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Long non-coding RNA SNHG1 suppresses cell migration and invasion and upregulates SOCS2 in human gastric carcinoma

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ABSTRACT

Gastric carcinoma (GC) is one of the most common malignancies and the third leading cause of cancer-related deaths worldwide. Long noncoding RNAs (lncRNAs) may be an important class of functional regulators involved in human gastric cancers development. In this study, we investigated the clinical significance and function of lncRNA SNHG1 in GC. SNHG1 was significantly downregulated in GC tumor tissues compared with adjacent noncancerous tissues. Overexpression of SNHG1 in BGC-823 cells remarkably inhibited not only cell proliferation, migration, invasion *in vitro*, but also tumorigenesis and lung metastasis in the chick embryo chorioallantoic membrane (CAM) assay *in vivo*. Conversely, inhibition of SNHG1 by transfection of siRNA in AGS cells resulted in opposite phenotype changes. Mechanically, SNHG1 was found interacted with ILF3, NONO and SFPQ. RNA-seq combined with bioinformatic analysis identified a serial of downstream genes of SNHG1, including SOCS2, LOXL2, LTBP3, LTBP4. Overexpression of SNHG1 induced SOCS2 expression whereas knockdown of SNHG1 decreased SOCS2 expression. In addition, knockdown of SNHG1 promoted the activation of JAK2/STAT signaling pathway. Taken together, our data suggested that SNHG1 suppressed aggressive phenotype of GC cells and regulated SOCS2/JAK2/STAT pathway.

1. Introduction

Gastric carcinoma (GC) is one of the most common malignancies and the third leading cause of cancer-related deaths worldwide [1]. Although great advances in surgical techniques and adjuvant treatment have been made, the prognosis of patients with gastric cancer remains poor [2]. Therefore, understanding the molecular mechanisms underlying GC tumorigenesis and metastasis is urgently needed.

Long noncoding RNAs (lncRNAs) are a class of noncoding RNAs with lengths greater than 200 nucleotides [3]. A growing number of studies have revealed that lncRNAs participate in a wide range of biological processes, and aberrant lncRNAs expression is involved in diverse human diseases, including cancers [4]. Many lncRNAs have been found to play pivotal roles in GC development, such as TINCR, GHET1, and HOTAIR, which were reported to regulate proliferation and/or metastasis in GC cells [5–7]. These findings suggested that lncRNAs play crucial roles in GC carcinogenesis and have great impacts on GC clinical application.

The lncRNA small nucleolar RNA host gene 1 (SNHG1), has been reported dysregulated in several types of cancers, such as glioma [8], osteosarcoma [9], colorectal cancer [10], hepatocellular carcinoma [11], esophageal cancer [12] and gastric cancer [13]. Mechanically, SNHG1 could regulate the expression of MYC through interacting with FUBP1 and regulate p53 expression and activity by competing with p53 for binding to hnRNPC [14,15]. SNHG1 could also mediates its biological functions through sequestration of microRNAs, including miR-361–3p in non-small-cell lung cancer [16], miR-145 in colorectal cancer [10], miR-195 in pancreatic cancer [17], etc. However, the biological role and underlying mechanisms of SNHG1 in GC have not yet been clearly clarified. In this study, we aim to investigate and identify the biological functions and gene regulatory networks of action of SNHG1 in GC.

2. Materials and methods

2.1. Cell lines and cell culture

Human GC cell lines (SGC-7901, AGS, BGC-823 and MGC-803) were

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purchased from the American Type Culture Collection (ATCC) and Chinese Academy of Medical Sciences & Peking Union Medical College. Cells were cultivated in RPMI 1640 containing 10% FBS, 100U/ml penicillin, and 100 mg/ml streptomycin in a 5% CO_2 humidified incubator at 37 °C.

2.2. Patients and clinical specimens

The primary paired samples were collected from Beijing Cancer Hospital, Beijing, China. This study was approved by the medical ethics committee of the Beijing Cancer Hospital & Institute for Medical Research Ethics and all patients have given informed consent for the use of material for research purposes.

2.3. Plasmid construction

SNHG1 cDNA was amplified by PCR and cloned into the vector plenti6-TR backbone (Invitrogen). The small interfering (si) RNA targeting SNHG1 was designed and synthesized by RiboBio (Guangzhou, China).

2.4. Western blotting analysis

Equal amounts of protein were subjected to SDS-PAGE and transferred to PVDF membranes (Millipore, Bedford, MA, USA). The membranes were incubated with primary antibodies, including rabbit anti-ILF3 (1:2000, ab93255, Abcam), rabbit anti-SFPQ (1:2000, ab133574, Abcam), rabbit anti-NONO (1:2000, ab177149, Abcam), rabbit anti-GAPDH (1:5000, 5174, Cell Signaling Technology), rabbit anti-H3 (1:5000, 9728, Cell Signaling Technology), rabbit anti-SOCS2 (1:2000, ab109245, Abcam), rabbit anti-pJAK2 (1:2000, ab32101, Abcam), rabbit anti-JAK2 (1:2000, ab108596, Abcam), rabbit anti-pSTAT5 (1:2000, ab32364, Abcam), rabbit anti-STAT5 (1:2000, ab200341, Abcam), rabbit anti-pSTAT3 (1:2000, ab76315, Abcam), rabbit anti-STAT3 (1:2000, ab68153, Abcam). HRP-linked secondary antibody was used as the secondary antibody. Signals were visualized with chemiluminescence (Millipore, MA, USA).

