GC effect of *ASAP1*.

In this study, we aim to investigate the impact of ASAP1 on the malignant behavior of GC cells and to uncover the underlying molecular mechanisms driving cancer development and progression.

2. Materials and methods

2.1. Study design

In this study, our initial step involved the selection of suitable cell lines for both culture and transferction purposes. Subsequently, we devised experiments targeting both ASAP1 overexpression and ASAP1 knockdown. Then, we evaluated the influence of ASAP1 modulation on gastric cancer cell behavior, encompassing proliferation, clonogenicity, apoptosis, invasion, and migration, employing both molecular and functional analyses. Futhermore, we examined the repercussions of ASAP1 knockdown on tumor growth utilizing a nude mouse xenograft model.

The experimental groups were delineated as follows: For the ASAP1 overexpression assay, the gastric cancer cells without any intervention served as the "Control group", the gastric cancer cells transfected with empty lentiviral vectors constituted the "Vector group", and the gastric cancer cells transfected with recombinant lentiviral vectors, inducing overexpression of ASAP1, comprised the "oeASAP1 group". For the ASAP1 knockdown study, the gastric cancer cells consisting of untreated served as the "Control group", the gastric cancer cells transfected with empty lentiviral vectors were designated as the "siNC group", and the gastric cancer cells transfected with recombinant ASAP1-shRNA lentiviral vectors was designed as the "siASAP1 group".

2.1.1. Cell culture

The GC cell lines, namely BGC823, MGC803, MKN45, and the GES-1 normal human epithelial cell line were procured from ShangHai Zishi Biotechnology Co., Ltd. (Shanghai, China). The cells were cultured in RPMI-1640 medium that contained 10 % fetal bovine serum (FBS) (Gibco, Gaithersburg, MD, USA) and 100 U/mL penicillin/streptomycin at 37 °C and 5 % CO_2 humidified incubator.

2.1.2. Quantitative PCR

Total RNA isolation from the GC cells was performed using TRIzol (Invitrogen, Carlsbad, CA, USA). Subsequently, we utilized the PrimeScript RT Reagent Kit (TaKaRa, Shiga, Japan) to synthesize the cDNA. Quantitative PCR (qPCR) was performed using SYBR Premix Ex TaqTM II (TaKaRa, Shiga, Japan) according to the manufacturer's protocols. The *ASAP1* expression was normalized to GAPDH, and gene expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method. The primers for *ASAP1* and GAPDH were synthesized by Shanghai Biotechnology Co., Ltd. (Shanghai, China). We used the following primer pairs: 5′-GAGAGGTATGAAGGTTATTCA-3' (forward) and 5′-ATCACCACCTTACGAGCCACC-3' (reverse) for *ASAP1* and 5′-GCGGGGCTCCAGAACATCAT-3' (forward) and 5′-CCAGCCCCAGCGTCAAGGTG-3′ for GAPDH.

2.1.3. Transfection

Kilton Biotechnology Co., Ltd (Shanghai, China) synthesized two small interfering RNAs (siRNAs) targeting ASAP1 and negative control (siNC) (Shanghai, China). Moreover, Kilton Biotechnology Co., Ltd. constructed the overexpression plasmid, pcDNA3.1-

ASAP1, and an empty plasmid. Finally, the cells were transfected using Lipofectamine 2000 (Invitrogen, USA) per the protocols. The following siRNA sequences were used in Transfection: 5′-CCAGGGAUUUACUUGCACUUU-3' (sense) and 5′-AGUGCAA-GUAAAUCCCUGGUU-3' (antisense) for siASAP1-1 and 5′-CAGUACUUUUGUGUAGUACAA-3' (sense) and 5′-UUGUACUACACAAAA-GUACUG-3' (antisense) for siNC.

2.1.4. Cell Counting Kit-8 assay

Cell Counting Kit-8 (CCK-8) (Beyotime Biotechnology, Beijing, China) was utilized to assess 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. Subsequently, 10 mL of CCK-8 solution was added to each well, followed by incubation for 1 h. The optical density was measured employing a microplate reader (Molecular Devices, Sunnyvale, CA, USA) 450 nm.

2.1.5. 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 for 14 days at 37 °C. Subsequently, the supernatants were discarded, and the cells were fixed in methanol and stained with 0.1 % crystal violet (Sigma-Aldrich, St. Louis, MO, USA). The colony numbers were determined using light microscopy.

2.1.6. Cell apoptosis assay

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

2.1.7. Transwell invasion assay

The Transwell invasion assay (BD Biosciences) was utilized in 24-well plates to assess cell invasion capability. The upper chambers were coated in advance with 60 μL of Matrigel (BD Biosciences) that had been diluted at a ratio of 1:3. About 6×10^4 cells were seeded into the upper chambers in 200 μL serum-free medium. Meanwhile, the lower chambers were filled with 600 μL complete medium containing 10 % FBS. After a 24-hour incubation at 37 °C with 5 % CO$_2$, the cells on the upper membranes were removed using cotton wool, fixed in 4 % paraformaldehyde, and stained with 0.1 % crystal violet. Lastly, invading cells were counted under a microscope in five randomly chosen fields (200×magnification). Each experiment was conducted in triplicate at least three times.

2.1.8. Wound healing assay

1×10^6 cells/well were seeded into 6-well plates and cultured for 12 h in a serum-free medium. A perpendicular scratch wound was then performed using a 10 μL pipette tip. Images were captured at 0 and 48 h to monitor the wound healing process employing an inverted microscope (100 × magnification; Olympus Corporation, Tokyo, Japan). ImageJ software was utilized to calculate the distance between the two edges of the wound. Healing rate = (0h average gap-24h average gap)/0h average gap.

2.1.9. Western blot (WB) analysis

WB was conducted following standard protocols. 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-Caspaes3 (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), AKT (1:2000; #4060; CST) and GAPDH (1:1000; #5174; CST). The total cellular proteins were extracted utilizing RIPA buffer, separated via 10 % sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and subsequent electro-transfer onto PVDF membranes. Following a 2-h blocking period at 37 °C using 5 % non-fat milk, the membranes were subjected to overnight incubation with primary antibodies at 4 °C and then probed with secondary antibodies for 2 h at 37 °C. Eventually, the protein bands were identified utilizing an ECL kit per the protocols. The quantification of each band was performed utilizing the ImageJ software.

