LncRNA ELFN1-AS1 Upregulates TRIM29 to Promote Gastric Cancer Progression by Suppressing miR-211-3p

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Research

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Abstract

Background

Extracellular leucine rich repeat and fibronectin type III domain containing 1-antisense RNA 1 (ELFN1-AS1) was upregulated in tumors. Nevertheless, the biological functions of ELFN1-AS1 in gastric cancer are not fully understood.

Methods

The ELFN1-AS1, miR-211-3p and TRIM29 expression levels were determined by reverse transcription-quantitative PCR. CCK8, EDU and colony formation assays were done to test the GC cell vitality. The migratory and invasive capabilities of GC cells were further measured by transwell invasion and cell scratch assays. The ceRNA activity of ELFN1-AS1 for TRIM29 via miR-211-3pp was ascertained through pull down, RIP and luciferase reporter assays.

Results

ELFN1-AS1 and TRIM29 were robustly expressed in gastric cancer tissues and negatively associated overall survival time of patients. The ELFN1-AS1 silence blocked the proliferation, migration and invasion of GC cells. The oncogenic role of ELFN1-AS1 was recognized to be modulated by miR-211-3pp, which competitively bind to 3'UTR TRIM29 and resulted in the reduced expression of TRIM29.

Conclusion

ELFN1-AS1 maintained the tumorigensis of GC cells by ELFN1-AS1/miR-211-3pp/TRIM29 axis, suggesting that intervention targeting this axis may be warranted for GC treatment.

Introduction

Gastric cancer (GC) is a frequently diagnosed gastrointestinal cancer, with more than one million new cases and about 770, 000 mortalities in 2020 globally [1]. Currently, surgical debulking in combination with radiotherapy and chemotherapy favors improved clinical outcomes of GC patients. Notwithstanding, the metastatic GC patients missed the optimal treatment window because of a late diagnosis [2]. According to epidemiologic studies, genetics and ethnicity are associated with GC incidence and mortality burden besides helicobacter pylori infection, diet and lifestyle [3]. Therefore, discovering novel effective target for GC occurrence and progression is clinically required.

Long noncoding RNAs (lncRNAs) are one type of non-coding transcriptome greater than 200 nucleotides. Various studies have demonstrated that lncRNAs are commonly dysregulated in GC and implicated in GC as tumor suppressors or as oncogenes. lncRNAs predominantly have a functionality to reorchestrate gene expression and thereby influence the cancer genesis and progression [4]. For example, lncRNA MT1JP is a tumor suppressive lncRNAs that lessen gene expression in transcription, which enhanced

malignant phenotypes of GC [5]. Ectopically expressed lncRNA NMRAL2P are thought to account for DNA methyltransferases 3 activity with reduced expression of Acyl-CoA thioesterase 7 (ACOT7), implicated in gastric cancer development [6]. lncRNA ANRIL was documented as a modular scaffold to spatially recruit several proteins and epigenetically regulate gene expression involved in gastric cancer progression [7]. Since lncRNA constitutes a major part of human genome, deeper insight into the function of lncRNA in GC is of paramount importance. Currently, ELFN1-AS has been found to be highly regulated in esophageal cancer, ovarian cancer, colon adenocarcinoma and conferred a predisposition for cancer progression. However, none has been focused on the role of ELFN1-AS1 in GC progression.

Tripartite motif (TRIM) containing 29 (TRIM29) belonging to the TRIM protein family is characterized by multiple zinc finger motifs and a leucine zipper motif. It is reportedly functioned as a putative scaffold or adaptor in DNA double-strand break repair. Therefore, TRIM29 dysregulation is proposed to be implicated in various pathological disorders including tumors [8]. Emerging evidence has documented that TRIM29 exerts pro-oncogenic and tumor-suppressor role in a specific cancer type. For example, TRIM29 deficiency in hepatocellular carcinoma cells elicit Wnt/ β -catenin signaling pathway, consequently promoting cancer cell malignant phenotypes and accelerating cancer growth [9]. In contrast, profound overexpression of TRIM29 is detected in lung squamous cell carcinoma and high-TRIM29 patients show shortest survival [10]. In gastric cancer, highly expressed TRIM29 was tightly associated with clinical pathological characteristics and unfavorable prognosis [11]. Furthermore, TRIM29 is defined as an oncoprotein which shows pro-proliferative, migratory and invasive characteristics in vitro, by hyperactivation of Wnt/ β -catenin signaling pathway [12]. However, the concrete regulatory mechanism in GC is still fully determined.

