GSE1 predicts poor survival outcome in gastric cancer patients by SLC7A5 enhancement of tumor growth and metastasis

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**ABSTRACT**

Gastric cancer remains a malignancy with poor survival outcome. We herein report that GSE1, a proline rich protein, possesses a role in progression of human gastric cancer. The expression of GSE1 was observed to be much higher in human gastric cancer tissues compared with normal gastric tissues, and GSE1 expression correlated positively with lymph node metastasis, histological grade, depth of invasion and clinical stage in gastric cancer patients. Moreover, GSE1 expression was also associated with decreased post-operative relapse-free survival (RFS) and overall survival.
survival (OS) in the cohort. The forced expression of GSE1 in gastric cancer cell lines resulted in increased cell proliferation, increased colony formation, enhanced cell migration and invasion. Furthermore, forced expression of GSE1 but also increased tumor size and enhanced lung metastasis in xenograft models. The depletion of endogenous GSE1 with shRNAs decreased the oncogenicity and invasiveness of gastric cancer cells both in vitro and in vivo. In addition, GSE1 was determined to be a direct target of miR-200b and miR-200c. Furthermore, GSE1 positively regulated the downstream gene SLC7A5 (also known as LAT-1), which was scanned and verified from mRNA sequencing. GSE1 therefore possesses an oncogenic role in human gastric cancer, and targeted therapeutic approaches to inhibit GSE1 function in gastric cancer warrant further consideration.

Gastric cancer is one of the leading causes of cancer-related mortality worldwide (1,2). Patients with gastric cancer are commonly diagnosed at an advanced stage and their median 5-year survival rates are no more than 20% (3). The current optimal approach for gastric cancer therapy is surgical resection with curative intent and adjuvant chemotherapy or radiotherapy. However, the recurrence rate of gastric cancer remains high, with poor prognosis (4,5). Therefore, further study on the molecular mechanisms promoting gastric cancer progression is essential to unveil new diagnostic or prognostic markers and therapeutic targets to improve clinical outcomes.

GSE1 (human Gse1 coiled-coil protein), a proline rich protein, also known as KIAA0182, was first isolated and identified using an ion trap mass spectrometry (6). Little is known about the roles of GSE1 in human diseases thus far. In our previous study, GSE1 was demonstrated to possess an oncogenic function in human breast cancer cells (7). We have also previously reported that GSE1 is negatively regulated by miR-489-5p, and promotes both proliferation and metastasis in breast cancer cells (7). However, the role of GSE1 in human gastric cancer remains unclear.

In this study, we have defined a functional role for GSE1 in human gastric cancer cells. We have determined that GSE1 promoted cell proliferation, colony formation, migration, invasion, tumor growth and metastasis both in vitro and in vivo. Moreover, gastric cancer patients with high tumor expression of GSE1 exhibited worse clinicopathological parameters and survival rates. SLC7A5 (solute carrier family 7 member 5, also named LAT-1 (L-type amino-acid transporter 1), system L-type transporters, important in cell maintenance and proliferation (8,9)) is positively regulated by GSE1, and partly mediates the role of GSE1 in progression of gastric cancer. In addition, we demonstrated that the expression of GSE1 is suppressed and directly targeted by miR-200b and miR-200c. Furthermore, GSE1 and SLC7A5 expression were positively correlated in gastric cancer tissues. GSE1 and miR-200b / miR-200c expression were significantly and inversely correlated in gastric cancer tissues. Hence, we have provided evidence that GSE1 possesses an important function in progression of
human gastric cancer. GSE1 could potentially be utilized as a novel clinical diagnostic and therapeutic target for gastric cancer.

RESULTS

Expression of GSE1 in human gastric cancer tissues and normal gastric tissues—We first determined the expression of GSE1 in 100 human gastric cancer tissues and 100 normal gastric tissues using immunohistochemistry. Immunoreactive GSE1 protein was mainly located in the cytoplasm of gastric cancer cells and glandular epithelial cells (Fig.1A). As shown in Table 1, 51 out of 100 cases of normal gastric tissues were negative for expression of GSE1, 34 out of 100 cases exhibited low expression of GSE1, 10 out of 100 cases exhibited moderate expression of GSE1, and 5 out of 100 cases exhibited high expression of GSE1. In contrast, 17 out of 100 cases of gastric cancer tissues were negative for expression of GSE1, 27 out of 100 cases exhibited low expression of GSE1, 37 out of 100 cases exhibited moderate expression of GSE1, and 19 out of 100 cases exhibited high expression of GSE1 (P<0.001). Therefore, the expression levels of GSE1 in human gastric cancer tissues were higher than that in normal gastric tissues.

Association of GSE1 expression with clinicopathological parameters and survival of gastric cancer patients—We next examined the association of GSE1 expression with clinicopathological parameters including patients’ age, gender, tumor size, lymph node metastasis, histological grade, depth of invasion and clinical stage. As shown in Table 2, the expression of GSE1 was positively correlated with patient lymph node metastasis (P=0.001), histological grade (P=0.037), depth of invasion (P=0.008) and clinical stage (P=0.001). However, there was no significant correlation between GSE1 expression and patients’ age, gender, or tumor size (P>0.05).

Furthermore, these gastric cancer patients were followed-up for more than 5 years, and the association of GSE1 expression with their survival rate was analyzed by means of Kaplan-Meier analysis. Zero and 1 scoring of GSE1 were designated as low expression of GSE1; 2 and 3 scoring were designated as high expression of GSE1. In the 100 gastric cancer tissues, 44 exhibited low expression of GSE1 and 56 exhibited high expression of GSE1. As shown in Fig.1B, both RFS rate (P<0.001) and OS rate (P<0.001) were significantly lower in tissues with high GSE1 expression compared with tissues with low GSE1 expression. This data suggested that GSE1 is associated with poor prognosis in human gastric cancer.

GSE1 stimulates cellular proliferation and oncogenicity of human gastric cancer cells—Gastric cancer cell lines BGC-823, HGC-27, AGS, MKN-45 were used in this study. As shown in Fig.2A, the basal level of GSE1 was high in HGC-27 and MKN-45 cells, and was low in BGC-823 and AGS cells. Therefore, we have selected HGC-27 and MKN-45 cells to perform GSE1 depletion -related studies and selected BGC-823 and AGS cells to perform studies with forced expression of GSE1. The protein level of GSE1 decreased significantly after transfection with shGSE1-1 or shGSE1-2 in HGC-27 and MKN-45 cells (Fig.2B, Fig.S1A),
and increased significantly after transfection with an expression plasmid pIRESneo3 encoding GSE1 in BGC-823 and AGS cells (Fig.2C, Fig.S1E).

In monolayer cultures, shGSE1-1 or shGSE1-2 decreased the total cell number dramatically over a period of 5 days (both HGC-27 and MKN-45 cell lines) (Fig.2D, Fig.S1B). Concordantly, MTT assay showed a significant decrease in the cell viability over a period of 5 days after transfection with shGSE1-1 or shGSE1-2 in both HGC-27 and MKN-45 cells (Fig.2E, Fig.S1C). Moreover, shRNA-mediated depletion of GSE1 in HGC-27 and MKN-45 cells significantly reduced cell colony formation as indicated in Fig.2F and Fig.S1D (HGC-27-shNC 257±33, HGC-27-shGSE1-1 38±4, HGC-27-shGSE1-2 45±5, P<0.01 and MKN-45-shNC 651±70, MKN-45-shGSE1-1 318±44, MKN-45-shGSE1-2 330±35, P<0.01).

