SPANDIDOS PUBLICATIONS

ONCOLOGY LETTERS 15: 7305-7311, 2018

Circulating SH2B1 is associated with an increased risk of gastric cancer

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Received September 19, 2016; Accepted September 1, 2017

DOI: 10.3892/ol.2018.8196

Abstract. Gastric cancer (GC) is one of the most common types of cancer in humans and the second leading cause of cancer-associated mortality worldwide. Identifying novel risk factors will facilitate the development of therapeutic strategies to prevent and treat GC. Increased expression of the Src homology 2 B adaptor protein 1 (SH2B1) may stimulate the malignant progression of lung cancer, esophageal cancer and neuroblastoma. However, its function in GC has not yet been investigated. To identify whether increased serum SH2B1 is a risk factor for GC, the present study performed a nested case-control study of patients within the Chinese cohort study. Levels of serum SH2B1 were measured in 563 patients diagnosed with GC during the follow-up period and in 1,126 matched healthy controls. The results demonstrated that high levels of serum SH2B1 were associated with an increased GC risk (odds ratio, 3.23; 95% confidence interval, 2.45-5.65). When analyses were stratified further by sex, age and smoking, an association between increased levels of SH2B1 and GC was identified in males but not in females. Furthermore, the association between SH2B1 levels and GC was more evident in younger than in older participants, and statistically significant in current smokers but not in nonsmokers. These results were not altered following the exclusion of outliers. Furthermore, it was demonstrated that overexpression of SH2B1 contributes to the malignant transformation of normal gastric epithelial cells. Thus, the present study demonstrated that elevated serum SH2B1 levels may increase the risk of GC.

Introduction

Gastric cancer (GC) is the fifth most common type of cancer in humans and the second leading cause of cancer-associated mortality worldwide (1). A total of 951,000 new cases of GC (accounting for 6.8% cases of cancer) were estimated to have occurred in 2012 (2). Its incidence is particularly high in East Asia, including China. In China, the majority of patients with GC are diagnosed at a late stage and have a poor prognosis (3). The incidence of GC is greater in males than in females (4). Elucidating the molecular mechanisms underlying the initiation of GC and identifying the risk factors of GC may aid the development of novel methods of preventing and treating patients with GC.

Clinical manifestations of the disease in patients with GC are well-known (4). For example, GC usually occurs in those >55 years of age with symptoms including abdominal pain, anorexia, satiety, vomiting and weight loss (4). Although early-stage tumors tend to be diagnosed in countries in which endoscopic screening is common, GC may be also diagnosed at an advanced stage (5). Patients with stage I disease have a good prognosis. However, patients with stage IV disease have a poor prognosis.

The Src homology 2 B adaptor protein 1 (SH2B1) belongs to an adaptor protein family that includes SH2B1, SH2B2 and SH2B3 (also known as the lymphocyte adapter protein) (6). All three members share a common domain structure that includes a dimerization domain, a pleckstrin homology domain, several proline-rich regions and a SH2 domain near the C-terminus (7). Previous studies have demonstrated that SH2B1 contributes to the malignant progression of tumors, including colon, ovary and non-small cell lung tumors (8-12). However, the effect of SH2B1 expression on GC has not yet been reported.

The present study investigated whether levels of circulating SH2B1 are associated with an increased GC risk and whether the overexpression of SH2B1 stimulates the malignant transformation of normal gastric epithelial cells.

Materials and methods

Study population and serum samples. A nested case-control study of participants in a Chinese Cohort (Lin-Xian) was conducted. A detailed description of the Lin-Xian cohort has been reported previously (13). Briefly, in 1984, a total of 29,584 individuals (14,591 male and 14,993 female), between 40 and 69 years of age, with no family history of cancer, were recruited. All participants were interviewed to

