Long non-coding RNA DDX11-AS1 facilitates gastric cancer progression by regulating miR-873-5p/SPC18 axis

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ABSTRACT
Gastric cancer (GC) is a malignant tumour with high lethality.Accruing evidence elucidates the critical adjusting role of long non-coding RNA (lncRNAs) in human cancers. DDX11 antisense RNA 1 (DDX11-AS1) was previously found to be involved in GC pathogenesis. However, the precise molecular mechanisms of DDX11-AS1 need to be further investigated. In this study, we found that DDX11-AS1 expression was up-regulated in GC tumour tissues and cells. Increased DDX11-AS1 expression was associated with advanced TNM stage and lymph node metastasis. Functionally, knockdown of DDX11-AS1 repressed cell proliferation and clone formation, while induced cell cycle arrest and apoptosis. As expected, DDX11-AS1 overexpression displayed the opposite effect. Mechanically, DDX11-AS1 enhanced SPC18 expression through acting as a ceRNA for miR-873-5p. Furthermore, the inhibitory effect of DDX11-AS1 silencing on malignant biological behaviour of GC cells was attenuated by either miR-873-5p inhibitor or SEC11A up-regulation. Moreover, suppression of DDX11-AS1 also decreased GC tumorigenesis in vivo. In conclusion, DDX11-AS1 may serve as an oncogene in GC progression by sponging miR-873-5p and promoting SPC18 expression, providing a new insight into the mechanisms of DDX11-AS1 and elucidating a promising therapy target in GC.

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Introduction
Gastric cancer (GC) ranks fifth (5.7%) in most frequently diagnosed cancer and occupies third (8.2%) in cancer-related death worldwide, even though its incidence and mortality have been falling over the past decades [1,2]. GC remains a primary contributor to the global cancer burden in men in terms of disability-adjusted life-years, behind lung and liver cancers [3]. Surgical resection combined with perioperative adjuvant chemotherapy, as well as chemoradiation, is widely accepted as the effective treatment for locally advanced gastric cancer [4]. However, patients with advanced gastric cancer always have a dismal prognosis, with a median overall survival of only 10–12 months [5]. GC is a complex disease involving environmental factors and genetic alterations [6]. Thus, understanding the molecular mechanisms of GC pathogenesis is of great significance for identifying novel therapeutic targets and improving patient outcomes.

Along with the development of high-throughput sequencing and microarray technologies, it is found that at least 75% of the human genomes are transcribed into non-coding RNAs, including lncRNAs and miRNAs [7]. LncRNAs, with over 200 nucleotides in length and no protein-coding capability, are able to affect genes associated with proliferation, cell cycle, migration, immune response or pluripotency in cancers by chromosomal, transcriptional, and post-transcriptional regulation [8,9]. Increasing lncRNAs have been discovered to be aberrantly expressed in GC and closely relevant to carcinogenesis, metastasis, diagnosis or prognosis [10,11]. For example, urothelial carcinoma-associated 1 (UCA1) played an accelerated role in tumour metastasis by interacting with microRNA-203 and releasing miR-203-targeted transcripts ZEB2 in GC [12]. Plasmacytoma variant translocation 1 (PVT1) could enhance angiogenesis via inducing signal transducers and activators of transcription 3/vascular endothelial growth factor A (STAT3/VEGFA) axis in GC [13]. Researches also demonstrated the tumour-suppressive effect of growth arrest-specific transcript 5 (GAS5) in GC by repressing cell proliferation and promoting apoptosis via modulating regulating E2F1 and P21 [14]. DDX11 antisense RNA 1 (DDX11-AS1), located at chromosome 12p11.21, was previously found to be increased in hepatocellular carcinoma patients compared with normal controls, suggesting its potential as therapy target for hepatocellular carcinoma [15]. Moreover, Liu et al. showed the up-regulation of DDX11-AS1 in GC tissues, and knockdown of DDX11-AS1 inhibited cell proliferation [16]. However, the detailed molecular mechanisms of DDX11-AS1 in human GC tumorigenesis are still unknown.

An abundance of evidence manifests that lncRNA can suppress miRNA functions and abate mRNA repression by sponging miRNA via interacting with miRNA via ceRNA. It is clear that DDX11-AS1 is an lncRNA which may act as a ceRNA to modulate miRNA expressions, so as to regulate GC tumorigenesis. In this study, we characterized the potential role of DDX11-AS1 in GC progression and tumorigenesis, which may shed insights on the understanding of GC and further developing novel therapeutic strategies.