2.5. Quantitative real-time PCR (qRT-PCR) analysis

Quantitative real-time PCR (qRT-PCR) was performed using SYBR Green PCR Master Mix (Toyobo, Osaka, Japan) on ABI7500 System (Applied Biosystems, CA, USA). The expression level of gene was calculated by the $2^{-\Delta Ct}$ method. The sequences of all primers are listed in Supplementary Table S1.

2.6. RNA pull-down and RNA immunoprecipitation (RIP)

RNA pull-down assay was performed using a Pierce Magnetic RNA-Protein Pull-Down Kit following the manufacturer's instructions (ThermoFisher Scientific). The purified SNHG1 interacting proteins were subjected to SDS-PAGE, visualized using Coomassie blue staining and analyzed by regular liquid chromatography-mass spectrometry (LC-MS) analysis (micrOTOFQ II LC/MS QTOF). Central laboratory of Peking University Cancer Hospital & Institute provided technical guidance and assistance for the specific procedures of LC-MS. RIP was performed as previously described [18]. The coprecipitated RNAs were detected by reverse transcription PCR and qRT-PCR.

2.7. Differential expression analysis by RNA-sequencing (RNA-seq)

RNA-sequencing was performed as previously described [19]. Differentially expressed genes were identified using a criterion of a fold change >1.5. The differentially expressed genes were listed in Supplementary Table S2.

2.8. RNA FISH assay

RNA FISH was performed using digoxin-conjugated SNHG1 probes generated by Boster Biological Technology following the manufacturer's protocol (Boster Biological Technology, Wuhan, China).

2.9. Cell proliferation assay

Cell proliferation was evaluated by Cell Counting Kit-8 (CCK; Dojindo, Kumamoto, Japan) following the manufacturer's instructions. In colony formation assay, 500 cells/well were seeded into 6-well plate and cultured in RPMI 1640 medium containing 10% FBS. The colonies were stained with crystal violet and counted after 2 weeks.

2.10. Ethynyl-2-deoxyuridine (EdU) incorporation assay

Cell proliferation ability was tested by EdU incorporation assay using iClickTM EdU Andy Fluor 555 Imaging Kit (GeneCopoeia, Rockville, Md, USA) according to manufacturer's instructions. The cells were visualized and photographed under a fluorescence microscopy. The percentage of EdU positive cells was calculated as (EdU staining cells/Hoechst staining cells) × 100%.

2.11. Transwell migration and invasion assays

Cells (1×10^5) were suspended in 100 μL serum-free RPMI 1640 medium and seeded into the upper chamber of the Transwell insert with or without Matrigel. RPMI 1640 containing 10% FBS in the lower chamber served as the chemoattractant. The cells on the lower side of the membrane were counted in five randomly selected microscopic fields and photographed.

2.12. In vivo tumor growth and metastasis assay

The growth and metastatic characteristics of the cells were measured by a modified chick embryo chorioallantoic membrane (CAM) assay as previously described [20]. The sex of chick embryo has no influence on the results in CAM assay, therefore the chick embryo was randomly used without sex identification in this study. Animal studies were approved by the institutional guidelines of Peking University Cancer Hospital Animal Care Committee, and all experiments were performed in accordance with the Principles of Laboratory Animal Care (NIH publication NO. 85Y23, revised 1996).

2.13. Statistical analysis

SPSS 16.0 software (SPSS Inc., Chicago, IL, USA) was used to perform the statistical analyses. All values are expressed as means ± SD of at least 3 independent experiments. All comparisons were analyzed with two-sided Student's t-test, unless specified. A p value of <0.05 was considered significant.

3. Results

3.1. Overexpression of SNHG1 inhibits cell proliferation, migration and invasion in BGC-823 cells in vitro and in vivo

We investigated the expression of SNHG1 in a cohort of 96 pair primary GC samples using qRT-PCR, and found that SNHG1 expression decreased in GC tumor tissues compared with adjacent non-neoplastic tissues (supplementary Fig. 1A, $p < 0.0001$). Meanwhile, we analyzed the expression data of GC from Gene Expression Omnibus (GEO), including GSE81948, GSE103236, GSE118916, GSE3438, GSE51575, GEO2R was used to compare the tumor and adjacent normal tissues in order to identify differentially expressed genes. The results showed that SNHG1 was significantly downregulated to 0.28, 0.60, 0.60, 0.78, and

0.75 in tumor tissues compared to adjacent normal tissues in these GEO datasets, respectively ($p < 0.05$) (supplementary Table S3, $p < 0.0001$). The results of qRT-PCR analysis of SNHG1 in GC cell lines showed that AGS cells expressed higher level of SNHG1, and SGC-7901, MGC-803, and BGC-823 cells expressed relative lower level of SNHG1 (supplementary figure 1B). Therefore, we performed gain-of-function with BGC-823 and SGC-7901 cells, whereas loss-of-function with AGS cells to examine the biological functions of SNHG1 in GC.