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Key words: gastric cancer, Src homology 2 B adaptor protein 1, risk factor, malignant transformation

complete a baseline questionnaire and the majority of the participants (>98%) donated a blood sample. Additionally, a total of 50 healthy participants, who donated one blood sample in January 2001, were asked to donate a second sample in January 2002. The same protocol was employed for the first and second round of blood collection. In brief, 30 ml venous blood was collected at the time of recruitment under fasting and non-fasting conditions in tubes containing citrate as an anticoagulant. Blood donations were processed within the same day. Following centrifugation at 1,500 x g for 20 min at 4˚C, blood fractions were separated to produce 0.5 ml aliquots of serum (8 straws), plasma (12 straws), buffy coat (4 straws) and erythrocytes (4 straws). Each participant's straws were stored in liquid nitrogen. After 15 years of follow-up, a total of 3,410 cases of upper gastrointestinal types of cancer were identified, including 1,958 esophageal squamous cell carcinoma and 1,452 GC. The project included 563 patients with GC each matched to two controls (n=1,126). For each case-subject match set, two control subjects were matched to the case based on the following matching criteria: Age at blood draw, sex and municipality. All control subjects had an available blood sample and were selected among appropriate risk sets consisting of all cohort members alive and free of cancer at the time of diagnosis of the index case. Serum aliquots of 100-500 μl were stored at -80˚C prior to measuring SH2B1.

Approval for the present study was obtained from the Ethics Committee of the Shandong Provincial Qianfoshan Hospital (Shandong, China) and the Huabei Medical Ethics Committee (Huabei, Shijiazhuang, China). Written informed consent was obtained from each individual.

Cell culture. Cells from the normal gastric epithelial cell line GES-1 were purchased from the Biochemistry and Cell Biology Institute of Shanghai, Chinese Academy of Sciences (Shanghai, China). Cells were cultured in Dulbecco Modified Eagle's medium (DMEM; Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS; Thermo Fisher Scientific, Inc.) and antibiotics (100 mg/ml penicillin and 100 U/ml streptomycin) in a 5% CO_2 incubator at 37˚C.

SH2B1-expressing plasmids/empty vectors and transfection. SH2B1-expressing plasmids (pcDNA3.1-SH2B1)/empty vectors (pcDNA3.1) (10 μg/100 mm dish) were purchased from Tiangen Biotech (Beijing) Co., Ltd. (Beijing, China). Prior to transfection, cells were cultured in serum-free DMEM without antibiotics until they reached 70% confluence and then cultured for a further 24 h. Cells were transfected with Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) following the manufacturer's protocol. Following incubation for 6 h, the medium was removed and replaced with serum-rich culture medium for 48 h.

ELISA. ELISA was performed to detect levels of SH2B1 in the pre-diagnostic serum in patients with GC and in matched controls (14,15). Serum SH2B1 levels were measured using an SH2B1 (human) ELISA kit (cat. no. ABIN4884508; Antibodies-online GmbH, Aachen, Germany), according to the manufacturer's protocol.

The intra-batch and inter-batch coefficients of variation for the SH2B1 protein were 3.81 and 5.34%, respectively. Multivariate unconditional logistic regression was performed to calculate odds ratios (OR) and corresponding 95% confidence intervals (CI) for GC, calculating ORs over the quartile levels and on a continuous ($\log_2$) scale of circulating SH2B1 levels. The final multivariate models presented include *Helicobacter pylori* infection and smoking, which altered the estimated effect by 10%. Tests for trend were based on $\log_2$ exposures. All P-values presented are two-sided and P<0.05 was considered to indicate a statistically significant difference. Statistical analyses were conducted using SAS software (version 9.3; SAS Institute, Inc., Cary, NC, USA).

Western blot analysis. GEC-1 cells were harvested and western blot analysis was performed following a previously described protocol (15). Proteins were incubated with rabbit anti-SH2B1, anti-c-myc, anti-p53, anti-runt-related transcription factor (RUNX)3, anti-signal transducer and activator of transcription (STAT)3, anti-vimentin, anti-epithelial (E-)cadherin and anti-β-actin primary antibodies (Abcam, Cambridge, MA, USA) overnight at 4˚C. Subsequently, proteins were incubated with IRDye®-800 conjugated anti-rabbit secondary antibodies (Li-COR Biosciences, Lincoln, NE, USA) for 30 min at room temperature. Specific proteins were visualized using the Odyssey Infrared Imaging System (Li-COR Biosciences, Lincoln, NE, USA) and β-actin was used as a loading control. Data were analyzed using Student's t-test and P<0.05 was considered to indicate a statistically significant difference.