In our present work, an unregulated lncRNA termed as ELFN1-AS1 was recognized in GC tissues from GEPIA database analysis. Following cellular functional assays validated ELFN1-AS1 as a pro-tumorigenic lncRNA with clinical importance. Mechanically, we examined that ELFN1-AS1 acted as a ceRNA of miR-211-3pp and enhanced the transcriptional activity of TRIM29 and promote GC cell progression. Our findings might enrich the understanding of the lncRNA function during GC malignancy and offer a novel clue to explore effective target for GC diagnosis and treatment.

Materials And Methods

Clinical specimens

GC tissues and paired adjacent noncancerous tissues were obtained from our hospital after patient informed consent was acquired. Ethics approval was received from the committee of our hospital's Ethics Committee. The patient data is available in Table 1. All surgically resected tissues were collected and promptly stored in -80°C for RNA isolation.

Cell culture, plasmid and transfection

GC cell lines (HGC-27, AGS and MKN74) and normal human gastric epithelial cell line GES-1 were purchased from ATCC (Manassas, VA, USA). All cells were maintained in RPMI-1640 (HGC-27, MKN74 and GES-1) and Ham's F-12 (AGS) containing supplemented with 10% fetal bovine serum and antibiotics, at 37°C with 5% CO₂. siRNA-ELFN1-AS1 (si-lnc), miR-211-3p mimics, miR-211-3p inhibitor, siRNA-TMIM29, and their negative controls (NC) were purchased from GenePharma (Guangzhou, China) and subjected to transfection or co-transfection using Lipofectamine 3000 (Invitrogen, MA, USA) as instructed. Untreated cells were used as blank.

The recombinant vectors: psiCHECK-2-ELFN1-AS1-wide type (WT), psiCHECK-2-ELFN1-AS1-mutant 1 (Mut1), psiCHECK-2-ELFN1-AS1-mutant 2 (Mut2), psiCHECK-2-ELFN1-AS1-mutant 1 and 2 (Co-mut), psiCHECK-2-TRIM29 3'UTR-wide-type (WT) and psiCHECK-2-TRIM29 3'UTR mutant (MUT) were synthesized by GenePharma Co. (Shanghai, China).

RT-qPCR

A PureLink RNA Mini Kit (ThermoFisher, USA) was used for RNA extraction. RNA quality was spectrophotometrically ascertained with the 260/280 ratio before reverse transcription through the QuantiTect Reverse Transcription Kit (Qiagen, China). RT-qPCR was performed for examination of the mRNA expression. U6 or GAPDH served as internal control. The $2^{-\Delta\Delta CT}$ method was used for analyzing data.

Western blot

Cells were lysed by adding protein lysis buffer. After microcentrifuged, transfer supernatant was collected and treated with BCA kit (Thermo Fisher Scientific) for assessment of protein dose. 20 µg protein was loaded 10 in SDS-PAGE gel and separated by electrophoresis following electrotransferred to PVDF membrane. Subsequently, the membrane subjected to blockage with blocking buffer for 1h at room temperature and detected at 4 °C with antibodies BAX (cat#: 2772; 1:1000; Cell signaling, USA), antibodies Bcl-2 (cat#: AB112; 1:1000; beyotime, China), antibodies GAPDH (cat#:AF5009; 1:1000; beyotime, China). Next day, the protein markers were examined by inculcation with appropriate secondary antibodies for another 1h at room temperature. Blots were detected with ECL (Amersham, Germany) and photographed on X-ray film (Thermo Scientific, USA).

Nuclear–cytoplasmic fractionation

A PARIS Kit (invitrogen, USA) was used for subcellular localization of ELFN1-AS1 according to the instruction of manufacture. U6 and GAPDH acted as a nuclear or cytoplasmic control, respectively.

RIP analysis

An EZ-Magna RIP RNA-binding protein immunoprecipitation kit (millipore sigma, USA) was used to analyze the interaction of ELFN1-AS1 and miR-211-3p. 5×10^3 AGS and MKN74 cells were transfected with miR-211-3p or NC. At 48h post-transfection, cells was processed by RIP Lysis Buffer and treated with

magnetic beads protein A/G at 4 °C. After 4h inoculation, RIP lysate underwent RNA purification for further quantification of ELFN1-AS1 in cells.

RNA-pull down analysis

5×10^3 AGS and MKN74 cells were transfected with miR-211-3p mimic or miR-211-3p NC end-labeled with desthiobiotin (RiboBio, China) for 48h. Cell pellets were continuously incubated with streptavidin magnetic beads at 4°C. Next day, RNA-binding protein complexes underwent washing and elution before RT-qPCR analysis of TRIM29.

