ORIGINAL ARTICLE
ANIMAL STUDY

Animal study on stanniocalcin 2 (STC2) and gastric cancer metastasis: discussing possible molecular mechanisms

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Abstract

Objective: To assess the role of stanniocalcin 2 protein in abdominal invasion and metastasis of gastric cancer, and its molecular mechanism.

Method: The study was conducted at the Department of General Surgery, Jiangxi Provincial People's Hospital, Nanchang, Jiangxi, China, from January 2020 to March 2022 and comprised female mice that were divided into the experimental group A and control group B. Satellite glial cells 7901 were inoculated into the stomach wall to induce metastasis. In group A, the cells were genetically modified using short hairpin ribonucleic acid to silence stanniocalcin 2 expression. The silencing effect was confirmed via Western blot. Tumour metrics, including size, weight and number, were measured, alongside cell migration and invasion using Transwell assays. Data was analysed using SPSS 21.

Results: Of the 36 mice, 18(50%) were in each of the two groups. There was a significant reduction in tumour volume, weight and number in group A compared to group B ($p<0.05$). Survival time was notably extended in group A than group B ($p<0.001$). Western blot analysis revealed significantly lower VEGF-C and interleukin-10 (IL-10) protein levels in group B (approximately a 50% reduction for VEGF-C and a 40% reduction for IL-10, $p< 0.01$). Transwell assay results suggested that stanniocalcin 2 (STC2) promotes cell invasion and metastasis, as the invasion rate in the STC2-overexpressing group was significantly higher compared to controls (increase of approximately 40%, $p< 0.05$).

Conclusion: The invasion and metastasis potential of gastric pain cells decreased after silencing stanniocalcin 2, suggesting that it may promote the invasion and metastasis of gastric cancer.

Keywords: Stanniocalcin 2, Gastric cancer metastasis, Molecular mechanism, Cancer tumours.

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Introduction

[1] Gastric cancer (GC) mortality accounts for 7.7% of all cancer deaths, which is the fourth leading malignant tumour in the world behind lung, colorectal and liver cancers, seriously threatening human physical and mental health, and causing severe social and financial burden.[1,2] Over the past few decades, as helicobacter pylori (H. pylori) infection has been effectively controlled, significant progress has been made in the early diagnosis and treatment of GC. H. pylori is a spiral-shaped bacterium that thrives in the mucous layer lining the stomach. It is uniquely adapted to survive the acidic environment of the stomach by neutralising the local acidity and burrowing into the mucous layer, which helps it evade immune responses. The morbidity and mortality of GC have been significantly reduced.

However, the prognosis of GC remains poor, with a low 5-year survival rate. GC is biologically and genetically heterogeneous, with patients exhibiting different genetic and molecular biological characteristics. Studies have shown that genes abnormally expressed in GC may be involved in the occurrence and progression of tumours, and are related to the adverse outcomes of GC.[3-5] Epithelial cell adhesion molecule (EpCAM) is involved in cell adhesion and signalling. Overexpression is linked to tumour progression and metastasis. Catumaxomab, a bispecific antibody targetting EpCAM, is being evaluated for its therapeutic potential in GC. However, the specific occurrence and development mechanism of GC has yet to be fully clarified.[6,7] The clinical treatment of GC faces many challenges, especially the treatment of patients who have lost the chance of surgery, and the screening of patients with early-stage cancer.[8]

Studies in recent years have found that stanniocalcin (STC) is abnormally expressed in tumours. Its role and mechanism in tumours have aroused researchers' interest. STC is a glycoprotein-secreted hormone with two genotypes:[9,10] STC1 and STC2.[11,12] STC1 and STC2 play crucial roles in cancer progression by enhancing tumour growth, survival and metastasis.[13] They help cancer cells adapt to stress conditions, promote angiogenesis, and

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contribute to treatment resistance.[14] These characteristics make STC1 and STC2 potential biomarkers and therapeutic targets in cancer research.

STC is widely expressed in tissues and organs in a paracrine or autocrine manner.[11,12] It promoted phosphate absorption in the kidney, inhibits calcium uptake, maintains the body's calcium and phosphorus balance, and prevent hypercalcaemia.[15,16] STC is also involved in a variety of physiological and pathological processes[9] including oxidative stress, damage repair, musculoskeletal development, cardiovascular diseases, neuronal differentiation, pregnancy and lactation, inflammation and tumours, etc.[17,18]

STC may have a role in promoting tumour occurrence and development. The mechanism by which STC promotes tumours is still unclear, but a standard view is that STC has the function of protecting cells from oxidative stress, which may be related to the hypoxic protection of tumours.[19] Solid tumours often experience hypoxia after growth.[20] Through a series of gene expressions and metabolic regulation, tumour cells can continue to survive and proliferate. In addition, hypoxia protection can also help tumours tolerate the killing effects of chemotherapy and radiotherapy.[21]

Other studies[22-24] have found that STC can also regulate endothelial cell function and promote the formation of tumour neovascularisation through the angiopoietin and vascular endothelial growth factor (VEGF)/ Vascular endothelial growth factor receptor 2 (VEGFR2) pathways. VEGFR2 is a crucial regulator of angiogenesis.[25] It binds to VEGF-A, initiating downstream signalling pathways that promote endothelial cell proliferation. In colon cancer cells, VEGFR2 plays a crucial role in endothelial differentiation. Poorly-differentiated colon cancer cells, such as human colorectal carcinoma (HCT116), can express endothelial markers and form tube-like structures when cultured in an endothelial-inducing conditioned medium.[26]