In contrast, the forced expression of GSE1 in BGC-823 and AGS cells dramatically increased total cell number and cell viability over a period of 5 days (Fig.2G, H, Fig.S1F, G). Concordantly, BGC-823-GSE1 and AGS-GSE1 cells exhibited significantly enhanced cell colony formation compared with BGC-823-Vec and AGS-Vec cells, respectively (Fig.2I, Fig.S1H). Thus, it is concluded that GSE1 stimulates proliferation and enhances viability of human gastric cancer cells.

**GSE1 promotes metastasis of human gastric cancer cells**—Next, we evaluated the role of GSE1 in migration and invasion of human gastric cancer cells. After transfection with shGSE1-1 or shGSE1-2, both cell migration (HGC-27-shNC 408±55, HGC-27-shGSE1-1 185±30, HGC-27-shGSE1-2 199±39, P<0.01 and MKN-45-shNC 156±22, MKN-45-shGSE1-1 62±15, MKN-45-shGSE1-2 78±19, P<0.01) and invasion (HGC-27-shNC 171±28, HGC-27-shGSE1-1 31±6, HGC-27-shGSE1-2 37±8, P<0.01 and MKN-45-shNC 88±20, MKN-45-shGSE1-1 33±8, MKN-45-shGSE1-2 48±11, P<0.01) were abrogated in both HGC-27 and MKN-45 cells (Fig.2J, L, Fig.S1I, K). Moreover, depletion of GSE1 with shGSE1-1 or shGSE1-2 resulted in a retarded wound closure in HGC-27 and MKN-45 cells as shown in Fig.2N and Fig.S1M.

In contrast, the forced expression of GSE1 in BGC-823 and AGS cells dramatically increased cell migration (BGC-823-Vec 18±5, BGC-823-GSE1 63±9, P<0.01 and AGS-Vec 35±7, AGS-GSE1 58±13, P<0.01) and invasion (BGC-823-Vec 11±5, BGC-823-GSE1 39±10, P<0.01 and AGS-Vec 9±6, AGS-GSE1 26±8, P<0.01) compared with control respectively (Fig.2K, M, Fig.S1J, L). Concordantly, forced expression of GSE1 promoted a faster wound closure compared with the respective control BGC-823 and AGS cells (Fig.2O and Fig.S1N). Therefore, GSE1 promotes the motile behavior of human gastric cancer cells.

**GSE1 promotes xenograft growth and metastasis of human gastric cancer cells**—To determine the effects of GSE1 on tumor growth in vivo, HGC-27-shNC / HGC-27-shGSE1-1 (designated as HGC-27-shGSE1) and BGC-823-Vec / BGC-823-GSE1 cells were subcutaneously injected into the dorsal flank of nude mice. As shown in Fig.3A, all of the 8 sites injected with
HGC-27-shNC cells formed palpable tumors, whereas only 6 of the 8 sites injected with HGC-27-shGSE1 formed palpable tumors even until termination of the experiment. We measured the tumor size on a 3-day interval. The growth curves showed HGC-27-shGSE1 generated tumors grew slower than HGC-27-shNC generated tumors. After 21 days, HGC-27-shNC generated tumors were more than 5 times the size of the HGC-27-shGSE1 generated tumors. Accordingly, the mean weight of tumors formed by HGC-27-shGSE1 cells was significantly lower than that of tumors formed by HGC-27-shNC cells (P<0.01) (Fig.3B). Moreover, the Ki-67-positive cell population in tumors formed by HGC-27-shGSE1 cells was significantly decreased compared with tumors formed by HGC-27-shNC cells (P<0.01) (Fig.3C).

In contrast, both BGC-823-Vec and BGC-823-GSE1 cells formed palpable tumors in all animals after less than a week. During the period of 21 days, BGC-823-GSE1 generated tumors grew much faster compared with BGC-823-Vec generated tumors (P<0.05). Tumors formed by BGC-823-GSE1 cells were more than 2 times the size of tumors formed by BGC-823-Vec cells at the end of the study (Fig.3D). As such, the mean weight of tumors formed by BGC-823-GSE1 cells was significantly higher than that of tumors formed by BGC-823-Vec cells (P<0.01) (Fig.3E). Moreover, the Ki-67-positive cell population in tumors formed by BGC-823-GSE1 cells was significantly increased compared with tumors formed by BGC-823-Vec cells (P<0.01) (Fig.3F). These findings suggest that GSE1 promotes cell proliferation of gastric cancer cells and tumor growth in vivo.

Furthermore, we examined the effects of GSE1 on tumor metastasis in vivo by injecting HGC-27-shNC / HGC-27-shGSE1 and BGC-823-Vec / BGC-823-GSE1 into the venous circulation of mice. After 40 days, mice were killed and their lungs were collected for histology. Five random sections of each mouse lung were examined for lung micrometastases. In the 8 mice injected with HGC-27-shGSE1 cells, no lung metastases were observed, whereas 4/8 mice injected with HGC-27-shNC cells exhibited lung metastases (P=0.021). Meanwhile, lung metastases were observed in 7/8 mice injected with BGC-823-GSE1 cells whereas only 3/8 mice injected with BGC-823-Vec cells exhibited metastases (P=0.039). Moreover, the total number of lung micrometastases was much lower in mice injected with HGC-27-shGSE1 cells compared with mice injected with HGC-27-shNC cells (P<0.05), whereas the number of lung micrometastases was much higher in mice injected with BGC-823-GSE1 cells compared with mice injected with BGC-823-Vec cells (P<0.01). (Fig.3G, H) Hence, GSE1 also promotes tumor metastasis of gastric cancer cells in vivo.

GSE1 regulates the expression of SLC7A5 in gastric cancer cells—To identify the downstream mechanism of GSE1 in human gastric cancer cells, we performed mRNA sequencing in HGC-27-shNC and HGC-27-shGSE1-1 cells (designated as HGC-27-shGSE1) to search for genes potentially regulated by GSE1. As shown in Fig.4A, the
expression of PER3, CTH, LAPTM5, DPM3, RAB3GAP2, AKR1C2 and SLC7A5 decreased significantly and the expression of TNFRSF1B, TINAGL1, ADC, IFI44L, IFI44, PALMD and CSF1 increased significantly after transfection with shGSE1-1 in HGC-27 cells. Among these genes, SLC7A5 showed the greatest reduction after depletion of GSE1. This is consistent with reports that SLC7A5 contributes to gastric cancer malignant behavior (8,10).

To confirm mRNA sequencing results, protein levels of SLC7A5 were examined in HGC-27 and MKN-45 cells after transfection with shGSE1 or shNC and in BGC-823 and AGS cells after transfection with GSE1 expressing plasmid or Vector control plasmid. The observations were consistent with previous results, whereby GSE1 and protein levels of SLC7A5 decreased significantly after transfection with shGSE1 compared with shNC both in HGC-27 and MKN-45 cells (Fig.4B, C). Concordantly, both GSE1 and SLC7A5 increased dramatically after forced expression of GSE1 compared with Vector transfected control cells (Fig.4D, E). As such, this suggests that GSE1 positively regulates the expression of SLC7A5 in human gastric cancer cells.

