Effects of stromal interacting molecule 1 gene silencing by short hairpin RNA on the biological behavior of human gastric cancer cells

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Abstract. Gastric cancer is one of the most common types of cancer worldwide. It has been reported that stromal interacting molecule 1 (STIM1) is associated with tumor progression and metastatic spread, including in cervical cancer, breast carcinoma and prostatic cancer. The present study investigated whether STIM1, an endoplasmic reticulum Ca^{2+} sensor and activator of store-operated channel entry, contributed to SGC7901 cell progression. The pGPU6-shSTIM1 recombinant plasmid was constructed, and the effects of downregulation of STIM1 on the proliferation, apoptosis, migration and invasion of SGC7901 cells were examined. Western blot analysis revealed that transfection with the pGPU6-shSTIM1 plasmid successfully inhibited the expression of STIM1. STIM1 silencing in the gastric cancer cells significantly inhibited cell proliferation by arresting the cell cycle at the G0/G1 phase, and increasing the apoptotic rate following treatment of the SGC7901 cells with pGPU6-shSTIM1, indicated using an MTT cell viability assay and flow cytometery, respectively. As expected, STIM1 knock down also reduced the migration and invasion of the SGC7901 cells, demonstrated using a Transwell assay. The possible molecular mechanism involved the regulation of several signaling pathways involved in the biological behavior of cell survival, apoptosis, migration and metastasis. Together, these finding suggested that the expression of STIM1 is crucial for the proliferation and invasion of SGC7901 cells, providing a foundation for the development of novel type-specific diagnostic strategies and treatments for gastric cancer.

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Introduction

Gastric cancer remains the second leading cause of cancer-associated mortality worldwide, with an overall 5-year survival rate of <25% (1,2). There are ~930,000 newly diagnosed cases and ~700,000 patients succumb to the condition each year (2). Therefore, novel strategies for gastric cancer treatment are urgently required.

Calcium (Ca^{2+}) is a multifunctional messenger, which controls several cellular processes ranging between short-term responses, including muscle contraction and secretion, and long-term regulation, including cell growth and proliferation (3,4). Store-operated Ca^{2+} entry (SOCE) is a major mechanism for Ca^{2+} entry across the cell membrane, which is stimulated in response to the depletion of Ca^{2+} from intracellular Ca^{2+} stores, primarily the endoplasmic reticulum (ER), and mediated via the activation of specific plasma membrane channels, termed store-operated channels (SOCs) (5). Stromal interacting molecule 1 (STIM1) is a highly conserved type-I membrane and ER-resident protein, containing a luminal EF-hand Ca^{2+}-binding domain and several cytosolic protein-protein interaction domains, and serves a dual role as an ER Ca^{2+} sensor and activator of SOCE (6-8).

The role of STIM1 in regulating cancer progression remains controversial. In previous studies, which were performed prior to the elucidation of its role in Ca^{2+} signaling, STIM1 was described as a tumor suppressor, as it caused growth arrest in human G401 rhabdoid tumor cells and human RD rhabdomyosarcoma cells (9,10). However, subsequent studies have revealed a potential role of STIM1 as an oncogene, as it is upregulated in several types of human cancers, including breast cancer (11), glioblastoma (12,13) and cervical cancer (14). Thus, further investigation is required to fully determine the role of STIM1 in tumorigenesis, which might vary in different types of tumor. In the present study, plasmid-mediated short hairpin (sh)RNA was used to suppress the expression of STIM1 in SGC7901 cells, and the effects of STIM1 knockdown on cell

proliferation, apoptosis, cell cycle progression, migration and invasion were examined. Elucidation of the role of STIM1 in regulating cancer cell progression provides aimed to establish whether STIM1 may serve as a therapeutic target for gastric cancer.

Materials and methods

Chemicals and suppliers. Disposable culture equipment was purchased from Corning Glass Works (Corning, NY, USA). Cell culture media and fetal bovine serum (FBS) were obtained from Gibco Life Technologies (Carlsbad, CA, USA). OPti-MEM medium, thapsigargicine (TG), Fura-2AM and Lipofectamine™ 2000 were obtained from Invitrogen Life Technologies (Carlsbad, CA, USA). All other biochemicals were obtained from Sigma-Aldrich (St. Louis, MO, USA). The rabbit polyclonal antibody against STIM1 (1:1,000; #4916) was purchased from Cell Signaling Technology, Inc. (Danvers, MA). Mouse β-actin monoclonal antibody (1:500; sc-47778) and the secondary antibodies, goat anti-mouse immunglobulin (Ig)G conjugated to horseradish peroxidase (1:2,000; sc-2005), and goat anti-rabbit IgG conjugated to horseradish peroxidase (1:5,000; sc-2004) were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA).

Cell culture. Human gastric cancer SGC7901 cells were obtained from American Type Culture Collection (Manassas, VA, USA) and cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% heat-inactivated FBS, 100 U/ml penicillin and 100 μg/ml streptomycin. The cells were maintained in a 95% humidified air, 5% CO_2 incubator at 37˚C.