MTT assay. Cell proliferation was assessed using an MTT assay, following a previously described protocol (16). Cells were plated at a density of $8x10^3$ cells/well in 96-well plates in DMEM containing 10% FBS and incubated at 37˚C for 12 h, in 5% CO_2. Following transfection of GES-1 cells with SH2B1-expressing plasmids, MTT (5 mg/ml) was added to the wells (20 μl/well). Plates were incubated in a cell incubator for 4 h, then the supernatant was removed and 150 μl dimethyl sulfoxide was added to each well. Following incubation for 10 min, the absorbance of each well was measured using a Synergy™ 4 microplate reader (BioTek Instruments, Inc., Winooski, VT, USA) at 570 nm. The reference wave length was set at 630 nm. Absorbance was directly proportional to the number of surviving cells. The viability of the control group (GES-1 cells transfected with pcDNA3.1) was considered to be 100%. The experiment was performed three times. Data are expressed as the mean ± standard error of the mean and were analyzed using Student's t-test. P<0.05 was considered to indicate a statistically significant difference.

Bromodeoxyuridine (BrdU) assay. A cell proliferation enzyme-linked immunosorbent assay was performed to analyze the incorporation of BrdU during DNA synthesis. A BrdU kit (Beyotime Institute of Biotechnology, Haimen, China) was used following the manufacturer's protocol. Absorbance was measured at 450 nm using a Spectra Max 190 ELISA reader (Molecular Devices, LLC, Sunnyvale, CA, USA). Images were acquired using the Axioskop 2 plus laser-scanning microscope (Carl Zeiss AG, Oberkochen, Germany). The experiment was performed three times. Data

Table I. ICCs for circulating SH2B1 in healthy participants.

Analyte	No. of participants/no. of time points	CV (%)	ICC (95% CI)
SH2B1	50/2	5.6	0.76 (0.67-0.89)

n=50; SH2B1, Src homology 2 B adaptor protein 1; CV, coefficient of variation; CI, confidence interval; ICC, intraclass correlation coefficient.

Table II. Baseline characteristics of patients with gastric cancer and matched controls.

Characteristics	Cases (n=563)	Controls (n=1,126)
Age (median, years)	62.5	61.2
Males (%)	62.4	63.5
BMI (median, kg/m^2)	24.0	24.8
Current smokers (%)	29.9	30.5
Hypertension (%)	19.9	15.3
Serum cholesterol (mg/dl)	265.4	271.3
Hematocrit (volume, %)	43.8	44.5
α-Tocopherol (mg/l)	9.1	9.8
β-Carotene (μg/l)	89.1	80.7
Retinol (μg/l)	630.4	630.2
Selenium (μg/l)	60.9	65.1

n=1,689. BMI, body mass index.

are expressed as the mean± standard error of the mean and were analyzed using a Student's t-test. P<0.05 was considered to indicate a statistically significant difference.

Migration and invasion assays. For Transwell migration assays, 2.5x10^4 cells were plated in the upper chamber with the non-coated membrane (24-well insert; pore size, 8 mm; BD Biosciences, San Jose, CA, USA). For invasion assays, 1.25x10^5 cells were plated in the upper chamber, which contained a Matrigel-coated membrane. In each assay, cells were plated in the upper chamber in DMEM. In the lower chamber, medium was supplemented with FBS which was used as a chemoattractant. Cells were incubated for 24 h and cells that did not migrate or invade through the pores were removed using a cotton swab. Cells on the lower surface of the membrane were stained using the Diff-Quik Staining Set (Dade Behring AG, Dudingen, Switzerland) (15) for 30 min at room temperature and images were captured using an Olympus inverted microscope (magnification, x40). Cell migration was quantified by counting the number of cells in 3 random fields. Three individual experiments were performed. Data are expressed as the mean ± standard error of the mean and were analyzed using Student's t-test. P<0.05 was considered to indicate a statistically significant difference.

Table III. Odds ratios[a] of gastric cancer between people with and without increased titers of circulating SH2B1 concentration in the total sample.

Variable	Threshold value, μg/l	Cases (n)	Controls (n)	Odds ratio	95% CI
SH2B1	<67	278	563	1	1
	≥67	285	563	3.23	2.45-5.65

n=1,689. [a]Adjusted for *Helicobacter pylori* infection and smoking. SH2B1, Src homology 2 B adaptor protein 1; CV, coefficient of variation; CI, confidence interval; ICC, intraclass correlation coefficient.

Semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR). Total RNA was isolated from cells or tissues using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc.). A total of 1 μg RNA was reverse-transcribed into cDNA using SuperScript III First-Strand Synthesis system (Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol. qPCR was then performed using an RT-PCR kit (Promega Corporation, Madison, WI, USA) in a 50 μl PCR system, following the manufacturer's protocol. The housekeeping gene GAPDH was used as an RNA loading control. The sequences of the primers used were as follows: GAPDH, forward, 5'-ATTCAACGGCACAGTCAAGG-3' and reverse, 5'-GCAGAAGGGGCGGAGATGA-3'; E-cadherin, forward, 5'-TCAACGATCCTGACCAGCAGTTCG-3', reverse, 5'-GGTGAACCATCATCTGTGGCGATG-3'; vimentin, forward, 5'-GACAATGCGTCTCTGGCACGTCTT-3' and reverse, 5'-TCCTCCGCCTCCTGCAGGTTCTT-3'. PCR products were analyzed by 1.5% agarose gel electrophoresis. Gels were stained with ethidium bromide and densities of the bands were determined using a computerized image analysis system (UV transillumination; Alpha Innotech, San Leandro, CA, USA). The area of each band was calculated as the integrated density value. Data were analyzed using Student's t-test and P<0.05 was determined to indicate a statistically significant difference.

Immunocytochemistry. Immunocytochemistry assays were performed as described previously (16). Following transfection with plasmids, GES-1 cells were fixed in 4% paraformaldehyde for 15 min, and then blocked with goat serum blocking solution for 20 min at room temperature. The samples were incubated with the following primary antibodies: Rabbit anti-vimentin (1:200; cat. no. ab92547; Abcam, Cambridge, UK) and rabbit anti-E-cadherin (1:200; cat. no. ab40772; Abcam) in a humid chamber overnight at room temperature. After washing three times with phosphate buffer containing NaCl (0.9% NaCl and 25 mM sodium phosphate, pH 7.4), cells were incubated with appropriate biotin-conjugated secondary antibodies (1:10,000; cat. no. ab222772; Abcam) for 30 min at 37˚C. After washing with phosphate buffer containing NaCl, the samples were observed under a laser-scanning confocal microscope (magnification, x40; Olympus, Tokyo, Japan). DAPI staining (blue) stained cell nuclei. Data were analyzed using Student's t-test and P<0.05 was determined to indicate a statistically significant difference.

Table IV. ORs[a] for gastric cancer by tertile levels and on a continuous log_2 scale of circulating SH2B1.

Analyte	Cases/controls	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P_{trend}
SH2B1	563/1,126	1	2.1 (1.01-3.45)	3.3 (1.87-4.32)	3.8 (2.01-5.46)	0.001

n=1,689. Values are presented as the OR (95% CI) unless otherwise specified. [a]Adjusted for *Helicobacter pylori* infection and smoking. Tests for P_{trend} were based on the continuous (log_2) variable among all groups. SH2B1, Src homology 2 B adaptor protein 1; CV, coefficient of variation; CI, confidence interval; ICC, intraclass correlation coefficient; OR, odds ratio.

Results

Reproducibility of serum SH2B1 in healthy participants. To identify the function of serum SH2B1 levels, their temporal variability was evaluated by calculating intraclass correlation coefficient (ICC) values. ICCs were calculated by dividing the between-individual variance by the sum of the within- and between-individual variances; 95% CI was also calculated as described previously (17). ICCs were interpreted as follows: >0.75 indicated excellent reproducibility; 0.40 to 0.75 indicated fair to good reproducibility and <0.40 indicated poor reproducibility (18). A total of 50 healthy participants, who donated one blood sample in January 2001, were asked to donate a second sample in January 2002. The coefficients of variation (CVs) and ICCs for SH2B1 are summarized in Table I. Overall, CVs in healthy participants were <10%, within the expected range. ICCs for SH2B1 were relatively high (ICC, 0.76; 95% CI, 0.67-0.89), indicating good to excellent reproducibility.

SH2B1 is associated with an increased risk of GC. Baseline characteristics of the 563 patients with GC were compared with those of the 1,126 matched controls, and it was determined that the baseline characteristics of patients with GC were similar to those of the controls (Table II). The OR of GC at concentrations of SH2B1 ≥67 μg/l was 3.23 (95% CI, 2.45-5.65) compared with 1 in the controls, indicating that serum SH2B1 levels are positively associated with an increased risk of GC (Table III). The association between circulating SH2B1 and GC risk in quartiles was also analyzed. An increased risk of GC was observed in patients with the three highest quartiles of SH2B1 concentrations, compared with controls (Table IV).

