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Up-regulated ONECUT2 and down-regulated SST promote gastric cell migration, invasion, epithelial-mesenchymal transition and tumor growth in gastric cancer

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Abstract. – OBJECTIVE: Gastric cancer is a common malignancy, with high metastasis and poor prognosis. Our purpose was to explore potential molecular mechanisms of gastric cancer.

PATIENTS AND METHODS: A total of 10 pairs of gastric cancer tissues and adjacent normal gastric tissues were collected for RNA sequencing (RNA-seq), followed by differential expression analysis. Combining qRT-PCR results, two novel genes were selected for in-depth analysis, including up-regulated ONECUT and down-regulated SST. To investigate the effects of ONECUT and SST on the biological behaviors of gastric cancer cells, gastric cancer cell lines were transfected by ONECUT2 knockdown and SST overexpression. Afterwards, cell migration and invasion were examined using transwell assays, and the expressions of epithelial-mesenchymal transition (EMT)-related proteins were measured by Western blot analysis. Furthermore, cell viability was detected by CCK-8 assay. Finally, tumorigenicity in nude mice was performed.

RESULTS: Gastric cancer cell migration and invasion were inhibited in BGC823 cells transfected by shONECUT2. Similar results were observed in SST overexpression in MGC803 cells. Silencing ONECUT2 or overexpressing SST reduced the expressions of mesenchymal markers (N-cadherin and vimentin), STAT3, fibronectin, Wnt2, β-catenin and increased epithelial marker (E-cadherin), p-STAT3, smad2/3, α-catenin protein levels. In addition, inhibiting ONECUT2 or elevated SST suppressed tumor cell viability *in vitro*. Moreover, ONECUT2 silencing or elevated SST significantly inhibited tumor growth *in vivo*.

CONCLUSIONS: Up-regulated ONECUT2 and down-regulated SST promote gastric cell migration, invasion, epithelial-mesenchymal transition and tumor growth in gastric cancer.

Key Words:

Gastric cancer, Migration, Invasion, Epithelial-mesenchymal transition, ONECUT2, SST.

Abbreviations

RNA-seq: RNA sequencing; EMT: epithelial-mesenchymal transition; qRT-PCR: quantitative real-time polymerase chain reaction; shRNAs: short hairpin RNAs; CCK-8: Cell Counting Kit-8.

Introduction

Gastric cancer is a common malignant tumor in the digestive system worldwide, with high morbidity and mortality[1,2]. Surgical resection is a main curative treatment[3,4]. Metastasis contributes to poor prognosis and high mortality in gastric cancer[5]. In the advanced stage of gastric cancer, tumor cells invade the blood or lymphatic vessels and transfer to distant organs[6]. Therefore, inhibition of metastasis is a key to improving gastric cancer patients' prognosis[7,8]. Although surgery and chemotherapy have improved over the past few decades, gastric cancer metastasis has not been resolved. High metastatic rate and low sensitivity chemotherapy in gastric cancer patients result in poor prognosis[9]. Therefore, more efforts are still needed to reveal the mechanism of gastric cancer metastasis and to promote the development of gastric cancer treatment.

EMT participates in gastric cancer metastasis[10]. It can induce the transformation of epithelial cells

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into cells with a mesenchymal phenotype, first described in embryonic development[11]. Several indicators such as epithelial markers (E-cadherin and β-catenin), mesenchymal markers (N-cadherin, vimentin, and fibronectin) and Wnt signaling pathways are involved in EMT process[12,13]. One of the most reliable risk factors of prognosis is tumor stage that may reflect invasion depth and metastasis degree. In addition, tumor size and histological type are also useful prognostic factors[14]. It has been considered that primary tumor invasion and lymph node metastasis are the most reasonable prognostic risk factors in a variety of clinical pathological parameters, however, the accuracy of TNM classification remains a controversy in the clinical application of gastric cancer[15]. In view of the biological features of malignancies, highly specific markers are capable of improving the accuracy to predict the prognosis of gastric cancer. Despite in-depth research on the mechanisms of gastric cancer, little is known about its metastasis and invasion. In our study, we aimed to explore new molecular markers that may lead to gastric cancer metastasis.

RNA-seq, a high-throughput technology, provides a comprehensive view of the entire transcriptome. It could recognize novel genes, allele-specific expression, fusion genes, disease-related single nucleotide polymorphisms (SNPs), post-transcriptional modifications and ncRNA[16-18]. Gastric cancer is characterized by heterogeneity, which evolves in a variety of genetic and epigenetic changes, such as transcriptional changes[19]. In our study, we took advantage of RNA-seq to choose differentially expressed genes. Two specific genes including overexpressed ONECUT2 and down-regulated SST were selected for further analysis. Silencing ONECUT2 or overexpressing SST could significantly inhibit gastric cancer cell migration and invasion and could affect the expression of EMT-associated proteins. Silencing ONECUT2 or overexpressing SST suppressed tumor growth *in vivo*. Up-regulated ONECUT2 and down-regulated SST could promote gastric cell migration, invasion, epithelial-mesenchymal transition and tumor growth in gastric cancer.

Patients and Methods

RNA-seq

From September 2017 to December 2017, 10 pairs of gastric cancer tissues and their corresponding adjacent normal tissues were collected from the First Affiliated Hospital of Guangxi Medical University (Nanning, Guangxi, China). Adjacent normal gastric tissues were 5 cm away from the edge of tumors. All specimens were immediately stored in liquid nitrogen before RNA extraction[20,21]. All patients were diagnosed by pathologists and histologically according to the Chinese Society of Clinical Oncology (CSCO). Gastric cancer patients who did not receive preoperative treatment (including chemotherapy, radiotherapy or target therapy) were included in this study. Our study was approved by the Ethics Committee of the First Affiliated Hospital of Guangxi Medical University (2016-KY-173), and all patients signed informed consent.

Total RNA was extracted from tissues. The quality and quantity evaluation, cDNA library preparation, and sequencing were achieved by BasePair BioTechnology Co., Ltd. (Suzhou, Jiangsu, China). Genes with $p<0.05$ and |fold change|>2 were considered as differentially expressed genes. Functional enrichment analysis was performed to predict the potential functions of differentially expressed genes. Based on the sequencing results, differentially expressed genes associated with cell migration were identified.

Cell Culture

Human gastric epithelial cells GES-1, human gastric cancer cells including SGC7901, SNU-1, HGC-27, MGC803, AGS, RATO3 and BGC823 were purchased from Sailan Biological Technology Co., Ltd. (Hangzhou, Zhejiang, China). All cells were cultured in 1640 medium with 10% fetal bovine serum (FBS) in a humidified atmosphere of 5% CO_2 at 37°C.

qRT-PCR

Total RNA was extracted from tissues or cells using TRIzol (TakaRa, Dailian, China). The RNA samples were reverse-transcribed into cDNA by a RevertAid First Strand cDNA synthesis Kit (Thermo Fisher Scientific, Waltham, MA, USA). The cDNA templates were amplified by qRT-PCR using SsoAdvance Universal SYBR Green Supermix (Bio-Rad, Hercules, CA, USA). GAPDH served as an endogenous control. Relative fold changes of target genes were calculated with the $2^{-\Delta\Delta Ct}$ method. The primers are listed in Table I.

Cell Transfection

ONECUT2 shRNAs, and SST overexpression lentivirus vector were synthesized by Sailan Biological Technology Co., Ltd (Hangzhou, Zheji-

Table I. The primers for qRT-PCR.

Target genes	Primer sequences
human ONECUT	5'-GGAATCCAAAACCGTGGAGTAA-3' 5'-CTCTTTGCGTTTGCACGCTG-3'
human SST	5'-ACCCAACCAGACGGAGAATGA-3' 5'-GCCGGGTTTGAGTTAGCAGA-3'
Human GAPDH	5'-CTCGCTTCGGCAGCACA-3' 5'-AACGCTTCACGAATTTGCGT-3'

ang, China). Transfection was performed using Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA). After transfection for 48 h, all cells were harvested. The target sequence of ONECUT2-shRNA1 was as follows: 5'-CAACCTCTACAGTCCCTACAA-3'. To examine transfection effects, qRT-PCR and Western blot were performed.