**GSE1 increased SLC7A5 transcript stability through a post-transcriptional mechanism**—RT-qPCR and mRNA decay assays were performed to determine the mechanism of regulation of SLC7A5 by GSE1 in HGC-27 and MKN-45 cells after transfection with shGSE1 or shNC and in BGC-823 and AGS cells after transfection with GSE1 expressing plasmid or Vector control plasmid. As shown in Fig.5A, shGSE1 dramatically decreased the mRNA levels of SLC7A5 in both HGC-27 and MKN-45 cells compared with shNC. Moreover, the decay rate of SLC7A5 mRNA dramatically increased after transfection with shGSE1 compared with shNC in both HGC-27 and MKN-45 cells (Fig.5B). Concordantly, the mRNA levels of SLC7A5 increased significantly after transfection with GSE1 expressing plasmid compared with Vector control in both BGC-823 and AGS cells (Fig.5C), and forced expression of GSE1 retarded SLC7A5 mRNA decay compared with Vector control (Fig.5D). Therefore, GSE1 positively regulated the expression of SLC7A5 in human gastric cancer cells by increasing transcript stability in a post-transcriptional manner.

In addition, ribonucleoprotein (RNP) immunoprecipitation (IP) assay was performed using anti-GSE1 antibody and the mRNA levels of SLC7A5 and negative control gene HER2 were detected. There was no significant change of SLC7A5 mRNA enriched between anti-GSE1 antibody and control IgG, and similarly with the negative control gene HER2. There was also no significant difference of SLC7A5 and HER2 mRNA total levels between anti-GSE1 antibody group and IgG group (Fig.5E). Therefore, we excluded that SLC7A5 mRNA was regulated by GSE1 through a direct binding mechanism. GSE1 might therefore regulate SLC7A5 indirectly.

**SLC7A5 stimulates cellular proliferation and metastasis of human gastric cancer cells**—Next, we used shSLC7A5-1 and shSLC7A5-2 to deplete endogenous SLC7A5 in HGC-27 and MKN-45 cells. Protein levels of SLC7A5 decreased
significantly after transfection with shSLC7A5-1 or shSLC7A5-2 in both HGC-27 and MKN-45 cells (Fig.6A). Total cell number assay and MTT assay determined that cell growth and cell viability decreased significantly after depletion of SLC7A5 with shSLC7A5-1 or shSLC7A5-2 in both HGC-27 and MKN-45 cells during a period of 5 days (Fig.6B, C). Moreover, colony formation was reduced dramatically in HGC-27 and MKN-45 cells after transfection with shSLC7A5-1 or shSLC7A5-2 (Fig.6D).

To determine the function of SLC7A5 in migration and invasion of human gastric cancer cells, cell migration, cell invasion and wound healing assays were carried out in HGC-27 and MKN-45 cells. After transfection with shSLC7A5-1 or shSLC7A5-2, both cell migration and invasion decreased significantly in HGC-27 and MKN-45 cells (Fig.6E, F). Accordingly, the depletion of SLC7A5 retarded wound closing both in HGC-27 and MKN-45 cells (Fig.6G). This indicates that SLC7A5 promotes both proliferation and metastasis of human gastric cancer cells.

**GSE1 stimulates oncogenic behaviors of human gastric cancer cells through SLC7A5**—To determine whether GSE1 enhanced oncogenic behaviors of human gastric cancer cells were mediated by SLC7A5, cell function experiments were performed in BGC-823 and AGS cells with forced expression of GSE1 and SLC7A5 depletion by shSLC7A5-1 (designated as shSLC7A5) / shNC, and in HGC-27 and MKN-45 cells with GSE1 depletion by shGSE1-1 (designated as shGSE1) / shNC and forced expression of SLC7A5.

In BGC-823 and AGS cells, protein levels of GSE1 increased significantly after transfection with GSE1 plasmid + shNC or GSE1 plasmid + shSLC7A5 compared with control. Whereas protein levels of SLC7A5 increased significantly after transfection with GSE1 plasmid + shNC compared with control, this increase was abrogated by transfection with GSE1 plasmid + shSLC7A5 (Fig.7A). Consistent with previous results, cell proliferation (measured using total cell number assay), cell viability (measured using MTT assay), cell colony formation, cell migration, cell invasion and cell wound closing all increased significantly in both BGC-823 and AGS cells with the forced expression of GSE1. However, the depletion of SLC7A5 specifically abolished the enhanced oncogenic behaviors of BGC-823 and AGS cells as a consequence of the forced expression of GSE1 (Fig.7B-G).

In HGC-27 and MKN-45 cells, protein levels of GSE1 decreased significantly after transfection with shGSE1 + Vec plasmid or shGSE1 + SLC7A5 plasmid compared with control. Whereas protein levels of SLC7A5 decreased significantly after transfection with shGSE1 + Vec plasmid compared with control, this decrease was abrogated by transfection with shGSE1 + SLC7A5 plasmid (Fig.8A). Consistent with previous results, cell proliferation (measured using total cell number assay), cell viability (measured using MTT assay), cell colony formation, cell migration, cell invasion and cell wound closing all decreased significantly in both HGC-27 and MKN-45 cells with the depleted expression of GSE1.
However, the forced expression of SLC7A5 specifically abolished the decreased oncogenic behaviors of HGC-27 and MKN-45 cells as a consequence of the depleted expression of GSE1 (Fig. 8B-G).

Therefore, we conclude that GSE1 stimulates oncogenic behaviors of human gastric cancer cells associated with tumor progression partly through specific regulation of SLC7A5.

**GSE1 is a direct downstream target of miR-200b and miR-200c in gastric cancer cells**—To determine upstream mechanisms involved in GSE1 regulation, we used TargetScans to search for potential miRNAs directly targeting GSE1 in gastric cancer cells. MiR-200b and miR-200c were predicted to possess direct binding sites at the GSE1 3’UTR (5’-CAGUAUU-3’). We generated luciferase reporter plasmids containing the GSE1 3’UTR and GSE1 3’UTR in which the binding site of miR-200b and miR-200c was mutated into 5’-CUCUACC-3’ (Fig.9A). Fig.9B showed that luciferase reporter activity in HGC-27 cells was significantly decreased after co-transfection with luciferase reporter plasmid containing GSE1 wild type 3’UTR and miR-200b or miR-200c mimics compared with control. There was no significant difference in the luciferase reporter activity between the cells co-transfected with luciferase reporter plasmid containing GSE1 mutated 3’UTR / miR-200b or miR-200c mimics and the cells co-transfected with luciferase reporter plasmid containing GSE1 mutational 3’UTR / NC miRNA mimics (Fig.9B). Furthermore, both miR-200b and miR-200c decreased the protein expression of GSE1 in HGC-27 and MKN-45 cells (Fig.9C). Hence, miR-200b and miR-200c regulated the expression of GSE1 through binding sites in the 3’UTR of GSE1.