Under hypoxic conditions simulating physiological tumour environments, colon cancer cells secrete more VEGF and express higher levels of VEGFR2.[27] Increased VEGFR2 expression correlates with differentiation, metastasis/ recurrence, and poor prognosis in human colon cancer samples.[28] A positive correlation exists between VEGFR2 and expression of vascular endothelial cadherin (VE-cadherin), another endothelial marker.[29,30]

STC expression is abnormal in a variety of tumour tissues. For example, the expression of STC1 in colon cancer tissues is >10 times higher than that in normal tissues. Multiple studies have found that STC1 is abnormally expressed in various tumours, such as nasopharyngeal carcinoma, ovarian cancer, GC, lung cancer, oesophageal cancer and bladder cancer.[31]

The expression of STC2 is significantly increased in GC,[32,33] colorectal cancer,[34] oesophageal squamous cell carcinoma (SCC)[35] and other malignant tumours.[36] These studies also found that abnormal expression of STC is associated with clinicopathological parameters in tumour patients.[37]

A study[33] found that the expression of STC2 in blood and cancer tissue cells of GC patients was also significantly increased. Its expression is correlated with patient age, depth of tumour invasion, lymph node metastasis, clinical stage, and venous invasion, and it was negatively related to the 5-year survival rate. Therefore, it is believed that STC2 can be used as a target molecule for the detection of circulating tumour cells (CTCs) in GC patients.[38]

In-depth research on STC2 in recent years has led to more and more results showing that STC2 can play a critical regulatory role in a variety of solid tumours, such as accelerating tumour cell invasion and metastasis, inhibiting cell apoptosis, etc.[39-41] However, the role of STC2 in GC, and the mechanism of its occurrence and action are still unclear.[42-44]

The current study was planned to assess the role of STC2 protein in abdominal invasion and metastasis of GC, and its molecular mechanism.

Materials and Methods

The study was conducted at the Department of General Surgery, Jiangxi Provincial People's Hospital, Nanchang, Jiangxi, China, from January 2020 to March 2022 and comprised female Balb/C nude mice. After approval from the institutional ethics review committee, satellite glial cells (SGC)7901 were inoculated into the stomach wall to induce metastasis.

After the SGC7901 cells were successfully revived, they were cultured in a 1640 culture medium containing 10% foetal bovine serum (FBS) at 37°C in a 5% carbon dioxide (CO_2) cell culture incubator. The cancer cells were taken in the logarithmic growth phase to make a single cell suspension. Trypan blue staining was used to determine cell viability >95%, and the cell concentration was adjusted to 2.5x1010 /L. They were divided into two groups: In group A, the cells were genetically modified using short hairpin ribonucleic acid (shRNA) to silence STC2 expression. The SGC7901 group was taken as the control.

The STC2-shRNA adenovirus expression vector was constructed using Saiye (Guangzhou) Biotechnology Co., Ltd. (Saiye Biotechnology, Guangzhou, China). SGC7901 were seeded into a 6-well cell culture plate at a density of 5x105/well. During the logarithmic growth phase of the cells, 200m purified virus culture medium, empty virus liquid, and culture medium were added to each cell culture medium, respectively. The medium was incubated for 48~72h, and the transfection was observed under a fluorescent microscope. The silenced STC2 cells after transfection were removed from the culture medium, and washed gently once with pre-chilled phosphate buffer saline (PBS). After that, 300µl protein extraction cell lysis buffer+3µl sodium metavanadate ($NaVO_3$)+3µl phenylmethylsulfonyl fluoride (PMSF)+3µl ion pair chromatography (PIC) reagent was added, and placed on ice for 1-2 hours for lysis at 4°C, 12,000rpm, with centrifuge radius 3cm. It was centrifuged for 15 minutes, and the supernatant was taken. An equal volume of 2-times sodium dodecyl sulfate (SDS) loading buffer and 5% β-mercaptoethanol was added to the protein sample and was heated at 100°C for 5 minutes. A 12% separation gel was prepared, a 5% stacking gel was cast, and SDS-polyacrylamide gel (PAGE) electrophoresis was performed. After electrophoresis, it was time to separate and transfer to the membrane, blocked with blocking buffer for 1h, incubated with primary antibody at room temperature for 1h, and incubated with secondary antibody at room temperature for 1h. Millipore's horseradish peroxidase (HRP) colour development kit (Millipore, Burlington, MA, USA) was used for colour development, and a gel scanning analysis system (Bio-Rad, Hercules, CA, USA) was used to scan and save the image, detecting the effects of silencing STC2.

The gastric tumour body was cut into pieces and the cells were lysed with radioimmunoprecipitation assay (RIPA) lysis buffer containing 1% phenylmethylsulfonyl gas (PMSF) at 400m for 30min. The lysates were separated by 10% SDS-PAGE electrophoresis, transferred to a membrane, buffered with blocking buffer for 1h and incubated with primary antibody at room temperature for 3h. After incubation with the secondary antibody for 1h, the colour was developed with a 5-bromo-4-chloro-3-indolyl-phosphate/vapourized nitro tetrazolium blue (BCIP/NBT) staining solution. A gel imaging system (Bio-Rad, Hercules, CA, USA) was used to detect the protein expression levels of VEGF-C and interleukin-10 (IL-10) in tumour tissues.