The present study also investigated the effect of stratifying for sex, age and smoking status (Table V). There was a statistically significant association between serum SH2B1 levels and GC risk among males but not among females. The association between GC risk and SH2B1 levels was stronger for people <60 years old than for participants ≥60 years old. Furthermore, there was an association between SH2B1 levels and current smokers, but not among non-smokers. These results were not altered following the exclusion of outliers.

SH2B1 promotes the proliferation of GES-1 cells. Given that circulating SH2B1 is associated with an increased risk of GC, the present study sought to determine whether SH2B1 has any impact on the proliferation of GES-1 cells. Firstly, the results of western blot analysis demonstrated that the expression of SH2B1 was increased following the transfection of SH2B1-expressing plasmids in GES-1 cells (Fig. 1A). The results of the MTT assay demonstrated that overexpression of SH2B1 significantly promoted the proliferation of GES-1 cells (P<0.05; Fig. 1B). To further determine the effects of SH2B1 on proliferation, a BrdU incorporation assay was used to detect whether SH2B1 expression affects DNA synthesis in GES-1 cells. The results identified that SH2B1 overexpression significantly promoted DNA synthesis (P<0.01; Fig. 1C). Western blotting was performed to determine whether the overexpression of SH2B1 affected c-myc, p53, RUNX3 and STAT3 expression in the cells. The results demonstrated that c-myc and STAT3 expression were increased and p53 and RUNX3 expression were decreased following transfection with SH2B1 (Fig. 1D).

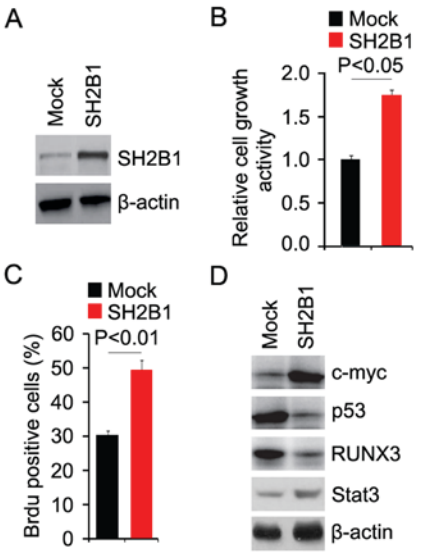

Figure 1. SH2B1 promotes the proliferation of GES-1 cells. (A) Western blot analysis detecting SH2B1 expression in GES-1 cells transfected with SH2B1-expressing plasmids and empty vectors (mock). (B) MTT assay of GES-1 cells transfected with SH2B1-expressing plasmids and empty vectors (mock). (C) A BrdU incorporation assay of GES-1 cells transfected with SH2B1-expressing plasmids and empty vectors (mock). (D) Western blot analysis of c-myc, p53, RUNX3 and STAT3 expression in GES-1 cells transfected with SH2B1-expressing plasmids compared with empty vectors (mock). BrdU, bromodeoxyuridine; SH2B1, Src homology 2 B adaptor protein 1; RUNX3, runt-related transcription factor 3; STAT3, signal transducer and activator of transcription 3.

SH2B1 promotes the migration and invasion of GES-1 cells. Subsequently, it was determined SH2B1 affects the migration and invasion of GES-1 cells. The results of the migration and invasion assays of GES-1 cells indicated that SH2B1 overexpression significantly increased the migration and invasion of GES-1 cells (P<0.01; Fig. 2A and B).

Table V. Circulating SH2B1 levels and gastric cancer incidence by sex, age and smoking status at the time of blood collection.

		SH2B1	
Variable	Cases/controls	Odds ratio	95% CI
Sex[a]			
Male	351/715	3.89	1.55-9.87
Female	212/411	0.98	0.78-3.45
Age, years[a]			
<60	214/697	4.13	1.01-10.06
≥60	349/429	1.98	0.62-3.65
Smoking[b]			
Non-smoker	346/687	2.34	0.54-22.5
Current smoker	217/439	3.65	1.99-18.98

n=1,689. [a]Adjusted for *Helicobacter pylori* infection and smoking; [b]adjusted for *Helicobacter pylori* infection. SH2B1, Src homology 2 B adaptor protein 1; CI, confidence interval.