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sequestering or competing with miRNAs [17]. miRNAs, a class of non-coding transcripts with 20–22 nucleotides, are well known to play a critical role in tumorigenesis by regulation of oncogenes or tumour-suppressor genes [18]. A previous document clarified that miR-873-5p expression was decreased in GC, and overexpression of miR-873-5p alleviated GC malignant phenotypes through targeting hedgehog-GLI signalling [19]. Nevertheless, whether miR-873-5p-mediated anti-cancer functions in GC are related to IncRNAs remain to be investigated.

Herein, we found that DDX11-AS1 expression was increased in GC tumour tissues, and associated with advanced TNM stage and lymph node metastasis. Furthermore, knockdown of DDX11-AS1 inhibited cell proliferation and facilitated apoptosis. More importantly, DDX11-AS1 could act as a sponge for miR-873-5p, thereby resulting in the derepression of signal peptidase complex 18 (SPC18). Together, this study elucidates a novel DDX11-AS1/miR-873-5p/SPC18 regulatory pathway, providing evidence for DDX11-AS1 as a promising molecular target for GC therapy.

Materials and methods

GC tissues collection

We obtained 72 pairs of tumour specimens and adjacent non-tumour tissues (>5 cm away from tumour location) from GC patients who were undergoing surgery at Huaihe Hospital of Henan University between May 2015 and Apr 2017. These patients consist of 44 males and 28 females with a median age of 60 years (range, 35–73 years). The pathological diagnosis of GC patients was performed by two experienced pathologists. None of the recruited participants received any local or systemic treatment before surgical resection. All the fresh tissues harvested in the study were maintained at −80°C until used. The research was approved by the Ethics Committee of Huaihe Hospital of Henan University and performed in compliance with the Helsinki declaration. The signed written informed consents were gained from each participant.

Cell culture

The cells used in the study, including gastric cancer cell lines (MKN45, SGC7901, BGC823, HGC27, AGS) and normal gastric epithelium cells GE5-1, were purchased from the Cell Bank of Chinese Academy of Sciences (Shanghai, China). All cells were grown in Roswell Park Memorial Institute (RPMI)-1640 medium (Gibco, Grand Island, CA, USA) with 10% foetal bovine serum (FBS; Gibco). Cells in this medium were placed into a humidified incubator with 5% CO₂ at 37°C.

Quantitative real-time polymerase chain reaction (qRT-PCR) assay

The total RNA was isolated from GC tissues and cell lines by using TRIzol (Invitrogen, Carlsbad, CA, USA). Then RNAs were reversely transcribed to the first-strand cDNA utilising PrimeScript RT reagent Kit (TaKaRa, Dalian, China). Next, the qRT-PCR assay was implemented with SYBR Premix Ex Taq™ II (TaKaRa) according to the producer’s manuals. After gently mixing the reaction solutions, the tubes were placed on the reactor of ABI Prism 7900HT system (Applied Biosystems, Foster City, CA, USA). U6 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) acted as the endogenous references to standardise the levels of DDX11-AS1, miR-873-5p, and SPC18. The relative gene levels were calculated using 2−ΔΔct method.

Transient transfection

The cDNA sequences of DDX11-AS1 and SEC11A were amplified by PCR and cloned into pcDNA3.1 vector (Invitrogen, Carlsbad, CA, USA), forming overexpression vectors of DDX11-AS1 (DDX11-AS1) and SEC11A (SEC11A), with the empty pcDNA3.1 as a control (pcDNA3.1). Small interfering RNA (siRNA) against DDX11-AS1 (si-DDX11-AS1) and its control (si-NC) were synthesised by Ribobio (Guangzhou, China). MiR-873-5p mimics (miR-873-5p) and inhibitor (anti-miR-873-5p), as well as their corresponding controls (miR-con and anti-miR-con) were also purchased from Ribobio. Finally, the constructed plasmids and oligonucleotides were introduced into GC cells using Lipofectamine™ 2000 (Invitrogen) based on the manufacturer’s manuals.

