Original Article

Inhibition of SRSF3 Alleviates Proliferation and Migration of Gastric Cancer Cells by Regulating the PI3K/AKT/mTOR Signalling Pathway

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Abstract. This study was aimed to investigate the impact of serine/arginine-rich splicing factor 3 (SRSF3) on the proliferation and migration of gastric cancer (GC) cells. SRSF3 levels in GC tissues and cell lines were measured by Western blotting. Functional assays were used for evaluation of GC cell proliferation, migration and invasion. The PI3K/AKT/mTOR pathway was then examined by Western blotting. *SRSF3* exhibits abnormal expression for the significantly increased levels in GC. *SRSF3* knockdown significantly suppressed GC progression. *SRSF3* knockdown significantly inhibited activation of PI3K/AKT/mTOR signalling. Inhibition of SRSF3 alleviates proliferation and migration of GC cells, and this process is mediated by inactivation of PI3K/AKT/mTOR signalling. Targeting SRSF3 may be a promising strategy to combat GC.

Introduction

Gastric cancer (GC) is the most common malignant tumour of the digestive system, and the mortality rate of GC ranks third among all malignant tumours in the world. Emerging studies have pointed out that recurrence and metastasis are the main causes of death from GC (GBD 2017 Stomach Cancer Collaborators, 2020; Sexton et al., 2020). Therefore, more studies focused on the molecular mechanism on GC cell proliferation and metastasis are crucial for the early diagnosis, treatment and prognosis of GC.

The PI3K/AKT/mTOR pathway is involved in the regulation of cell proliferation and migration, and is usually dysregulated in different tumours (Huang et al., 2019). Activated AKT is phosphorylated and inhibits TSC1/2, leading to activation of mTORC1 (Dibble and Cantley, 2015). Activated mTOR functions as a serine/threonine protein kinase, thereby promoting cell proliferation and migration (Laplante and Sabatini, 2009). Moreover, the PI3K/AKT/mTOR pathway is often overactivated in many cancers, including GC (Fattahi et al., 2020).

The serine/arginine (SR)-rich protein family is an important class of splicing regulators (Shepard and Hertel, 2009). Its members include SR splicing factor (SF) 1, SRSF3 and SRSF6, which show abnormal expression patterns in various cancer cells (Che and Fu, 2020). According to studies in different tumour pathologies, *SRSF3* is identified as an oncogene (Che and Fu, 2020). SRSF3 promotes the aggressive characteristics of several tumour types by regulating the alternative splicing of pre-mRNAs (Shepard and Hertel, 2009). Dysregulation of SRSF3 has proved to be implicated in the tumorigenesis of various tumours (Che and Fu, 2020). Studies have shown that silencing *SRSF3* leads to inhibition of U2OS and HeLa cell invasion and migration (Kim et al., 2017). Knockout of *SRSF3* in colorectal cancer cells can activate ArhGAP30/Ace-p53 and suppress cell proliferation, migration and survival (Wang et al., 2020). *SRSF3* knockdown reduced the abundance of phosphorylated AKT (p-AKT) (Shen et al., 2019). However, there are few studies on SRSF3 in GC, and the related mechanism is still unclear. Therefore, this study was aimed to clarify the potential role and underlying mechanism of SRSF3 in GC.

Material and Methods

Clinical samples

Tumour and matched adjacent non-tumour tissues were collected from 30 patients diagnosed with gastric cancer in the First People's Hospital of Jingzhou under the approval of Institutional Review Board and Human Ethics Committee of the hospital. All tissues were stored

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Abbreviations: GC – gastric cancer, SF – splicing factor, SD – standard deviation, SR – serine/arginine.

at −80 °C until use and all the patients have signed the informed consent form.

Cells and transfection

Human gastric mucosal epithelial cell line GES-1 was obtained from Procell Co., Ltd (Wuhan, China). Human GC cell lines including HGC27, AGS and MKN45 were from Huiying Biotechnology Co., Ltd (Shanghai, China). For knockdown of *SRSF3*, siRNAs (5′-GGTTGCTTCTGACCTCCATGT-3′) and negative control were purchased from TSINGKE (Beijing, China). Transfection was performed using Lipofectamine™ 3000 Transfection Reagent (Invitrogen, Waltham, MA) as the users' construction.