Plasmid construction and transfection. For plasmid construction, three shRNAs targeting the STIM1 gene were designed and synthesized by Invitrogen Life Technologies. The shRNA deemed most applicable by comparison of interference effects, shSTIM1, was used in the subsequent experiments. The sequences of shSTIM1 were as follows: Sense 5'-CA C CAGAAGGAGCTAGAATCTCACTTCAAGAGAGTGAGA TTCTAGCTCCTTCTTTTTTG-3 and antisense 5'-GATCCA AAAAAGAAGGAGCTAGAATCTCACTCTCTTGAAGTG AGATTCTAGCTCCTTCT-3'. The sequence of the scrambled shRNA (negative control) was sense 5'-CACCGACGCTGA AGACTCTTGGCTTCAAGAGAGCCAAGAGTCTTCAGC GTCTTTTTTG-3' and antisense: 5'-GATCCAAAAAAG ACGCTGAAGACT CTTGGCTCTCTTGAAGCCAAG AGTCTTCAGCGTC-3'. The shRNA was constructed by annealing the synthetic DNA oligonucleotide primers, which were cooled to room temperature and inserted between the BbsI and BamHI sites of the pGPU6/green fluorescent protein (GFP)/Neo eukaryote expression vector, which contained the GFP gene as a reporter, with an internal cytomegalovirus promoter. The recombinant vector was then transfected into competent DH5α cells. Clone identity was verified by sequencing at Invitrogen Life Technologies (Shanghai, China), and analyzing the results with DNAssist version 2.2 (http://dnassist.en.softonic.com/).

The SGC7901 cells (0.8~1x10^6 cells/dish) were transiently transfected using Lipofectamine™ 2000, according to the manufacturer's instructions. The medium containing the transfection reagents was replaced 4-6 h following transfection with DMEM supplemented with 10% FBS. The cells were collected 48 h after transfection, processed in the following experiments and prepared for protein extraction. The silence efficiency of STIM1 was assessed using western blot analysis.

Protein extraction and western blot analysis. Protein extraction and western blot analysis were performed, as previously described (15). In brief, total cell lysates were obtained by scraping the plates and centrifuging at 450 x g for 5 min at 4˚C. The supernatant was removed and the pellet was rinsed with 4 ml cold PBS, then centrifugation was conducted at 450 x g for 5 min at 4˚C. Lysis buffer (Pierce Biotechnology, Inc., Rockford, IL, USA) was added in the pellet and the cells were sonicated, incubating on ice for 40 min and subsequently centrifuging at 10,460 x g for 12 min at 4˚C. The protein concentrations in the supernatant were calculated using a bicinchoninic acid protein assay (Pierce Biotechnology, Inc.). The supernatants were boiled for 5 min and were then subjected to electrophoresis on 10% SDS-PAGE gels (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The proteins were transferred to polyvinylidene difluoride filters, which were incubated at room temperature (~25˚C) for 1 h in 5% non-fat dry milk and Tris-buffered saline with 0.5% Tween 20 (TBS-T). Immunological evaluation was performed either for 1 h at room temperature or overnight in 4˚C using 5% milk TBS-T containing 1-2 μg/ml the STIM1, antibody, according to the manufacturer's instructions. The filters were then washed with 1X TBS-T and incubated for 1 h at room temperature with the goat anti-rabbit HRP-conjugated secondary antibody. Following multiple washes with TBS-T, the filters were developed using enhanced chemiluminescence.

Intracellular calcium imaging. To visualize intracellular Ca^{2+}, the SGC7901 cells (0.8~1x10^6 cells/dish), transfected at 90% confluence with either 1.6 μg pGPU6-shSTIM1 or pGPU6-shNC/well were cultured with 6 μM Fura-2 AM and 0.02% pluronic acid for 60 min at 37˚C. The medium was then removed, and the cells were plated onto glass-bottomed perfusion chambers, which were washed three times in isotonic buffer without Ca^{2+} (KH buffer: 132 mM NaCl, 5 mM KCl, 10 mM dextrose, 10 mM HEPES, 1.05 mM $MgCl_2$). Ringer's solution, containing 2 mM Ca^{2+} (150 mM NaCl, 4.5 mM KCl, 10 mM d-glucose, 2 mM $CaCl_2$, 1 mM EGTA, 1 mM $MgCl_2$, and 5 mM HEPES) was used to induce intracellular Ca^{2+} flux; and 1 μM thapsigargin in Hanks' balanced salt solution was used to deplete ER Ca^{2+} stores. Images were captured every 20 sec using an IX71 inverted microscope (Olympus, Center Valley, PA, USA), at xcitation wavelengths of 340 nm and 380 nm, and emission wavelngth of 508 nm, and monitored using Cell^R (Olympus). The data, comprising relative intracellular Ca^{2+} concentrations are reported as 340/380 ratios.

Cell viability and proliferation assay. The cell viability and proliferation activity was examined using an MTT assay, as described previously (16). Following transfection of the cells with either pGPU6-shSTIM1 or scrambled shRNA for 48 h, 100 ml of the cell suspension (1x10^3 cells) was plated in a 96-well microtitre plate in triplicate. At 0, 24, 48 and

72 h, the cells were washed with warm PBS, and 20 μl MTT (5 mg/ml in PBS) was added into each well, following incubation for 4 h at 37˚C. The media was then removed and 200 μl dimethyl sulfoxide (Sigma-Aldrich) was added to dissolve the formazan crystals. The absorbance of the blue formazan derivative was measured at 570 nm using a microplate reader (Bio-Rad Laboratories, Inc.). The results are expressed as the mean ± standard deviation of three independent experiments.