**Expression of SLC7A5, miR-200b and miR-200c in human gastric cancer tissues and normal gastric tissues**—We next examined the expression of SLC7A5 in the cohorts of 100 human gastric cancer tissues and 100 normal gastric tissues using immunohistochemistry. Positive for SLC7A5 protein was mainly located in the cytoplasm with membranous enhancement of gastric cancer cells and glandular epithelial cells. A higher expression of SLC7A5 was observed in gastric cancer tissues compared with normal gastric tissues (gastric cancer tissues: 20% negative, 32% low, 30% moderate, 19% high; normal gastric tissues: 48% negative, 36% low, 10% moderate, 6% high. \( P<0.001 \) (Fig.10A) (Table 3). MiR-200b and miR-200c expression levels were also examined in fresh gastric cancer tissues and normal gastric tissues using RT-qPCR. As shown in Fig.10B, the expression of both miR-200b and miR-200c were much lower in gastric cancer tissues compared with normal gastric tissues (both \( P<0.01 \)).

Moreover, we analyzed the correlation between GSE1 and SLC7A5 protein levels in the 100 paraffin-embedded gastric cancer tissues (Table 4) and the correlation between GSE1 mRNA and miR-200b / miR-200c levels in the 60 fresh gastric cancer tissues (Fig.10C). There was a statistically positive correlation between GSE1 and SLC7A5 expression in gastric cancer tissues (\( P=0.003 \), Pearson’s correlation coefficient: 0.297) (Table 4).
In contrast, GSE1 and miR-200b expression were negatively correlated in fresh gastric cancer tissues ($P<0.0001$, Pearson’s correlation coefficient: -0.65047). GSE1 and miR-200c expression were also negatively correlated in these gastric cancer tissues ($P<0.0001$, Pearson’s correlation coefficient: -0.54332) (Fig.10C).

**DISCUSSION**

Herein, we systematically examined the functional role of GSE1 in human gastric cancer. The forced expression of GSE1 in gastric cancer cells was observed to promote cell proliferation, colony formation, migration and invasion *in vitro*, and increase tumor growth and metastasis in xenograft models. Concordantly, the depletion of GSE1 in gastric cancer cells reduced the oncogenic properties of gastric cancer cells both *in vitro* and *in vivo*. Moreover, the expression of GSE1 protein was higher in gastric cancer tissues compared with normal gastric tissues and the expression level of GSE1 positively associated with lymph node metastasis, histological grade, depth of invasion and clinical stage in gastric cancer patients. High levels of GSE1 were also associated with both low relapse-free survival (RFS) and overall survival (OS) rates of gastric cancer patients. As reported previously, GSE1 possesses an oncogenic function in human breast cancer cells with promotion of both cell proliferation and metastasis. Moreover, the expression level of GSE1 was observed to be increased in breast cancer tissues and predicted poor prognosis in breast cancer patients (7). These published results are consistent with our current findings. To the best of our knowledge, this study is the first to report that GSE1 possesses an oncogenic role in human gastric cancer.

SLC7A5 was observed to be positively regulated by GSE1 and to partly mediate the functions of GSE1 in gastric cancer. Consistent with our results, Wang et al. have reported previously that suppression of the expression of SLC7A5 in the MKN-45 gastric cancer cell line dramatically decreased cell proliferation, migration and invasion (8). Furthermore, it has been reported that SLC7A5 expression was positively regulated by CRKL and mediated the oncogenic role of CRKL in the gastric cancer cell line SGC-7901 (11). SLC7A5 was also reported to be a direct target of miR-126 and to mediate the tumor suppressive function of miR-126 in MKN-45 gastric cancer cells (10). Furthermore, we have observed that the protein level of SLC7A5 was significantly higher in gastric cancer tissues compared with normal gastric tissues. GSE1 and SLC7A5 were positively correlated in gastric cancer tissues. Ichinoe et al. reported that gastric cancer cases with lymph node metastasis exhibit significantly higher SLC7A5 expression than cases without lymph node metastasis, and high expression of SLC7A5 correlated with high Ki-67 level and a significantly poorer prognosis compared with low SLC7A5 group (12). SLC7A5 expression has also been reported to be significantly associated with clinicopathologic features such as tumor size, lymph node metastasis, local invasion and TNM stage in gastric cancer patients (8). These reports are concordant with our data herein. In
addition, SLC7A5 was also reported to promote growth of human breast cancer cells (13). Furthermore, Furuya et al. documented that SLC7A5 expression correlated with tumor size, nuclear grade and Ki-67 labeling index in tissues from breast cancer patients (14). In T-cell lymphoblastic lymphoma/T-cell acute lymphoblastic leukemia, SLC7A5 supports tumor cell growth and survival (15). Moreover, SLC7A5 acts as a tumor promoter in other human cancers including non-small cell lung cancer (16), colon cancer (17), prostate cancer (18) and cutaneous melanoma (19). In a complex study of various human cancers, Kaira et al. reported that SLC7A5 correlated significantly with cell proliferation and angiogenesis at primary and metastatic sites of human neoplasms (20). Hence, SLC7A5 is an important tumor promoter in human cancers and it mediates the oncogenic role of GSE1 in human gastric cancer cells. Moreover, we attempted to delineate the mechanisms for the regulation of SLC7A5 by GSE1 and determined that GSE1 increased the transcript stability of SLC7A5 in a post-transcriptional manner not dependent on direct binding.

MiR-200b and miR-200c were herein demonstrated to directly target and suppress the expression of GSE1. In this study, the expression levels of miR-200b and miR-200c were observed dramatically lower in gastric cancer tissues compared with normal gastric tissues. Moreover, in gastric cancer tissues, expression levels of GSE1 and miR-200b / miR-200c were significantly and inversely correlated. As now established, miRNAs play important roles in tumor initiation, development, and progression including in human gastric cancer (21-25). For example, miR-27a, miR-520c and miR-224 possess oncogenic roles in human gastric cancer (26-28), whereas miR-132-3p, miR-134 and miR-29c exhibit tumor suppressive functions in human gastric cancer (29-31). Herein, we identified that miR-200b and miR-200c potentially suppress the oncogenic behaviors of human gastric cancer cells by targeting GSE1. It has been previously reported that miR-200b decreases cell proliferation, invasion, and migration by targeting ZEB2 in human gastric cancer (32); miR-200c also suppresses gastric cancer progression by targeting ZEB1/2 and FN1 (33,34). Our findings are consistent with these observations. Moreover, Chang et al. reported that the expression levels of the miR-200 family including miR-200b and miR-200c were significantly lower in clinical samples of gastric cancer compared with paired non-cancerous tissues. Lower levels of miR-200b and miR-200c were associated with high histological grade and the presence of an intravascular cancer embolus (35). This report is also concordant with our data herein. In addition, miR-200b and miR-200c also contribute to tumor suppression in other human cancers, such as colon cancer, pancreatic cancer, breast cancer and prostate cancer (36-42). Therefore, miR-200b and miR-200c are tumor suppressors that, at least in gastric cancer, act partly by decreasing the expression of GSE1.

In summary, we have shown for the first time, the functional roles of GSE1 in human gastric cancer cells in vitro and in xenograft models in vivo. High
expression of GSE1 correlated with poor survival outcome for gastric cancer patients. GSE1 was identified as a direct target of miR-200b and miR-200c, and increased SLC7A5 expression was demonstrated to mediate the oncogenic functions of GSE1. GSE1 is therefore a potential diagnostic and therapeutic target in human gastric cancer.