The mice were divided into two groups with 12h fasting before surgery. They were injected with 1% Tobbi (30mg/kg). The middle incision was taken, the abdominal cavity was opened, the stomach tissue was pulled, and the cancer cell suspension was injected with the concentration of 2.5x1010/L on the sizeable curved side of the front wall of the stomach. After the injection site was white and swollen, the needle was pulled out. After surgery, the mice were observed daily.

Survival time, survival rate and tumour suppression rate of the mice were recorded. Survival extension rate was calculated as average survival time of STC2 group - SGC7901 average survival time/SGC7901 average survival time. Tumour suppression rate was calculated as SGC7901 group tumour body weight - STC2 group tumour weight/SGC7901 group tumour body weight. Once the mice were dead, the abdominal cavity was opened, and the tumour condition and tumour nodules of various abdominal organs was checked. After removing the tissue around the tumour, the tumour body was stripped, and the tumour weight was accurately referred to as an electronic scale.

Transwell migration and invasion were used to measure cell migration and invasion. A total of 1×104 inocous cells in 200µL of non-serum medium to the upper side of the Corning Incorpolet (Corning, NY, USA), and the quet or not applying Matrigels (BD BIOSCIENCES, CA, USA). Then, 10% FBS RPMI 1640 medium was added to the lower chamber. After 48 hours, the cells under the surface of the 0.1% crystalline purple dyeing chamber were counted separately and the remaining cells were counted. Each experiment was repeated at least three times.

Data was analysed using SPSS 21. Data was presented as mean ± standard deviation. Independent samples t-test and one-way analysis of variance (ANOVA) were used as appropriate. P<0.05 was considered significant.

Results

Of the 36 mice, 18(50%) were in each of the two groups. Increased expression of STC2 was found (Figure 1).

The amount of green fluorescence expressed was sparse, but at 72h after transfection, it increased significantly

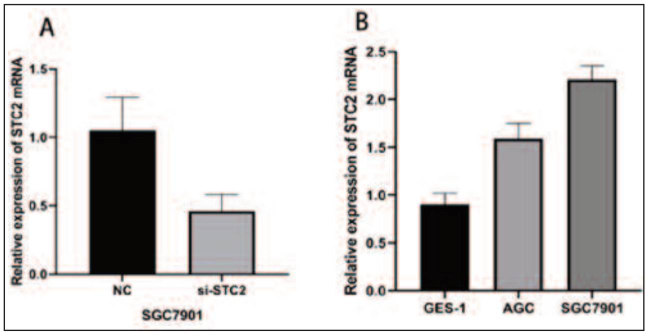

Figure-1: (A): Relative expression of STC2 mRNA after transfusion si-STC2. (B): STC2 expression in SGC7901, AGS and GES-1 cells. (NC: Negative Control. si-STC2: small interfering RNA targeting Stanniocalcin-2 (STC2). GES-1: normal gastric epithelial cell line. AGC: Advanced Gastric Cancer. SGC7901: human gastric carcinoma cell line.)

without showing an obvious cytopathic effect (CPE) (Figure 2A).

Western blot experiments showed that after silencing the STC2 gene, the relative expression of STC2 protein in cells in the STC2-shRNA group was (0.563±0.020 compared to 1.233±0.059 in the control group ($p<0.05$) (Figure 2B).

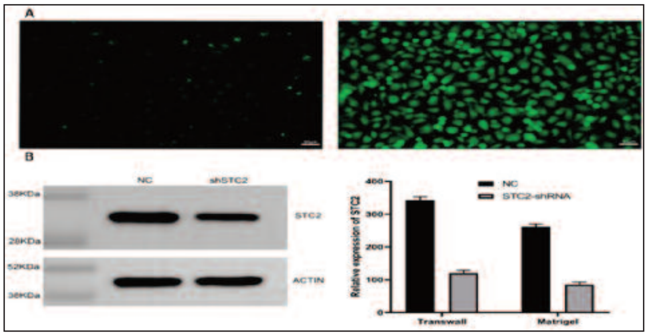

Figure-2: (A): Transwell migration and invasion used to measure the green fluorescence expression 24 hours after the STC2-shRNA adenoviral vector transfected gastric cancer cell SGC7901. (B): Representative images and bar graphs. (NC: Negative Control. shSTC2: short hairpin RNA targeting Stanniocalcin-2 (STC2). STC2: Stanniocalcin-2. ACTIN: beta-actin. Transwell: Transwell migration assay. Matrigel: Matrigel invasion assay.)

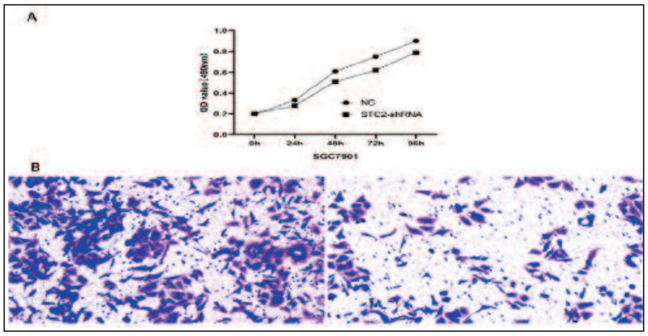

Figure-3: (A): Cell proliferation containing cells in SH-STC2. (The lower OD values in the STC2-shRNA group suggest that silencing STC2 reduced cell proliferation in gastric cancer cells. This indicated that STC2 played a role in promoting cell growth, and its inhibition could effectively reduce tumour cell proliferation. (B): The use of SH-STC2 (40×) to analyse cell invasion on SGC7901 cells ($p<0.01$). (NC: Negative Control. STC2-shRNA: short hairpin RNA targeting Stanniocalcin-2 (STC2). OD value: optical density (OD) value. SGC7901: human gastric carcinoma cell line.)