2.1.10. Animal experiments

Five-week-old male nude mice (Shanghai, China) were randomly assigned to three groups (Control, siNC, and siASAP1 groups, n = 5/group). The animal experiments were performed in accordance with the 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 at 8, 14, 17, and 20 days (volume = $\pi/6 \times$ length × width^2). The mice were euthanized, and the tumors were excised and quantified in terms of weight.

2.2. Statistical analysis

GraphPad Prism software (version 6.0; San Diego, CA, USA) was employed for the statistical analyses, presenting the three independent experiment data as mean ± standard deviation. Student's t-test was used for comparisons between two groups, while one-way analysis of variance (ANOVA) was used for multigroup comparisons. $P < 0.05$ was considered statistically significant.

3. Results

3.1. ASAP1 expression is upregulated in GC cells

The results of qPCR and WB assays in determining *ASAP1* expression in the three GC cell lines and GES-1 normal gastric epithelial cell lines revealed that *ASAP1* was overexpressed in the GC lines (especially BGC823 and MKN45 cell lines) compared to GES-1 cells at the mRNA and protein levels (Fig. 1). Therefore, BGC823 and MKN45 cells were selected for the knockdown, and BGC823 was selected for overexpression.

3.2. Silencing and overexpression of ASAP1 in GC cells

To modulate the expression of *ASAP1*, targeted siRNA molecules were employed to silence or overexpress the gene in BGC823 and MKN45 cell lines, and a plasmid overexpressing *ASAP1* (oe*ASAP1*) was transfected into BGC823 cells. qPCR and WB results revealed that *ASAP1* was successfully suppressed in BGC823 and MKN45 and overexpressed in BGC823 cells (Fig. 2A–C).

3.3. Silencing of ASAP1 inhibits GC cell proliferation and induces apoptosis

For elucidating the *ASAP1* role in GC cell proliferation and apoptosis *in vitro*, CCK-8, colony formation, and flow cytometry assays were conducted. The CCK-8 and colony formation assay results showed that *ASAP1* silencing reduced BGC823 and MKN45 cell growth, whereas *ASAP1* overexpression enhanced BGC823 cell proliferation (Fig. 3A and B). Flow cytometry test showed that silencing of *ASAP1* significantly enhanced apoptotic rate in BGC823 and MN45 cells, whereas overexpression of*ASAP1* had the contrary influences in BGC823 cells (Fig. 3C). These findings revealed that *ASAP1* possesses a negative function in GC cell proliferation.

3.4. ASAP1 knockdown suppresses GC cell invasion and migration

For investigating the involvement of *ASAP1* in GC cell invasion and migration, both transwell invasion and wound-healing assays were performed on BGC823 and MKN45 cells. *ASAP1* inhibition in BGC823 and MKN45 cells decreased in their respective capacities for cellular invasion and migration. Conversely, *ASAP1* overexpression in BGC823 cells resulted in enhanced cell migration and invasion capabilities (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. ASAP1 knockdown changes the protein levels associated with GC cell malignant behaviors

VEGFA is involved in transporting nutrients for tumors before angiogenesis. MMPs are a class of proteolytic enzymes that are closely associated with malignant tumor invasion and metastasis. Cysteinyl aspartate-specific proteinases (caspases) are a group of proteases related to apoptosis and cytokine maturation.

WB was conducted to examine the *ASAP1* silence impact on the tumor cell malignant behavior at the protein level of VEGFA, Cleaved-Caspase 3 (Casp3), Cleaved-PARP, and MMP2/9. The alterations in protein levels that were observed in conjunction with *ASAP* knockdown (Fig. 5A–C) suggest that the impairment of malignant biological behaviors in GC cells may be attributed to *ASAP1* knockdown.

For investigating the mechanism behind *ASAP1* silence inhibiting the malignant phenotype of GC further, EMT markers and AKT (*p*-AKT) protein levels were detected. WB results showed that *ASAP1* downregulation significantly suppressed N-cadherin and Vimentin expression while significantly overexpressing E-cadherin (Fig. 5D). Additionally, *ASAP1* downregulation significantly suppressed AKT and *p*-AKT expression (Fig. 5E).

These findings suggest that knockdown of *ASAP1* may suppress malignant behaviors of GC cells through reducing VEGFA, overexpressing cleaved-Casp3 and cleaved-PARP MMPs, and reducing MMPs, EMT, AKT, and *p*-AKT activity.
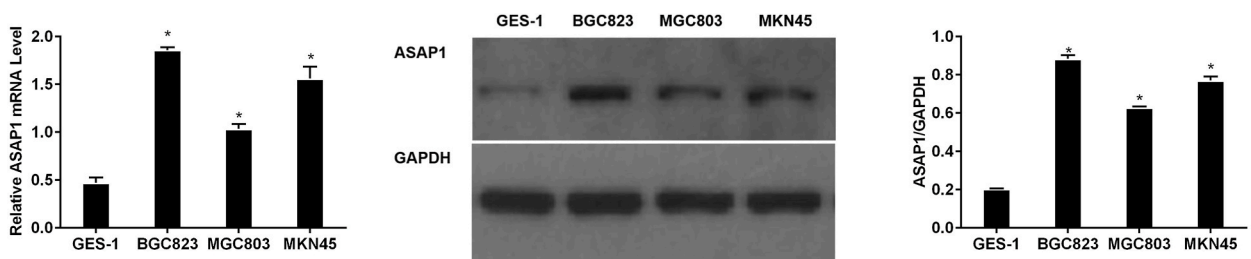