Apoptosis assay. The apoptosis of the SGC7901 cells was analyzed using an Annexin V-fluorescein isothiocyanate (FITC)/Propidium iodide (PI) Apoptosis Detection kit (KeyGEN Biotech Co, Ltd., Nanjing, China). Briefly, the cells were cultured in 6 cm dishes (0.8~1x10^6 cells/well) and transfected with pGPU6-shSTIM1 or scrambled shRNA for 48 h. The cells were then trypsinized using 0.25% Typsin. The cells were collected and washed twice with PBS, and suspended in 200 μl binding buffer and 10 μl annexin V-FITC for 20 min in the dark. Thereafter, 300 μl binding buffer and 5 μl PI were added to each sample. The apoptotic cells were determined using a flow cytometer (FACSCalibur; BD Biosciences) with FCS Express V3 (De Novo Software, Glendale CA, USA).

Cell cycle. The effect of STIM1 on cell cycle distribution was determined using flow cytometry (17). Briefly, the plasmid-transfected SGC7901 cells were harvested and washed with PBS and fixed overnight with 75% ice-cold ethanol at -20˚C. The fixed cells were stained with a solution containing PI (50 μg/ml), RNase A (100 μg/ml) and Triton X-100, and analyzed using flow cytometry (BD Biosciences, USA). The fraction of cells in G0/G1, S, and G2/M phases were analyzed with FCS Express V3 (De Novo Software, CA).

Cell migration and invasion assay. Transwell invasion and migration assays were performed, as described previously (18,19). Invasion assays were Performed at 37˚C for 12 h using a 12-well Transwell apparatus (8-μm pore size with a polycarbonate membrane; Corning Costar, Lowell, MA, USA), coated with 200 μg Matrigel (BD Biosciences). Following rehydration of the chambers, the transfected cells were trypsinized and seeded into the upper Transwell chamber. The lower chamber contained 500 μl DMEM with 10% FBS. The number of migratory cells, which invaded the Matrigel and migrated through the membrane, was measured as the number of cells, which invaded from a defined area of the microfilter through the micropores in 24 h. The micropore filters were fixed in paraformaldehyde (Sigma-Aldrich) and stained with crystal violet. Image of four randomly-selected fields were captured, and the number of cells were counted to calculate the average number of cells/field that had transmigrated. The cell migration assays were performed in a similar manner, but without the Matrigel coating.

Statistical analysis. All data are expressed as the mean ± standard deviation. Statistical analysis was performed using SPSS 13 software. Comparisons were assessed using Student's t-test, and differences between three or more groups were assessed using Bonferroni's test. P<0.05 was considered to indicate a statistically significant difference.