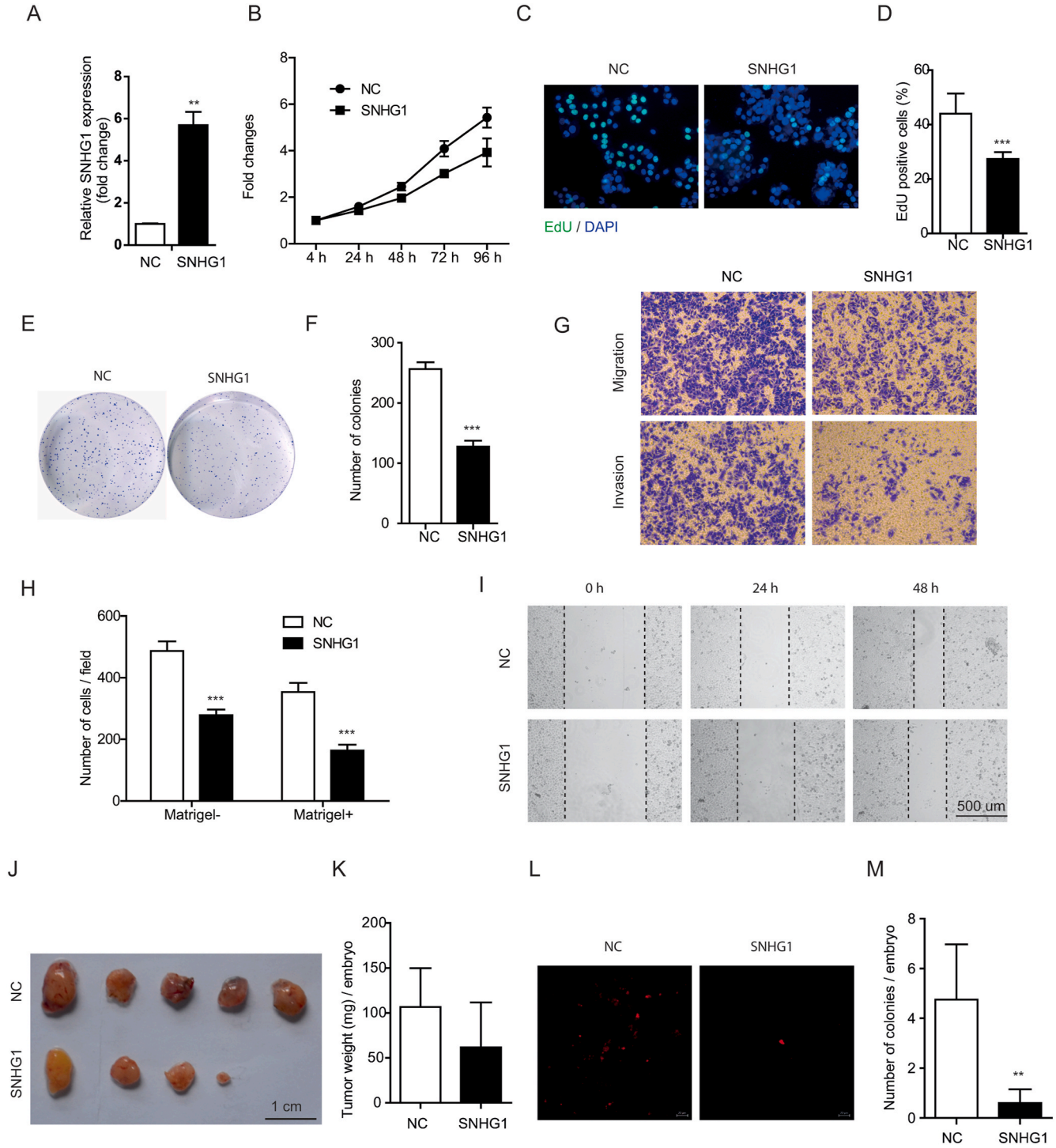

Fig. 1. Overexpression of SNHG1 inhibits migration and invasion in GC cells. (A) SNHG1 expression was detected by qRT-PCR. (B) The effect of SNHG1 overexpression on BGC-823 cells viability was detected by CCK8 analysis. (C, D) Proliferation of cells was measured by EdU (Green) uptake. The nucleus was stained with DAPI (blue) and images were taken on a Nikon fluorescence microscope (magnification × 200). (E, F) The effect of SNHG1 overexpression on BGC-823 cells colony formation. (G, H) The effects of SNHG1 overexpression on migration and invasion were determined by Transwell assay. Quantitative results are illustrated in H. (I) Images of the wound closure of monolayer SNHG1 overexpressing BGC-823 cells and control cells. (J, K) The effect of SNHG1 overexpression on tumor growth was measured by CAM assay. Weights of tumors were measured and showed in K. (n = 5) (L, M). Lung metastasis was identified by Dil-staining cell colonies under a fluorescence microscope, and the quantitative results are illustrated in M. $^{**}p < 0.01$ and $^{***}p < 0.001$. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

BGC-823 cells were infected with SNHG1-overexpression lentivirus, and then selected with blasticidin to establish stable SNHG1 overexpressing cell line. QRT-PCR analysis verified that SNHG1 was successfully overexpressed in BGC-823 cells (Fig. 1A). CCK8 assays showed that the proliferation of BGC-823 cells was inhibited by SNHG1 overexpression (Fig. 1B). In EdU incorporation assays, SNHG1-overexpressing cells showed lower EdU staining positive percentage, indicating a decreased number of S phase cells in SNHG1 overexpression group (Fig. 1C and D). Moreover, fewer colonies were observed in SNHG1 overexpressing cells compared to control cells in colony