Real-time polymerase chain reaction (qPCR)

RNA was extracted by TRIzol® Reagent (Invitrogen). Then 1 μg of RNA was used for reverse transcription into cDNA using PrimeScript™ RT reagent Kit with gDNA Eraser (TakaRa, Kusatsu, Shiga, Japan). PCR reactions were performed by the CFX96 Real-Time PCR system (Bio-Rad, Hercules, CA).

Primers are as follows:
5′-TGCTGTCAGTGGCTTTCACA-3′
(*SRSF3* forward)
and 5′-GCCTGGAGTCTGGCTGTATC-3′
(*SRSF3* reverse)
5′-AAAGCCTGCCGGTGACTAAC -3′
(*GAPDH* forward)
and 5′-AGGAAAAGCATCACCCGGAG -3′
(*GAPDH* reverse)

Western blotting

Cell samples with different treatments were harvested, and Western blotting was then performed as the protocols previously described (Zhao et al., 2019). Primary antibodies used in this study including phosphorylated PI3K (#17366; 1 : 1000), total PI3K (#4257; 1 : 1000), phosphorylated AKT (#4060; 1 : 1000), total AKT (#9272; 1 : 1000), phosphorylated mTOR (#2971; 1 : 1000), total mTOR (#2972; 1 : 1000), and β-actin (#4970; 1 : 1000) were all purchased from Cell Signaling Technology (Beverly, MA). All the antibodies were diluted in a 1 : 1000 ratio.

MTT assay

Cell viability of the AGS cell line was measured by MTT assays and cell proliferation was measured by colony formation assays, as the protocols previously described (Ruan et al., 2020).

Cell migration and invasion assays

Cell migration was assessed using the wound-healing assays. Cells in the amount of 5×10^5 were seeded in a 6-well plate. When the cell confluence reached 100 %, a 20 μl sterile pipette tip was used to make a scratch perpendicular to the cell plane. After the scratch had been completed, non-adherent cells were washed away by sterile PBS and then replaced with fresh serum-free medium. After the cells were cultured for another 12 h, the width of the scratch was measured under a microscope, and representative pictures were taken. Trans-well chambers solidified with Matrigel (Corning Biocoat, Corning, NY) were used for measurement of the invasion abilities of AGS cells. At the upper chamber, 1×10^5 cells were suspended in serum-free medium. At the same time, medium supplemented with 10 % FBS was added to the lower chamber. After 24 h, invasive cells were stained by crystal violet and counted.

Statistical analysis

Results were presented as mean ± standard deviation (SD). For data analysis, the method of independent Student's *t*-test was conducted via the GraphPad Prism 7.0 software.

Results

SRSF3 is significantly up-regulated in gastric cancer

To investigate the potential role of SRSF3 in gastric cancer (GC), 30 GC tissues and matched adjacent non-tumour tissues were collected. SRSF3 levels were measured by Western blotting, and the data suggested that SRSF3 exhibits significantly increased levels in GC tissues (Fig. 1A). Moreover, compared to SRSF3 levels in human gastric mucosal epithelial cells (GES-1), SRSF3 was significantly up-regulated in GC cells (HGC27, AGS and MKN45; Fig. 1B).

SRSF3 knockdown significantly inhibits the growth of GC cells

To determine the effect of SRSF3 on the malignant biological properties of GC cells, *SRSF3* knockdown was performed by siRNAs in AGS cells and the efficiency was confirmed by qRT-PCR (Fig. 2A). Cell viability was examined by MTT assays and the results indicated that *SRSF3* knockdown inhibits the proliferation of AGS cells (Fig. 2B; $P < 0.01$). Furthermore, the data of colony formation assay demonstrated significantly decreased colonies as a result of SRSF3 inhibition (Fig. 2C; $P < 0.01$). These results suggested that *SRSF3* knockdown significantly inhibits the growth of GC cells.