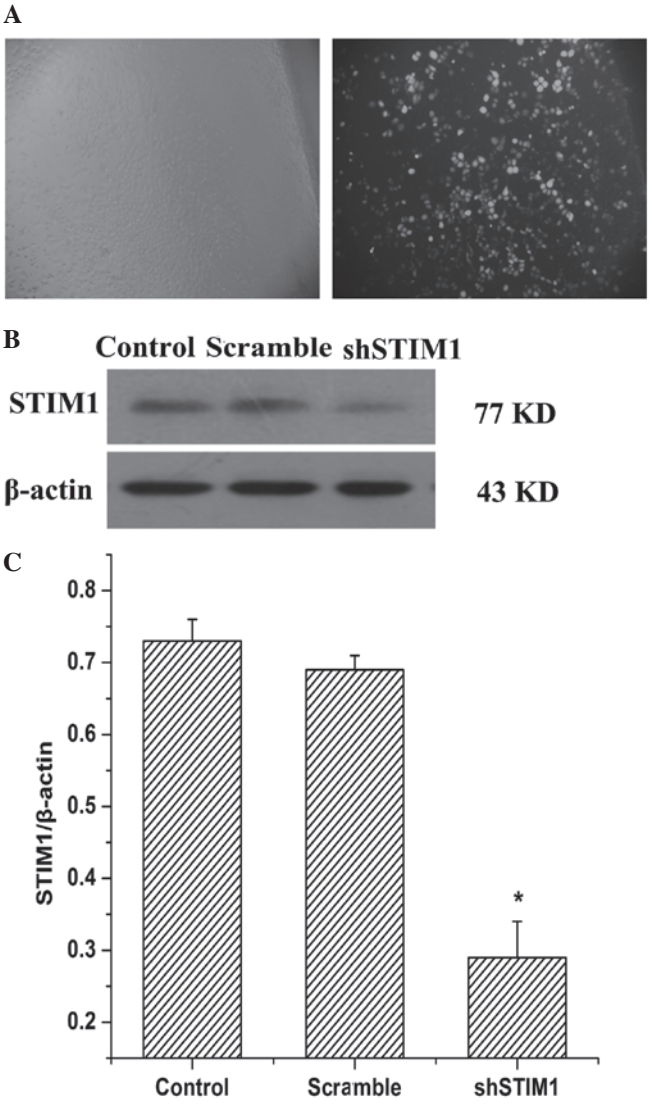

Figure 1. Plasmid-mediated shRNA inhibits the expression of STIM1 in SGC7901 cells. (A) Transfection efficiency was estimated 48 h after transfection. Expression of green fluorescent protein was observed in the cells under a light microscope (left) and fluorescence microscope (right). (Magnification, x100). (B and C) Total cellular proteins were extracted 48 h after transfection and their quantities were determined using western blot analysis with antibodies against STIM1, and β-actin as an internal control. Data represent one of three separate experiments. Scramble, cells transfected with a negative plasmid; shSTIM1, cells transfected with PGPU6-shSTIM1. Data are expressed as the mean ± standard deviation of duplicate determinations from three independent experiments. *P<0.01, compared with the control (untransfected parental SC7901 cells). STIM1, stromal interacting molecule 1; sh short hairpin.

Results

Plasmid-mediated shRNA targeting STIM1 inhibits the expression of STIM1 in SGC7901 cells. To determine whether STIM1 offers potential use a therapeutic target for gastric cancer, the present study used RNA interference (RNAi) to inhibit the expression of STIM1 in the SGC7901 cells. The efficiency of plasmid transfection in the SGC7901 cells was examined using fluorescent microscopy, and >80% of the cells were infected with shSTIM1 after 48 h (Fig. 1A). To determine the knockdown efficiency of STIM1, western blot analysis was performed. As shown in Fig. 1B, western blot analysis was

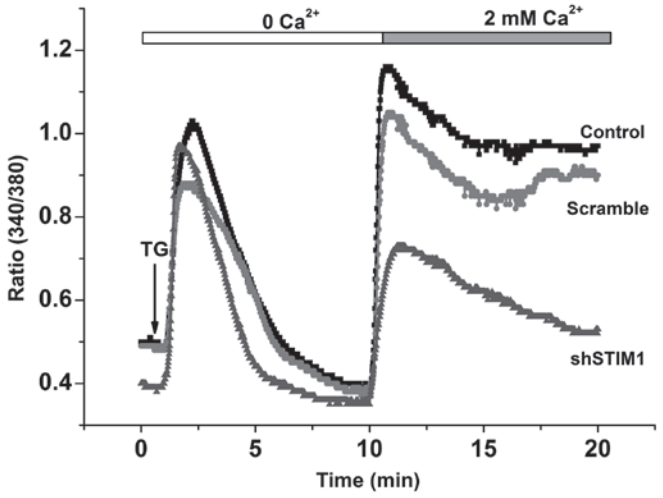

Figure 2. Effect of STIM1 knockdown on TG-induced Ca^{2+} entry. The cells were transiently transfected with negative control (scramble), or shRNA against STIM1. Representative traces of Ca^{2+} concentrations demonstrate the time-course of store-operated $Ca2^{+}$ entry. Data are expressed as the mean ± standard deviation of duplicate determinations from three independent experiments. *P<0.01 compared with untransfected parental SC7901 cells. STIM1, stromal interacting molecule 1; sh, short hairpin; TG, thapsigargicine.

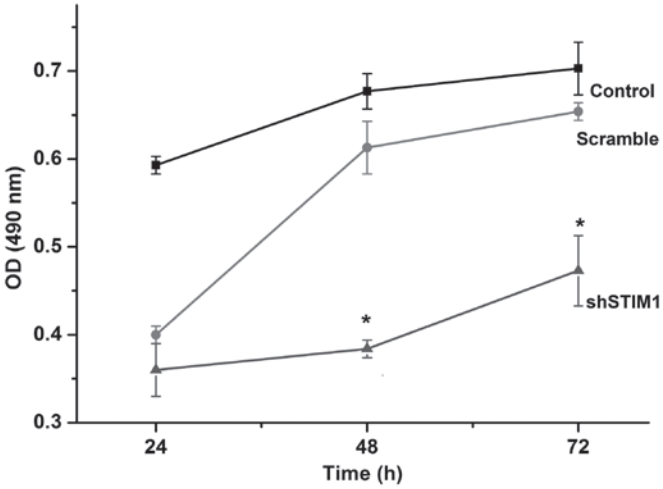

Figure 3. Effect of STIM1 silencing on SGC7901 cell proliferation. Cell proliferation was measured using an MTT assay and expressed as absorbance values. Data are expressed as the mean ± standard deviation of duplicate determinations from three independent experiments. *P<0.01, compared with the control (untransfected parental SC7901 cells). STIM1, stromal interacting molecule 1; sh, short hairpin; OD, optical density.

also performed 48 h after plasmid transfection. The protein expression level of STIM1 was significantly reduced in the shSTIM1 group compared with the scramble group. These results indicated that plasmid-mediated shRNA suppressed the expression of STIM1 in the SGC7901 cells, efficiently and specifically.