Luciferase reporter assay

Indicated constructed luciferase reporter vectors (100 ng/well) were co-introduced into AGS and MKN74 cells (2×10^5 cells/well) with the miR-211-3p inhibitor, inhibitor NC and pRL-CMV (1ng/well). At post-cotransfection 48h, luciferase activity in total cell lysates was measured as described previously. [13]

CCK8 assays

A CCK8 kit was utilized to measure the cell viability as instructed. Briefly, indicated cells (1×10^3 cells/well) were maintained in a 96-well plate. At the 24h, 48h, 72h and 96h after culturing, cells were treated by 10 uL CCK8 solution for 2h. Finally, the plate was read at 450nm using a spectrophotometer (ELx800, BioTek, USA).

EDU proliferation assay

An EdU Staining Proliferation kit (abcam, USA) was utilized to assay the proliferation of GC cells. Briefly, indicated cells (1×10^3 cells/well) were seed in a 24-well plate and then treated with 50 μ M/well EDU for 2h. The cells were subjected to fixation with 4% paraformaldehyde for another 15min before permeabilization with 0.5% Triton X-100. The resulting complex was sequentially stained with DAPI for 30min before photographed and calculated though a fluorescence microscope (Olympus DP80).

Colonic assay

500 GC cells were plated on 6-well plates for 14 days. The plate continuously underwent fixation with 4% paraformaldehyde and staining with 0.02% crystal violet for 15min. The cells containing more than 50 cells were counted and imaged by a scanner.

Scratch wound healing assay

A scratch wound healing assay was performed to test the GC cell migration. Briefly, 5×10^3 AGS and MKN74 cells were incubated in a 6-well plate. After 24 h of growth, a new 1 ml pipette tip was used to scratch across the center of the well and then the detached cells were removed. The cells underwent continuous 24-h incubation following fixation 3.7% paraformaldehyde for 30 min. The fixed cells were

stained with 1% crystal violet for additional 30 min. The stained monolayer was captured under a microscope and quantitative evaluation of the gap distance was performed by ImageJ.

Cell Invasion Assay

1×10^3 cells was plated into the Chill Millicell insert (Merck KGaA, Germany) pre-coated ECM gel. This insert was positioned into the well of a plate containing DMEM supplemented with 10% FBS as attractant. After 24h, the inserts were taken out and noninvasive cells were discarded. The lower sider of the insert were fixed 5% glutaraldehyde for 10 min before staining with 1% crystal violet for 20 min. The invasive cells were quantitatively assessed and imaged under a microscope.

Statistical analysis

All in vitro assays were conducted in triple independent repeats. Results are shown as mean $\pm$ SD. Statistical evaluation were performed using GraphPad Prism. Compression of two groups was conducted through unpaired Student's t-test and correlation of mRNA expression was examined through Spearman correlation test. One-way analysis of variance (ANOVA) with Bonferroni post hoc analysis was carried out for comparison among multiply groups. $P < 0.05$ was considered statistically significant.

Results

ELFN1-AS1 and miR-211-3p regulates TRIM29 to affect gastric cancer progression

TRIM29 is significantly upregulated in GC (data obtained from GEPIA database, (Figure 1A), and its upregulation is significantly associated with poor prognosis of GC patients (data obtained from KMplot.com, Figure 1B). Similarly, we found that ELFN1-AS1 is upregulated in GC (Figure 1C), and its upregulation predicts a poor overall survival outcome of patients with GC (Figure 1D). Then, to find a linking miRNA between ELFN1-AS1 and TRIM29, we predicted the target miRNAs of ELFN1-AS1 using miRDB and TRIM29 using TargetScan and miRWalk. 5 miRNAs are the targets of both ELFN1-AS1 and TRIM29 mRNA (Figure 1E). Finally, miR-221-3p was identified as the miRNA of our interest due to its low expression in GC samples (Figure 1F).

Frequent upregulation of ELFN1-AS1 in GC tissues and cell lines

Firstly, we substantiated the high expression of ELFN1-AS1 in 29 GC tissues and 29 noncancerous gastric mucosal tissues by RT-qPCR. As showed in figure 2A, the expression of ELFN1-AS1 was prominently elevated in GC tissues. Furthermore, we compared its expression in four widely-used GC cell lines (HGC-27, AGS, MKN74, and GTL-16) with GSE-1 cells. The obviously enhanced expression of ELFN1-AS1 was detected in GC cell lines, especially in AGS and MKN74 cells (Figure 2B). For further investigating the function of ELFN1-AS1, we analyzed the subcellular localization of ELFN1-AS1 in AGS and MKN74 and found that ELFN1-AS1 is predominant in cytoplasm (Figure 2C), implying the role of ELFN1-AS1 as competing endogenous RNA (*ceRNA*). These data suggested the potential function of ELFN1-AS1

enabling GC progression. According to the above results, we successfully established ELFN1-AS1-silencing AGS and MAK74 cells (Figure 2D) for the subsequent in vitro functional assays.