Cell counting kit-8 (CCK-8) assay

Cell proliferation capacity was measured using a CCK-8 reagent kit (Dojindo, Kumamoto, Kyushu, Japan) according to the producer’s descriptions. Firstly, 3 × 10³ cells transfected with indicated plasmids or oligonucleotides were seeded into 96-well plates to grow for 24, 48, 72 and 96 h. Then, the supernatant was replaced by 100 µL complete medium, and 10 µL CCK-8 solution was added to each well. The plates were incubated for 4 h at 37°C prior to absorbance measurement at 450 nm with a Spectramax M4 microplate reader (Molecular Devices, Sunnyvale, CA, USA).

EdU assay

Cell proliferation was also evaluated using Cell-Light™ EdU Apollo 567 In Vitro Imaging Kit (Ribobio). Briefly, cells were plated into 96-well plates and incubated with 50 µM EdU reagent for 2 h at 37°C. Then, cells were fixed using 4% paraformaldehyde, neutralised with 2 mg/mL glycine, permeabilized with 0.5% TritonX-100, and washed with PBS. Subsequently, cells were treated with 1 × Apollo staining solution for 30 min in dark place. After being incubation with 1 × Hoechst for 30 min, cells were imaged using a fluorescence microscopy (Nikon Eclipse 80i, Nikon, Tokyo, Japan).

Clone formation assay

This assay was used to examine GC cells’ cloning ability. Cells were seeded into 6-well plates and cultured in media containing 10% FBS for 12 days. During this period, the medium
was refreshed every 4 days. After being washed with PBS, fixed with methanol and stained by crystal violet (0.1%; Sigma-Aldrich, St. Louis, MO, USA), cells were subjected to a microscopy to count the number of visible colonies.

**Cell cycle distribution**

For cell cycle assay, cells were seeded into 6-well plates at a density of $1 \times 10^5$/mL and collected at 48 h. Subsequently, cells were fixed with 75% ethanol at $-20^\circ C$, treated with RNase A for 30 min, at 37°C and stained with propidium iodide (PI; BD Biosciences, San Jose, CA, USA) for 30 min at room temperature. Cell cycle distribution was identified by a FACSCalibur flow cytometer (BD Biosciences) and ModFit 3.0 (Verity Software House, Topsham, ME, USA).

**Apoptosis detection**

Cellular apoptosis was detected using an Annexin V-FITC apoptosis detection kit (BD Biosciences) following the manufacturer’s protocols. Briefly, harvested cells were double stained with Annexin V-FITC and PI at the dark for 15 min and subjected to a FACSCalibur flow cytometer (BD Biosciences) for determining the percentage of apoptotic cells (Annexin V-FITC-positive).

**In vivo experiments**

Six-week-old male BALB/c nude mice were purchased from Shanghai SLAC Laboratory Animal Co. Ltd, Shanghai, China) and kept in specific pathogen-free environment. All procedures were performed with approval of Animal Ethics Committee of Huaihe Hospital of Henan University in accordance with the guide for the Care and Use of Laboratory Animals of National Institutes of Health. BGC823 cells (1 $\times 10^5$) stably transfected with lentivirus vectors encoding shRNA against DDX11-AS1 (sh-DDX11-AS1) or a non-silencing control (sh-NC) were subcutaneously injected into the left armpit of every mouse. Tumour growth was measured every 4 day, and tumour volume was calculated using the formula $V = 0.5 \times \text{length} \times \text{width}^2$. Twenty-five days later, mice were all sacrificed for tumour resection and analysis.

**Isolation of cytoplasmic and nuclear RNA**

Cytoplasmic and nuclear RNA were separated and purified utilising the Cytoplasmic and Nuclear RNA Purification Kit (Norgen, Belmont, CA, USA) in the accordance with the manufacturer’s instructions. Then, qRT-PCR was conducted to detect the level of DDX11-AS1 extracted from each of the fractions. GAPDH and U6 were respectively used as cytoplasmic and nuclear control.

**Dual-luciferase reporter assay**

The binding sequences for miR-873-5p in DDX11-AS1 or SEC11A-3’UTR were amplified and inserted into the downstream of the luciferase gene in pMIR-Reporter vector (Promega, Madison, WI, USA), generating DDX11-AS1-wt and SEC11A-wt. The corresponding mutants were constructed using QuikChang site-directed mutagenesis kit (Agilent Technologies, Santa Clara, CA, USA), named as DDX11-AS1-mut and SEC11A-mut. Then, 293 T cells were co-transfected with the constructed reporters, renilla luciferase plasmid pRL-TK (Promega) and miR-con or miR-873-5p by utilising Lipofectamine 3000 transfection reagent (Invitrogen) according to the producer’s descriptions. After 36 h, cells were collected for luciferase detection by using a Dual-Luciferase Reporter Assay System (Promega). Renilla activity was applied to normalise the relative activity of firefly.