Suppression of STIM1 inhibits Ca^{2+} entry in SGC7901 cells. To examine the role of STIM1 protein on SOCE, the SGC7901 cells were transfected with shRNA, designed against STIM1. No significant difference was observed between the basal Ca^{2+} entry in the absence of TG stimulation and the control cells. The addition of TG in Ca^{2+}-free medium elicited similar responses in the control and STIM1-knockdown cells, whereas the response elicited by Ca^{2+} re-addition was inhibited by 55% in the STIM1-knockdown cells (Fig. 2). These results demonstrated that the STIM1 protein contributed to SOCE in the SGC7901 gastric cancer cell line.

Suppression of STIM1 inhibits SGC7901 cell proliferation. The effect of downregulation of STIM1 on the proliferation of gastric cancer cells *in vitro* was assessed using an MTT assay. As shown in Fig. 3, silencing of the STIM1 gene inhibited SGGC7901 cell proliferation in a time-dependent manner. When compared with the scramble group, the number of cells in the shSTIM1 group was reduced significantly by 41% 48 h after transfection. These results demonstrated that knockdown of STIM1 by plasmid-mediated shRNA inhibited SGC7901 cell proliferation *in vitro.*

Knockdown of the expression of STIM1 induces gastric cancer cell apoptosis. As shown in Fig. 4, the percentage of apoptotic cells infected with STIM1 shRNA was significantly higher compared with that observed in the scramble group (P<0.05). No significant differences were observed between the control shRNA plasmid-infected cells and the untransfected cells. These data indicated that knockdown of the expression of STIM1 induced apoptosis in the gastric cancer cells.

Suppression of STIM1 induces cell cycle arrest at the G0/G1 phase in SGC7901 cells. To further elucidate the growth suppression effect of si-STIM1 on SGC7901 cells, cell cycle distribution analysis was performed, using flow cytometry, 48 h after transfection. As shown in Fig. 5, STIM1 knockdown induced cell cycle arrest at the G0/G1 phase in the SGC7901 cells. Compared with the scramble group, the percentage of cells at the G0/G1 phase in the shSTIM1 group was 24.92% higher at 48 h. This result demonstrated that STIM1 silencing induced cell cycle arrest at the G0/G1 phase.

Silencing of STIM1 inhibits the migration and invasion of SGC7901 cells. To evaluate the effects of STIM1 on cell migration and invasion, a Matrigel invasion assay was performed (Fig. 6A). The results of the assay revealed that the decreased expression of STIM1 inhibited the migration of the SGC790 cells by 67.25%, compared with the scramble group. The invasion of the cancer cells was also significantly reduced following transfection with shSTIM1, as determined by the invasion assay. Decreased expression of STIM1 inhibited cell invasion by 72.24% in the SGC7901 cells compared with the scramble group (Fig. 6B). Taken together, these results indicated that the suppression of STIM1 inhibited the migration and invasion ability of the SGC7901 cells.

Discussion

Gastric cancer is one of the most common types of solid tumor worldwide and is the second highest cause of cancer-associated mortality, despite decreases in its incidence and mortality rates (20). Despite curative resection treatment, patients with advanced stage gastric cancer have poor prognoses (21). The