Table-1: Intergroup comparison of abdominal tumour number, weight and volume.

Group	**Number of tumours**	**Total weight** (g)	**Volume** (mm^3)
SGC7901	18.86±1.83	3.98±0.23	3.62±0.41
STC2-shRNA	5.27±1.14	1.24±0.12	0.75±0.13
***p*-value**	<0.001	<0.001	<0.001

Table-2: Intergroup comparison of survival time, survival rate and life extension rate.

Group	**Survival time** (d)	**Mortality** n (%)	**Survival rate** n (%)	**Life extension rate** [n (%)]
SGC7901 (n=18)	24.03±1.26	13 (72.22)	5 (27.78)	4 (22.22)
STC2-shRNA (n=18)	40.11±2.64	4 (22.22)	14 (77.78)	11 (61.11)
***t*-test**	23.321	9.028	9.028	5.600
***p*-value**	<0.001	0.003	0.003	0.018

Cell proliferation in the STC2-shRNA group was significantly reduced compared to the SGC7901 group (Figure 3A). STC2-shRNA also inhibited cell invasion in SGC7901 cells (Figure 3B).

Tumour growth occurred in all mice 8-15 days after inoculation, and hard and movable undesirable nodules were palpable in the surgical area. Intergroup comparison of tumour size, weight, and number of tumours showed significant differences ($p<0.001$) (Table 1), and tumour

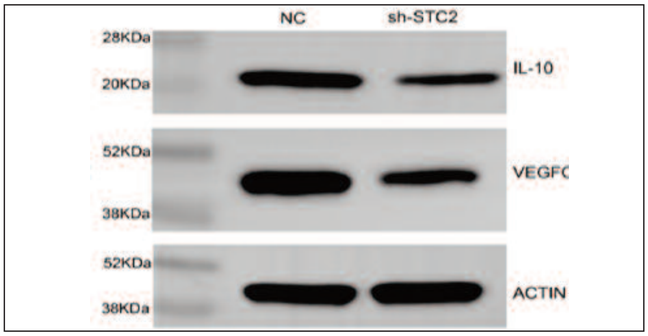

Figure-4: Western blot detection of tumour tissue VEGF-C, IL-10 protein expression. (NC: Negative Control. sh-STC2: short hairpin RNA targeting Stanniocalcin-2 (STC2). IL-10: Interleukin-10. VEGFC: Vascular Endothelial Growth Factor C. ACTIN: beta-actin).

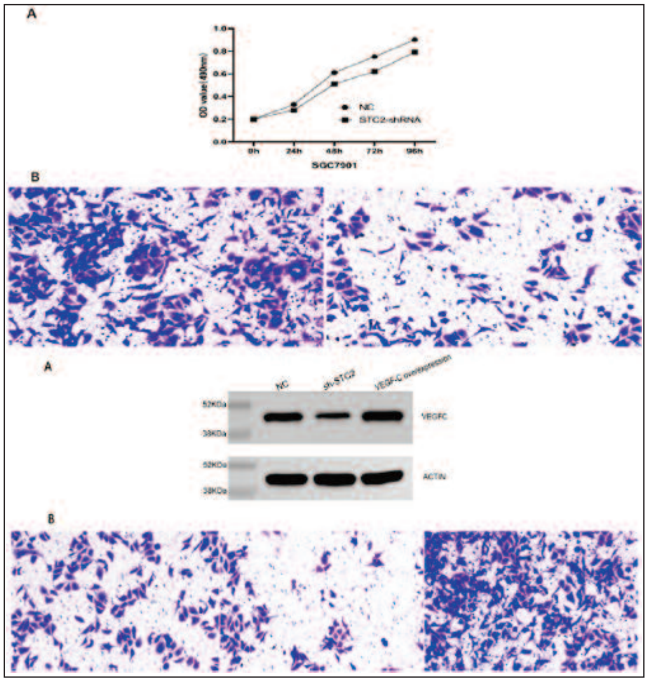

Figure-5: (A): Western blot result of VEGF-C. (B): The influence of the expression of VEGF-C on cell invasion and metastasis. (NC: Negative Control. sh-STC2: short hairpin RNA targeting Stanniocalcin-2 (STC2). VEGFC: Vascular Endothelial Growth Factor C. ACTIN: beta-actin. OD value: optical density (OD) value.)

formation in various organs were lower in the STC2-shRNA group compared to the SGC7901 group (*p*<0.05).

Also, the survival data of mice in the STC2-shRNA group was significantly better than in the control group (Table 2).

The relative expression levels of VEGF-C and IL-10 proteins were 0.300±0.040 and 0.387±0.015 in groups A and B, respectively (*p*<0.05). The value of VEGF-C and IL-10 was also significantly reduced in the STC2-shRNA group compared to the SGC7901 group (*p*<0.05).