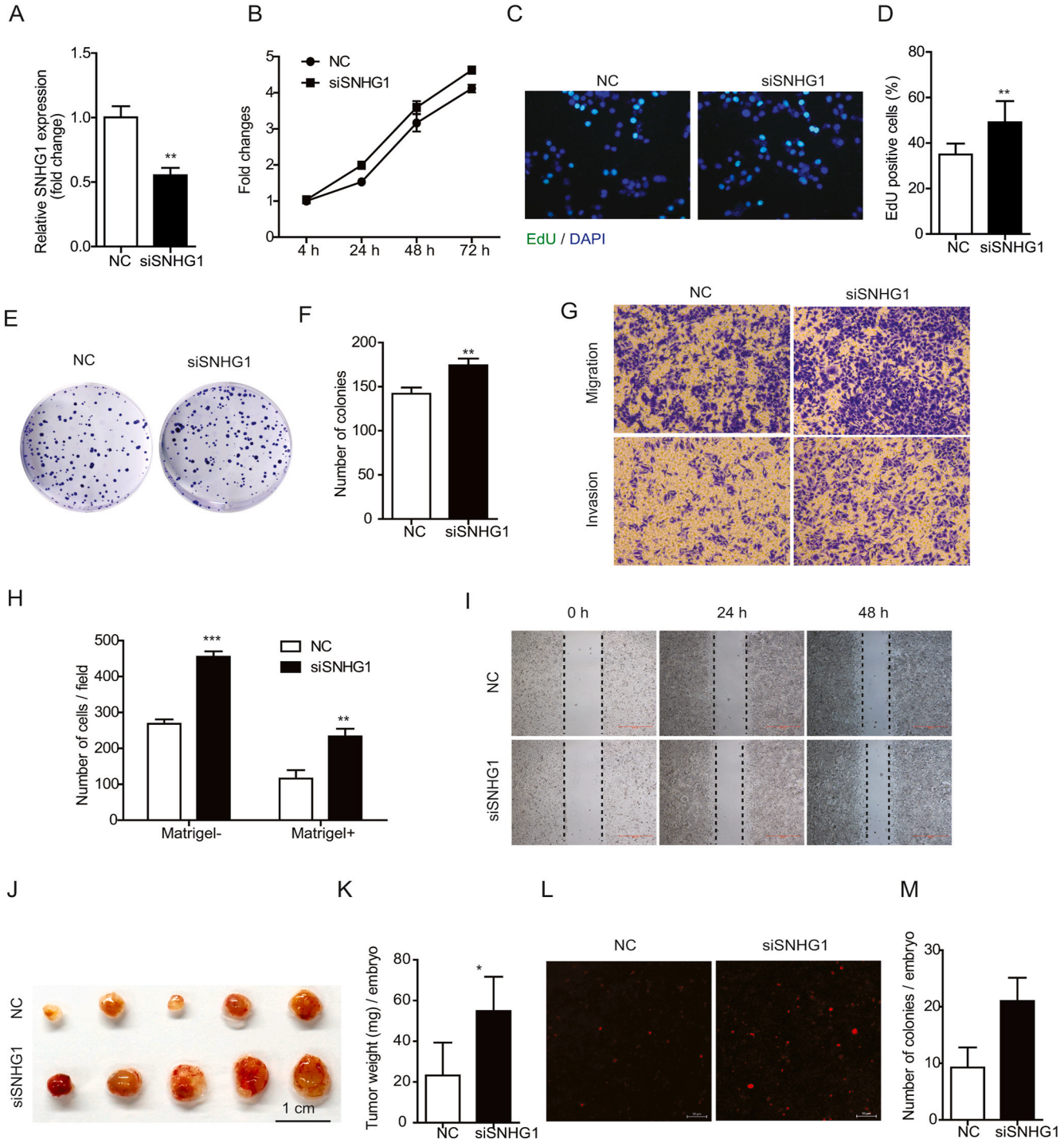

Fig. 2. Knockdown of SNHG1 enhances GC cell migration and invasion. (A) SNHG1 expression was detected by qRT-PCR. (B) The effect of SNHG1 knockdown on AGS cells viability was detected by CCK8 analysis. (C, D) Proliferation of cells was measured by EdU (Green) uptake. The nucleus was stained with DAPI (blue) and images were taken on a Nikon fluorescence microscope (magnification × 200). (E, F) The effect of SNHG1 knockdown on AGS cells colony formation. (G, H) The effects of SNHG1 knockdown on cell migration and invasion were determined by Transwell assay. Quantitative results are illustrated in H. (I) Images of the wound closure of monolayer AGS cells transfected with siSNHG1 or siNC. (E) (J, K) The effect of SNHG1 knockdown on tumor growth was measured by CAM assay. Weights of tumors were measured and showed in K. (n = 5) (L, M). Lung metastasis was identified by Dil-staining cell colonies under a fluorescence microscope, and the quantitative results are illustrated in M. $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