Fig. 1. Expression levels of *ASAP1* in normal GES-1 cells and three GC cell lines were determined by qRT-PCR and WB (*$P < 0.001$ *vs.* GES-1, n = 3/group).
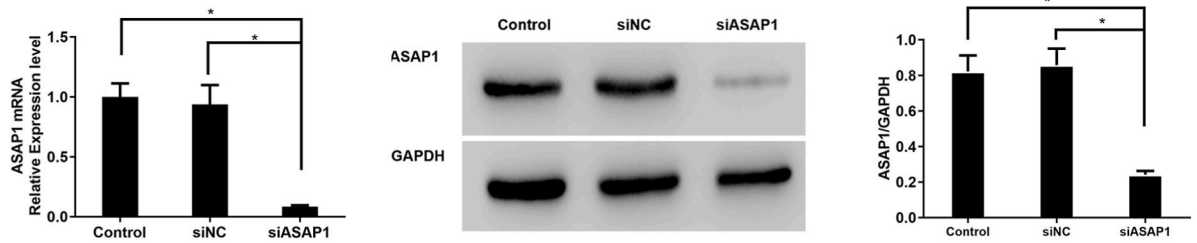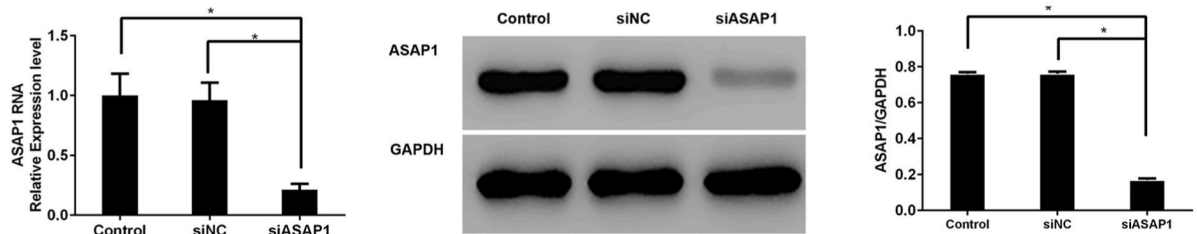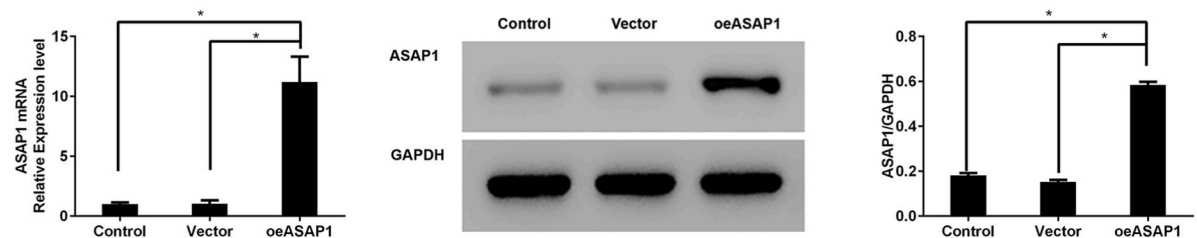
Fig. 2. Silencing and overexpression of *ASAP1* in GC cells. (*P < 0.001, n = 3/group) **(A)** The efficiency of *ASAP1* silencing in BGC823 was detected by qRT-PCR and WB. **(B)** The efficiency of *ASAP1* silencing in MKN45 was detected by qRT-PCR and WB. **(C)** The efficiency of *ASAP1* overexpression in BGC823 was detected by qRT-PCR and WB.

3.6. ASAP1 silencing represses GC subcutaneously xenografted growth in vivo

Next, to examine the *ASAP1* function *in vivo*, mice xenograft models of GC were established, and 8 days after subcutaneous cancer cell inoculation, all nude mice transfected with cells developed tumors. The results revealed that tumor volumes and weights were significantly lower in the siASAP1 group than in the control and siNC groups (Fig. 6A–C). At 20 days, the tumor volume and weight inhibition rates were 77.23 % and 78.67 %, respectively. These findings validated that ASAP1 knockdown could hinder tumor growth.

4. Discussion

The 5-year survival rate of GC remains below 30 %. The primary reason for this low survival rate in advanced GC therapy is the lack of effective target proteins and their corresponding inhibitors [16,17]. Hence, studies have endeavored to explore new cancer treatments by identifying novel GC targets. In our prior research, *ASAP1* overexpression was found to be correlated to the poorer overall survival of GC [14,15]. Therefore, we demonstrate that *ASAP1* is overexpressed in GC cells compared to GES1 cells. *ASAP1* knockdown suppressed GC cell proliferation, invasion, and migration while promoting apoptosis. Conversely, *ASAP1* overexpression has opposite effects. Additionally, we observed tumor growth suppression *in vivo* upon *ASAP1* knockdown, consistent with findings in breast, bladder, non-small cell lung, and ovarian cancers [18–20]. Therefore, we propose *ASAP1* as an innovative strategy for GC-targeted therapy.