Western blot analysis confirmed that compared to the SGC7901 group, the STC2-shRNA group showed a significant increase in VEGF-C expression (approximately 2-fold, as shown in Figure 4, *p*<0.01). Transwell assay results indicated that after overexpression of VEGF-C in the STC2-shRNA group, cell invasion and metastasis were significantly aggravated (invasion rate increased by approximately 40%, *p*<0.05, as shown in Figure 5).

Discussion

STC was found in the STC small body in hard bonefish in 1839, mainly STC1 and STC2.[9] STC2 is confirmed as a kind of glycoprotein hormone widely expressed in a variety of human tissue cells, such as the stomach, intestines, kidneys, uterus, and ovaries, and participates in various physiological functions, such as calcium and phosphorus metabolism.[45] Studies have confirmed that the proliferation invasion of STC2 and tumour cells, such as liver cancer, stomach pain, laryngeal cancer, and colorectal cancer, are closely related.[46,47] The level of expression can change the biological characteristics of cancer cells in the body, affect the internal quality mesh function of cancer cells, damage the mitochondrial function, cause abnormal regulation of the tumour cell cycle, and increase the cell ratio in the G1/S phase transition. In one study, researchers[48] applied immunohistochemical methods to test the expression of STC2 protein in normal gastric tissue, GC tissue, and cancer tissue. The results showed that STC2 protein was higher than normal gastric mucosa tissue in the GC tissue and cancer tissue. The analysis of clinical pathological factors further showed that STC2's high expression was closely related to infiltration depth and lymphatic metastasis. Another study examined STC2 in rectal cancer.[49]

In the current study, after silencing the STC gene, the tumour formation rates of the stomach, liver, spleen, kidney, intestine, mesentery, and peritoneum of nude mice in the STC2-shRNA group were 50%, 33.3%, 11.1%, 16.7%, 22.2% and 5.6%, respectively. The tumour rates of SGC7901 were 100%, 100%, 55.6%, 67.7%, 72.2%, and 67.7%, respectively. The intraperitoneal metastasis rate in the STC2-shRNA group was significantly lower than that in the control group. The number of abdominal metastases, tumour size and tumour weight in the STC2-shRNA group was significantly reduced compared to the control group, and the tumour inhibition rate reached 68.84%. In terms of survival, the survival time of nude mice in the STC2-shRNA group was significantly longer than that of the control group, and the life extension rate reached 66.92%. The results showed that, after silencing STC2, the ability of GC cells to invade and metastasise was reduced, and STC2 could play a specific role in the process of peritoneal invasion and metastasis of GC cells.

A study has shown that IL-10 is responsible for the invasion and metastasis of GC cells, especially one of the possible mechanisms of lymphatic metastasis.[50] The results of the current study showed that, after silencing STC2, the ability of GC cells SGC7901 to induce the production of IL-10 was reduced, its anti-inflammatory and immune tolerance-inducing effects were weakened, and the metastatic potential of tumour cells was reduced, resulting in reduced invasion and metastasis capabilities.

The primary transfer path of the GC is lymphatic transfer. VEGF-C is an important lymphatic vessel-generating factor with special-specific receptor VEGFR-3 and VEGFR-2. VEGF-C acts on the versatility of the special receptor VEGFR-3, mediating the proliferation of lymphatic endothelial cells and the hyperplasia of lymphatic vessels. The formation of neonatal blood vessels is combined with VEGFR-2. Studies have shown that the positive expression rate of VEGF-C is significantly higher than that of adjacent cancer tissues and normal tissues.[51] VEGF-C expression is closely related to tumour invasion depth and lymph node metastasis and is positively correlated.[52] These research results indicate that VEGF-C may be involved in the occurrence and development of GC. The results of the current study showed that, after silencing STC2, the ability of GC cells SCC7901 to secrete VEGF-C was reduced, and its ability to mediate lymphatic endothelial cell proliferation, migration and microangiogenesis was weakened. This resulted in a significant decrease in tumour proliferation and metastasis capabilities. The intraperitoneal metastasis rate and metastatic tumour size of nude mice in the STC2-shRNA group were significantly reduced compared to the control group. This is consistent with the expression level of IL-10 in metastatic tumours, indicating that the metastatic potential of GC cells after silencing STC2 is reduced, and the invasion and metastasis abilities are reduced. Overexpression analysis of VEGF-C showed that, compared to the control group, the degree of cell invasion and metastasis in the STC2-shRNA group was inhibited. After overexpression of VEGF-C, the degree of cell invasion and metastasis was aggravated. Studies have shown that STC2 behaves differently in different cancers.[53]

They current study speculates that STC2 has a role in regulating the expression of VEGF-C/IL-10 in GC. In the study, STC2-shRNA silenced STC2, and the expression of VEGF-C also decreased. In other words, STC2 may induce cell invasion and metastasis by promoting the expression of VEGF-C/IL-10. However, the result needs to be verified by future experiments. Research on the molecular mechanism of STC2 may provide new directions for the treatment of GC in the future.

In summary, the results of this study indicate that the metastatic potential of GC cells is reduced after silencing STC2. STC2 may promote peritoneal invasion and metastasis of GC by promoting the expression of VEGF-C. VEGF-C and IL-10 may play vital roles in the regulatory molecular mechanism of STC2.

Conclusion

The metastatic potential of GC cells was reduced after silencing STC2. STC2 may promote peritoneal invasion and metastasis of GC by promoting the expression of VEGF-C. VEGF-C and IL-10 may play vital roles in the regulatory molecular mechanism of STC2.

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Conflict of Interest: None.