ELFN1-AS1 silence impedes the proliferation, migration and invasion and activates the apoptosis of GC cells in vitro

CCK8 assays demonstrated that ELFN1-AS1 silencing AGS and MAK74 cells showed a significantly lower proliferative capacity compared with the si-NC cells (Figure 3A). This reduced proliferation of si-NC cells was also observed in Edu cell proliferation assay (Figure 3B). As expected, ELFN1-AS1 silence led to the diminished colony forming rate of two GC cells (Figure 3C). The apoptosis-related factors, BAX and Bcl-2 were tested by western blots. As showed in figure 3D, ELFN1-AS1 silence upregulated Bax and downregulated Bcl-2 in AGS and MAK74 cells.

We also evaluated the migratory and invasive capacities of AGS and MAK74 cells. As described in figure 4A, quantification of wound healing scratch assay witnessed an impaired migration distance of AGS and MAK74 cells (about 0.5 fold) when ELFN1-AS1 was silenced. Similarly, the invasion tranwell assays showed the MAK74 knockdown significantly lessened invasion of two GC cells compared with NC group (Figure 4B). Accordingly, these findings in vitro indicated MAK74 might act as an oncogenic RNA in GC progression.

ELFN1-AS1 functions as a miR-211-3p “sponge” in GC cells

Considering the preferential accumulation of ELFN1-AS1 in the cytoplasm of GC cells, we tried to address the underlying mechanism of ELFN1-AS1 as a ceRNA in GC progression. According to the above bioinformatics prediction, we noticed miR-211-3p shared the binding sites in the 3'UTR sequence of ELFN1-AS1 (Figure 5A). Following luciferase reporter were adopted to verify whether the miR-211-3p is a target physically bind by ELFN1-AS1 in GC cells. Four ELFN1-AS1 *luciferase reporter* vectors were generated including wild-type (WT), one mutant-type ELFN1-AS1 (mut1 and mut2), two mutant-type ELFN1-AS1 (Co-mut). Following cotransfection, synthetic miR-211-3p overexpression resulted in the lowest WT-ELFN1-AS1-mediated fluorescence signals compared with other groups (Figure 5B), suggesting that miR-211-3p physically interacts with ELFN1-AS1. This miRNA-mRNA interaction was also certified by RIP assays (Figure 5C) which evidenced the ELFN1-AS1 enrichment displayed in AGO2 IP of miR-211-3p. An inverse association between ELFN1-AS1 and miR-211-3p (Figure 5D). By RT-qPCR, we also turned out a lower miR-211-3p expression in two GC cells compared with GSE cells (Figure 5E). Additionally, we co-transfected si-NC and miR-211-3p inhibitor to validate the inverse correlation. ELFN1-AS1 knockdown resulted in a 0.5-fold elevated expression of miR-211-3p while this effect was abrogated in two GC cells upon transfection with miR-211-3p inhibitor (Figure 5F). Collectively, ELFN1-AS1 functions as a ceRNA for miR-211-3p during GC progression.

MiR-211-3p inhibitor alleviates the repressive effect of ELFN1-AS1 silence on proliferation, migration and invasion of GC cells in vitro

Subsequent in vitro functional assays were performed to validate the interaction between ELFN1-AS1 and miR-211-3p in GC cells. As showed in figure 6A, ELFN1-AS1 silence manifested an obvious inhibition on the cell viability while miR-211-3p inhibitor treatment promoted the viability of the two GC cells. As expected, the inhibition caused by ELFN1-AS1 silence was offset by miR-211-3p inhibitor in GC cells (Figure 6A). Therefore, promotion of miR-211-3p inhibitor could alleviate the inhibitory effect of ELFN1-AS1 knockdown on cell proliferation, evidenced by the results of EDU proliferation assay (Figure 6B). Furthermore, a promoting clone formation ability of GC cells induced by miR-211-3p inhibitor is reversible by ELFN1-AS1 knockdown in GC cells (Figure 6C). In addition to modulating the caspase-3 activity, we detected that miR-211-3p inhibitor treatment could reduce BAX expression and elevate Bcl-2 expression, while cotransfection with miR-211-3p inhibitor and si-ELFN1-AS1 led in no significant alternation of both typical apoptotic-related proteins in the two GC cells (Figure 6D).