SRSF3 knockdown suppresses the invasion and migration of GC cells

Moreover, the impact of SRSF3 on the invasion and migration of GC cells was assessed. As shown in Fig. 3A, *SRSF3* knockdown significantly decreased the capacities of AGS cells passing through the Matrigel-solidified trans-well chambers. In addition, *SRSF3* knockdown significantly decreased the speed of wound closure (Fig. 3B). *SRSF3* knockdown suppresses the invasion and migration of GC cells.

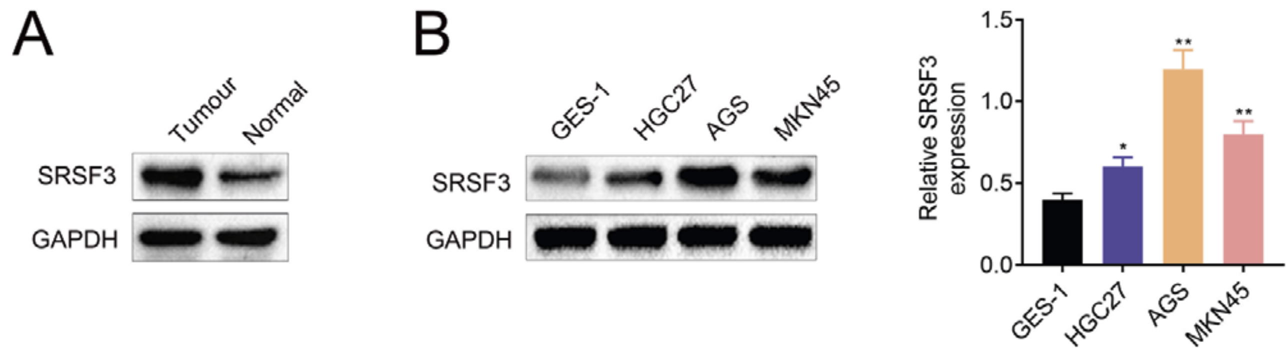

Fig. 1. SRSF3 is significantly up-regulated in GC tissues and cell lines. (**A**) Representative immunoblot of SRSF3 in GC tissues and matched adjacent non-tumour tissues. (**B**) Western blotting was performed to examine the expression of SRSF3 in human gastric mucosal epithelial cells (GES-1) and GC cell lines (HGC27, AGS and MKN45). $^{*}P < 0.05$, $^{**}P < 0.01$.