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References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin 2021;71:209-4. doi: 10.3322/caac.21660
2. Farrokhi M, Taheri F, Farrokhi M, Heydari Z, Darbani R, Salbi M, et al. Advancements and innovations in cancer management: a comprehensive perspective. Kindle 2024;4:1–161. doi: 10.5281/zenodo.11108886.
3. Naeli P, Pourhanifeh MH, Karimzadeh MR, Shabaninejad Z, Movahedpour A, Tarrahimofrad H, et al. Circular RNAs and gastrointestinal cancers: Epigenetic regulators with a prognostic and therapeutic role. Crit Rev Oncol Hematol 2020;145:102854. doi: 10.1016/j.critrevonc.2019.102854
4. Shen Y, Ding Y, Ma Q, Zhao L, Guo X, Shao Y, et al. Identification of Novel Circulating miRNA Biomarkers for the Diagnosis of Esophageal Squamous Cell Carcinoma and Squamous Dysplasia. Cancer Epidemiol Biomarkers Prev 2019;28:1212-20. doi: 10.1158/1055-9965.EPI-18-1199
5. Amirahmadi S, Farimani FD, Akbarian M, Mirzavi F, Eshaghi Ghalibaf MH, Rajabian A, et al. Minocycline attenuates cholinergic dysfunction and neuro-inflammation-mediated cognitive impairment in scopolamine-induced Alzheimer's rat model. Inflammopharmacology 2022;30:2385-97. doi: 10.1007/s10787-022-01071-2
6. Sarbaz P, Beigoli S, Payami B, Eshaghi Ghalibaf MH, Amirahmadi S, Hosseini M, et al. Curcuma longa impact on behavioral, brain oxidative stress, and systemic inflammation in rats exposed to inhaled paraquat. Toxicol Environ Health Sci 2024;16:287–98. doi: 10.1007/s13530-024-00225-9.
7. Monemi M, Garrosi L, Mirzaei S, Farhadi B, Ataee Disfani R, Zabihi MR, et al. Identification of proteins' expression pathway and the effective miRNAs for the treatment of human papillomavirus-induced cervical cancer: in-silico analyses-experimental research. Ann Med Surg (Lond) 2024;86:5784-92. doi: 10.1097/MS9.0000000000002513
8. Efati Z, Shahangian SS, Darroudi M, Amiri H, Hashemy SI, Aghamaali MR. Green chemistry synthesized zinc oxide nanoparticles in Lepidium sativum L. seed extract and evaluation of their anticancer activity in human colorectal cancer cells. Ceram Int 2023;49:32568–76. doi: 10.1016/j.ceramint.2023.07.221.
9. In: Ando H, Ukena K, Nagata S, eds. Handbook of Hormones: Comparative Endocrinology for Basic and Clinical Research, 2nd ed. Massachusetts, USA: Academic Press; 2021.
10. Amirahmadi S, Hosseini M, Ahmadabady S, Akbarian M, Abrari K, Vafaee F, et al. Folic acid attenuated learning and memory impairment via inhibition of oxidative damage and acetylcholinesterase activity in hypothyroid rats. Metab Brain Dis 2021;36:2393-40. doi: 10.1007/s11011-021-00815-3
11. Zhao F, Yang G, Feng M, Cao Z, Liu Y, Qiu J, et al. Expression, function and clinical application of stanniocalcin-1 in cancer. J Cell Mol Med 2020;24:7686-9. doi: 10.1111/jcmm.15348
12. Joshi AD. New Insights Into Physiological and Pathophysiological Functions of Stanniocalcin 2. Front Endocrinol (Lausanne) 2020;11:172. doi: 10.3389/fendo.2020.00172
13. Rotherham M, Moradi Y, Nahar T, Mosses D, Telling N, El Haj AJ. Magnetic activation of TREK1 triggers stress signalling and regulates neuronal branching in SH-SY5Y cells. Front Med Technol 2022;4:981421. doi: 10.3389/fmedt.2022.981421
14. Saberi R, Mirazi N, Amirahmadi S, Darbandi ZK, Vafaee F, Rajabian A, et al. Ameliorative effects of thiamin on learning behavior and memory dysfunction in a rat model of hypothyroidism: implication of oxidative stress and acetylcholinesterase. Metab Brain Dis 2023;38:2603-1. doi: 10.1007/s11011-023-01317-0
15. Chanpaisaeng K, Teerapornpuntakit J, Wongdee K, Charoenphandhu N. Emerging roles of calcium-sensing receptor in the local regulation of intestinal transport of ions and calcium. Am J Physiol Cell Physiol 2021;320:C270-8. doi: 10.1152/ajpcell.00485.2020
16. Forqani MA, Akbarian M, Amirahmadi S, Khorrami MB, Hosseini M, Forouzanfar F. Protective Effect of Carvacrol against Oxidative Damage in Aged Rats. Cent Nerv Syst Agents Med Chem 2024. doi: 10.2174/0118715249303906240729074821. [ahead of print].
17. Dabouri Farimani F, Hosseini M, Amirahmadi S, Akbarian M, Shirazinia M, Barabady M, et al. Cedrol supplementation ameliorates memory deficits by regulating neuro-inflammation and cholinergic function in lipopolysaccharide-induced cognitive impairment in rats. Heliyon 2024;10:e30356. doi: 10.1016/j.heliyon.2024.e30356
18. Akbarzadeh I, Poor AS, Khodarahmi M, Abdihaji M, Moammeri A, Jafari S, et al. Gingerol/letrozole-loaded mesoporous silica nanoparticles for breast cancer therapy: In-silico and in-vitro studies. Micropor Mesopor Mater 2022;337:111919. doi: 10.1016/j.micromeso.2022.111919.
19. Moradi Y, Atyabi SA, Ghiassadin A, Bakhshi H, Irani S, Atyabi SM, et al. Cold Atmosphere Plasma Modification on Beta-Carotene-Loaded Nanofibers to Enhance Osteogenic Differentiation. Fibers Polym 2022;23:18–27. doi: 10.1007/s12221-021-0033-y.
20. Nashtahosseini Z, Sadeghi F, Aghamaali MR. Changes in expression of miRNA-320a and miRNA-497-5p in early stage of breast cancer. Iran Red Crescent Med J 2021;23:e17034. doi: 10.32592/ircmj.2021.23.6.17034.
21. Hu S, Jiang S, Qi X, Bai R, Ye XY, Xie T. Races of small molecule clinical trials for the treatment of COVID-19: An up-to-date comprehensive review. Drug Dev Res 2022;83:16-54. doi: 10.1002/ddr.21895
22. Wang Y, Qi Z, Zhou M, Yang W, Hu R, Li G, et al. Stanniocalcin 1