Besides modulating GC cell proliferation, we further evaluated the invasive and migratory behaviors of GC cells after contranfecting siRNA-ELFN1-AS1 and miR-211-3p inhibitor. As showed in figure 7A, the elevation of miR-211-3p downregulation on migration was nullified when we knockout ELFN1-AS1 expression in GC cells transfected miR-211-3p inhibitor. Likewise, introducing miR-211-3p inhibitor into ELFN1-AS1 silencing GC cells remarkably offset the inhibitory effect resulted from ELFN1-AS1 silencing on GC cell invasion (Figure 7B). Collectively, ELFN1-AS1 on the proliferation of GC cells were at least partially modulated by miR-211-3p inhibitor in GC cells.

MiR-211-3p targets TMIM29 and lessens its expression in GC cells

MiRNA degrades its target mRNA and lessens target gene expression, thereby implicating in the pathogenesis of neoplasias. Therefore, we performed bioinformatics analysis and identified the 3'UTR of TMIM29 contained putative binding sites of miR-211-3p (Figure 8A). This direct targeting relationship was corroborated by luciferase reporter assays which showed an obviously decreased luciferase activity in GC cells upon transfection with luciferase- TMIM29 3' UTR-WT and miR-211-3p mimic (Figure 8B). In parallel, RNA pulldown confirmed the directed binding relationship of MiR-211-3p with TMIM29 in GC cells (Figure 8C). Next, RT-qPCR analysis was performed to compare the TMIM29 expression in GC tissues and normal tissues. Opposite to the low expression of miR-211-3p in GC tissues, robust expressed TMIM29 was detected in GC tissues (Figure 8D). Pearson correlation analysis demonstrated TMIM29 expression negatively correlated with miR-211-3p expression (Figure 8E). The highly expressed TMIM29 was also examined in GC cells line compared with GSE cells (Figure 8F). To further examine the specificity of their negative correlation, AGS and MAK74 cells were transfected alone with si- TMIM29, miR-211-3p inhibitor or their NC or cotransfected with si- TMIM29 and miR-211-3p inhibitor. As displayed in figure 8G, transient transfection of miR-211-3p inhibitor could not only accelerate the TMIM29 expression in GC cells but also rescue the TMIM29 expression in TMIM29-silencing GC cells. Overall, all results support the high specificity of miR-211-3p targeting TMIM29.

MiR-211-3p acts a pro-tumor factor by targeting TMIM29

Subsequently, we asked whether miR-211-3p affects neoplastic phenotypes by targeting TMIM29. According to CCK8 assays, a low proliferative rate of si-TMIM29 GC cells was measured, and miR-211-3p inhibitor resulted in a high proliferative rate, which was nullified accompanied with miR-211-3p downregulation in si-TMIM29 GC cells (Figure 9A). Identical results were found in Edu proliferation assays (Figure 9B). We further demonstrated that the promotion effects of miR-211-3p on colony forming capacities of two GC cells were abolished following TMIM29 silence (Figure 9C). Similarly, opposite to the effect of miR-211-3p inhibitor on BAX and bcl-2 expression, the TMIM29 knockdown enhanced the BAX expression but lessen the Bcl-2 expression. More importantly, upon co-transfection, the effect of the miR-211-3p inhibitor on both apoptotic-related proteins can be offset to the level almost comparable to the NC groups (Figure 9D). Moreover, the interaction between TMIM29 and miR-211-3p was essential for the suppressive function of miR-211-3p in migratory capacities of GC cells because downregulated migration rates of GC cells after miR-211-3p inhibitor treated were abolished by TMIM29 knockdown (Figure 10A). Additionally, the miR-211-3p inhibitor treatment on TMIM29 silencing GC cells hardly altered the invasion in vitro, completely different with the effects of alone GC cells transfection of miR-211-3p inhibitor or si-TMIM29 (Figure 10B). Hence, miR-211-3p suppresses GC progression in vitro by targeting TMIM29.