**RNA immunoprecipitation (RIP) assay**

To verify the interaction between DDX11-AS1 and miR-873-5p, RIP assay was performed as the protocols of EZMagna RNA immunoprecipitation Kit (Millipore, Billerica, MA, USA). SG7901 cells were lysed in freshly prepared RIP lysis buffer, and then the whole-cell lysate was incubated with magnetic beads conjugated with anti-Ago2 antibody (Millipore) or control IgG (Millipore) for 6 h at 4°C. After washing the beads, Proteinase K was added to digest the proteins. qRT-PCR analysis was carried out to measure the relative level of DDX11-AS1 and miR-873-5p in immunoprecipitated RNA.

**RNA pull-down assay**

The pull-down assays with biotinylated miRNAs were carried out as described previously [20]. The bound RNAs were purified and subjected to qRT-PCR for the measurement of DDX11-AS1 enrichment.

**Western blot assay**

Total protein was isolated from GC cell lines using RIPA reagent (Beyotime, Beijing, China), separated on 12% sodium dodecyl sulphate (SDS)-polyacrylamide gels, and transferred onto the PVDF membrane (Millipore). After blocking with 5% skim milk for 2.5 h at room temperature, the membranes were incubated with SPC18 (1:1000, ab174794, Abcam), followed by further probed with HPR-labeled with secondary antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA). The antibody-antigen complexes were examined using an ECL kit (Millipore) according to the manufacturer’s direction.

**Statistical analysis**

The data obtained from three independent experiments were analysed with the SPSS 16.0 software and expressed as Mean ± Standard Deviation (SD). Student t-test was employed to assess the differences between two groups. The
comparisons among groups were assessed by one-way ANOVA followed by the Tukey-Kramer's post hoc test. Pearson's coefficient correlation was applied to analyse the expression correlation. *p*-values less than .05 were considered as statistically significant.

**Results**

**DDX11-AS1 expression was increased in GC tissues and cell lines**

To explore the biological role of DDX11-AS1 in GC, we firstly analysed the expression of DDX11-AS1 in TCGA Data Portal from starBase V3.0. The results showed that DDX11-AS1 expression was higher in GC tumour tissues (n = 375) than that of normal samples (n = 32) (Figure 1(A)). Consistently, high expression of DDX11-AS1 was confirmed in GC tissues from 72 patient samples in our hospital via qRT-PCR analysis (Figure 1(B)). Also, DDX11-AS1 expression was assessed in GC tissues at different TNM stages. As displayed in Figure 1(C), DDX11-AS1 expression was up-regulated in advanced-stage (III + IV) compared with that in early-stage (I + II). Moreover, high DDX11-AS1 level was associated with lymph node metastasis in GC patients (Figure 1(D)). Simultaneously, we determined DDX11-AS1 expression in five GC cell lines (MKN45, SGC7901, HGC27, BGC823, and AGS) and normal gastric epithelium cells GES-1. As expected, DDX11-AS1 level was increased in all five types of GC cells when compared to GES-1 cells (Figure 1(E)). As control, we also analysed whether knockdown of DDX11-AS1 could affect the growth of normal gastric epithelial cell. DDX11-AS1 expression was successfully suppressed in GES-1 cells by transfection with si-DDX11-AS1 (Supplemental Figure 1(A)). However, knockdown of DDX11-AS1 couldn't inhibit GES-1 cell proliferation (Supplemental Figure 1(B,C)). All the data indicated a potential oncogenic role of DDX11-AS1 in GC progression.