Transwell Assay

Transwell assay was carried out based on 24-well transwell chambers (Corning, NY, USA). The upper chambers were uncoated (migration) and coated Matrigel (invasion; BD Biosciences, Franklin Lakes, NJ, USA). Cells were added to the upper chamber for 48 h. The upper chambers were fixed and stained with 0.1% crystal violet for 2 min. After that, migrated and invaded cells were counted in five random fields under a microscope, respectively.

Western Blot Analysis

Total protein was extracted from cells using radio immunoprecipitation (RIPA) buffer containing protease inhibitor cocktail (Beyotime Biotechnology Co., Ltd., Shanghai, China). The extracted protein was separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). The PVDF membranes were then blocked with a 5% non-fat milk for 2 h at room temperature. After that, the membranes were incubated with primary antibodies at 4°C overnight, followed by secondary antibody. The target protein was detected with a Clarity™ Western ECL Substrate Kit (Beyotime Biotechnology Co., Ltd., Shanghai, China)[22]. The primary antibodies included fibronectin, E-cadherin, N-cadherin, α-catenin, p-STAT3, STAT3, β-catenin, p-smad2/3, smad2/3, vimentin, Wnt2, SST, onecut2 and GAPDH antibody (1: 5000, Cell Signaling, Danvers, MA, USA). GAPDH served as an internal control.

Cell Counting Kit-8 (CCK-8)

After incubating BGC823 and MGC803 cells for 48 h, 10 ul of CCK-8 solution was added to each well, mixed and incubated at 37°C for 4 h. Then, 10 ul of stop solution was added to each well. Finally, cell viability was measured at OD450 with a microplate reader.

Tumorigenicity in Nude Mice

BGC823 cells transfected by sh-ONECUT2 and MGC803 cells transfected by SST overexpression were harvested and inoculated subcutaneously into male nude mice (4-5 weeks old; four nude mice each group). Tumor size was measured every four days with a caliper. Mice were sacrificed after 4 weeks. Tumor volume (mm^3) was then calculated as follows: volume (mm^3=1/2×length×width^2). Our study was approved by the Ethics Committee of the First Affiliated Hospital of Guangxi Medical University (2016-KY-173).

Statistical Analysis

All experiments were performed at least three times independently. All statistical analyses were performed using GraphPad Prism 7 (La Jolla, CA, USA). Data were expressed as the mean ± SD. Comparisons were evaluated using Student's *t*-test or one-way analysis of variance. $p \leq 0.05$ was considered statistically significant.

Results

Highly Expressed ONECUT2 and Lowly Expressed SST in Gastric Cancer Tissues

Differentially expressed genes were identified between 10 pairs of gastric cancer tissues and adjacent normal tissues using RNA-seq. According to bioinformatics analysis, it was predicted that ONECUT2 and SST could be associated with cancer cell migration. To detect RNA-seq results, qRT-PCR was carried out. Consistent with RNA-seq results, the expression levels of ONECUT2

were lower in normal tissues than in tumor tissues (Figure 1A), while the expression levels of SST in normal tissues were higher than those in gastric cancer tissues (Figure 1B). However, there was no correlation between ONECUT2 and SST in gastric cancer. Therefore, we further analyzed the expression patterns of these differentially expressed genes in gastric cancer using GEPIA. ONECUT2 was significantly up-regulated in gastric cancer compared with normal samples (Figure 2A), furthermore, high ONECUT2 expression was in association with poorer disease-free survival time (Figure 2B). In addition, the results confirmed that SST was significantly down-regulated in gastric cancer compared with normal samples (Figure 2C). Moreover, low SST expression indicated better disease-free survival time (Figure 2D). Therefore, ONECUT2 and SST were both abnormally expressed in gastric cancer and were associated with prognosis of gastric cancer.

BGC823 and MGC803 Cells Successfully Transfected by ONECUT2 and SST

To select an appropriate cell line to construct interference strain of ONECUT2 and overexpression strain of SST, we detected the expression of SST and ONECUT2 in 8 cells including GES-1, SGC7901, SNU-1, AGS, HGC-27, MGC803, RATO3 and BGC823 cells after transfection. qRT-PCR results showed that SST had the highest mRNA relative expression level in MGC803 cells. ONECUT2 had the lowest mRNA relative expression level in BGC823 cells (Figure 3). Therefore, we chose BGC823 cells transfected by ONECUT2, and MGC803 cells transfected by SST.

To evaluate the effects of ONECUT2 knockdown in BGC823 cells transfected by its shONECUT2, qRT-PCR and Western blot results suggested that ONECUT2 expression was significantly decreased after transfection at the mRNA and protein levels (Figure 4A-4C). Similarly, to assess the effects of SST overexpression in MGC803 cells, the results demonstrated that the mRNA and protein expression levels of SST were both elevated after transfection (Figure 4D-4F).

Up-Regulated ONECUT2 and Down-Regulated SST Promote Gastric Cancer Cell Migration and Invasion In Vitro

To further investigate the roles of ONECUT2 and SST in gastric cancer cell migration, invasion and EMT, transwell assays were performed. The results showed that cell migrated, and invaded abilities were suppressed in BGC823 cells transfected by shONECUT2 (Figure 5A-C). Similar results were observed in MGC803 cells transfected by SST overexpression (Figure 6A-C).

Up-Regulated ONECUT2 and Down-Regulated SST Promote EMT in Gastric Cancer Cell In Vitro

EMT is a critical event in the metastasis of tumor cells. Western blotting analysis was performed to detect the expression levels of EMT-associated proteins in gastric cancer cells. Intriguingly, inhibiting ONECUT2 decreased the expression of mesenchymal markers (N-cadherin and vimentin), STAT3, fibronectin, Wnt2, β-catenin and increased the expression of epithelial

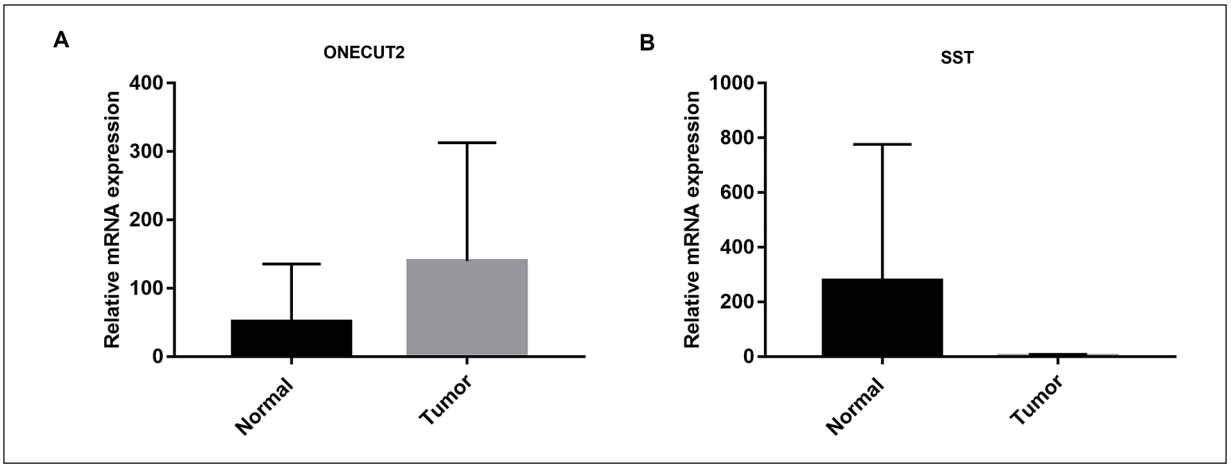

Figure 1. The mRNA relative expression levels of ONECUT2 and SST between gastric cancer tissues and normal tissues by qRT-PCR. (**A**) ONECUT2; (**B**) SST.