promotes cell proliferation, chemoresistance and metastasis in hypoxic gastric cancer cells via Bcl 2. Oncol Rep 2019;41:1998-200. doi: 10.3892/or.2019.6980

23. Gan L, Zhao L, Yang C, Wang S, Gao Z, Ye Y. Systematic Pan-Cancer Analysis of the Oncogenic and Immunological Function of Stanniocalcin-1 (STC1). Res Sq 2023. doi: 10.21203/rs.3.rs-3043100/v1. [Preprint]
24. Ren S, Yang Y. The proliferation and angiogenesis in hemangioma-derived endothelial cells is affected by STC2 medicated VEGFR2/Akt/eNOS pathway. Pak J Med Sci 2023;39:1119-23. doi: 10.12669/pjms.39.4.7384
25. Nie Y, Li D, Peng Y, Wang S, Hu S, Liu M, et al. Metal organic framework coated MnO2 nanosheets delivering doxorubicin and self-activated DNAzyme for chemo-gene combinatorial treatment of cancer. Int J Pharm 2020;585:119513. doi: 10.1016/j.ijpharm.2020.119513
26. Akbarian M, Hosseini M, Mirzavi F, Amirahmadi S, Arab FL, Rajabian A. Punica granatum peel supplementation attenuates cognitive deficits and brain injury in rat by targeting the Nrf2-HO-1 pathway. Food Sci Nutr 2022;11:168-80. doi: 10.1002/fsn3.3049
27. Li WQ, Tan SL, Li XH, Sun TL, Li D, Du J, et al. Calcitonin gene-related peptide inhibits the cardiac fibroblasts senescence in cardiac fibrosis via up-regulating klotho expression. Eur J Pharmacol 2019;843:96-10. doi: 10.1016/j.ejphar.2018.10.023
28. Khazaei M, Khabiri M, Mohseni Tabrizi A. Qualitative study of effective biomedical factors in tendency of retired elite wrestlers to drug abuse; A grounded theory study. Sport Sci Health Res 2020;12:135–44.
29. Assaran AH, Akbarian M, Amirahmadi S, Salmani H, Shirzad S, Hosseini M, et al. Ellagic Acid Prevents Oxidative Stress and Memory Deficits in a Rat Model of Scopolamine-induced Alzheimer's Disease. Cent Nerv Syst Agents Med Chem 2022;22:214-27. doi: 10.2174/1871524923666221027100949
30. Sulkowska M, Famulski W, Wincewicz A, Moniuszko T, Kedra B, Koda M, et al. Levels of VE-cadherin increase independently of VEGF in preoperative sera of patients with colorectal cancer. Tumori 2006;92:67-71. doi: 10.1177/030089160609200111
31. Lin X, Liao Y, Chen X, Long D, Yu T, Shen F. Regulation of Oncoprotein 18/Stathmin Signaling by ERK Concerns the Resistance to Taxol in Nonsmall Cell Lung Cancer Cells. Cancer Biother Radiopharm 2016;31:37-43. doi: 10.1089/cbr.2015.1921
32. Yokobori T, Mimori K, Ishii H, Iwatsuki M, Tanaka F, Kamohara Y, et al. Clinical significance of stanniocalcin 2 as a prognostic marker in gastric cancer. Ann Surg Oncol 2010;17:2601-7. doi: 10.1245/s10434-010-1086-0
33. Arigami T, Uenosono Y, Ishigami S, Yanagita S, Hagihara T, Haraguchi N, et al. Clinical significance of stanniocalcin 2 expression as a predictor of tumor progression in gastric cancer. Oncol Rep 2013;30:2838-44. doi: 10.3892/or.2013.2775
34. Watanabe T, Shiozawa M, Kimura Y, Hiroshima Y, Hashimoto I, Komori K, et al. Clinical Significance of Stanniocalcin2 mRNA Expression in Patients With Colorectal Cancer. Anticancer Res 2021;41:2117-22. doi: 10.21873/anticanres.14983
35. Wang Y, Wu J, Xu J, Lin S. Clinical significance of high expression of stanniocalcin-2 in hepatocellular carcinoma. Biosci Rep 2019;39:BSR20182057. doi: 10.1042/BSR20182057
36. Qie S, Sang N. Stanniocalcin 2 (STC2): a universal tumour biomarker and a potential therapeutical target. J Exp Clin Cancer Res 2022;41:161. doi: 10.1186/s13046-022-02370-w
37. Lin X, Yu T, Zhang L, Chen S, Chen X, Liao Y, et al. Silencing Op18/stathmin by RNA Interference Promotes the Sensitivity of Nasopharyngeal Carcinoma Cells to Taxol and High-Grade Differentiation of Xenografted Tumours in Nude Mice. Basic Clin Pharmacol Toxicol 2016;119:611-20. doi: 10.1111/bcpt.12633