formation assay (Fig. 1E and F). Transwell assay demonstrated that SNHG1 overexpression attenuated BGC-823 cells migration and invasion through Matrigel (Fig. 1G and H). Overexpression of SNHG1 also inhibited the migration of BGC-823 cells in wound-healing assay (Fig. 1I). Consistent with the results in BGC-823 cells, overexpression of SNHG1 in SGC-7901 cells also repressed the proliferation, migration and invasion of SGC-7901 cells *in vitro* (supplementary figure 2). To validate the result of studies *in vitro*, we examined the role of SNHG1 on tumor growth and metastasis *in vivo* using modified chick embryo chorioallantoic membrane (CAM) assay. The results showed that tumor weight was decreased in SNHG1 overexpression group compared with control group (Fig. 1J and K). Furthermore, SNHG1 overexpression decreased the metastastic lung lesions of BGC-823 cells (Fig. 1L and M). Collectively, these data indicated that ectopic overexpression of SNHG1 suppressed GC tumorigenesis and metastasis *in vitro* and *in vivo*.

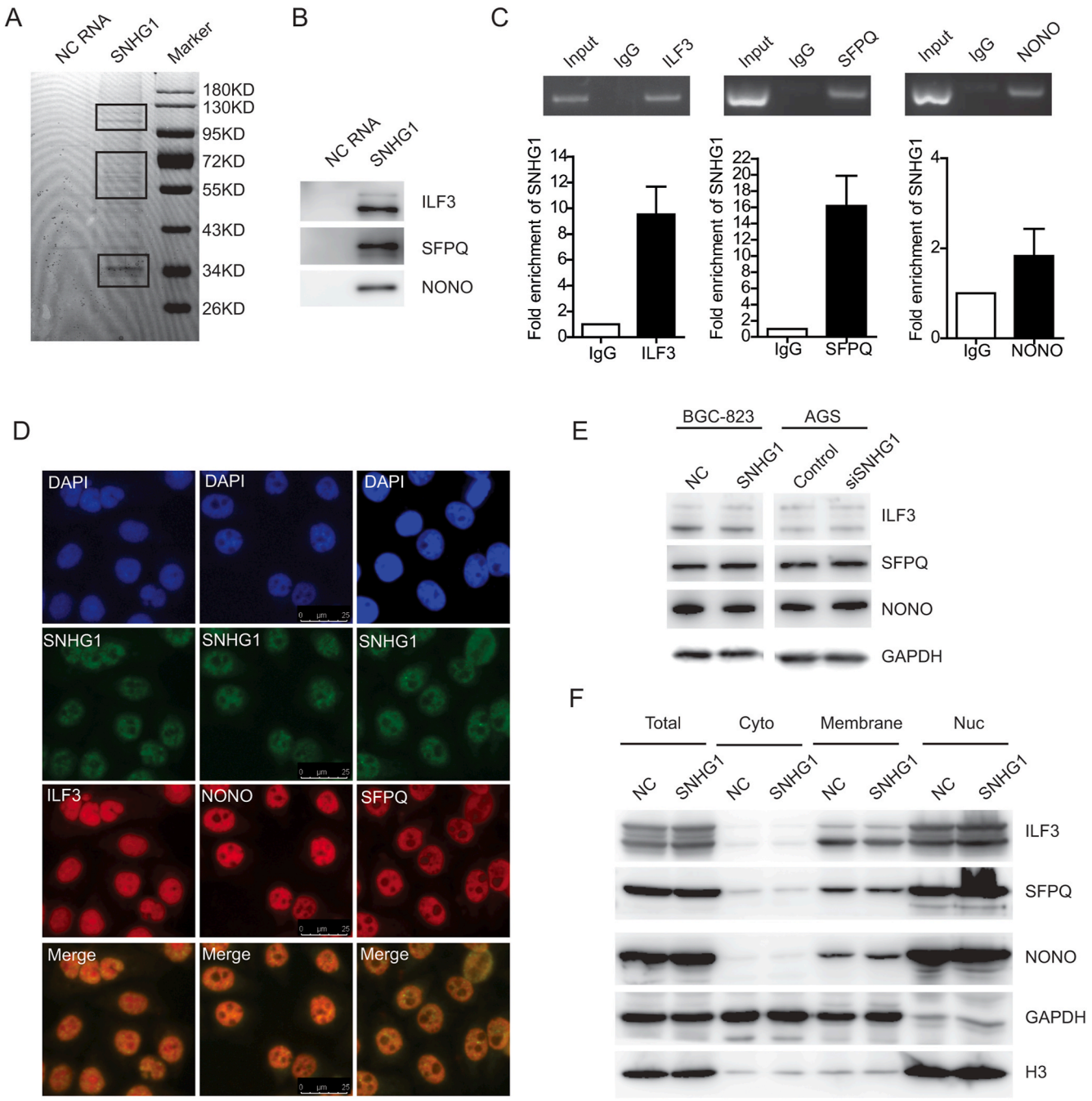

Fig. 3. SNHG1 interacts with ILF3, SFPQ, and NONO. (A) Coomassie blue staining of the SDS-PAGE gel containing proteins pulled down by SNHG1 and negative control RNA. The boxes indicate the gels cutted for mass-spectrum. (B) ILF3, SFPQ and NONO protein levels in immunoprecipitates were evaluated by western blots. (C) SNHG1 RNA levels in RIP were determined by qRT-PCR. SNHG1 RNA expression levels are presented as fold enrichment values relative to IgG immunoprecipitate. (D) FISH and IF analysis of the co-localization of SNHG1 (green) and ILF3, SFPQ and NONO protein (red) in BGC-823 cells. (E) ILF3, SFPQ and NONO protein levels in BGC-823 cells transfected with SNHG1 overexpressing vector or empty vector and AGS cells transfected with siSNHG1 and siNC. (F) The protein levels of ILF3, SFPQ and NONO in the cytoplasmic, nuclear, and integral membrane fraction of BGC-823 cells overexpressing SNHG1 cells and control cells were detected by western blots. $^{**}p < 0.01$ and $^{***}p < 0.001$. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