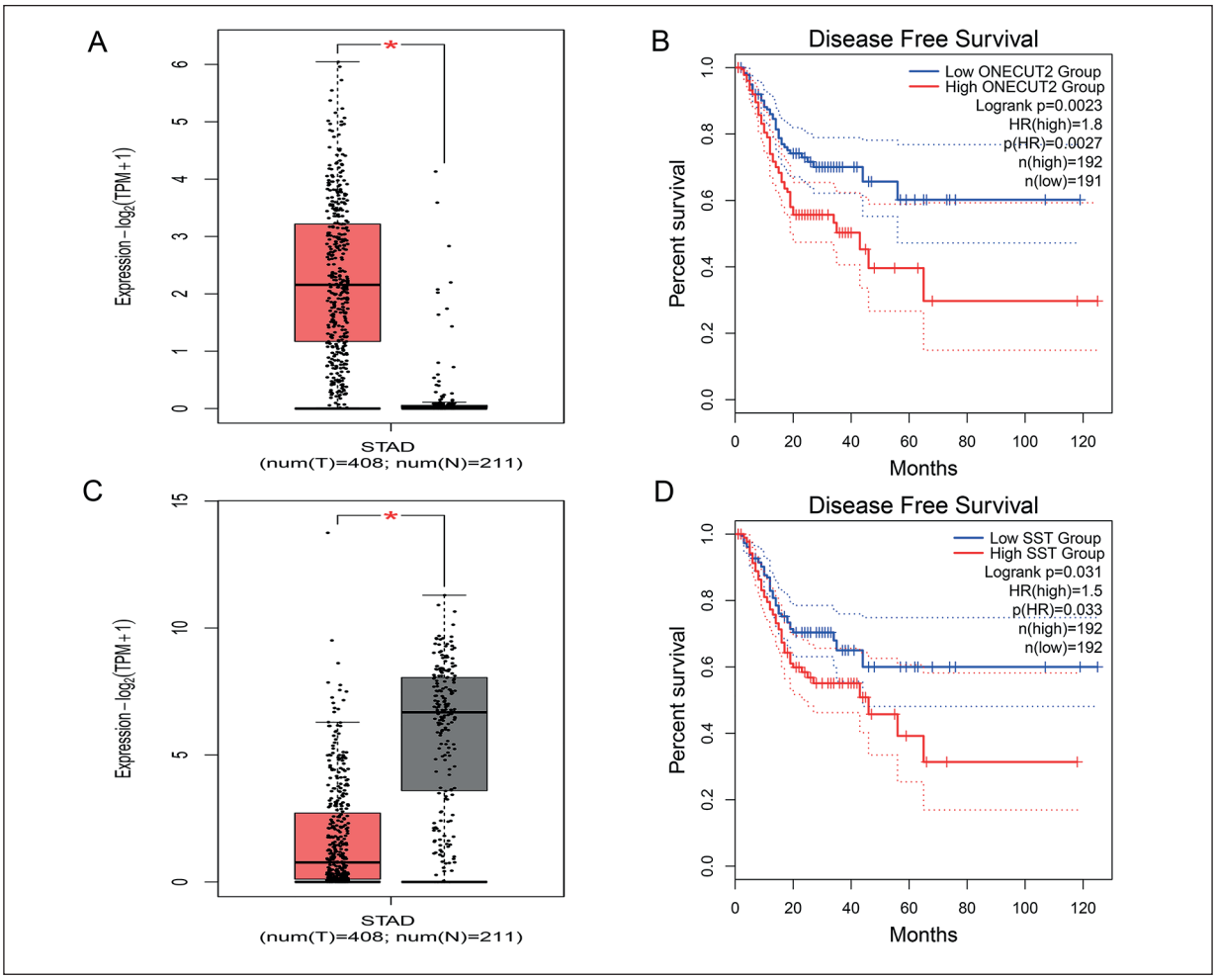

Figure 2. Up-regulated ONECUT2 and down-regulated SST are associated with gastric cancer prognosis. **A,** ONECUT2 is up-regulated in gastric cancer compared to normal samples. **B,** Disease free survival analysis of ONECUT2. **C,** SST is down-regulated in gastric cancer compared to normal samples. **D,** Disease free survival analysis of SST. *p<0.05.

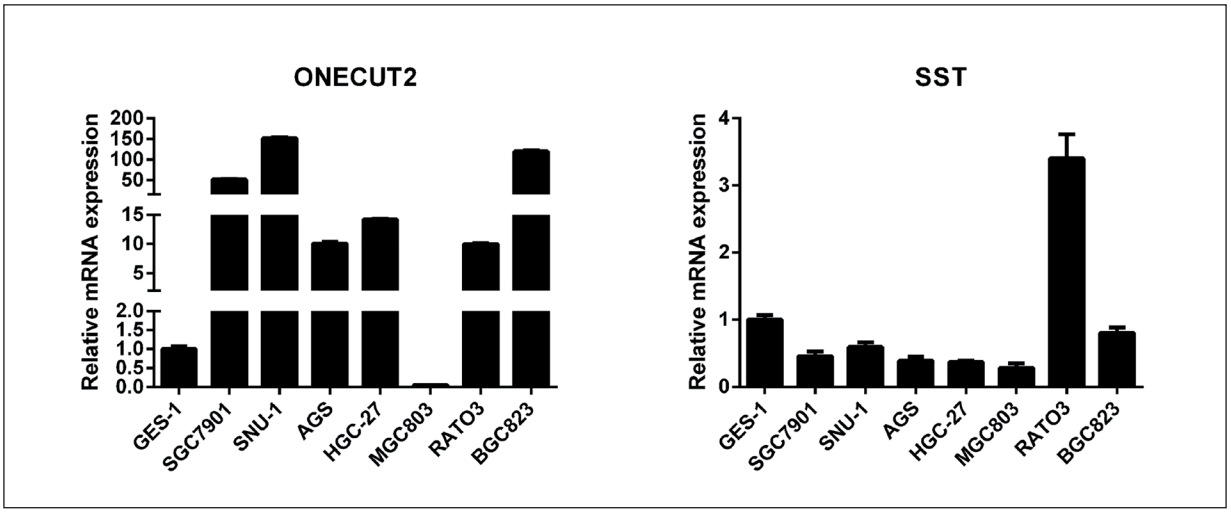

Figure 3. The mRNA expression levels of SST and ONECUT2 in 8 cells by qRT-PCR.

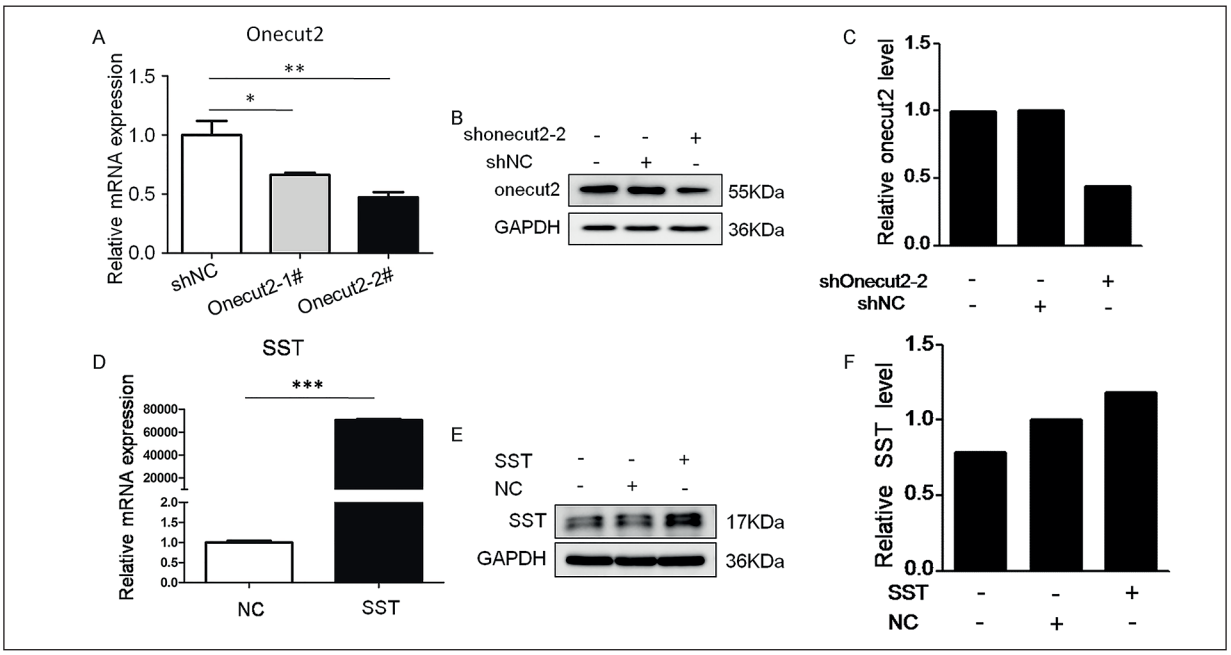

Figure 4. Successful construction of cell transfection. **A-C,** The mRNA and protein expression levels of ONECUT2 in BGC823 cells after transfection; **D-F,** The mRNA and protein expression levels of SST in MGC803 cells after transfection. **p*<0.05; ***p* <0.01; ****p*<0.001.