38. Wang K, Yin J, Chen J, Ma J, Si H, Xia D. Inhibition of inflammation by berberine: Molecular mechanism and network pharmacology analysis. Phytomedicine 2024;128:155258. doi: 10.1016/j.phymed.2023.155258
39. Li Q, Zhou X, Fang Z, Pan Z. Effect of STC2 gene silencing on colorectal cancer cells. Mol Med Rep 2019;20:977-84. doi: 10.3892/mmr.2019.10332
40. Cui J, Liu X, Yang L, Che S, Guo H, Han J, et al. MiR-184 Combined with STC2 Promotes Endometrial Epithelial Cell Apoptosis in Dairy Goats via RAS/RAF/MEK/ERK Pathway. Genes (Basel) 2020;11:1052. doi: 10.3390/genes11091052
41. Lin C, Sun L, Huang S, Weng X, Wu Z. STC2 Is a Potential Prognostic Biomarker for Pancreatic Cancer and Promotes Migration and Invasion by Inducing Epithelial-Mesenchymal Transition. Biomed Res Int 2019;2019:8042489. doi: 10.1155/2019/8042489
42. Peng J, Ge C, Shang K, Liu S, Jiang Y. Comprehensive profiling of the chemical constituents in Dayuanyin decoction using UPLC-QTOF-MS combined with molecular networking. Pharm Biol 2024;62:480-98. doi: 10.1080/13880209.2024.2354341
43. Bemidinezhad A, Radmehr S, Moosaei N, Efati Z, Kesharwani P, Sahebkar A. Enhancing radiotherapy for melanoma: the promise of high-Z metal nanoparticles in radiosensitization. Nanomedicine (Lond) 2024;19:2391-41. doi: 10.1080/17435889.2024.2403325
44. Radmehr S, Dehghani F, Bai Y, Yang X, Li J. The impact of intermittent and continuous training on the levels of CIDE and Perilipin-1 proteins and their effect on the size of lipid droplets in the visceral adipose tissue of obese male rats. Eur J Hum Mov 2024;52:43-53. DOI: 10.21134/eurjhm.2024.52.4.
45. Bu Q, Deng Y, Wang Q, Deng R, Hu S, Pei Z, et al. STC2 is a potential biomarker of hepatocellular carcinoma with its expression being upregulated in Nrf1α-deficient cells, but downregulated in Nrf2-deficient cells. Int J Biol Macromol 2023;253:127575. doi: 10.1016/j.ijbiomac.2023.127575
46. Lai R, Ji L, Zhang X, Xu Y, Zhong Y, Chen L, et al. Stanniocalcin2 inhibits the epithelial-mesenchymal transition and invasion of trophoblasts via activation of autophagy under high-glucose conditions. Mol Cell Endocrinol 2022;547:111598. doi: 10.1016/j.mce.2022.111598
47. Niu X, Zhan Y, Zhang S, Liu Z, Qu C. Research progress of STC2 in breast cancer. Biophys Rep 2021;7:185-92. doi: 10.52601/bpr.2021.210002
48. Quispe R, Martin SS, Michos ED, Lamba I, Blumenthal RS, Saeed A, et al. Remnant cholesterol predicts cardiovascular disease beyond LDL and ApoB: a primary prevention study. Eur Heart J 2021;42:4324-32. doi: 10.1093/eurheartj/ehab432
49. Huang F, Li H, Qin Z, Wang A, Zhang Y, Guo J, et al. SNHG17 Serves as an Oncogenic lncRNA by Regulating the miR-361-3p/STC2 Axis in Rectal Cancer. Front Genet 2021;12:654686. doi: 10.3389/fgene.2021.654686
50. Tang J, Pan R, Xu L, Ma Q, Ying X, Zhao J, et al. IL10 hypomethylation is associated with the risk of gastric cancer. Oncol Lett 2021;21:241. doi: 10.3892/ol.2021.12502
51. Lian L, Li XL, Xu MD, Li XM, Wu MY, Zhang Y, et al. VEGFR2 promotes tumorigenesis and metastasis in a pro-angiogenic-independent way in gastric cancer. BMC Cancer 2019;19:183. doi: 10.1186/s12885-019-5322-0
52. Zhao WX, Liu ZF, Li XL, Li Z. Correlations of serum homocysteine, VEGF and gastrin 17 with gastric cancer and precancerous lesions. Eur Rev Med Pharmacol Sci 2019;23:4192-8. doi: 10.26355/eurrev_201905_17922
53. Hu L, Zha Y, Kong F, Pan Y. Prognostic value of high stanniocalcin 2 expression in solid cancers: A meta-analysis. Medicine (Baltimore) 2019;98:e17432. doi: 10.1097/MD.0000000000017432