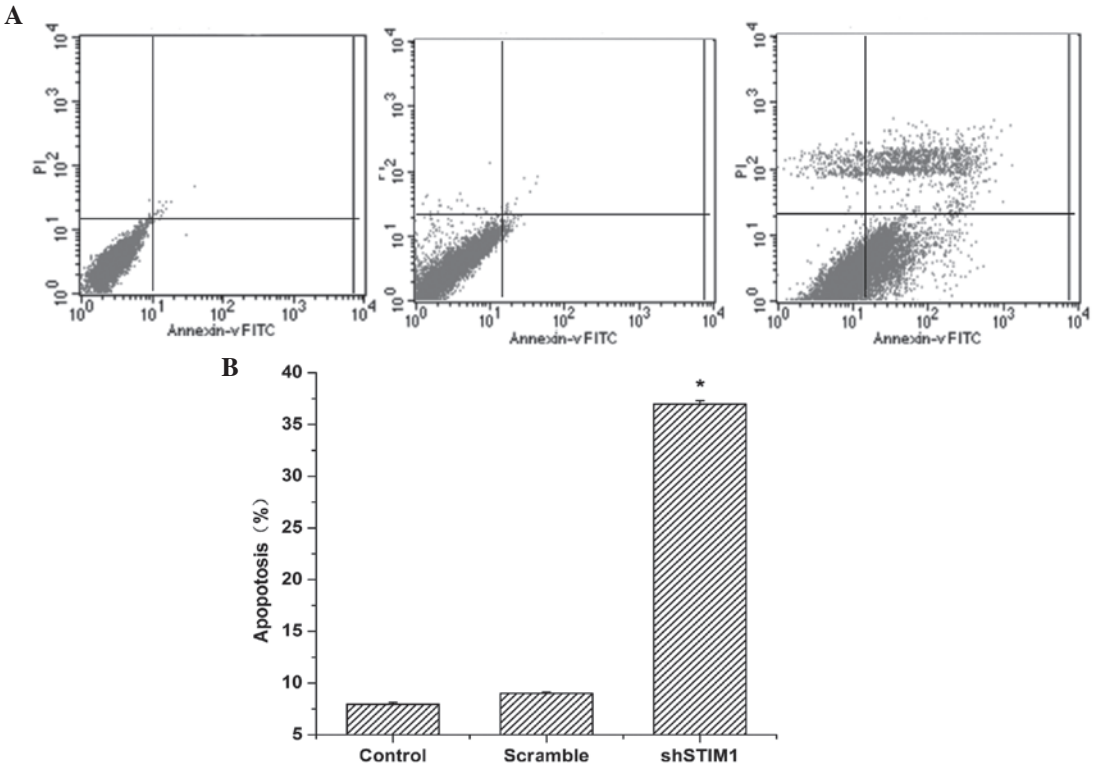

Figure 4. Effect of STIM1 knockdown in the SGC7901 gastric cancer cell line on apoptosis. (A) Cell apoptosis was measured using annexin V-FITC-PI assays. (B) Percentages of apoptosis. Data are expressed as the mean ± standard deviation of duplicate determinations from three independent experiments. *P<0.01, compared with the control (untransfected parental SC7901 cells). STIM1, stromal interacting molecule 1; sh, short hairpin; FITC, fluorescein isothiocyanate; PI, propidium iodide.

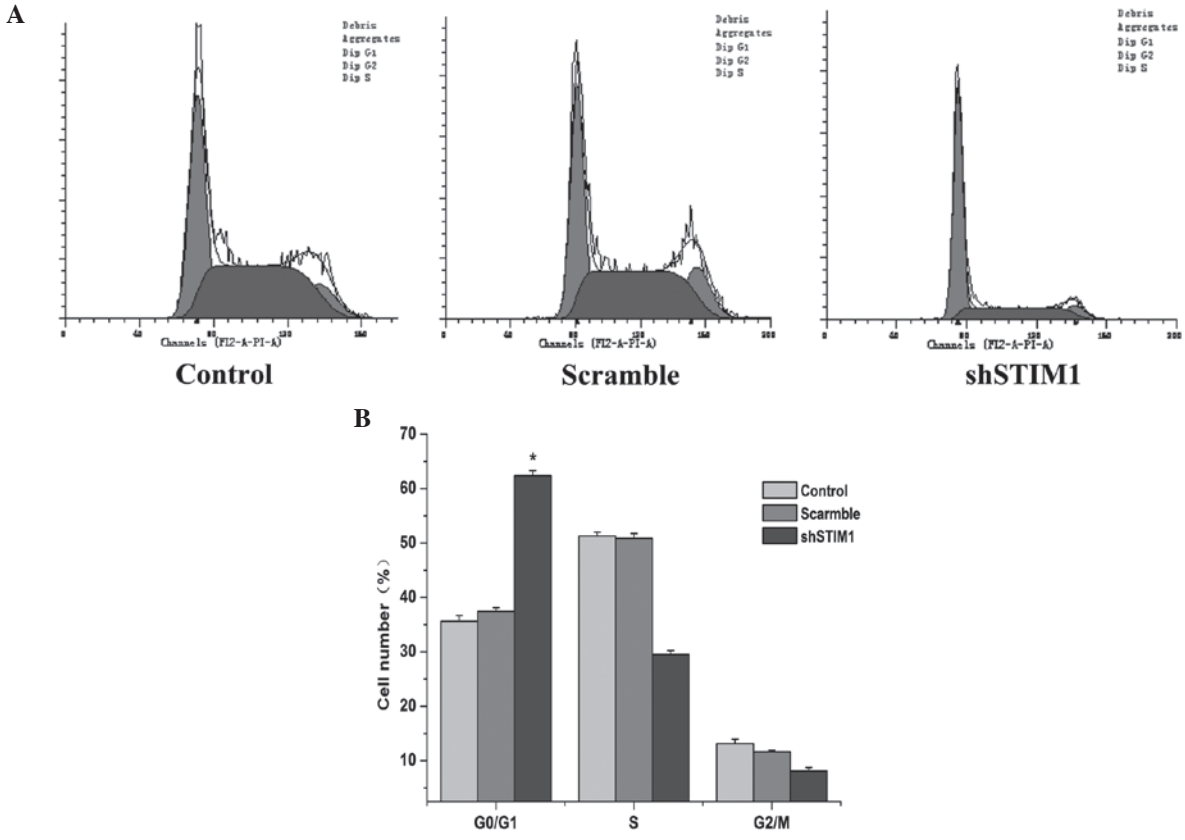

Figure 5. Effect of downregulation of STIM1 on cell cycle progression in the SGC7901 cells. (A) Cell cycle distribution was analyzed using flow cytometric analysis. (B) Representative flow cytometric histograms at 48 h, indicating the distribution of the cell cycle. Data are expressed as the mean ± standard deviation of duplicate determinations from three independent experiments. *P<0.01, compared with the control (untransfected parental SC7901 cells). STIM1, stromal interacting molecule 1; sh, short hairpin.