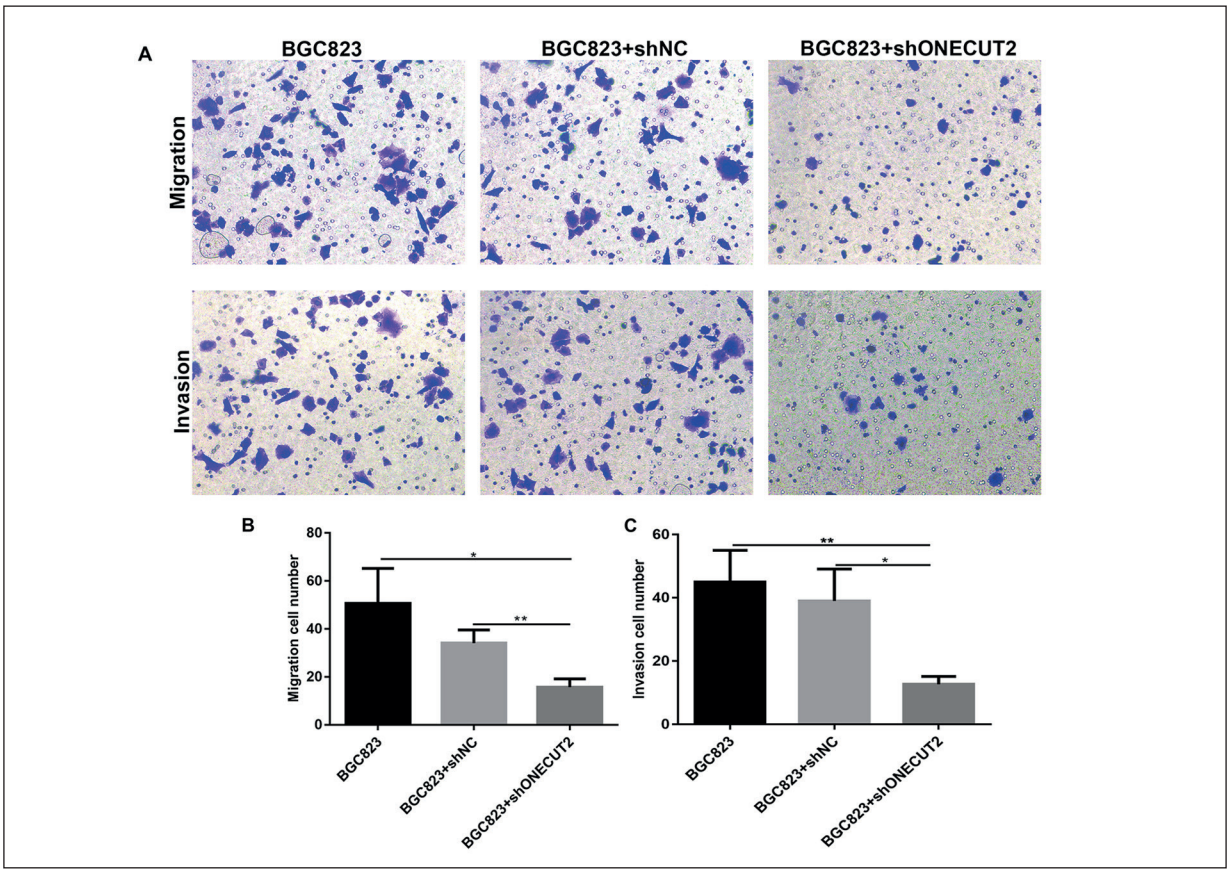

Figure 5. Inhibiting ONECUT2 expression decreases migration and invasion of BGC823 cells. **A,** Representative images (×200). **B,** Migration cell number. **C,** Invasion cell number. **p*<0.05; ***p*<0.01.

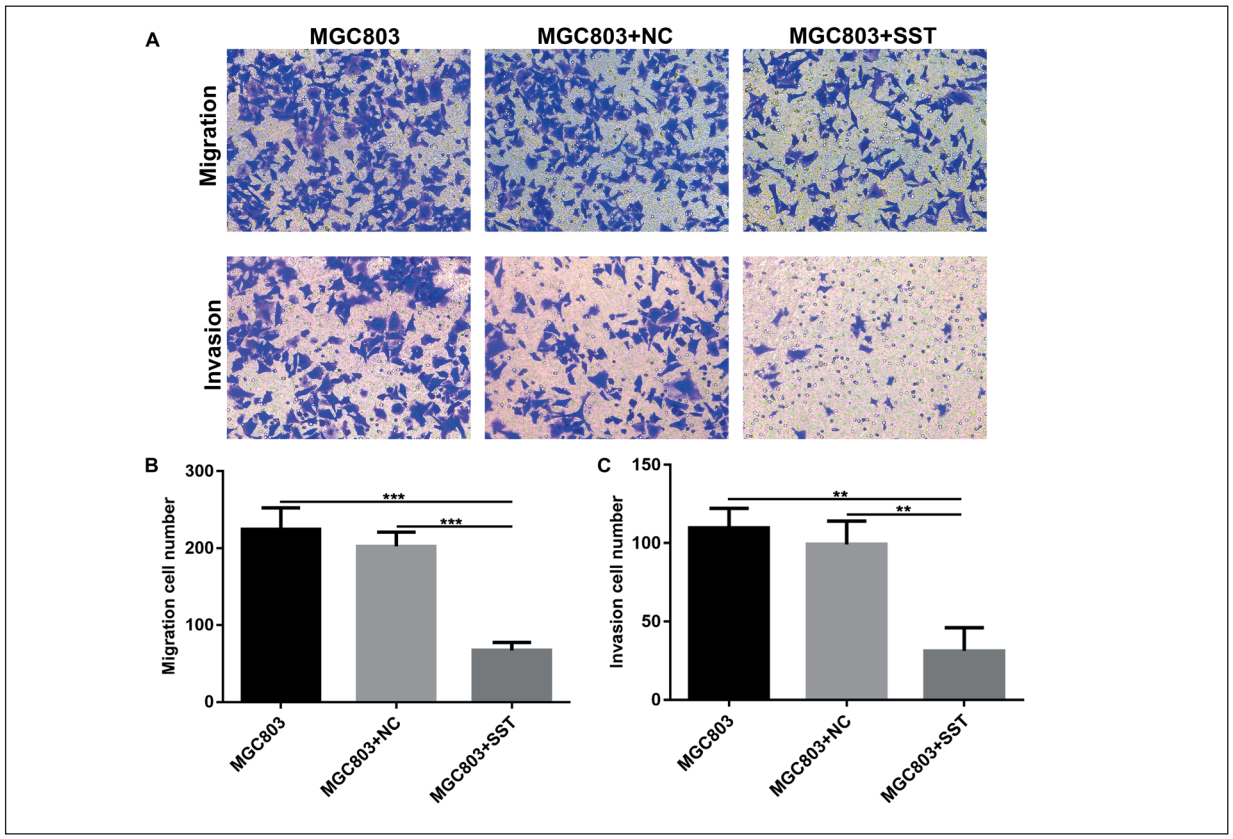

Figure 6. Overexpressed SST expression decreases migration and invasion of MGC803 cells. **A,** Representative images (×200). **B,** Migration cell number. **C,** Invasion cell number. ***p* <0.01; ****p*<0.001.

marker (E-cadherin), p-STAT3, smad2/3, α-catenin (Figure 7A-K), while the similar results were detected when SST overexpression in MGC803 cells (Figure 8A-K). The results strongly suggested that up-regulated ONECUT2 and down-regulated SST could promote EMT in gastric cancer cells.