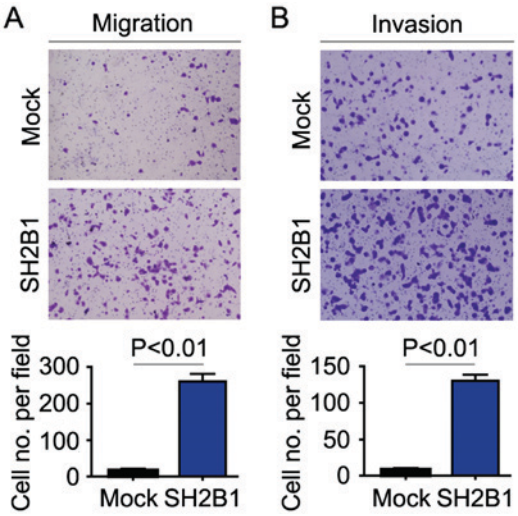

Figure 2. SH2B1 overexpression promotes the migration and invasion of GES-1 cells. (A) Transwell migration assay for GES-1 cells transfected with SH2B1-expressing plasmids compared with empty vectors (mock). Images were captured using an Olympus inverted microscope (magnification, x40) and migrated cells were quantified in 3 random fields. (B) Matrigel invasion assay for GES-1 cells transfected with SH2B1-expressing plasmids compared with empty vectors (mock). Images were captured using an Olympus inverted microscope (magnification, x40) and invasive cells were quantified in 3 random fields.

SH2B1 promotes the epithelial-mesenchymal transition (EMT) in GES-1 cells. To assess the function of SH2B1 in EMT of GES-1 cells, GES-1 cells were transfected with SH2B1-expressing plasmids. It was determined that SH2B1 expressed induced an evident phenotype change in cells from cobblestone-like cells to spindle-like cells (Fig. 3A). To confirm that the changes of cell morphology were a result of the EMT, immunofluorescence was used to measure the expression of the epithelial marker E-cadherin and mesenchymal marker vimentin in GES-1 cells. It was

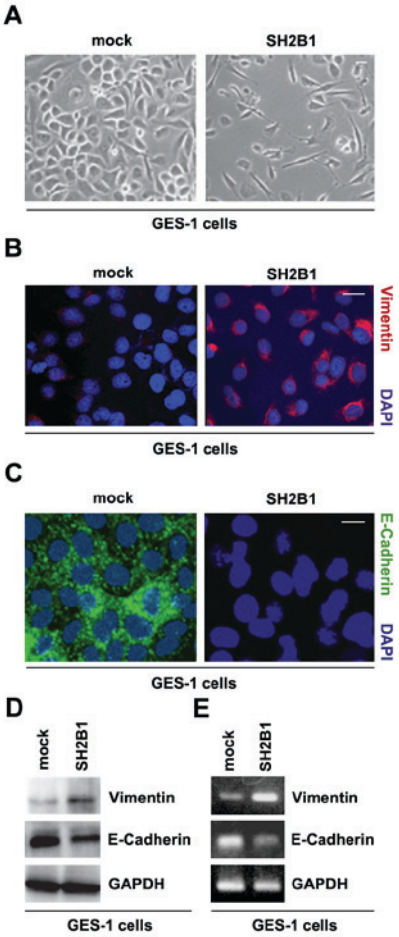

Figure 3. SH2B1 promotes the epithelial-mesenchymal transition in GES-1 cells. (A) Cells transfected with SH2B1-expressing plasmids or empty vectors (mock) were photographed following 72 h transfection (scale bars, 25 μm). (B) Representative photographs of vimentin immunofluorescence staining in transfected GES-1 cells. Nuclei were counterstained with DAPI. Red indicates vimentin fluorescence and blue indicates DAPI (scale bars, 25 μm). (C) Representative photographs of E-cadherin immunofluorescence staining of GES-1 cells transfected as indicated. Nuclei were counterstained with DAPI. Green indicates E-cadherin fluorescence and blue indicates DAPI (scale bars, 25 μm). (D) Western Blot analysis measuring the expression of E-cadherin and vimentin in GES-1 cells transfected with SH2B1-expressing plasmids compared with empty vectors (mock). (E) Semi-quantitative reverse transcription-polymerase chain reaction measuring the expression of E-cadherin and vimentin mRNA expression in GES-1 cells transfected with SH2B1-expressing plasmids compared with empty vectors (mock). SH2B1, Src homology 2 B adaptor protein 1; RUNX3, runt-related transcription factor 3; STAT3, signal transducer and activator of transcription 3; E-cadherin, epithelial cadherin.

demonstrated that SH2B1 promotes vimentin expression (Fig. 3B) and inhibits E-cadherin expression (Fig. 3C). To confirm the results of immunofluorescence, western blotting was performed to measure the expression of vimentin and E-cadherin in GES-1 cells. The results demonstrated that the expression of vimentin was increased and the expression E-cadherin was decreased following transfection with SH2B1 (Fig. 3D). Semi-quantitative RT-PCR was also performed to analyze the expression of vimentin and E-cadherin mRNA in cells transfected with SH2B1. Consistent with the results of western blotting, it was demonstrated that vimentin mRNA was upregulated and E-cadherin mRNA was downregulated by SH2B1 (Fig. 3E).