**EXPERIMENTAL PROCEDURES**

*Clinical samples*—One hundred paraffin-embedded gastric cancer tissues and 100 normal gastric tissues were collected at the Department of Pathology in the First Affiliated Hospital of Anhui Medical University (Hefei, Anhui, China). These tissues were from patients who underwent surgical resection between 2009 and 2010. Patients with other diseases or patients who underwent special therapies prior to surgery were excluded. The clinic-pathological parameters of these patients were determined according to the 2003 World Health Organization (WHO) classification system. These gastric cancer patients were followed-up for more than 5 years, and the deadline is December 2, 2016. Another 60 fresh gastric cancer tissues and 60 fresh normal gastric tissues were collected for miRNA and mRNA study. These tissues were from patients who underwent surgical resection in 2016. This study plan was approved by the Institutional Review Boards of Anhui Medical University and was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki). All patients had signed an informed consent form.

*Immunohistochemistry (IHC)* analyses of GSE1 and SLC7A5 protein expression in paraffin sections of tissues from gastric cancer patients, and Ki-67 protein expression in paraffin sections of tumors formed from HGC-27-shNC / HGC-27-shGSE1 and BGC-823-Vcc / BGC-823-GSE1 cells in mice were carried out using the Ultra Sensitive-SP kit (Maixin-Bio, Fuzhou, China) as described in our former study (43,44). Antibodies used in this study were rabbit polyclonal antibody against GSE1, rabbit polyclonal antibody against SLC7A5 (both 1:100, Proteintech Group, Inc., Chicago, USA), mouse polyclonal antibody against Ki-67 (1:1, Zhongshan Goldenbridge Biotechnology Co, Beijing, China). The stained sections were evaluated using an Olympus microscopy (Olympus America, Inc., Melville, NY). For examination of the protein levels of GSE1 and SLC7A5 in patient tissues, one sample for each patient was utilized. The IHC stained sections were reviewed and scored independently by two experienced pathologists who had no knowledge of the patients’ identities or clinical status, and both pathologists had similar accuracy rates. Expression levels of GSE1 and SLC7A5 in patients’ tissues were evaluated using the 0, 1, 2, 3 scoring system. Sections with 0% stained cells were designated as no expression of GSE1 or SLC7A5 (0 scoring); sections with 1%-33% stained cells were designated as low expression of GSE1 or SLC7A5 (1 scoring); sections with 34%-66% stained cells were designated as moderate expression of GSE1 or SLC7A5 (2 scoring); Sections with 67%-100% stained cells were designated as high expression of
GSE1 or SLC7A5 (3 scoring). In Kaplan-Meier analysis, 0 and 1 scoring of GSE1 were designated as low expression of GSE1; 2 and 3 scoring were designated as high expression of GSE1.

**Cell lines and Cell culture**—Human gastric cancer cell lines BGC-823, HGC-27, AGS and MKN-45 were used in this study. All cells were obtained from ATCC (the American Type Culture Collection) (Rockville, MD) and cultured at 37°C in a humidified atmosphere of 5% CO₂ as recommended.

**Transfection of plasmid constructs and RNA oligonucleotides**—In this study, we used mammalian expression vector pIRESneo3 (Invitrogen) to construct GSE1 and SLC7A5 over-expressing plasmids. GSE1 coding sequence transcript (GenBank accession no. NM_001134473.2) was cloned into pIRESneo3 and was designated as pIRESneo3-GSE1. SLC7A5 coding sequence transcript (GenBank accession no. NM_003486.6) was cloned into pIRESneo3 and was designated as pIRESneo3-SLC7A5. shRNAs (including shGSE1-1, shGSE1-2, shSLC7A5-1 and shSLC7A5-2) and miR-200b, miR-200c mimics were synthesized by GenePharma (Shanghai, China). As previously described (43,44), all plasmids, shRNAs and miRNA mimics were transfected using lip2000 (QIAGEN). Specific shRNA was used: shGSE1-1, 5'-GCCUACAUCAUGAU GAGUTT-3'; shGSE1-2, 5'-GAGAUG AACAACAGUCCATT-3'; shSLC7A5-1, 5'- GGAAGGGUGA UUGUCCAATT -3'; shSLC7A5-2, 5'-GCAUUUAACAGCGGCCUCUTT -3'.

**Western blot analysis**—Western blot analysis was performed as described in our previous studies (43,44). Rabbit polyclonal antibody against GSE1, rabbit polyclonal antibody against SLC7A5 (both 1:1000, Proteintech Group, Inc., Chicago, USA), mouse β-actin monoclonal antibody (1:5000, Sigma) were used.

**Cell proliferation and metastasis assays**—Cell proliferation assays including total cell number assay, 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay and colony formation assay were performed essentially as described previously (43-45). Briefly, in total cell number assay, cells were seeded into 6-well plates with an original cell number of 1x10⁵ and subsequently counted daily for 5 days. In MTT assay, cells were seeded into 96-well plates with an original cell number of 2000 and cell viability tested (OD570 nm) every day for 5 days. In colony formation assay, cells were seeded into 6-well plates with an original cell number of 1000 and tested after 10-15 days. Meanwhile, cell metastasis assays including cell migration assay, cell invasion assay and wound healing assay were also performed as described previously (43-45). For migration assay, HGC-27 derived cells were tested after 18 hours; BGC-823 derived cells, MKN-45 derived cells and AGS derived cells were tested after 24 hours. For invasion assay, HGC-27 derived cells were tested after 24 hours; BGC-823 derived cells, MKN-45 derived cells and AGS derived cells were tested after 48 hours. Wound healing assay were tested after 24 hours.

**Xenograft analyses**—HGC-27-
shNC / HGC-27-shGSE1-1 (designated as HGC-27-shGSE1) and BGC-823-Vec / BGC-823-GSE1 cells (500×10^4 per 125μl per site) were subcutaneously injected into the dorsal flank of 4-week-old BALB/c-nu/nu mice (Shanghai Slaccas Co, Shanghai, China). Each cell was injected into 8 sites and palpable tumors formed after about a week. The length and width of the tumors were measured every 3 days. Tumor volume was calculated according to the formula: Volume (mm^3) = L×W^2×Π/6 (46). After 21 days, mice were sacrificed and tumors were harvested. Weights of the tumors were measured and Ki-67 protein expression in paraffin sections of these tumors was examined using immunohistochemistry (IHC).

For tail vein injection, HGC-27-shNC / HGC-27-shGSE1 and BGC-823-Vec / BGC-823-GSE1 cells (500×10^4 cells in 250 μL PBS) were injected directly into the lateral tail vein of 4-week-old BALB/c-nu/nu mice. Every group contained 8 mice. After 40 days, mice were sacrificed and their lungs were harvested and prepared for histologic examination. Five random hematoxylin and eosin–stained sections of each mouse lung were reviewed and examined for lung micrometastases from injected tumor cells. The exact number of micrometastases in each mouse lung of the 5 random sections was also calculated.

Experiments on animals in this study were carried out according to the Institutional Animal Care and Use Committee guidelines (available at www.iacuc.org). Local institutional approval from the Animal Ethics Committee of Anhui Medical University was obtained prior to commencement of work (No. LLSC20160331).