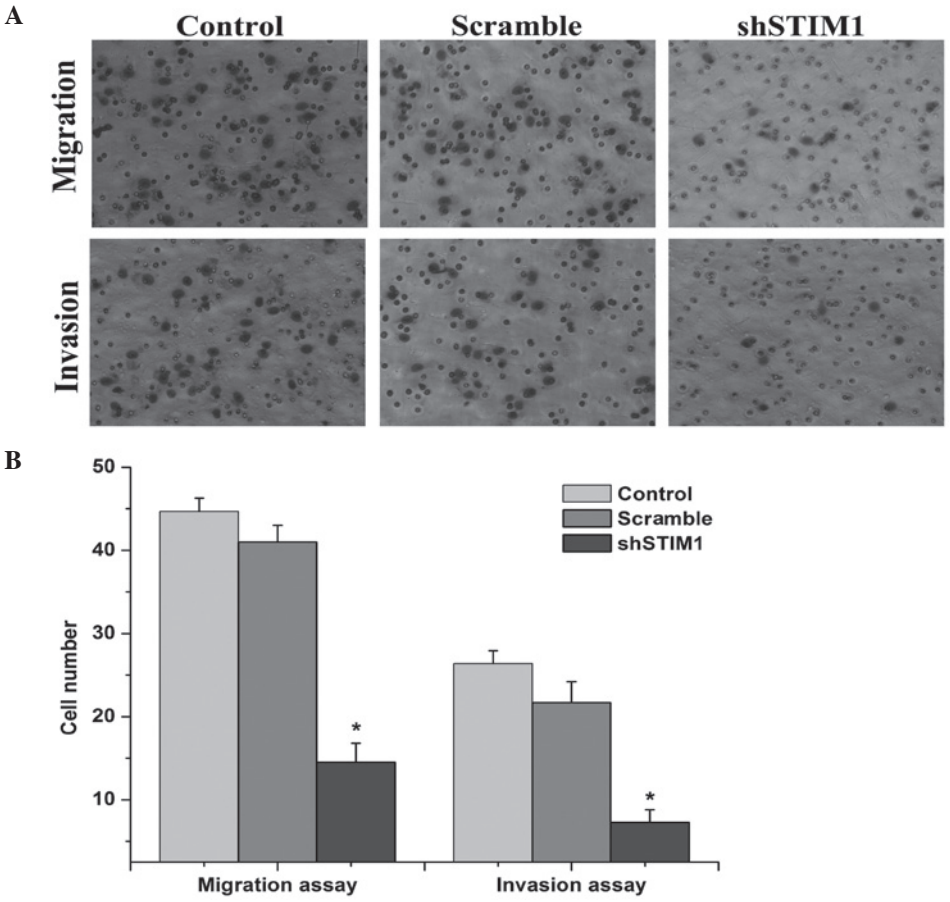

Figure 6. Effects of STIM1 inhibition on SGC7901 cell migration and invasion *in vitro*. (A) Representative photographs of migrating or invading cells using Transwell migration and invasion assays. The number of cells migrating through the inserts were counted. Magnification, x200. (B) Quantification of the Transwell assay data demonstrating average migration or invasion cell number. Data are expressed as the mean ± standard deviation of duplicate determinations from three independent experiments. *P<0.05, compared with the scramble cells. STIM1, stromal interacting molecule 1; sh, short hairpin.

effects of comprehensive treatment, which combines radical resection with chemotherapy, immunotherapy and traditional Chinese medicine treatment, are far from ideal. The local recurrence rate is as high as 50%and the incidence of lymph node metastasis is up to 60% (22). Therefore, identifying relevant therapeutic target for gastric cancer has become an urgent requirement. Gastric cancer is a heterogeneous, multifactorial disease. Recurrence and metastasis are the leading causes of failure in the clinical treatment of gastric cancer (23). If certain important genes can be regulated to inhibit the biological behavior of tumor invasion and metastasis, the survival rates and life quality of patients with gastric cancer can be improved (24).

SOCE is the principal Ca^{2+} entry mechanism in non-excitable cells., and STIM1 is an ER Ca^{2+} sensor, which triggers SOCE (5).

STIM1 has gained attention due to its tumor inhibition properties and role in immunity. Previous studies have found that STIM1 can inhibit cell growth and cause cell death in G401 rod-shaped tumor and rhabdomyosarcoma cell clines (25); and inhibiting the expression of STIM1 effectively relieves symptoms of spontaneous autoimmune diseases, including rheumatoid arthritis and autoimmune encephalomyelitis (26) in a mouse model. STIM1 has been observed to be associated with the proliferation and migration of vascular smooth muscle cells, endothelial progenitor cells and normal cells (27), however, it is also closely associated with the development of tumor cells, including metastatic melanoma cells (28), cervical cancer cells (14), glioblastoma (29) and breast cancer cells (11). This suggested that the biological functions of STIM1 may differ from different cell types. Abdullaev *et al* (30) found that the expression of STIM1 mediates SOCE, which is involved in the proliferation of human umbilical vein endothelial cells. Knockout of STIM1 arrests endothelial cells at the S or G2/M phases, thereby inhibiting the proliferation of endothelial cells. In clinical specimens from patients with a diagnosis of cervical cancer, Chen *et al* (14) observed that the Ca^{2+} inflow, mediated by STIM1 protein, is important in regulating the metastasis of cervical cancer cells, otherwise, the downregulation of STIM1 inhibits the proliferation of cervical cancer cells, arresting the cells at the G1/S or G2/M phases. In addition, through random ribozyme detection, Suyama E *et al* (31) demonstrated that STIM1 is linked to the migration of metastatic melanoma. In a previous study on breast cancer cells *in vitro* and *in vivo*, Yang *et al* (11) found that STIM1 is involved in SOCE, promoting the metastasis of cancer cells, and inhibiting the expression of STIM1 effectively inhibits biological behaviors, including the proliferation and metastasis of tumor cells.