The malignant behavior of tumor cells is intricately linked to angiogenic factors, with VEGFA standing out as a crucial positive regulator of angiogenesis. VEGFA facilitates tumor cell proliferation and growth by nourishing them and aiding in the formation of vascular networks through increased blood vessel permeability. Before angiogenesis, VEGFA plays a role in transporting nutrients for tumors. In a study by Hashimoto et al. [21], it was demonstrated that RNAi targeting *ASAP1* significantly impeded VEGFA-induced lumen formation, suggesting a potential impact on breast cancer angiogenesis via the GEP100-Arf6-*ASAP1*-cortacin pathway. Moreover, this investigation revealed that knocking down *ASAP1* in BGC823 GC cells led to a notable reduction in VEGFA protein
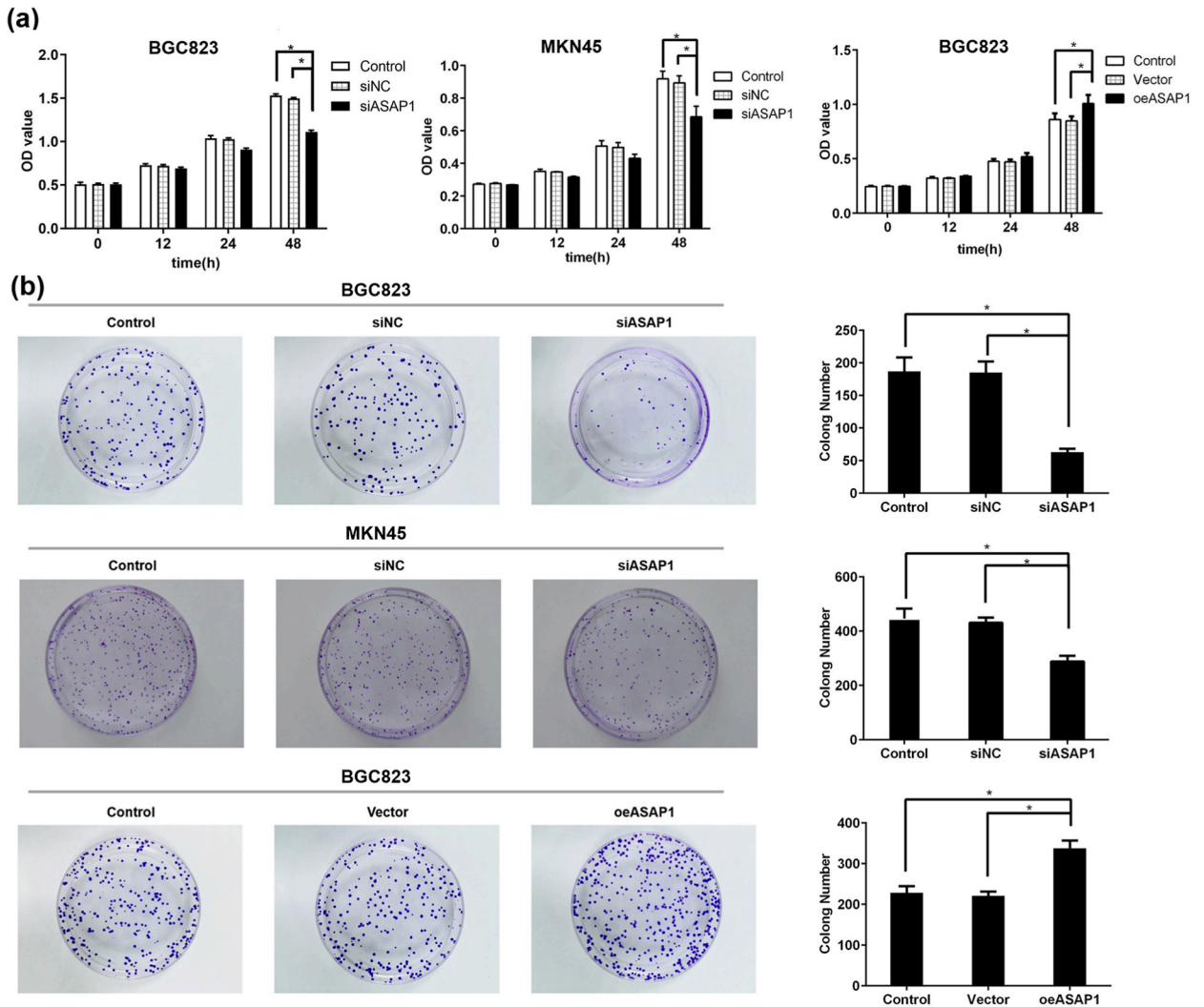
Fig. 3. *ASAP1* silencing inhibited, while overexpression promoted the cell progression of GC cells. (*P < 0.001, n = 3/group) **(A)** The CCK-8 assay for the cell proliferation of BGC823 and MKN45 cells with *ASAP1* silencing and BGC823 cells with *ASAP1* overexpression. **(B)** The clone formation 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.

expression, consequently obstructing relevant signal transduction pathways. As s result, this inhibition suppressed malignant GC angiogenesis, as well as proliferation, growth, invasion, and metastasis.

The malignant behavior of tumor cells is closely intertwined with programmed cell death. Cysteinyl aspartate-specific proteinases (caspases) constitute a group of proteases associated with apoptosis and cytokine maturation. Casp3, a pivotal protease, serves as an executioner of apoptosis, typically existing as an inactive zymogen. The activation of Casp3 signifies an irreversible step in the apoptosis process [22]. Cleaved-Casp3 respresnets the active fragment of activated Casp3. Song et al. [23] demonstrated that Casp3 activation induces apoptosis in GC cells. The expression rate of cleaved-Casp3 in cervical cancer tissues was observed to be low and negatively correlated with cervical cancer tissue differentiation. Multiple studies have confirmed that cleaved-Casp3 expression is closely related to prognosis [24–26]. PARP, a DNA repair enzyme, plays a crucial role in maintaining genetic materials stability and is activated during DNA damage and repair, thereby regulating apoptosis. Following cleavage by activated Casp3, PARP is transformed into cleaved-PARP, subsequently initiating apoptosis [27]. Wang et al. [28] demonstrated PARP overexpression in GC tissues compared to healthy gastric tissues. Our study revealed that cleaved-Casp3 and cleaved-PARP protein expression were downregulated in BGC823 *ASAP1* knockdown GC cells. This suggests that knocking down *ASAP1* enhances the expression of these two proteins, further confirming that *ASAP1* regulates the apoptosis of GC cells.