Up-Regulated ONECUT2 and Down-Regulated SST Promote Gastric Cancer Cell Viability In Vitro

To observe whether ONECUT2 and SST could affect gastric cancer cell viability, CCK-8 assay was performed after enforcing SST expression or inhibiting ONECUT2 expression. The results revealed that cell viability of BGC823 cells transfected by shONECUT2 was inhibited compared with control group (Figure 9A). The cell viability of MGC803 cells transfected by overexpressed SST was also suppressed (Figure 9B). Therefore, up-regulated ONECUT2 and down-regulated SST could promote gastric cancer cell viability *in vitro*.

Up-Regulated ONECUT2 and Down-Regulated SST Promote Tumor Growth In Vivo

We further investigated whether ONECUT2 and SST could affect tumor growth *in vivo*. BGC823 cells transfected by sh-ONECUT2 and MGC803 cells transfected by SST overexpression were inoculated subcutaneously into male nude mice. The results showed that tumor volumes in BGC823 cells transfected by sh-ONECUT2 groups were smaller compared with control group (Figure 10A, 10C). Furthermore, tumor volumes in MGC803 cells transfected by SST group were smaller compared with control group (Figure 10B, 10D). Tumor growth curves were drawn (Figure 10E, F). Therefore, ONECUT2 and SST could affect tumor growth *in vivo*. The hypothesis diagram of our study is shown in Figure 11.

Discussion

In the present study, we identified two novel differentially expressed genes (ONECUT2 and

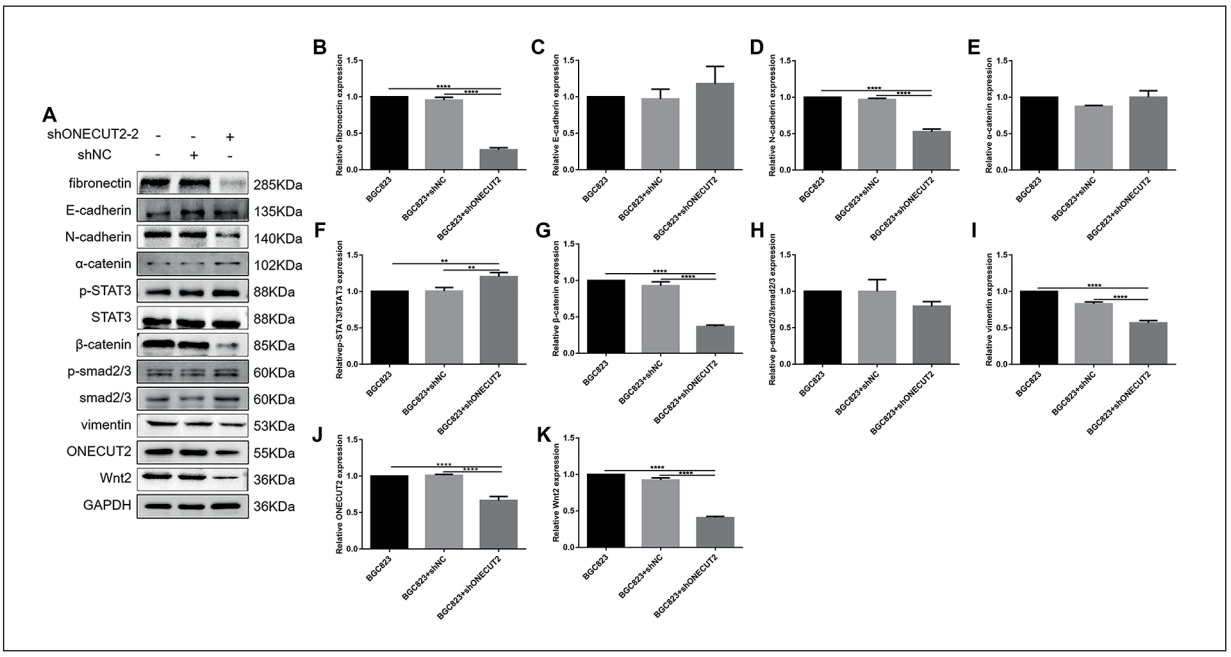

Figure 7. The expressions of EMT-related marker in BGC823 cells transfected by shONECUT2 by Western blot. **A,** Representative images; **B,** fibronectin; **C**, E-cadherin; **D,** N-cadherin; **E,** α-catenin; **F,** p-STAT3/STAT3; **G,** β-catenin; **H,** p-smad2/3/smad2/3; **I,** vimentin; **J,** ONECUT2; **K,** Wnt2. ***p*<0.01; *****p*<0.0001.

SST) associated with gastric cancer cell migration, invasion and EMT *in vitro*. Furthermore, up-regulated ONECUT2 and down-regulated SST could promote tumor growth *in vivo*. Our findings revealed that ONECUT2 and SST could be involved in the development of gastric cancer.

In this study, we analyzed 10 pairs of gastric cancer tissues and corresponding adjacent normal tissues using RNA-seq. qRT-PCR results further confirmed RNA-seq results. Finally,

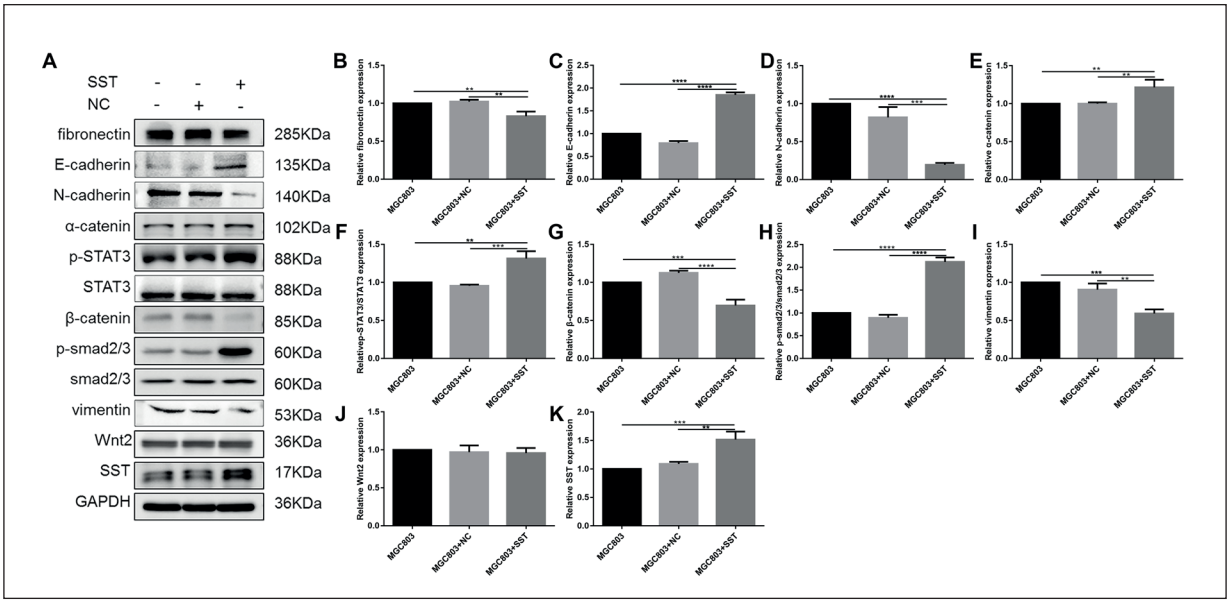

Figure 8. The expressions of EMT-related marker in MGC803 cells with overexpressed SST by Western blot. **A,** Representative images; **B,** fibronectin; **C,** E-cadherin; **D,** N-cadherin; **E,** α-catenin; **F,** p-STAT3/STAT3; **G,** β-catenin; **H,** p-smad2/3/smad2/3; **I,** vimentin; **J,** Wnt2; **K**, SST. ***p*<0.01; ****p*<0.001; *****p*<0.0001.