Table 1 The sequences of the PCR primers in this study

Primer	Sequences
Inc-ELFN1-AS1	Forward: 5'-AAAAGTTGACGCCGCATTCT-3'
	Reverse: 5'-GAGAATGGATTGTGGGTGCC-3'
TRIM29	Forward: 5'-CACCGCCACTCATTGCCCCGCGAAC-3'
	Reverse: 5'-AAACGTTCGCGGGCAATGAGTGGC-3'
miR-211-3p	Forward: 5'-ACACTCCAGCTGGGGCAGGGACAGCAAAG-3'
	Reverse: 5'-CTCAACTGGTGTCTGTGGAGTCGGCAATTCAGTTGAGTCCCCACG-3'
GAPDH	Forward: 5'-CCAGGTGGTCTCCTCTGA-3'
	Reverse: 5'-GCTGTAGCCAAATCGTTGT-3'
U6	Forward: 5'-CTCGCTTCGGCAGCACA-3'
	Reverse: 5'-AACGCTTCACGAATTTGCGT-3'

Discussion

In the current investigation, using GEPIA database, we found high expression of ELFN1-AS1 in GC tissues and its negative correlation with the prognosis of GC patients, suggesting that the significant involvement of ELFN1-AS1 in GC. We also found that ELFN1-AS1 silence blocked the proliferation, migration and

invasion of GC cells along with increment of apoptotic rate of GC cells. The subsequent mechanical assays showed that ELFN1-AS1 sequestered miR-211-3pp and resulted in an increment of TRIM29 expression, which in turn, promoted GC progress. Further evidence of this ELFN1-AS1, miR-211-3pp and TRIM29 interaction is provided by the examined negative association between miR-211-3pp expression and ELFN1-AS1 or TRIM29 expression in GC tissues.

The important regulatory role of LncRNAs is identified in various pathological conditions, such as cancers [14]. Currently, mounting evidence assign GC progression to the deregulation of lncRNAs. For example, lncRNA AK023391 is robustly expressed in GC, critical to maintain the malignant phenotypes in vitro and in vivo, and induced the PI3K/Akt signaling pathway [15]. Furthermore, high expression of ARHGAP5 is examined in GC tissues and confers to chemotherapy resistance [16]. Herein, we revealed that ELFN1-AS1 is profoundly higher in GC than normal tissues and negatively associated with clinical outcome of GC patients. ELFN1-AS1 has been reported to be highly expressed in various cancer and function as a pro-tumorigenic role in esophageal cancer, colorectal cancer, ovarian cancer, ect. And loss of LFN1-AS1 impairs the cancer cell proliferation, migration and invasion and then constrain the cancer progression [17-20]. Nevertheless, its expression status and role in GC have not been previously reported. In line with previous report, we also found that LFN1-AS1 silence can attenuate the GC cell malignant behaviors and trigger apoptosis in GC cells. To our knowledge, this is the first evidence about LFN1-AS1 role in GC.

To further unravel the mechanism of ELFN1-AS1 in GC, we performed subcellular localization of lncRNA and found that ELFN1-AS1 had high abundance in the cytoplasm, indicating its ceRNA activity in GC. In predicted competitive platform of ELFN1-AS1 for miRNA and mRNA, we focused on miR-211-3pp. The binding of miR-211-3pp to ELFN1-AS1 was also further validated by luciferase reporter and RIP assays. MiR-211-3pp has been found to possess anti-carcinogenic potential in various cancers [21-23]. However, its role in GC have been ignored. Outcome of our investigation showed that anti-miR-211-3p resulted from miR-211-3p inhibitor transfection promotes the proliferation, migration and invasion as well as inhibited apoptosis. It also can completely reversed the alternation caused by anti-miR-211-3p. The downregulation of miR-211-3p in GC tissue and negative correlation with ELFN1-AS1 further suggested the implication of miR-211 in the ELFN1-AS1's ceRNA activity.

According to the bioinformatics analysis, TRIM29 is characterized as another member of the competitive platform for miR-211-3p and ELFN1-AS1 because of its miRNA/mRNA complementarity. Its capacity to assemble homo- or heterodimers allow them to participate in nucleic acid binding as effector or scaffold proteins, thereby influencing genomic stability. Therefore, their dysregulation is recognized to impact broad and diverse cellular functions, particularly in cancer. Previous studies have revealed that the role of TRIM29 is cell and tissue-type dependent [24-26]. However, their cellular functions in GC is still under bleak. Clinically, we found that TRIM29 was upregulated in GC and the high-TRIM29 patients showed a worse prognosis, indicating the pro-tumor role in GC. Next, we further explored the cell function of GC cells transfected with si-TRIM29 and found that TRIM29 silence curbed the malignant potential of GC cells. These outcome confirmed the pro-oncogenic role of TRIM29 in GC. More importantly, TRIM29

silence could inhibited miR-211-3p inhibitor-mediated GC promoter function. The negative TRIM29 mRNA-vs-miR-211-3pp association further reinforced their interaction in GC.