Tumor cell invasion and metastasis constitute continuous processes driven by the intricate interplay between cancer cells and host cells. MMPs, a class of proteolytic enzymes, play a crucial role in facilitating malignant tumor invasion and metastasis. Among these,
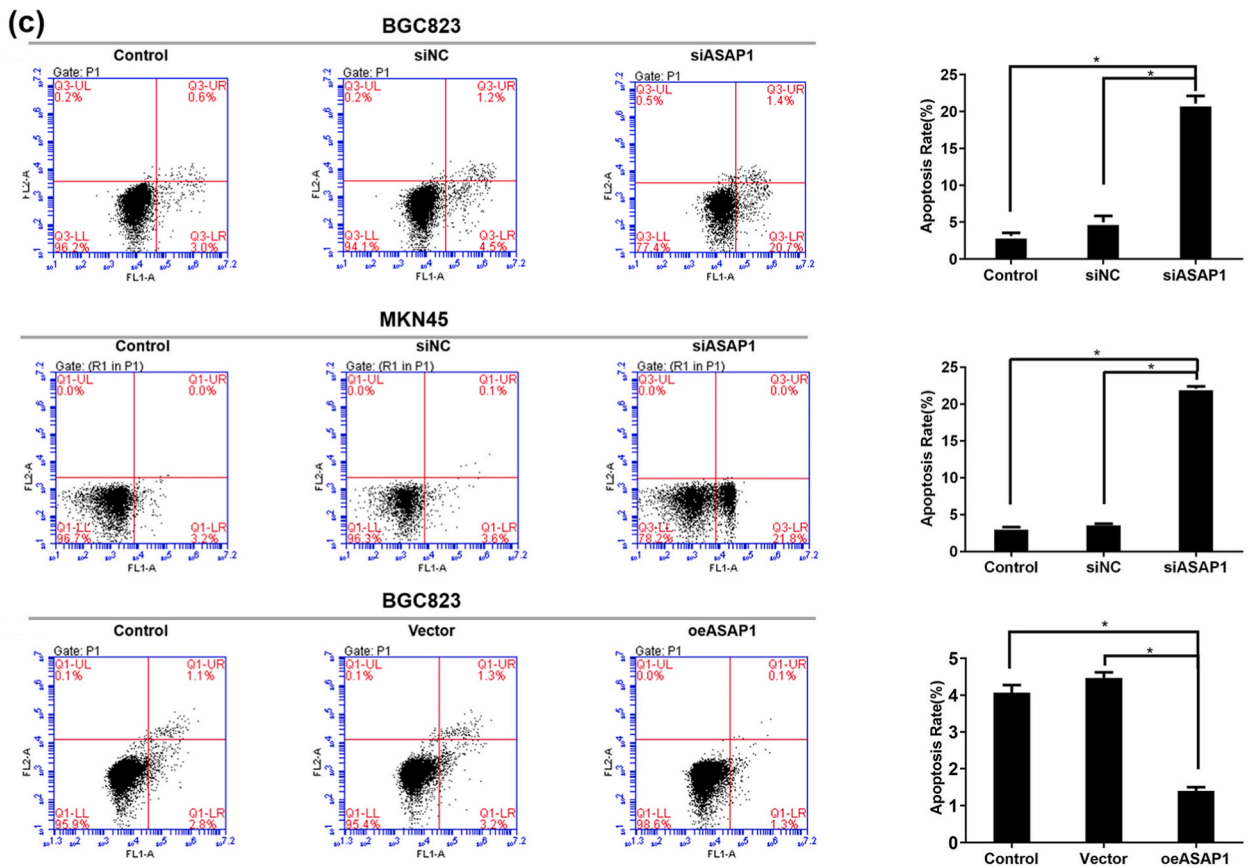
Fig. 3. (*continued*).

MMP2/9 are particularly noteworthy as they both induce and enhance the formation of blood vessels and infiltrate blood vessels and lymphatic vessels through processing involving degradation, destruction, and infiltration, ultimately culminating in tumor invasion and metastasis [29]. Downregulation of MMP2/9 has been shown to effectively inhibit the invasion and metastasis of tumor cells [30–32]. Additionally, *ASAP1* knockdown significantly downregulated MMP2/9 protein expression and inhibited GC cell invasion and migration.

Tumor cell invasion and metastasis necessitate a distinct cellular phenotype. The EMT is a transient and reversible phenomenon that takes place in distinct physiological and pathological contexts. It involves the loss of epithelial cell polarity and tight cell-cell connection, leading to a conversion from an epithelial to a mesenchymal phenotype. This transition mainly manifests as the suppression of the epithelial marker E-cadherin, the overexpression of the mesenchymal marker N-cadherin, and the transformation of keratin into vimentin, a core component of the cytoskeleton [33]. E-cadherin and N-cadherin play crucial roles in maintaining the integrity of the epithelial cell monolayer [34]. Vimentin, the primary component of the intermediate filament protein family, maintains cell integrity, provides stress resistance, and promotes tumor growth and invasion [35]. In a study by Li et al. [36] in 2017, *ASAP1* expression blockade by RNAi reversed the EMT process of breast cancer cells. Additionally, In 2018, Zhang et al. [20] showed that *ASAP1* overexpression enhanced tumor cell motility and invasiveness of ovarian cancer cells. In our study, *ASAP1* knockdown suppressed the loss of phenotype in GC epithelial cells and reduced the appearance of the mesenchymal cell phenotype, as evidenced by E-cadherin overexpression and N-cadherin and Vimentin suppression. This confirms that the knockdown of *ASAP1* effectively reverses the EMT process and inhibits the invasion and metastasis of malignant tumors.

The AKT serine/threonine kinase is a protein with oncogenic properties that is significantly implicated in the pathogenesis of malignant neoplasms. The study conducted by Zhang and colleagues revealed that the *ASAP1*-IT1 gene regulates the PTEN/AKT signaling pathway, which in turn affects NSCLC cell proliferation, invasion, and metastasis [12,19]. Our study found that knocking down *ASAP1* significantly reduces the levels of both *p*-Akt and Akt. Like other malignant tumors, GC is influenced by multiple targets and signaling pathways, with Akt central to many of these pathways. The potential effects of *ASAP1* on AKT-associated signaling pathways are noteworthy, suggesting the possibility of other intermediary targets that may involve additional signaling pathways. Our study represents an initial exploration into the role and mechanism of *ASAP1* in GC. However, a comprehensive understanding of the complete mechanism of *ASAP1* in this context has yet to be elucidated.