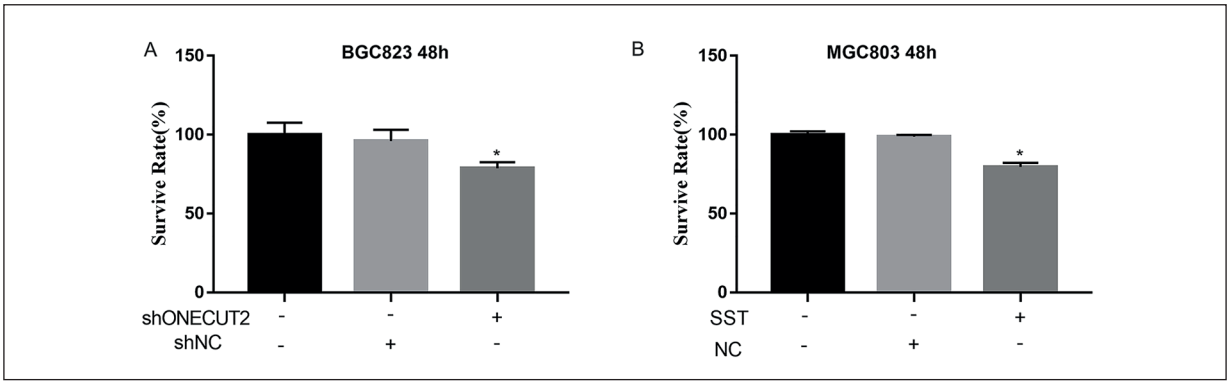

Figure 9. Silencing ONECUT2 and elevated SST suppress gastric tumor cell viability *in vitro*. **A,** The cell viability of BGC823 cells transfected by shONECUT2. **B,** The cell viability of MGC803 cells transfected by SST overexpression. *p<0.05.

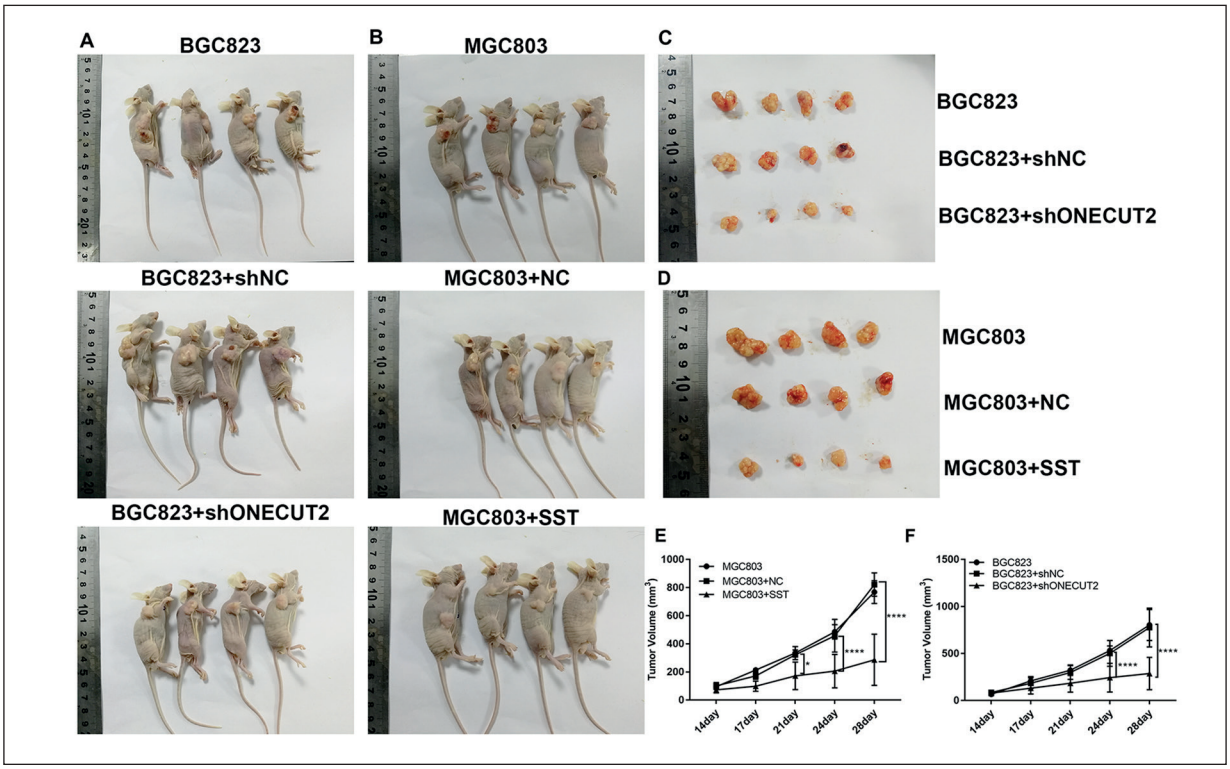

Figure 10. Inhibiting ONECUT2 and elevated SST suppress tumor growth *in vivo*. **A, C,** ONECUT2 silencing suppressed tumor growth; **B, D,** SST overexpression suppressed tumor growth; **E, F,** Tumor growth curves. *p<0.05; ****p<0.0001.

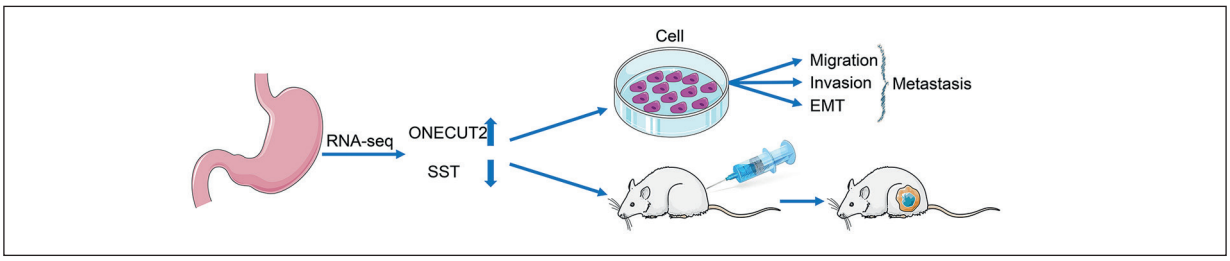

Figure 11. Hypothesis diagram illustrates function and mechanisms of ONECUT2 and SST in gastric cancer metastasis.

two novel genes up-regulated ONECUT2 and down-regulated SST were selected for further analysis. Correlation analysis revealed that there was no significant correlation between ONECUT2 and SST in gastric cancer. ONECUT2, one cut homeobox 2, encodes a member of the one cut family of transcription factors characterized by a cut domain and an atypical homeodomain[23]. ONECUT2 participates in the EMT, migration, and invasion of colorectal cancer cells[24]. ONECUT2 knockdown suppresses ovarian tumor cell proliferation, migration, invasion and angiogenesis[25]. In addition, ONECUT2 inhibits the androgen axis in prostate cancer[26]. SST, somatostatin, encodes the hormone somatostatin, which has active 14 aa and 28 aa forms. SST has anti-tumor and anti-secretion activities in a few human cancers like prostate cancer, cervical cancer, small cell lung cancer[27-29]. In addition, it has significant differences in gene expression and DNA methylation in gastric cancer[30].