3.2. Knockdown of SNHG1 enhances AGS cells proliferation, migration and invasion

We further examined the effects of SNHG1 knockdown in AGS cells. QRT-PCR showed decreased SNHG1 expression in AGS cells transfected with siRNA targeting SNHG1 (Fig. 2A). CCK8 assays demonstrated that AGS cells proliferation was increased by SNHG1 knockdown (Fig. 2B). Meanwhile, the EdU staining positive percentage was increased in SNHG1 knockdown cells compared with the control cells (Fig. 2C and D). The colony number was also increased in SNHG1 knockdown group (Fig. 2E and F). In transwell assay, the migration and invasive ability of the AGS cells was also dramatically enhanced by SNHG1 knockdown (Fig. 2G and H). Wound-healing assay results also showed that knockdown of SNHG1 promoted the migration of AGS cells (Fig. 2I).

To further confirm the results *in vitro*, AGS cells transfected with negative control or siSNHG1 RNA were seed onto CAM. The results showed that SNHG1 knockdown increased the tumor weight of AGS cells (Fig. 2J and K), and the metastatic cancer cells to embryo lungs were also increased in SNHG1 knockdown group compared to the control group (Fig. 2L and M). These data indicated that knockdown of SNHG1 enhances the AGS cell migration and invasion both *in vitro* and *in vivo*.

3.3. SNHG1 interacts with ILF3, SFPQ and NONO

Accumulating evidence suggests that many lncRNAs could participate in molecular regulation pathways via binding with proteins. To search the interacting proteins of SNHG1, we performed RNA pull-down assay using desthiobiotinylation-labelled SNHG1 and BGC-823 cell lysates. As shown in Fig. 3A, three clusters of protein bands were found to interact with SNHG1. The proteins that bound to SNHG1 were identified using liquid chromatography-mass spectrometry and listed in supplementary Table S4. Western blot results showed that ILF3, SFPQ and NONO were detected in SNHG1-pulled down sample but can't be detected in negative control RNA group (Fig. 3B). RNA immunoprecipitation (RIP) assays were performed to confirm the interaction between SNHG1 and ILF3, SFPQ and NONO. As expected, qRT-PCR analysis revealed that SNHG1 was enriched in the RIP samples obtained from the specific antibodies against ILF3, SFPQ and NONO (Fig. 3C).

Since the subcellular localization of lncRNA is critical in providing valuable information regarding its potential functions, we applied FISH combining immunofluorescence staining to localize SNHG1 in GC cells. The results showed that SNHG1 mainly localizes in the nuclei of BGC-823 cells (supplementary figure 3A). FISH combined with immunofluorescence was used to detected the colocalization of SNHG1 and the interacting proteins. The results showed that SNHG1 was colocalized