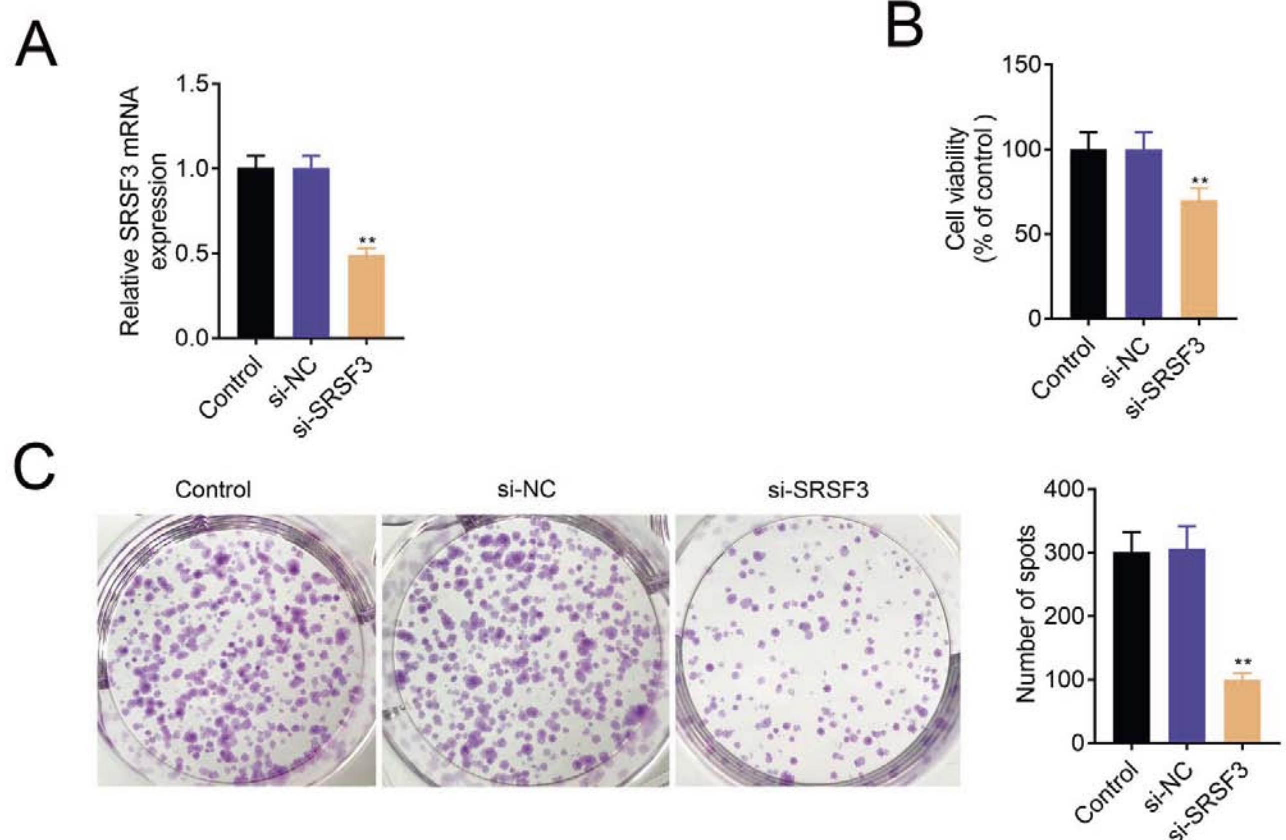

Fig. 2. SRSF3 knockdown significantly inhibits the growth of GC cells. (**A**) The efficiency of *SRSF3* knockdown was confirmed by qRT-PCR in AGS cells. (**B**) Cell viability was examined by MTT assays. (**C**) Cell proliferation was determined by colony formation assay. $^{**}P < 0.01$.

SRSF3 affects the malignant biological properties of GC cells by regulating the PI3K/AKT/mTOR signalling pathway

To determine whether the PI3K/AKT/mTOR axis was implicated in the regulation of GC progression by SRSF3, the PI3K/AKT/mTOR axis was then examined by Western blotting in AGS cells. *SRSF3* knockdown had no significant effects on the levels of PI3K, AKT, and mTOR. However, *SRSF3* knockdown significantly inhibited activation of the PI3K/AKT/mTOR axis (Fig. 4). These results suggested that SRSF3 affects the malignant behaviour of GC cells and this process is mediated by inactivation of the PI3K/AKT/mTOR pathway.