Additionally, this study employed nude mouse xenograft models to evaluate the effects of *ASAP1* knockdown on BGC823 gastric tumorigenesis *in vivo* and to verify the relationship between *ASAP1* and GC. The animal experiments revealed that tumors with *ASAP1*
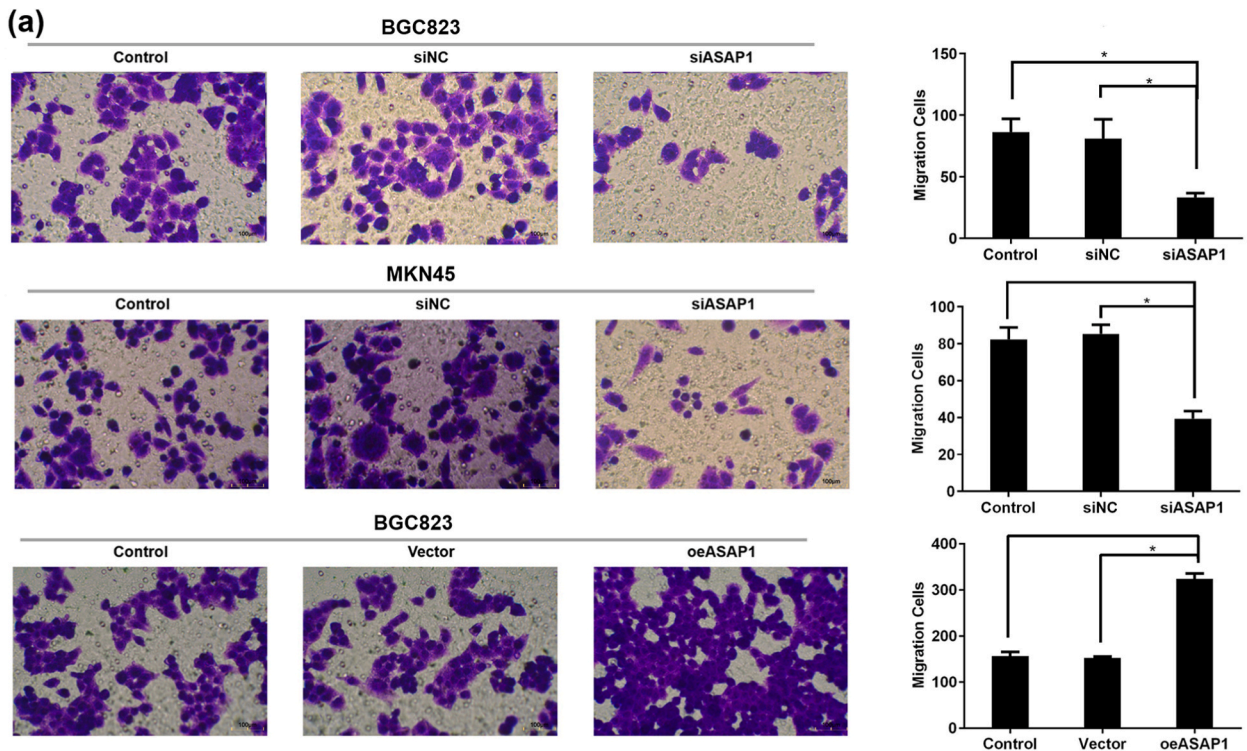
Fig. 4. *ASAP1* knockdown suppressed, while overexpression promoted the invasion and migration of GC cells. (*P < 0.001, n = 3/group) **(A)** The Transwell assay for the invasion of BGC823 and MKN45 cells with *ASAP1* silencing and BGC823 cells with *ASAP1* overexpression. **(B)** Wound healing assay for the migration of BGC823 and MKN45 cells with *ASAP1* silencing and BGC823 cells with *ASAP1* overexpression.

knockdown grew significantly slower and weighed less, confirming that the proliferation and growth of *ASAP1*-knockdown BGC823 GC cells were significantly inhibited.

5. Limitation

This study has several limitations that should be considered when interpreting its findings. Firstly, our research primarily focused on the expression patterns of ASAP1 and its influence on the malignant behaviors of gastric cancer cells. While these findings provide valuable insights, we did not extensively explore the specific signaling pathways through which ASAP1 contributes to tumor development. This limits a comprehensive understanding of the molecular mechanisms at play, thereby paving the way for future studies to delve deeper into these processes. Secondly, the experimental observations were largely restricted to the measurement of tumor volume and weight in nude mice. We did not document or compare changes in the activity or mental state of the mice throughout the experimental period. This omission hinders a full evaluation of the physiological and psychological impacts of ASAP1 on gastric cancer progression in an animal model, which could be crucial for understanding the broader implications of tumor growth and treatment responses. Lastly, our investigation into the role of ASAP1 also included a preliminary analysis of MMP expression levels to assess their potential influence on the migration and invasion capabilities of gastric cancer cells. However, the study did not extend to examining how ASAP1 regulates MMP activity and functionality. This area remains underexploited and warrants further exploration to clarify the regulatory mechanisms of ASAP1 in cancer cell dynamics. These limitations underscore the need for additional research to build upon our findings and address the gaps in understanding the role of ASAP1 in gastric cancer, which could contribute to the development of targeted therapies and improved clinical outcomes.