Conclusion

Conclusively, we carried out a bioinformatics analysis to discover a highly expressed ELFN1-AS1 and TRIM29 in GC. ELFN1-AS1 knock down curbed GC progression in vitro. Our further mechanical investigation found that ELFN1-AS1 functioned as a ceRNA for TRIM29 via miR-211-3pp, potentiating GC progression. Taken together, our study expanded our horizon to discover the promising biomarkers and therapeutic targets against GC.

Declarations

Acknowledgements

None.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of The Affiliated Cancer Hospital of Zhengzhou University (Zhengzhou, China). The processing of clinical tissue samples is in strict compliance with the ethical standards of the Declaration of Helsinki. All patients signed written informed consent.

Patient consent for publication

All patients signed written informed consent.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

JH performed the experiments and data analysis. WY and BC conceived and designed the study. GL made the acquisition of data. XC did the analysis and interpretation of data. All authors read and approved the manuscript.

Competing interests

The authors declare that they have no conflict of interests.

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Figures

Figure 1

The expression and prognosis significance of ELF1-AS1 and TRIM29 in GC. A. The expression of TRIM29 in GC. Data obtained from GEPIA database. STAD: stomach adenocarcinoma. T: tumor. N: healthy. B. The overall survival outcome of patients with GC according to the expression level of TRIM29. Data obtained from KMplot.com. C. The expression of ELF1-AS1 in GC. D. The overall survival outcome of patients with GC according to the expression level of ELF1-AS1. E. The intersection between the

targets of ELFN1-AS1 and TRIM29 mRNA 3'UTR. The targets for ELFN1-AS1 were predicted using miRDB. The targets for ELFN1-AS1 were predicted using TargetScan and miRWalk (3'UTR position and score=1). F. The expression of miR-447, miR-211-3p, miR-7111-5p, miR-6870-5p and miR-5698 in GC and adjacent normal samples.

Figure 2

Frequent upregulation of ELFN1-AS1 in GC tissues and cell lines. A. Expression level of ELFN1-AS1 was measured in 29 GC tissues and 29 normal tissues. B. RT-qPCR analysis of expression level of ELFN1-AS1 in GC cell lines (HGC-27, AGS, MKN74, and GTL-16) and GSE-1 cells. ** $p < 0.0001$ vs. GSE-1 cells. C. Levels of ELFN1-AS1 in the nuclear and cytoplasmic fractions in AGS and MKN74 cells. D. Expression level of ELFN1-AS1 in AGS and MKN74 cells transfected with si-NC or si-lncRNA-ELFN1-AS1 (si-lnc). ** $p < 0.0001$ vs si-NC.

Figure 3

ELFN1-AS1 silence impedes the proliferation and activates the apoptosis of GC cells in vitro. A. CCK8 assays assessing the proliferation of si-lnc and si-NC AGS and MKN74 cells (n=6). B. Proliferation of si-lnc and si-NC AGS and MKN74 cells assayed by EdU cell proliferation assay (n=3). C. Colony formation assays of AGS and MKN74 cells with ELFN1-AS1 silence (n=3). D. The levels of Bax and Bcl-2 were measured by western blot analysis. **P < 0.001 by unpaired two-tailed Student's test.

Figure 4

ELFN1-AS1 silence impedes the migration and invasion of GC cells in vitro A. Wound healing assays of AGS and MAKN74 cells with silencing of ELFN1-AS1. B. Transwell assays of the two GC cells with silencing of ELFN1-AS1. **P < 0.001 by unpaired two-tailed Student's test.

Figure 5

ELFN1-AS1 functions as a miR-211-3p "sponge" in GC cells. A. StarBase analysis predicting the binding sequences of miR-211-3p for ELFN1-AS1. B. AGS and MKN74 cells were cotransfected with miR-211-3p inhibitor and four ELFN1-AS1 luciferase reporter vectors including wild-type (WT), one mutant-type ELFN1-AS1 (mut1 and mut2), two mutant-type ELFN1-AS1 (Co-mut) for assessing luciferase activity. C. RIP analysis using AGO2 antibody detecting the enrichment of ELFN1-AS1 in AGS and MKN74 cells.

lines transfected with miR-211-3p mimics or mimics NC. D. Pearson correlation between miR-211-3p and MAK74 in GC tissues. E. RT-qPCR analysis measuring miR-211-3p expression in AGS and MAK74 cells and GSE-1 cells. F. AGS and MAK74 cells were transfected with si-lnc ELFN1-AS1 or miR-211-3p inhibitor and examined for their expression by real-time PCR. * $P < 0.05$; ** $P < 0.001$. NC, negative control; si-lnc, ELFN1-AS1; inhibitor, miR-211-3p inhibitor; si-lnc+inhibitor, siRNA- ELFN1-AS1-AS1+ miR-211-3p inhibitor.