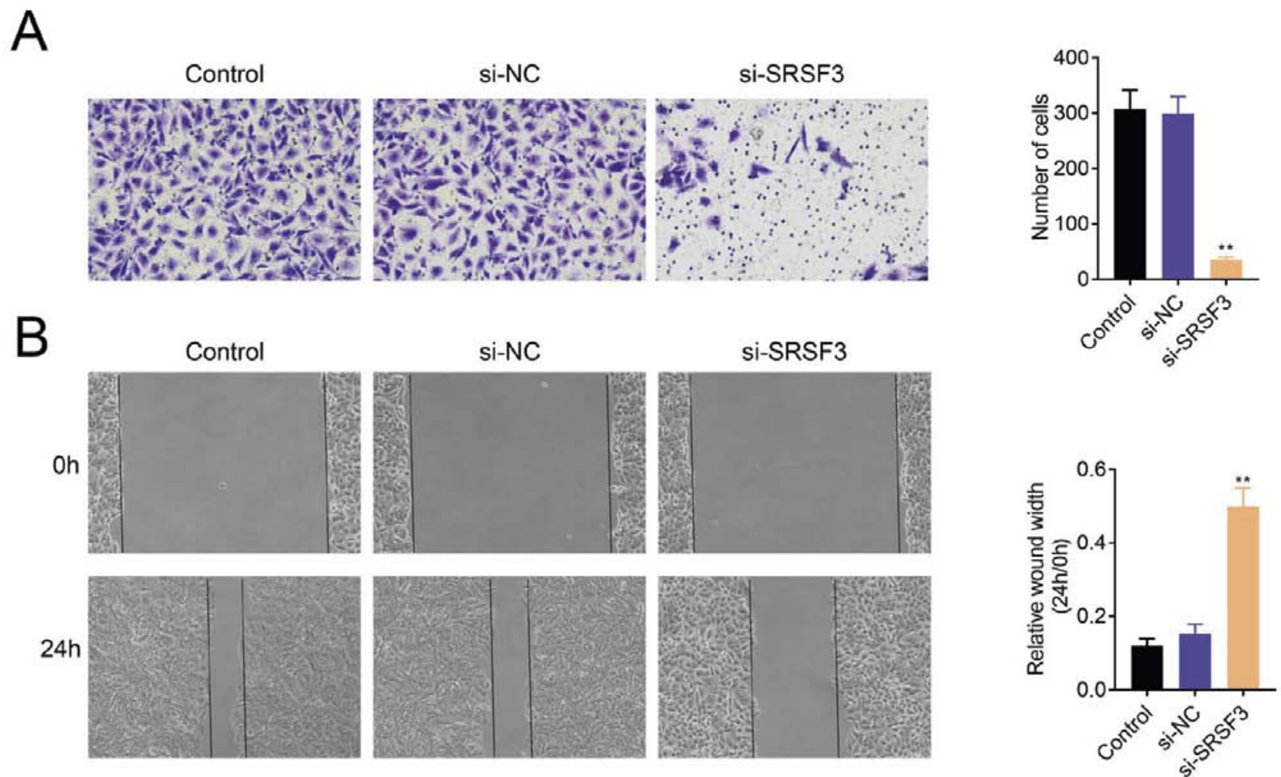

Fig. 3. *SRSF3* knockdown suppresses the invasion and migration of GC cells. (**A**) Cell migration assays were performed to examine the invasive abilities of AGC cells. (**B**) Wound-healing assays were performed to determine the migration abilities of AGC cells. **P < 0.01.