6. Conclusion

Our research has substantiated the overexpression of *ASAP1* in GC cells, identifying its pivotal role in modulating various malignant attributes. Specifically. Overexpression of *ASAP1* markedly enhances cell proliferation, diminishes apoptosis activity, and bolsters both invasion and migration capabilities within GC cells. Conversely, silencing *ASAP1* expression leads to a significant reduction in cellular proliferation and an increase in apoptosis, alongside weakened invasion and migration capabilities. Furthermore, our findings reveal that downregulation of VEGFA curtails angiogenesis and increases the expression of apoptosis markers cleaved-Casp3 and cleaved-PARP, while simultaneously reducing MMPs and EMT activity. These cellular effects are mediated through AKT-related
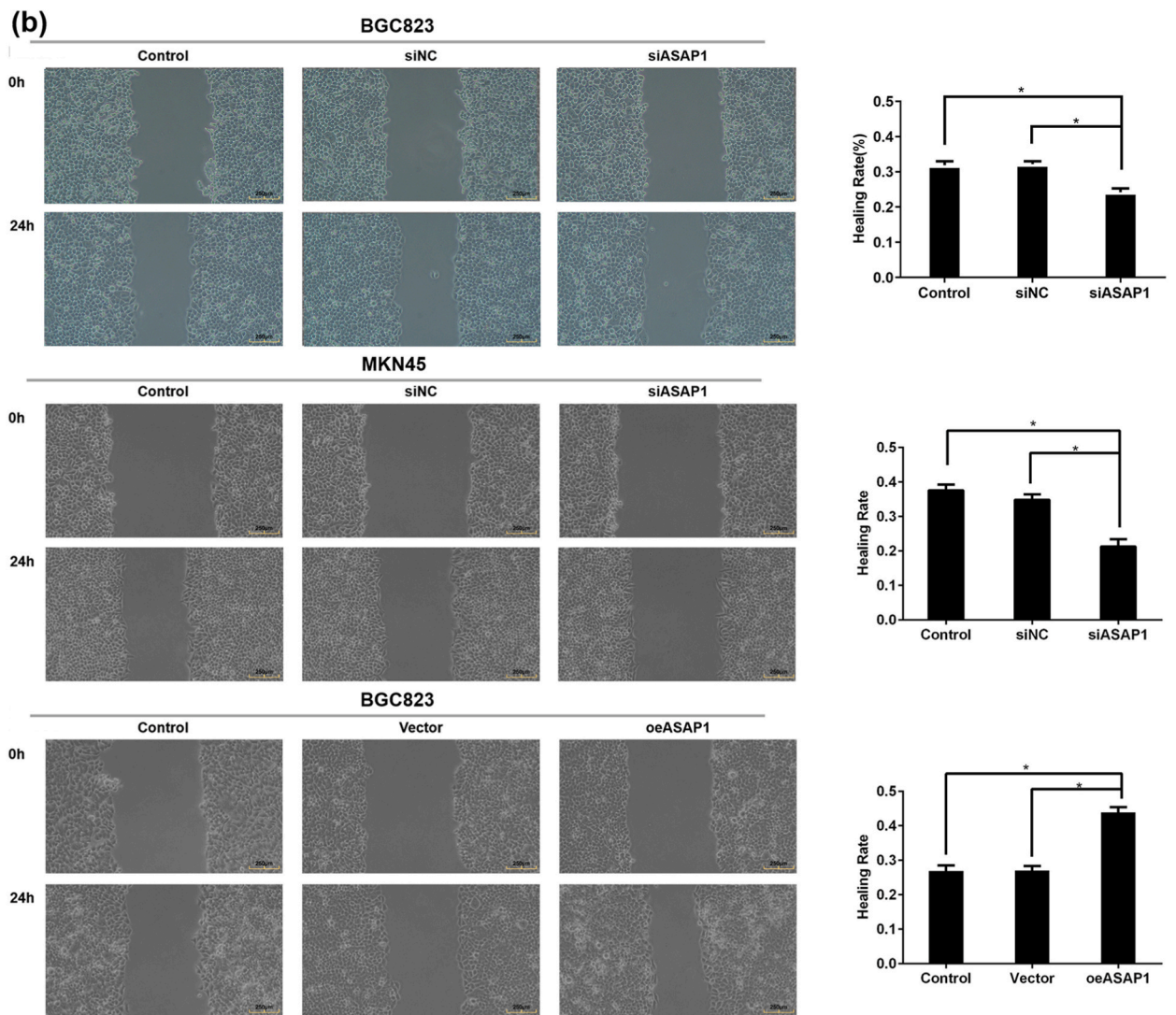
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signaling pathways, which are instrumental in suppressing the malignant behaviors of GC cells. These findings suggest potential avenues for the prevention and treatment of GC, warranting further investigation in future studies.
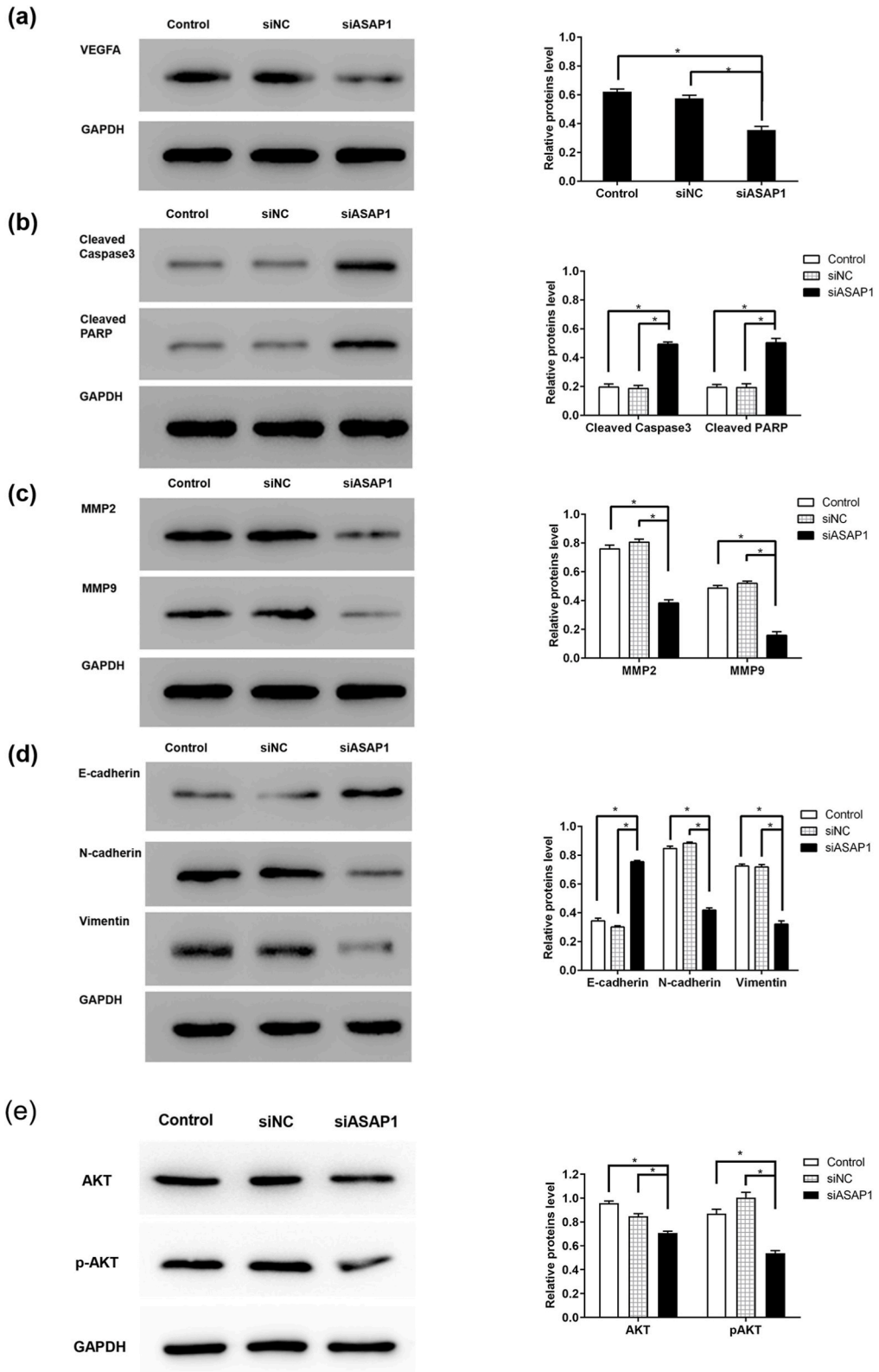
Funding