Figure 6

MiR-211-3p inhibitor alleviates the repressive effect of ELFN1-AS1 silence on proliferation of GC cells in vitro. AGS and MAKN74 cells were transfected with si-lnc NC, si-lnc, miR-211-3p inhibitor, and si-lnc+inhibitor. A. CCK-8 cell proliferation assay. B. Edu cell proliferation assay. C. Colony formation assay. D. Western blot analysis of BAX and bcl-2 expression. **, P< 0.001 compared with blank or inhibitor. NC, negative control; si-lnc, siRNA-ELFN1-AS1; inhibitor, miR-211-3p inhibitor; si-lnc+inhibitor, siRNA- ELFN1-AS1 + miR-211-3p inhibitor.

Figure 7

MiR-211-3p inhibitor alleviates the repressive effect of ELFN1-AS1 silence on migration and invasion of GC cells in vitro. AGS and MAKN74 cells were transfected with si-lnc NC, si-lnc, miR-211-3p inhibitor, and si-lnc+inhibitor. A. Scratch assay of AGS and MAKN74 cells. B Transwell invasion of AGS and MAKN74 cells. *, P< 0.05; **, P< 0.001 compared with NC or inhibitor. NC, negative control; si-lnc, siRNA- ELFN1-AS1; inhibitor, miR-211-3p inhibitor; si-lnc+inhibitor, siRNA-ELFN1-AS1+ miR-211-3p inhibitor.

Figure 8

MiR-211-3p targets TRIM29 and lessens its expression in GC cells. A. StarBase analysis predicting the predicted binding sequences of TRIM29 and miR-211-3p. B. Dual luciferase assay was performed in GC cells co-transfected with plasmids TRIM29 3'UTR-WT or TRIM29 3'UTR-MUT and NC mimics or miR-211-3p mimic in AGS and MKN74 cells. The luciferase activities were examined by a luciferase assay kit. C. The enrichment of TRIM29, miR-211-3p, and by RNA pull-down assay. D. RT-qPCR detection of TRIM29

expression in GC tissues and normal tissues. E The correlation between the relative expression level of miR-211-3p targets TMIM29. F RT-qPCR detection of TMIM29 expression in GC cells (AGS and MKN74 cells) and normal cell (GSE-1). G. RT-qPCR detection of TMIM29 expression in GC cells transfected with si-NC, inhibitor-NC, si-TRIM29 and si-TRIM29 +inhibitor. *, $P < 0.05$; **, $P < 0.001$. NC, negative control; WT, wild-type; MUT, Mutant; si-TMIM29, siRNA-TMIM29; inhibitor, miR-211-3p inhibitor; si-TMIM29+inhibitor, siRNA-TMIM29+ miR-211-3p inhibitor.

Figure 9

MiR-211-3p impairs GC cell proliferation and trigger apoptosis by targeting TMIM29. AGS and MAKN74 cells were transfected with NC, si-TMIM29, miR-211-3p inhibitor, and si-TMIM29+ miR-211-3p inhibitor. A CCK8 assays assessing cell viability. B. EdU assay was used to detect cell proliferation. C. In vitro colony formation assay confirmed the in vivo observation. D. Western blotting determine the expression of Bax and Bcl-2. **, P< 0.001 compared with blank or inhibitor. NC, negative control; si-TMIM29, siRNA-TMIM29; inhibitor, miR-211-3p inhibitor; si- TMIM29+inhibitor, siRNA-TMIM29+ miR-211-3p inhibitor.

Figure 10

A. MiR-211-3p impairs GC cell migration and invasion by targeting TMIM29. AGS and MAKN74 cells were transfected with NC, si-TMIM29, miR-211-3p inhibitor, and si-TMIM29+ miR-211-3p inhibitor. A. migration by a scratch assay at posttransfection of 24 h (n=3). B. Cell invasion was analyzed using Transwell invasion assays (n=3). *, P< 0.05; **, P< 0.001 compared with NC or inhibitor. NC, negative control; si-TMIM29, siRNA-TMIM29; inhibitor, miR-211-3p inhibitor; si- TMIM29+inhibitor, siRNA-TMIM29+ miR-211-3p inhibitor.