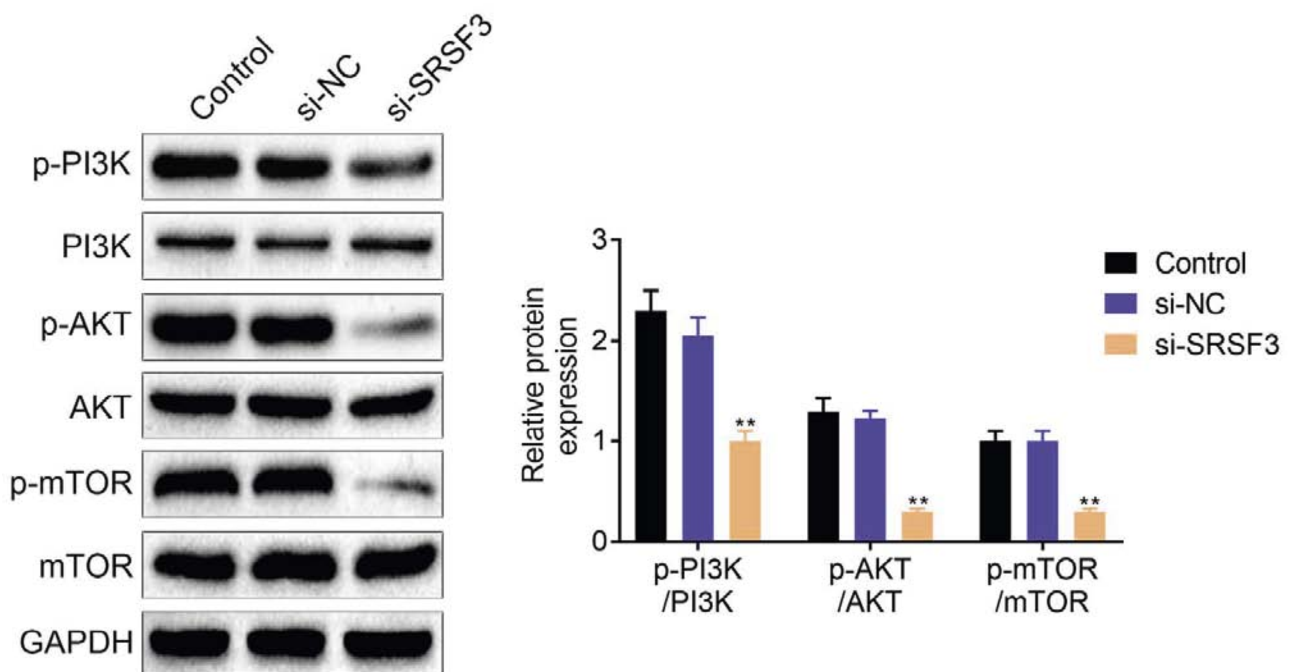

Fig. 4. SRSF3 affects the malignant biological properties of GC cells by regulating the PI3K/AKT/mTOR signalling pathway. The protein levels of phosphorylated PI3K (p-PI3K), total PI3K (PI3K), phosphorylated AKT (p-AKT), total AKT (AKT), phosphorylated mTOR (p-mTOR) and total mTOR (mTOR) were measured by Western blotting (left). Quantification of the relative expression of proteins determined by Western blotting (right). **P < 0.01.

Discussion

Serine/arginine-rich splicing factor 3 (SRSF3) is up-regulated in various cancer types, and its dysregulation has proved to be implicated in development of different tumours (Che and Fu, 2020; Zhou et al., 2020). The function of SRSF3 in GC was first elucidated in this study. The data indicated that SRSF3 was significantly increased in gastric cancer.

SRSF3 has been widely studied as an oncogene. The knockout of *SRSF3* in colorectal cancer cells can significantly activate ArhGAP30/Ace-p53 while inhibiting the malignant behaviour of tumour cells (Wang et al., 2020). In U2OS and HeLa cells, knocking down *SRSF3* can also significantly inhibit the malignant behaviour of tumour cells, which is mediated by the down-regulated Hippo/YAP signalling pathway (Kim et al., 2017). Not only that, *SRSF3* has also been proved to be an oncogene in glioblastoma and oral squamous cell carcinoma (Guo et al., 2015; Song et al., 2019). In this study, *SRSF3* was also confirmed as an oncogene, which further proves its functions in tumours.

Studies indicated that SRSF3 can participate in the regulation of tumour progression (Jia et al., 2019). Therefore, the effect of SRSF3 on the growth and aggressiveness of gastric tumours was examined in this article. A series of *in vitro* functional assays were conducted for the evaluation of the function of SRSF3 in regulating GC cell proliferation, migration and invasion. The results suggested that *SRSF3* knockdown significantly inhibited the growth and aggressiveness of the AGS cancer cells studied. These data thus support a critical role of SRSF3 in gastric cancer.

PI3K/AKT/mTOR signalling is involved in the regulation of cell proliferation and migration and is often dysregulated in cancer (Huang et al., 2019). Moreover, *SRSF3* knockdown inhibited phosphorylation of AKT (Shen et al., 2019). The current study pointed out that *SRSF3* knockdown significantly inhibited activation of PI3K/AKT/mTOR signalling. Collectively, *SRSF3* knockdown inhibited the malignant biological properties of gastric cancer cells, and its action might be mediated by inactivation of the PI3K/AKT/mTOR signalling.

In conclusion, the inhibition of SRSF3 alleviates the proliferation and migration of gastric cancer, and this process is mediated by the inactivation of PI3K/AKT/mTOR signalling. Targeting SRSF3 may be a promising strategy to combat gastric cancer in future.

Competing interests

The authors state that there are no conflicts of interest to disclose.

Ethics approval

This study was performed under the approval of the Institutional Review Board and Human Ethics Committee of the First People's Hospital of Jingzhou. All the patients have signed the informed consent form.

Availability of data and materials

All data generated or analysed during this study are included in this published article.

Authors' contributions

J. X. designed the study, supervised the data collection, Y. S. analysed the data, interpreted the data, Q. X. prepared the manuscript for publication and reviewed the draft of the manuscript. All authors have read and approved the manuscript.

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