**mRNA sequencing** —mRNA sequencing in HGC-27-shNC and HGC-27-shGSE1-1 cells (designated as HGC-27-shGSE1) to screen differentially expressed mRNAs were performed in Kangcheng Biotechnology Company (Shanghai, China).

**Luciferase reporter assay**—Luciferase reporter assay was carried out to examine the targeted regulation of miR-200b and miR-200c on GSE1 in gastric cancer cells and was performed as described in our previous publications using the Dual Luciferase Reporter Assay System (Promega Corp.) (44,47). Both Renilla luciferase activity and Firefly luciferase activity were measured in each sample. Firefly luciferase activity was used as a base control.

**RT-quantitative PCR (qPCR)** —miRNAs were isolated from fresh tissues of gastric cancer patients. MiR-200b and miR-200c expression levels were examined using RT-qPCR. As described previously, this was performed using TaqMan® MicroRNA Assays (Applied Bio systems, Foster City, CA) (44,46). U6 was used as an endogenous miRNA control. mRNAs were isolated from cells after transfection for 48 hours or directly from human tissues. mRNA levels of GSE1 and SLC7A5 were examined by RT-qPCR using SYBR green Master MIX (Applied Biosystem) as described earlier (44,47). GAPDH was used as an endogenous control. The primers used were: miR-200b, forward 5'- GCCGCTAATACTGCCGGTAAT -3’ and reverse 5’- GTGCCAGGGTCCAGGT-3’; miR-200c, forward 5’- GCCGCTAATACTGCCGGTAATG -3’ and reverse 5’- GTGCAGGGTGCGAGGT-3’; U6, forward 5’-TGGGAACGA
TACAGAGAAGATTAGCA-3’ and reverse 5’-AACGCTTCACGAATT TGCGT-3’; GSE1, forward 5’-AGAGCACCACAGGCGAGGCAGGAC -3’ and reverse 5’-CGTGCGGTGCAGC ATGGAGC TGCGT-3’; SLC7A5, forward 5’-CCTGCCTGTGTTCTCCTACCTG -3’ and reverse 5’-GACCACCTGCA TGAGCTTCTG-3’; GAPDH, forward 5’-TGCACCACCACTGCTTAGC -3’ and reverse 5’-GGCATGGACTGT GGTCATGAG-3’.

mRNA decay assay—mRNA decay assay was performed to detect the interaction between GSE1 protein and SLC7A5 mRNA. HGC-27, MKN-45, BGC-823 and AGS cells were treated with actinomycin D (10 μg/mL) 48 hours after shGSE1 / GSE1 expressing plasmid transfection, and then the cells were harvested at 0, 2, 4, 6 and 8 hours after actinomycin D treatment. mRNA levels of SLC7A5 were determined using RT-qPCR. GAPDH was used as an endogenous control.

Ribonucleoprotein (RNP) immunoprecipitation (IP) RT-PCR (RNP-IP RT-PCR) —The binding between GSE1 protein and SLC7A5 mRNA was examined using RNP-IP RT-PCR assay as described earlier (48). GSE1 protein- SLC7A5 mRNA complex was captured by anti-GSE1 antibody, and the SLC7A5 mRNA was examined using RT-qPCR. IgG and HER2 mRNA were used as negative controls.

Statistical analyses—At least three replicates were performed for each experiment and the results represented the average. RT-qPCR, luciferase reporter assay, cell proliferation and metastasis assays, tumor growth in xenograft, expression of miR-200b / miR-200c in tissues were analyzed using unpaired two-tailed t test. Immunohistochemistry and clinicopathological parameters study were analyzed using Pearson’s chi-square test. Relapse-free survival (RFS) and overall survival (OS) rate in patients were analyzed through Kaplan-Meier curves and the significance of the differences was analyzed using log-rank test. It was considered statistically significant when \( P<0.05 \).

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Conflict of interest
The authors declare that they have no conflict of interest.
Authors’ contributions

KD carried out all of the tissue study, participated in the cell functional study, ran RT-qPCR and western blot, performed the in vivo study, was involved in the design of the study and participated in the manuscript writing. ST performed all of the cell functional study, ran RT-qPCR and western blot, participated in the in vivo study, designed the mRNA sequencing, was involved in the design of the study and critically revised the manuscript. XH participated in all of the experiments in the revision and critically revised the manuscript. XW and XL performed the luciferase assays, participated in the tissue study and data analysis. RF participated in the tissue study. YZ participated in the in vivo study. PL participated in data analysis and critically revised the manuscript. WW designed this study, participated in data analysis and critically revised the manuscript. ZW designed this study and wrote the manuscript.

FOOTNOTES
Abbreviations GSE1: human Gse1 coiled-coil protein; IHC: Immunohistochemistry; LAT-1: L-type amino-acid transporter 1; miRNA: microRNA; MTT: 3-(4, 5-dimethylthiazol -2-yl)-2, 5-diphenyltetrazolium bromide; OS: overall survival; RFS: relapse-free survival; WHO: World Health Organization.
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Table 1. Expression of GSE1 in gastric cancer and normal tissues

| Group  | n  | None | Low | Moderate | High |
|--------|----|------|-----|----------|------|
| GC     | 100| 17   | 27  | 37       | 19   |
| Normal | 100| 51   | 34  | 10       | 5    |

Note: $P < 0.001$. 
Table 2. Association of GSE1 expression with clinicopathological parameters from gastric cancer patients

| Parameter                           | n   | GSE1 expression |   |   |   | P value |
|-------------------------------------|-----|-----------------|---|---|---|---------|
|                                     |     | None            | Low| Moderate | High|         |
| **Age (years)**                     |     |                 |    |          |    |         |
| ≤ 60                                | 34  | 10              | 7  | 13       | 4  | 0.075   |
| > 60                                | 66  | 7               | 20 | 24       | 15 |         |
| **Gender**                          |     |                 |    |          |    |         |
| male                                | 54  | 11              | 14 | 20       | 9  | 0.760   |
| female                              | 46  | 6               | 13 | 17       | 10 |         |
| **Tumor size (cm)**                 |     |                 |    |          |    |         |
| ≤ 5                                 | 60  | 10              | 17 | 25       | 8  | 0.317   |
| > 5                                 | 40  | 7               | 10 | 12       | 11 |         |
| **Lymph node metastasis**           |     |                 |    |          |    |         |
| No                                  | 34  | 15              | 17 | 1        | 1  | 0.001   |
| Yes                                 | 66  | 2               | 10 | 36       | 18 |         |
| **Grade**                           |     |                 |    |          |    |         |
| I                                   | 11  | 1               | 6  | 1        | 3  | 0.037   |
| II                                  | 48  | 7               | 10 | 25       | 6  |         |
| III                                 | 41  | 9               | 11 | 11       | 10 |         |
| **Depth of invasion**               |     |                 |    |          |    |         |
| T1,2                                | 40  | 13              | 9  | 13       | 5  | 0.008   |
| T3,4                                | 60  | 4               | 18 | 24       | 14 |         |
| **Stage**                           |     |                 |    |          |    |         |
| I - II                              | 32  | 13              | 13 | 5        | 1  | 0.001   |
| III - IV                            | 68  | 4               | 14 | 32       | 18 |         |
Table 3. Expression of SLC7A5 in gastric cancer and normal tissues

| Group   | n   | None | Low | Moderate | High |
|---------|-----|------|-----|----------|------|
| GC      | 100 | 20   | 32  | 30       | 18   |
| Normal  | 100 | 48   | 36  | 10       | 6    |

Note: * $P < 0.001$. 