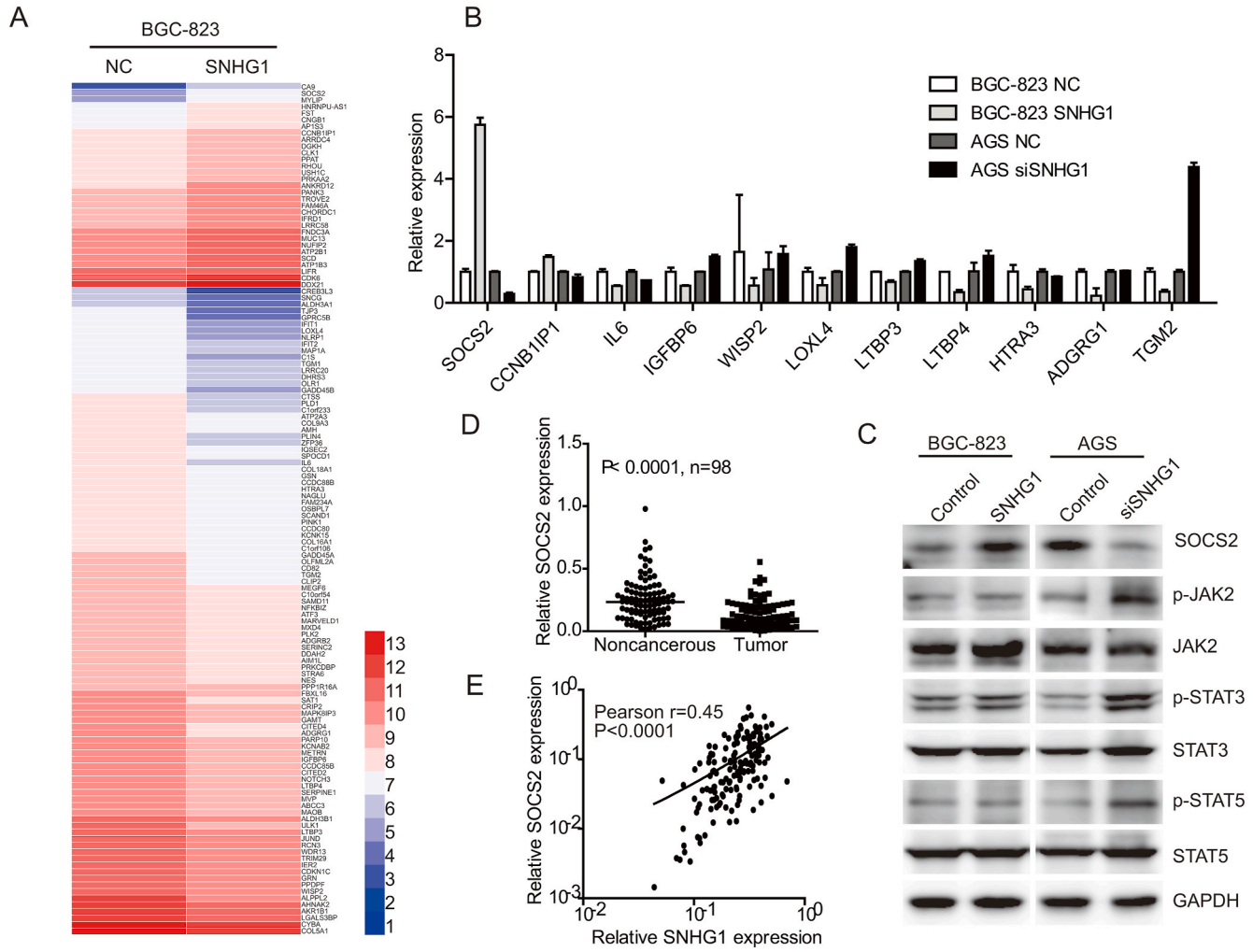

Fig. 4. SNHG1 increases SOCS2 expression and regulate the JAK/STAT signaling pathway in AGS cells (A) Hierarchically clustered heatmap of differentially expressed genes in SNHG1 overexpressing BGC-823 cells and control cells. (B) qRT-PCR analysis of differentially expressed genes in SNHG1 overexpressing BGC-823 cells and control cells and AGS cells transfected with siSNHG1 and siNC. (C) Western blots of SOCS2 and JAK/STAT signaling pathway proteins in SNHG1 overexpressing BGC-823 cells and control cells and AGS cells transfected with siSNHG1 and siNC. (D) Relative expression of SOCS2 in 98 paired GC tissues and adjacent noncancerous tissues. (E) Correlation analysis of the relationship between SNHG1 and SOCS2 expression levels in 150 GC tumor tissues. $^{**}p < 0.01$ and $^{***}p < 0.001$.

with ILF3, SFPQ and NONO in nucleus of BGC-823 cells (Fig. 3D). The specificity of the antibody was confirmed in mmunofluorescence assay using rabbit IgG and rabbit anti-ILF3 antibodies (supplementary figure 3B). To determine whether SHNG1 regulates the interacting proteins expression levels or subcellular localization, we performed cytoplasmic/nuclear fractionation and Western blot analysis. The results showed that SNHG1 overexpression in BGC-823 cells or knockdown in AGS cells have no effect on the total protein expression level (Fig. 3E). Moreover, the cytoplasmic/nuclear distribution of ILF3, SFPQ and NONO proteins showed no obvious changes in SNHG1 overexpressing BGC-823 cells compared to control cells (Fig. 3F).

3.4. SNHG1 increases SOCS2 expression and regulates the JAK/STAT signaling pathway in AGS cells

To explore the whole-transcriptome changes induced by SNHG1, differential gene expression analysis (DGE) was conducted in SNHG1 overexpressing BGC-823 cells and control cells by RNA-seq. Expression of 129 genes were found to be altered following SNHG1 overexpression, with 98 genes downregulated and 31 genes upregulated (Fig. 4A). Gene ontology analysis of differentially expressed genes showed that the biological processes including aging, positive regulation of response to external stimulus, extracellular matrix organization, collagen metabolic process and regulation of epithelial cell apoptotic process were significantly enriched. Then, we selected 11 key regulator genes involved in these pathways and verified whether these genes could respond to SNHG1 overexpression and knockdown. The results showed that overexpression of SNHG1 elevated the mRNA level of tumor suppressor genes including SOCS2 and CCNB1IP1, and downregulated the mRNA level of oncogenes including IL6, IGFBP6, LOXL4, LTBP3, LTBP4, WISP2, and TGM2 (Fig. 4B). Meanwhile, knockdown of SNHG1 in AGS cell resulted in the opposite expression pattern of these genes. Moreover, we analyzed their correlations with SNHG1 using the data download from TCGA for stomach adenocarcinoma (STAD). Consistent with the qRT-PCR results, the correlation analysis displayed positive correlation of CCNB1IP1 with SNHG1, and negative correlations between SNHG1 and IL6, IGFBP6, LOXL4, LTBP3, LTBP4, WISP2 and TGM2 (supplementary Figure 3C).