Our findings revealed that inhibiting ONECUT2 or overexpressing SST suppressed in gastric cancer migration and invasion *in vitro*. The cell migration and invasion were inhibited in BGC823 cells transfected by shONECUT2. Similar results were observed in MGC803 cells transfected by SST overexpression. Furthermore, inhibiting ONECUT2 or elevated SST affected the expression of EMT-associated proteins. EMT is a critical cellular process in gastric cancer metastasis[31]. In our study, silencing ONECUT2 reduced mesenchymal marker (N-cadherin and vimentin), STAT3, fibronectin, Wnt2, β-catenin and increased epithelial marker (E-cadherin), p-STAT3, smad2/3, α-catenin protein levels. Intriguingly, similar results were observed when overexpressing SST in MGC803 cells. Previous studies have confirmed that these proteins are involved in EMT processes. Cadherins involve in cellular invasion and migration. Over-expressed E-cadherin can significantly inhibit gastric cancer cell migration and invasion[32]. Silencing Cav-1 may induce EMT of gastric cancer cell via mediating E-cadherin[33]. Silencing N-cadherin inhibits cancer cell invasion and migration. Down-regulated vimentin can inhibit gastric cancer migration[34]. α-catenin participates in cadherin-mediated epithelium development and tissue maintenance, as well as cancer progression and metastasis[35,36]. Furthermore, cell-cell adhesion depends on α-catenin·β-catenin-binding interface[37]. STAT3 aggravates epithelial-to-mesenchymal transition and migration[38]. Up-regulated fibronectin participates in the migration of gastric cancer cells[39]. Several genes have been confirmed to regulate fibronectin. So, miR-200c, binding with fibronectin 1, can inhibit gastric cancer cell migration[40]. In our study, we found that inhibiting ONECUT2 or overexpressing SST could decrease the expression level of fibronectin. The Wnt/β-catenin pathway is the classical part of the Wnt signaling pathway, which is marked by the accumulation of β-catenin and is transferred into the nucleus. The Wnt/β-catenin pathway is well known for regulating cell proliferation and migration, and its aberrant activation is involved in gastric carcinogenesis and progression[41]. Smad2/3 signaling pathway contributes to gastric cancer metastasis[42,43]. More importantly, our results were consistent with above findings. Therefore, our findings suggested that ONECUT2 and SST were involved in gastric cancer cell metastasis. Furthermore, we also found that inhibiting ONECUT2 or elevated SST significantly suppressed gastric tumor cell viability, suggesting the two genes could affect gastric cell proliferation and apoptosis. We also investigated the effects of inhibiting ONECUT2 or elevated SST for *in vivo*. The results revealed that up-regulated ONECUT2 and down-regulated SST promoted tumor growth.

This study has several novelties. First, our study identified two novel genes including up-regulated ONECUT2 and down-regulated SST for gastric cancer. Second, we identified the functions of the two genes in gastric cancer. Inhibiting ONECUT2 or overexpressing SST could significantly inhibit gastric cancer migration and invasion *in vitro* and suppress tumor growth *in vivo*. Therefore, ONECUT2 and SST might possess potential value as therapeutic targets for gastric cancer, which deserve further analysis.

Conclusions

Our findings showed up-regulated ONECUT2 and down-regulated SST in gastric cancer tissues. Inhibiting ONECUT2 and overexpressing SST suppressed gastric cancer cell migration, invasion and EMT process. Furthermore, gastric cancer cell viability was inhibited. Inhibiting ONECUT2 and overexpressing

SST inhibited tumor growth *in vivo*. Therefore, ONECUT2 and SST could be involved in the development of gastric cancer, which worth further research.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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Ethics Approval and Consent to Participate

The study was approved by the Ethics Committee of the First Affiliated Hospital of Guangxi Medical University (2016-KY-173).

Authors' Contribution

Lei Tian, Zhen Wang conceived and designed the study. Gonghe Wang, Yiming Zhou, Zhu Yu and Jianping Deng conducted most of the experiments and data analysis and wrote the manuscript. Senfeng Liu, Chengzhi Wei, Yue Feng and Mao Mao participated in collecting data and helped to draft the manuscript. All authors reviewed and approved the manuscript..

References

1) Siegel RL, Miller KD, Jemal A. Cancer statistics, 2017. CA Cancer J Clin 2017; 67: 7-30.
2) Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, Jemal A, Yu XQ, He J. Cancer statistics in China, 2015; CA Cancer J Clin 2016, 66: 115-132.
3) Ito S, Sano T, Mizusawa J, Takahari D, Katayama H, Katai H, Kawashima Y, Kinoshita T, Terashima M, Nashimoto A, Nakamori M, Onaya H, Sasako M. A phase II study of preoperative chemotherapy with docetaxel, cisplatin, and S-1 followed by gastrectomy with D2 plus para-aortic lymph node dissection for gastric cancer with extensive lymph node metastasis: JCOG1002. Gastric Cancer 2017; 20: 322-331.
4) Chang PC, Tai CM, Hsin MC, Hung CM, Huang IY, Huang CK. Surgical standardization to prevent gastric stenosis after laparoscopic sleeve gastrectomy: a case series. Surg Obes Relat Dis 2017; 13: 385-390.
5) Fan Y, Wang YF, Su HF, Fang N, Zou C, Li WF, Fei ZH. Decreased expression of the long noncoding RNA LINC00261 indicate poor prognosis in gastric cancer and suppress gastric cancer metastasis by affecting the epithelial-mesenchymal transition. J Hematol Oncol 2016; 21: 9: 57.
6) Yan Y, Wang LF, Wang RF. Role of cancer-associated fibroblasts in invasion and metastasis of gastric cancer. World J Gastroenterol 2015; 21: 9717-9726.
7) Song S, Ajani JA. The role of microRNAs in cancers of the upper gastrointestinal tract. Nat Rev Gastroenterol Hepatol 2013; 10: 109-118.
8) Obermannová R, Lordick F. Management of metastatic gastric cancer. Hematol Oncol Clin North Am 2017; 31: 469-483.
9) Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer 2010; 127: 2893-2917.
10) Wei YL, Hua J, Liu XY, Hua XM, Sun C, Bai JA, Tang QY. LncNEN885 inhibits epithelial-mesenchymal transition by partially regulation of Wnt/β-catenin signalling in gastroenteropancreatic neuroendocrine neoplasms. Cancer Sci 2018; 109: 3139-3148.
11) Wu Y, Ding ZY, Jin GN, Xiong YX, Yu B, Sun YM, Wang W, Liang HF, Zhang B, Chen XP. Autocrine transforming growth factor-β/activin A-Smad signaling induces hepatic progenitor cells undergoing partial epithelial-mesenchymal transition states. Biochimie 2018; 148: 87-98.
12) Chen SC, Liao TT, Yang MH. Emerging roles of epithelial-mesenchymal transition in hematological malignancies. J Biomed Sci 2018; 25: 37.
13) Wei Z, Shan Z, Shaikh ZA. Epithelial-mesenchymal transition in breast epithelial cells treated with cadmium and the role of Snail. Toxicol Appl Pharmacol 2018; 344: 46-55.
14) Pan YM, Wang CG, Zhu M, Xing R, Cui JT, Li WM, Yu DD, Wang SB, Zhu W, Ye YJ, Wu Y, Wang S, Lu YY. STAT3 signaling drives EZH2 transcriptional activation and mediates poor prognosis in gastric cancer. Molecular Cancer 2016; 15: 79.
15) Deng J, Liang H, Zhang R, Hou Y, Liu Y, Ying G, Pan Y, Hao X. Clinical and experimental role of ring finger protein 180 on lymph node metastasis and survival in gastric cancer. Br J Surg 2016; 103: 407-416.
16) Hrdlickova R, Toloue M, Tian B. RNA-Seq methods for transcriptome analysis. Wiley Interdiscip Rev RNA 2017; 8.
17) Byron SA, Van Keuren-Jensen KR, Engelthaler DM, Carpten JD, Craig DW. Translating RNA sequencing into clinical diagnostics: opportunities and challenges. Nat Rev Genet 2016; 17: 257-271.
18) Wang J, Dean DC, Hornicek FJ, Shi H, Duan Z. RNA sequencing (RNA-Seq) and its application in ovarian cancer. Gynecol Oncol 2019; 152: 194-201.
19) Ye G, Huang K, Yu J, Zhao L, Zhu X, Yang Q, Li W, Jiang Y, Zhuang B, Liu H, Shen Z, Wang D, Yan L, Zhang L, Zhou H, Hu Y, Deng H, Liu H, Li G, Qi X. MicroRNA-647 targets SRF-MYH9 axis to suppress invasion and metastasis of gastric cancer. Theranostics 2018; 8: 6350-6351.