Ethics approval and consent to participate

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.

(caption on next page)

Fig. 5. *ASAP1* silencing inhibited the protein levels related to malignant behaviors in BGC823 cells. (*P < 0.001, n = 3/group) **(A)** WB was used to assess the effect of *ASAP1* silencing on VEGFA. **(B)** WB 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)** WB was performed to evaluate the effect of *ASAP1* silencing on the protein levels of MMP2 and MMP9 in BGC823 cells. **(D)** WB was used to assess the effect of *ASAP1* silencing on E-cadherin, N-cadherin, and vimentin. **(E)** WB was used to assess the effect of *ASAP1* silencing on AKT and *p*-AKT.
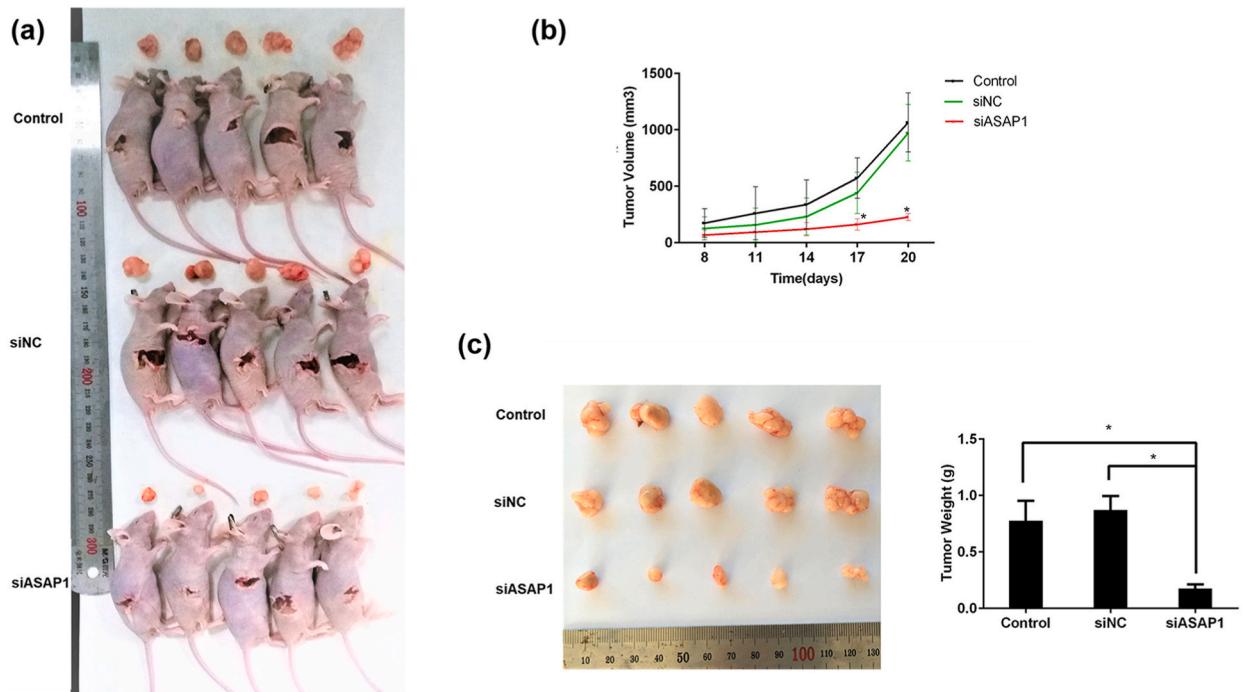

Fig. 6. *ASAP1* silencing suppressed GC tumor growth *in vivo*. Nude mice were injected with BGC823 cells transfected with siNC or si*ASAP1*. (*P < 0.001, n = 5/group)**(A)** Image of tumor-bearing nude mice. **(B)** Tumor growth curves. **(C)** Average tumor weights in the si-*ASAP1* group were greatly lower than those in the control and siNC groups.

Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Qiong Luo: Writing – original draft, Visualization, Software, Formal analysis, Data curation, Conceptualization. **Suyun Zhang:** Software, Methodology, Investigation, Formal analysis, Conceptualization. **Fan Yang:** Software, Data curation. **Rui Feng:** Visualization, Software, Data curation. **Qian Xu:** Visualization, Software, Methodology. **Xiangqi Chen:** Writing – review & editing, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Sheng Yang:** Writing – review & editing, Resources, Project administration, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare no conflict of interest.

Acknowledgements

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