The preliminary stages of the present study found that the expression of STIM1 was almost absent in normal gastric

tissues, however, marked expression was observed in gastric cancer tissues on primary sites, and expression levels in metastatic gastric cancer tissues were significantly higher than those in the primary sites ($P<0.05$). SGC7901 gastric cancer cells are cells lines with high metastatic potential, isolated and cultured from metastatic lymph nodes of gastric cancer. For this reason, the present study selected this cell line as target cells for transfection with shRNAs of the STIM1 gene, and the STIM1 genes were silenced in the SGC7901 cells using an shRNA technique. Subsequent to depleting Ca^{2+} in the calcium stores of the ER and increasing the concentration of extracellular Ca^{2+} between 0 and 2Mm, the extracellular Ca^{2+} influx in the single-cell calcium ions was observed using an imaging system. The results revealed rapid extracellular Ca^{2+} influxes in the control and scarmble groups, while the extracellular Ca^{2+} influxe was significantly inhibited in the STIM1 knockdown group. The MTT assay demonstrated that, following silencing of the STIM1 gene, proliferation of the SGC7901 cells was significantly inhibited, while proliferation of the SGC7901 cells were unaffected in the scramble and control groups. Takahashi *et al* also found that inhibiting the expression of STIM1 inhibits Ca^{2+} influx, thereby inhibiting the proliferation of vascular smooth muscle cells cultured *in vitro* (32). The results of are were consistent with those of the present study. This preliminarily indicated that STIM 1 may regulate the proliferation and growth of SGC7901 gastric cancer cells by regulating of extracellular Ca^{2+} influx.

To further examine the causes for the slow growth and proliferation of cells following RNAi, the present study investigated the cell cycle of the three groups of gastric cancer cells using flow cytometry. The results revealed that all the gastric cancer cells in STIM1 silencing group were arrested at the G0/G1 phases, and the numbers of cells at the S and G2/M phases were significantly decreased. These results suggested that, in the STIM1 silencing group, gastric cancer cells increased at stationary stages, but significantly decreased at active proliferative phases. These results differed from those observed in the scramble and control groups. In clinical specimens diagnosed with cervical cancer, Chen *et al* (14) found that the Ca^{2+} inflow, mediated by STIM1 protein, is important in the regulation of metastasis in cervical cancer cells, and the downregulation of STIM1 inhibits the proliferation of cervical cancer cells, and arrest cells at the G1/S or G2/M phases. This suggested that STIM1 silencing may inhibit the proliferation of gastric cancer cells through arresting the tumor cells at the G0/G1 phase.

Cell apoptosis is the process in which certain factors trigger a stored procedure, which leads to active cell death. Similar to the process of mitosis, apoptosis has a regulatory role in the body's vital functions and a stable internal environment, while tumor development is closely associated with the decline or failure of the mechanism of cell apoptosis (33). In the present study, flow cytometry revealed that the apoptotic rates of gastric cancer cells were increased significantly in the STIM1 silencing group, compared with those in the scramble and control groups. This result was consistent with the findings of Sun *et al* (34), which reported that downregulation of STIM1 promotes the apoptosis of colon cancer cells.

The biological behaviors of malignant tumors include invasion and metastasis, which depends predominantly on the invasion and migration of the cells. The invasion of cells through the basement membrane is considered an early event and predominant feature of metastasis (35). Through random ribozymal detection, Suyama E *et al* found that STIM1 is associated with the migration of metastatic melanoma (31); whereas a study by Yang *et al*, investigating breast cancer cells *in vitro* and in animal experiments, found that STIM1 is involved in SOCE and promotes the metastasis of cancer cells (11). The results of the present study demonstrated that, compared with the scramble and control groups, the metastasis and invasion of SGC7901 gastric cancer cells were significantly decreased following silencing of the STIM1 gene ($P<0.05$).

However, further investigations to elucidate the signaling pathway, which regulates the function of STIM1 in gastric cancer.

The present study demonstrated that STIM1 is expressed in human gastric cancer cell lines. The RNAi-mediated gene silencing of STIM1 suppressed SGC7901 cell growth *in vitro* and inhibited cell cycle progression at the G0/G1 phase. Inhibiting the activity of STIM1 also inhibited SGC7901 cell migration and invasion. In conclusion, the findings of the present study indicate the significance of STIM1 in the biological behavior of gastric cancer, and provide evidencethat STIM1 may be a potential therapeutic target for human gastric cancer.

Acknowledgements

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