20) Peng Y, Zhang X, Ma Q, Yan R, Qin Y, Zhao Y, Cheng Y, Yang M, Wang Q, Feng X, Huang Y, Huang W, Zhao Z, Wang L, Wei Y, He Z, Fan X, Li S, Jin Z, Meltzer SJ. MiRNA-194 activates the Wnt/β-catenin signaling pathway in gastric cancer by targeting the negative Wnt regulator, SUFU. Cancer Lett 2017; 385: 117-127.

21) Zhang J, Liu X, Yu G, Liu L, Wang J, Chen X, Bian Y, Ji Y, Zhou X, Chen Y, Ji J, Xiang Z, Guo L, Fang J, Sun Y, Cao H, Zhu Z, Yu Y. UBE2C is a potential biomarker of intestinal-type gastric cancer with chromosomal instability. Front Pharmacol 2018; 9: 847.

22) Liang R, Xiao G, Wang M, Li X, Li Y, Hui Z, Sun X, Qin S, Zhang B, Du N, Liu D, Ren H. SNHG6 functions as a competing endogenous RNA to regulate E2F7 expression by sponging miR-26a-5p in lung adenocarcinoma. Biomed Pharmacother 2018; 107: 1434-1446.

23) Pike BL, Greiner TC, Wang X, Weisenburger DD, Hsu YH, Renaud G, Wolfsberg TG, Kim M, Weisenberger DJ, Siegmund KD, Ye W, Groshen S, Mehrian-Shai R, Delabie J, Chan WC, Laird PW, Hacia JG. DNA methylation profiles in diffuse large B-cell lymphoma and their relationship to gene expression status. Leukemia 2008; 22: 1035-1043.

24) Sun Y, Shen S, Liu X, Tang H, Wang Z, Yu Z, Li X, Wu M. MiR-429 inhibits cells growth and invasion and regulates EMT-related marker genes by targeting Onecut2 in colorectal carcinoma. Mol Cell Biochem 2014; 390: 19-30.

25) Lu T, Wu B, Yu Y, Zhu W, Zhang S, Zhang Y, Guo J, Deng N. Blockade of ONECUT2 expression in ovarian cancer inhibited tumor cell proliferation, migration, invasion and angiogenesis. Cancer Sci 2018; 109: 2221-2234.

26) Rotinen M, You S, Yang J, Coetzee SG, Reis-Sobreiro M, Huang WC, Huang F, Pan X, Yáñez A, Hazelett DJ, Chu CY, Steadman K, Morrissey CM, Nelson PS, Corey E, Chung LWK, Freedland SJ, Di Vizio D, Garraway IP, Murali R, Knudsen BS, Freeman MR. ONECUT2 is a targetable master regulator of lethal prostate cancer that suppresses the androgen axis. Nat Med 2018; 24: 1887-1898.

27) Pedraza-Arévalo S, Hormaechea-Agulla D, Gómez-Gómez E, Requena MJ, Selth LA, Gahete MD, Castaño JP, Luque RM. Somatostatin receptor subtype 1 as a potential diagnostic marker and therapeutic target in prostate cancer. Prostate 2017; 77: 1499-1511.

28) Verlaat W, Snijders PJF, Novianti PW, Wilting SM, De Strooper LMA, Trooskens G, Vandersmissen J, Van Criekinge W, Wisman GBA, Meijer CJLM, Heideman DAM, Steenbergen RDM. Genome-wide DNA methylation profiling reveals methylation markers associated with 3q gain for detection of cervical precancer and cancer. Clin Cancer Res 2017; 23: 3813-3822.

29) Tartarone A, Lerose R, Aieta M. Somatostatin analog therapy in small cell lung cancer. Semin Nucl Med 2016; 46: 239-242.

30) Jackson K, Soutto M, Peng D, Hu T, Marshal D, El-Rifai W. Epigenetic silencing of somatostatin in gastric cancer. Dig Dis Sci 2011; 56: 125-130.

31) Huang L, Wu RL, Xu AM. Epithelial-mesenchymal transition in gastric cancer. Am. J. Transl. Res 2015; 7: 2141–2158.

32) Yan HB, Wang XF, Zhang Q, Tang ZQ, Jiang YH, Fan HZ, Sun YH, Yang PY, Liu F. Reduced expression of the chromatin remodeling gene ARID1A enhances gastric cancer cell migration and invasion via downregulation of E-cadherin transcription. Carcinogenesis 2014; 35: 867-876.

33) Zhang K, Yang G, Wu W, Zhang J, Xia X, Jiang T, Cao J, Huang K, Qiu Z, Huang C. Decreased expression of Caveolin-1 and E-Cadherin correlates with the clinicopathologic features of gastric cancer and the EMT process. Recent Pat Anticancer Drug Discov 2016; 11: 236-244.

34) Shi Y, Shi H, Zhang B, Yan Y, Han X, Jiang W, Qian H, Xu W. MiR-373 suppresses gastric cancer metastasis by downregulating vimentin. Mol Med Rep 2018; 17: 4027-4034.

35) Bajpai S, Correia J, Feng Y, Figueiredo J, Sun SX, Longmore GD, Suriano G, Wirtz D. α-catenin mediates initial E-cadherin-dependent cell-cell recognition and subsequent bond strengthening. Proc Natl Acad Sci U S A 2008; 105: 18331-18336.

36) Lin TC, Su CY, Wu PY, Lai TC, Pan WA, Jan YH, Chang YC, Yeh CT, Chen CL, Ger LP, Chang HT, Yang CJ, Huang MS, Liu YP, Lin YF, Shyy JY, Tsai MD, Hsiao M. The nucleolar protein NIFK promotes cancer progression via CK1α/β-catenin in metastasis and Ki-67-dependent cell proliferation. eLife 2016; 5. pii: e11288.

37) Shao X, Kang H, Loveless T, Lee GR, Seok C, Weis WI, Choi HJ, Hardin J. Cell-cell adhesion in metazoans relies on evolutionarily conserved features of the α-catenin·β-catenin-binding interface. J Biol Chem 2017; 292: 16477-16490.

38) Wang B, Liu T, Wu JC, Luo SZ, Chen R, Lu LG, Xu MY. STAT3 aggravates TGF-β1-induced hepatic epithelial-to-mesenchymal transition and migration. Biomed Pharmacother 2018; 98: 214-221.

39) Wang W, Chin-Sheng H, Kuo LJ, Wei PL, Lien YC, Lin FY, Liu HH, Ho YS, Wu CH, Chang YJ. NNK enhances cell migration through α7-nicotinic acetylcholine receptor accompanied by increased of fibronectin expression in gastric cancer. Ann Surg Oncol 2012; 19: S580-S588.

40) Zhang H, Sun Z, Li Y, Fan D, Jiang H. MicroRNA-200c binding to FN1 suppresses the proliferation, migration and invasion of gastric cancer cells. Biomed Pharmacother 2017; 88: 285-292.

41) Ooi CH, Ivanova T, Wu J, Lee M, Tan IB, Tao J, Ward L, Koo JH, Gopalakrishnan V, Zhu Y, Cheng LL, Lee J, Rha SY, Chung HC, Ganesan K, So J, Soo KC, Lim D, Chan WH, Wong WK, Bowtell D, Yeoh KG, Grabsch H, Boussioutas A, Tan P. Oncogenic pathway combinations predict clinical prog-

nosis in gastric cancer. PLoS Genet 2009; 5: e1000676.

42) He Z, Dong W, Li Q, Qin C, Li Y. Sauchinone prevents TGF-β-induced EMT and metastasis in gastric cancer cells. Biomed Pharmacother 2018; 101: 355-361.

43) Wang JK, Wang WJ, Cai HY, Du BB, Mai P, Zhang LJ, Ma W, Hu YG, Feng SF, Miao GY. MFAP2 promotes epithelial-mesenchymal transition in gastric cancer cells by activating TGF-β/SMAD2/3 signaling pathway. Onco Targets Ther 2018; 11: 4001-4017.