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Table 4. Correlations of GSE1 and SLC7A5 expression in gastric cancer patients.

| GSE_1_2 * SL_1 Cross tabulation |
|----------------------------------|
| None  | Low  | Moderate | High | Total |
|-------|------|----------|------|-------|
| **GSE_1_2** | Count | 2 | 11 | 2 | 2 | 17 |
| % within GSE_1_2 | 11.8% | 64.7% | 11.8% | 11.8% | 100.0% |
| % within SL_1 | 10.0% | 34.4% | 6.7% | 11.1% | 17.0% |
| % of Total | 2.0% | 11.0% | 2.0% | 2.0% | 17.0% |
| **Low** | Count | 12 | 6 | 8 | 1 | 27 |
| % within GSE_1_2 | 44.4% | 22.2% | 29.6% | 3.7% | 100.0% |
| % within SL_1 | 60.0% | 18.8% | 26.7% | 5.6% | 27.0% |
| % of Total | 12.0% | 6.0% | 8.0% | 1.0% | 27.0% |
| **Moderate** | Count | 4 | 10 | 14 | 9 | 37 |
| % within GSE_1_2 | 10.8% | 27.0% | 37.8% | 24.3% | 100.0% |
| % within SL_1 | 20.0% | 31.3% | 46.7% | 50.0% | 37.0% |
| % of Total | 4.0% | 10.0% | 14.0% | 9.0% | 37.0% |
| **High** | Count | 2 | 5 | 6 | 6 | 19 |
| % within GSE_1_2 | 10.5% | 26.3% | 31.6% | 31.6% | 100.0% |
| % within SL_1 | 10.0% | 15.6% | 20.0% | 33.3% | 19.0% |
| % of Total | 2.0% | 5.0% | 6.0% | 6.0% | 19.0% |
| **Total** | Count | 20 | 32 | 30 | 18 | 100 |
| % within GSE_1_2 | 20.0% | 32.0% | 30.0% | 18.0% | 100.0% |
| % within SL_1 | 100.0% | 100.0% | 100.0% | 100.0% | 100.0% |
| % of Total | 20.0% | 32.0% | 30.0% | 18.0% | 100.0% |

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FIGURE LEGENDS

Fig.1 Expression of GSE1 in tissues from gastric cancer patients and the association between GSE1 expression and patients’ survival rates.
A, Protein levels of GSE1 in gastric cancer tissues and normal gastric tissues were examined using immunohistochemistry. The magnifications of the photographs were 200 and 400. B, Kaplan-Meier curves were made to show the RFS and OS rates of gastric cancer patients with different GSE1 expression level.

Fig.2 GSE1 promotes cell proliferation and metastasis of human gastric cancer cells.
A, Protein levels of GSE1 were examined in human gastric cancer cells BGC-823, HGC-27, AGS, MKN-45 by using western blot. B, C, HGC-27 cells were transfected with shGSE1-1, shGSE1-2 or shNC. BGC-823 cells were transfected with GSE1 over-expressing pIRESneo3 plasmid or Vec control plasmid. Protein levels of GSE1 were examined using western blot. β-actin was used as a control. In HGC-27-shNC, HGC-27- shGSE1-1, HGC-27- shGSE1-2, BGC-823-Vect and BGC-823-GSE1 cells, D,G, total cell number assay was performed with an original cell number of 1x10^3 and tested every day for 5 days; E, H, MTT assay was performed to evaluate cell viability; F, I, cell colony formation assay was performed with an original cell number of 1000 and tested after 10-15 days; J, K, L, M, cell migration and invasion were determined using Transwell chamber; N, O, wound-healing assay was performed, with the photographs showing significant differences after 24 hours. * P<0.05. ** P<0.01.

Fig.3 GSE1 promotes tumor growth and metastasis of human gastric cancer cells in nude mice.
A, D, HGC-27-shNC / HGC-27-shGSE1-1 (designated as HGC-27-shGSE1) and BGC-823-Vect / BGC-823-GSE1 cells were subcutaneously injected into nude mice. Every cell was injected at 8 sites. Tumor volumes were evaluated every 3 days and tumors were harvested and tumor growth curves were made after 21 days. B, E, Weights of transplanted tumors were evaluated. C, F, Ki-67-stained percentage in sections of xenograft tumors was evaluated. G, HGC-27-shNC / HGC-27-shGSE1 and BGC-823-Vect / BGC-823-GSE1 cells were injected into the venous circulation of mice. After 40 days, mice were killed and their lungs were collected. Hematoxylin and eosin–stained sections showed the lung micrometastases as indicated by arrows. H, Numbers of mice with lung micrometastases of tumors and numbers of lung micrometastases in 5 random sections of each mouse were calculated. * P<0.05. ** P<0.01.

Fig.4 GSE1 regulates the expression of SLC7A5 in gastric cancer cells.
A, Heat map depicting the mRNA expression profile of mRNA sequencing in HGC-27-shNC and HGC-27-shGSE1-1 (designated as HGC-27-shGSE1) cells. Yellow squares correspond to base level of mRNAs; red squares correspond to down-regulated mRNAs; and green squares correspond to up-regulated mRNAs. B, C, Protein levels of GSE1 and SLC7A5 were examined using western blot in HGC-27 and MKN-45 cells transfected with shGSE1-1(designated as shGSE1) or shNC. D, E,
Protein levels of GSE1 and SLC7A5 were examined using western blot in BGC-823 and AGS cells transfected with GSE1 over-expressing plasmid or Vec control. \(\beta\)-actin was used as a control.

Fig. 5 GSE1 increased SLC7A5 transcript stability through a post-transcriptional manner.

A, mRNA levels of SLC7A5 after transfected with shGSE1 and shNC in HGC-27 and MKN-45 cells were examined using RT-qPCR. B, mRNA decay assay was performed in HGC-27 and MKN-45 cells after transfected with shGSE1 and shNC. mRNA levels of SLC7A5 were detected using RT-qPCR at 0, 2, 4, 6 and 8 hours after actinomycin D (10 \(\mu\)g/mL) treatment. C, mRNA levels of SLC7A5 after transfected with GSE1 over-expressing plasmid or Vec control in BGC-823 and AGS cells were examined using RT-qPCR. D, mRNA decay assay in BGC-823 and AGS cells after transfected with GSE1 over-expressing plasmid or Vec control. GAPDH was used as a control for RT-qPCR. E, Ribonucleoprotein (RNP) immunoprecipitation (IP) assay. mRNAs of SLC7A5 and negative control gene HER2 were enriched by anti-GSE1 antibody or control IgG and were analyzed by RT-qPCR. The total input mRNAs of SLC7A5 and HER2 were also examined. * \(P<0.05\); ** \(P<0.01\).

Fig. 6 SLC7A5 promotes cell proliferation and metastasis of human gastric cancer cells.