SOCS2, the suppressor of cytokine signaling 2, has been shown to inhibit signaling by IL-6, GH, insulin-like growth factor (IGF-1), and prolactin, and can regulate the cytokine-dependent JAK/STAT signaling pathway in several systems [21]. Western blot analysis confirmed that SOCS2 protein was increased in SNHG1 overexpressing BGC-823 cells and decreased in SNHG1 knockdown AGS cells compared to control cells (Fig. 4C). Furthermore, SOCS2 expression was downregulated in GC tumor tissues compared to adjacent noncancerous tissues (Fig. 4D), and was positively correlated with SNHG1 in GC tumor tissues (Fig. 4E). Consistently, the phosphorylation of JAK2, STAT3 and STAT5 was increased in SNHG1 knockdown AGS cells in comparison to control cells, although the phosphorylation of JAK2, STAT3 and STAT5 showed no obvious difference between SNHG1 overexpressing BGC-823 and control cells (Fig. 4C). These data suggested that SNHG1 acts as a regulator involved in the SOCS2/JAK/STAT signaling pathway, and SOCS2 may contribute to the inhibitory effect of SNHG1 on GC metastasis. However, the molecular mechanisms between them need to be further explored.

4. Discussion

LncRNAs have been reported as critical players in tumorigenesis and cancer progression processes [22]. In this study, we found that SNHG1 was significantly downregulated in GC tissues and exerted inhibitory effects in GC cell proliferation, migration and invasion using loss- and gain-of-function assays, suggesting that SNHG1 functions as a tumor suppressor gene in GC.

Recent studies suggested that SNHG1 might be served as a novel regulator of GC prognosis and potential therapeutic target for GC treatment [23]. Hu et al. demonstrated that SNHG1 expression was significantly higher in GC tissues compared to adjacent tissues and was correlated with TNM stage and lymph node metastasis [13]. SNHG1 accelerated the proliferation of GC cells via increasing the expression of DNMT1 [13]. However, the molecular mechanism underlying the upregulating of DNMT1 by SNHG1 was not explored. Guo et al. showed that SNHG1 promoted HGC-27 cell growth and migration via the miR-140/ADAM10 axis [24]. Liu et al. showed that lncRNA SNHG1 could sponge miR-15 b and regulate the effects of DCLK1/Notch1 on EMT process [25]. Proper subcellular localization of a lncRNA is essential for its functions, thus SNHG1 functions as a ceRNA regulating the expression of its target genes in GC cells implied its cytoplastic localization. In this study, we observed that SNHG1 transcript was mainly abundant in the nucleus of GC cell line and colocalized with the nucleus proteins including ILF3, NONO and SFPQ, which was consistent with the previous report showing the chromatin-association of SNHG1 [26]. Shen et al. demonstrated that nucleus-overexpressed SNHG1 increased the p53 protein level and its transcriptional activity, subsequently induced the expression of p21 and PUMA, which make nucleus-retained SNHG1 dominates cell proliferation inhibition [14]. Consistent with Shen's study, overexpression of SNHG1 inhibited the proliferation and migration of BGC-823 and SGC-7901 cells. These results suggested that the subcellular localization is critical in SNHG1 mediating its functional role in GC cells.

ILF3, NONO and SFPQ are well-known RNA-binding proteins [27,28]. Overexpression of ILF3 was reported in non-small cell lung carcinoma [29], nasopharyngeal carcinoma [30], ovarian cancer [31], and hepatocellular carcinoma [32]. SFPQ forms a heterodimer with NONO to build the nuclear paraspeckle complex and regulate mRNA processing, DNA repair, and transcriptional activity [33]. Previous study showed that SFPQ and NONO involved in prostate cancer progression through promoting splicing events of AR transcripts and AR-V7 production [34]. In this study, we found SNHG1 interacted with ILF3, NONO and SFPQ without affecting their expression level and localization. Therefore, the functional significances of the RNA-protein interaction between SNHG1 and ILF3, NONO and SFPQ still need to be further explored.

RNAseq of SNHG1 overexpressing BGC-823 and control cells demonstrated a panel of differentially expressed genes regulated by SNHG1. SOCS2 showed the highest fold change among the upregulated genes. SOCS2 is well known to regulate the growth hormone (GH), insulin-like growth factor 1 (IGF-1) and prolactin signaling pathways, and mainly characterized as a negative feedback regulator of cytokine receptor signal transduction via the JAK/STAT pathway [35]. SOCS2 has been reported to act as a tumor suppressor gene in numerous tumors, including pulmonary adenocarcinoma, ovarian cancer, breast cancer, and anal cancers, hepatocellular carcinoma, prostate cancer and gastric cancer [36]. We observed that SOCS2 was positively correlated with SNHG1 expression in GC tumor tissues. Consistently, Knockdown of SNHG1 in AGS cells decreased the SOCS2 mRNA and protein levels and elevated the *p*-JAK2, p-STAT3 and p-STAT5 expression levels. These data suggested that SNHG1 might inhibit the proliferation and metastasis of GC cells through promoting the expression of the SOCS2 gene.

In conclusion, SNHG1 was significantly downregulated and acted as a tumor suppressor in GC. SNHG1 inhibited GC cell growth, migration and invasion and regulated the JAK2/STAT signaling pathway. For the first time we demonstrated SNHG1 can bind with SFPQ, NONO, and ILF3. These findings further the understanding of lncRNAs function and mechanism in GC pathogenesis and progression.

Declaration of competing interest

The authors declare no conflict of interests.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrep.2021.101052.

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