Discussion

In the majority of epidemiological studies, including the present study, only one type of biological specimen (e.g., blood or urine) is collected from each participant and analyzed. However, in studies investigating diseases with long latency periods, such as cancer, it is critical to determine whether a single biomarker measurement accurately reflects levels of this biomarker in the long-term. The lack of temporal stability including natural fluctuation serves an important function in interpreting risk estimates and may attenuate effect estimates (the association between the levels of pre-diagnostic circulating SH2B1 and the risk of GC) when prospectively evaluating the association between a biomarker and disease risk (19) and it has yet not been evaluated for circulating SH2B1. When variability is moderate, error correction methods use estimates of variability to correct measures of association (19). The ICC value is a good measure of reproducibility. An ICC ≥0.75 indicates that a single measurement of serum SH2B1 effectively reflects long-term SH2B1 levels and indicates that these levels remain relatively stable in individuals over the period of a year. This is indicative of relatively low within-individual and/or high between-individual variation over time. By contrast, a low ICC (<0.40) suggests poor reproducibility (20). The ICC for SH2B1 was relatively high (ICC, 0.76; 95% CI, 0.67-0.89), indicating good to excellent reproducibility.

It has been demonstrated that SH2B1 acts as an oncogene in lung cancer, esophageal cancer and neuroblastoma (9-12). However, its functions in GC remain unknown. It has been demonstrated that the overexpression of SH2B1 is associated with malignant progression, invasion and metastasis of esophageal cancer (11). The present prospective study indicated that serum SH2B1 is a risk factor for GC and that overexpression of SH2B1 contributes to the malignant transformation of normal gastric epithelial cells. To the best of our knowledge, the present study is the first to combine to indicate that SH2B1 expression is associated with an increased risk of GC. The results of the present study indicated that this association is limited to males, suggesting that sex hormones may serve an important function in determining serum levels of SH2B1. Furthermore, SH2B1 expression is associated with an increased risk of GC in current smokers, which makes sense as smoking has been identified as a risk factor for GC (21). These results indicate that smoking may upregulate serum SH2B1 concentrations and promote the malignant transformation of normal gastric epithelial cells. It has also been demonstrated *in vivo* that STAT3 activation may promote gastric tumorigenesis (22). The results of the present study demonstrated that overexpression of SH2B1 may significantly upregulate STAT3 expression in normal gastric epithelial cells.

RUNX3 has been identified as a major regulator of the proliferation of gastric epithelial cells. The tumorigenicity of human GC is negatively associated with RUNX3 expression and in ~50% of patients with GC, RUNX3 is not significantly expressed (23). The present study demonstrated that the overexpression of SH2B1 significantly inhibits RUNX3 expression in normal gastric epithelial cells. c-myc promotes the initiation of GC (24) and in the present study, it was demonstrated that SH2B1 overexpression significantly promotes c-myc expression in normal gastric epithelial cells. Furthermore, it has been demonstrated that the EMT serves an important function in tumorigenesis (25,26) and the present study indicated that SH2B1 overexpression significantly promotes the EMT.

In conclusion, the present study investigated the function of SH2B1 in the tumorigenesis of GC. To the best of our knowledge, the present study is the first to demonstrate that serum SH2B1 concentrations are stable over a 1-year period and that elevated serum SH2B1 concentrations are associated with an increased risk of GC. Furthermore, the present study demonstrated that SH2B1 overexpression promoted the malignant transformation of normal gastric epithelial cells.

Acknowledgements

The present study was supported by Natural Science Foundation of Shandong Province (grant no. ZR2015HL081), the Science and Technology Development Project of Shandong Province (grant no. 2011YD21035), Natural Science Foundation of Shandong Province (grant no. ZR2015HL070).

Competing interests

The authors declare that they have no competing interests.

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