HGC-27 and MKN-45 cells were transfected with shGSE1-1, shGSE1-2 or shNC. A, Protein levels of SLC7A5 were examined using western blot. \(\beta\)-actin was used as a control. B, total cell number assay, C, MTT assay, D, cell colony formation assay were performed to evaluate cell proliferation. E, Cell migration assay, F, cell invasion assay, G, wound-healing assay were carried out to evaluate cell metastasis. * \(P<0.05\). ** \(P<0.01\).

Fig. 7 SLC7A5 mediates the oncogenic role of GSE1 in human gastric cancer cells (BGC-823 and AGS).

BGC-823 and AGS cells with forced expression of GSE1 were co-transfected with shSLC7A5-1 (designated as shSLC7A5) or shNC. A, Protein levels of GSE1 and SLC7A5 were examined using western blot. \(\beta\)-actin was used as a control. B, total cell number assay, C, MTT assay, D, cell colony formation assay were performed to evaluate cell proliferation. E, Cell migration assay, F, cell invasion assay, G, wound-healing assay were carried out to evaluate cell metastasis. * \(P<0.05\). ** \(P<0.01\).

Fig. 8 SLC7A5 mediates the oncogenic role of GSE1 in human gastric cancer cells (HGC-27 and MKN-45).

HGC-27 and MKN-45 cells were co-transfected with shGSE1-1 (designated as shGSE1) / shNC and SLC7A5 plasmid / Vec plasmid. A, Protein levels of GSE1 and SLC7A5 were examined using western blot. \(\beta\)-actin was used as a control. B, total cell number assay, C, MTT assay, D, cell colony formation assay were performed to evaluate cell proliferation. E, Cell migration assay, F, cell invasion assay, G,
wound-healing assay were carried out to evaluate cell metastasis. *P<0.05. **P<0.01.

Fig. 9 GSE1 is a direct target of miR-200b and miR-200c in gastric cancer cells.
A, Predicted binding site and mutant binding site between miR-200b / miR-200c and the 3’-UTR of GSE1. B, Luciferase assay of HGC-27 cells co-transfected with miR-200b / miR-200c mimics and a luciferase reporter-containing GSE1 wild-type 3’-UTR (3’UTR-WT) or GSE1 mutant 3’-UTR (3’UTR-MUT). C, Protein levels of GSE1 were examined using western blot in HGC-27 and MKN-45 cells transfected with miR-200b, miR-200c and NC mimics.

Fig.10 SLC7A5 and miR-200b / miR-200c expression levels in tissues from gastric cancer patients.
A, Protein levels of SLC7A5 in gastric cancer tissues and normal gastric tissues were examined using immunohistochemistry. The magnifications of the photographs were 200 and 400. B, miR-200b / miR-200c expression levels in gastric cancer tissues and normal gastric tissues were examined using RT-qPCR. C, GSE1 mRNA levels in fresh gastric cancer tissues were examined using RT-qPCR, and the correlation between GSE1 mRNA and miR-200b / miR-200c levels was analyzed. * P<0.05. **P<0.01.
Fig. 1

A

| Gastric cancer | Normal |
|----------------|--------|

GSE1

B

Relapse-free survival

Overall survival

Month after surgery

$P < 0.001$
**Fig. 3**

### A

**Tumor volume (mm³)**

| Days after injection | HGC-27-shNC | HGC-27-shGSE1 |
|----------------------|-------------|---------------|
| 7                    |             |               |
| 10                   |             |               |
| 13                   |             |               |
| 16                   |             |               |
| 19                   |             |               |
| 21                   |             |               |

### B

**Tumor weight (g)**

| shNC | shGSE1 |
|------|--------|
| 1.5  | 1.2    |
| 1.0  | 0.8    |

### C

**Ki-67 positive cells (%)**

| HGC-27-shNC | HGC-27-shGSE1 |
|-------------|---------------|
| 30%         | 40%           |

### D

**Tumor volume (mm³)**

| Days after injection | BGC-823-Vec | BGC-823-GSE1 |
|----------------------|-------------|--------------|
| 7                    |             |              |
| 10                   |             |              |
| 13                   |             |              |
| 16                   |             |              |
| 19                   |             |              |
| 21                   |             |              |

### E

**Tumor weight (g)**

| Vec | GSE1 |
|-----|------|
| 1.5 | 1.2  |
| 1.0 | 0.8  |

### F

**Ki-67 positive cells (%)**

| BGC-823-Vec | BGC-823-GSE1 |
|-------------|--------------|
| 30%         | 40%          |

### G

- **HGC-27-shNC**
- **HGC-27-shGSE1**
- **BGC-823-Vec**
- **BGC-823-GSE1**

### H

|                          | HGC-27-shNC | HGC-27-shGSE1 |
|--------------------------|-------------|---------------|
| **Lung metastasis**      | 4/8         | 0/8           |
| *P value χ² test*        |             | 0.021         |

|                          | BGC-823-Vec | BGC-823-GSE1 |
|--------------------------|-------------|--------------|
| **Lung metastasis**      | 3/8         | 7/8          |
| *P value χ² test*        |             | 0.039        |
Fig. 4

A

shNC  shGSE1

PER3  CTH  LAPTM5  DPM3  RAB3GAP2  AKR1C2  SLC7A5  TNFRSF1B  TINAGL1  ADC  IFI44L  IFI44  PALMMD  CSF1

Low  High

B

shNC  shGSE1

GSE1  1.00  0.24
SLC7A5  1.00  0.28
β-ACTIN  1.00  0.35

GSE1  1.00  2.11
SLC7A5  1.00  2.82

C

shNC  shGSE1

GSE1  1.00  0.31
SLC7A5  1.00  3.26

D

Vec  GSE1

GSE1  1.00  2.17
SLC7A5  1.00  3.26
Fig. 8

| shNC+Vec | shGSE1+Vec | shGSE1+SLC7A5 |
|----------|------------|---------------|
| HGC-27   | 0h         | 24h           |
| MKN-45   | 0h         | 24h           |

**A**

| GSE1  | 1.00   | 0.07   | 0.06   |
|-------|--------|--------|--------|
| SLC7A5| 1.00   | 0.58   | 1.96   |

**B**

**C**

**D**

**E**

**F**

**G**

**HGC-27**

**MKN-45**

**Cell Migration**

**Cell Invasion**

**Wound Healing**
A

GSE1-3'UTR-WT  5'- CUCAGACGAAUGUACCGCCAGUAUUA-3'
miR-200b  3'- AGUAGUAAUGGUCCGUCAUAAU -5'
miR-200c  3'- AGGUAGUAAUGGGCGCGUCAUAAU -5'
GSE1-3'UTR-MUT  5'- CUCAGACGAAUGUACCGCCUCUACCA-3'

B

C

Fig. 9
Fig. 10

SLC7A5
Gastric cancer                Normal
200X
400X
rs = -0.65047
P < 0.0001

rs = -0.54332
P < 0.0001

200μm 200μm
100μm 100μm

C

GSE1 mRNA levels

miR-200b levels

r_s = -0.65047
P < 0.0001

miR-200c levels

r_s = -0.54332
P < 0.0001
GSE1 predicts poor survival outcome in gastric cancer patients by SLC7A5 enhancement of tumor growth and metastasis
Keshuo Ding, Sheng Tan, Xing Huang, Xiaonan Wang, Xiaocan Li, Rong Fan, Yong Zhu, Peter E. Lobie, Wenbin Wang and Zhengsheng Wu

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