**Silencing of DDX11-AS1 suppressed the progression of GC in vitro**

In view of the high expression of DDX11-AS1 in GC, we further focussed on its biological roles in GC progression. To manipulate the expression level of endogenous DDX11-AS1, SGC7901 and BGC823 cells were transfected with si-DDX11-AS1, and DDX11-AS1-overexpressing plasmid was introduced into AGS cells. As demonstrated by qRT-PCR analysis, DDX11-AS1 expression was successfully down-regulated in SGC7901 and BGC823 cells, while up-regulated DDX11-AS1 expression was observed in AGS cells (Figure 2(A)). CCK-8 assay exhibited that DDX11-AS1 deficiency efficiently reduced cell growth in SGC7901 and BGC823, while DDX11-AS1 up-regulation resulted in an increase of proliferative ability in AGS cells (Figure 2(B)). Edu staining results manifested that DDX11-AS1 expression was higher in GC tissues and cell lines. (A) DDX11-AS1 expression of gastric cancer tissues (n = 375) and normal controls (n = 32) in the TCGA data portal from Starbase vers.0 Pan-cancer analysis. (B) Level of DDX11-AS1 was assessed by qRT-PCR analysis in tumour tissues and adjacent normal tissues from 72 GC patients. (C) DDX11-AS1 expression in GC tissues at different TNM stages (I + II versus III + IV). (D) DDX11-AS1 expression difference in GC patients with different lymph node metastasis status (N0 + N1 versus N2 + N3). (E) DDX11-AS1 expression analysis in five GC cell lines and normal GES-1 cells. N0 and N1 indicates no or low metastasis; N2 and N3 represents middle or high metastasis. *p < .05, **p < .01, ***p < .001.
silencing significantly decreased the rate of proliferating cells in SGC7901 and BGC823, while DDX11-AS1 overexpression exerted the opposite effect in AGS cells (Figure 2 (C–E)). Similarly, clone formation assay suggested that cell clonality was impaired by knockdown of DDX11-AS1, but was improved in AGS cells transfected with pcDNA3.1-DDX11-AS1 (Figure 2(F–H)). In addition, flow cytometry was performed to detect the effects of DDX11-AS1 on cell cycle distribution and apoptosis. As presented in Figure 2(I–K), DDX11-AS1 down-regulation caused cell cycle arrest in SGC7901 and BGC823, but increased DDX11-AS1 led to significant cell cycle progression in AGS cells. Moreover, SGC7901 and BGC823
cells with DDX11-AS1 knockdown displayed an evident enhancement of apoptotic rate (Figure 2(L,M)). Furthermore, western blot analysis was conducted to examine the expression of cell cycle- and apoptosis-related proteins in si-DDX11-AS1-transfected GC cells. As displayed in Figure 2(N), knockdown of DDX11-AS1 resulted in a decrease of cyclin D1 protein, while an increase of p27, cleaved PAPR and cleaved caspase-3 proteins in SGC7901 and BGC823 cells, indicating the induction of DDX11-AS1 knockdown on cell cycle arrest and apoptosis. These data suggested that knockdown of DDX11-AS1 inhibited cell proliferation and induced apoptosis in GC in vitro.

**DDX11-AS1 acted as a molecular sponge for miR-873-5p**

As we all know, the functions and mechanisms of IncRNAs are associated with their specific cellular localisation [21]. To gain insight into the molecular mechanisms of DDX11-AS1 involved in GC tumorigenesis, we firstly predicted the subcellular location of DDX11-AS1 using online database InLocater (http://www.csbio.sjtu.edu.cn/bioinf/InLocater/). As exhibited in Figure 3(A), DDX11-AS1 was mainly located in cytoplasm. Subsequent nucleus-cytoplasm fractionation assay verified the prediction result in GC cells (Figure 3(B)). According to online platform starBase V3.0, six miRNAs (miR-3605-3p, miR-4731-5p, miR-766-5p, miR-499b-5p, miR-2355-5p, miR-873-5p) were predicted to containing the complementary sequences in DDX11-AS1. To verify the binding between DDX11-AS1 and candidate miRNAs, dual-luciferase reporter assays were conducted in 293 T cells co-transfected DDX11-AS1 reporter plasmid and candidate miRNAs. Among these miRNAs, miR-873-5p significantly decreased the luciferase activity of DDX11-AS1 reporter (Figure 3(C)). Thus, miR-873-5p was selected for further experiments. Bioinformatic tool starBase V3.0 displayed the detailed binding sites of DDX11-AS1 and miR-873-5p (Figure 3(D)). Moreover, miR-873-5p expression was evidently boosted by DDX11-AS1 knockdown, while was obviously hampered following DDX11-AS1 overexpression in SGC7901 and BGC823 cells (Figure 3(E,F)). To confirm the actual binding between DDX11-AS1 and miR-873-5p, dual-luciferase reporter, RIP, and RNA pull-down assays were performed in SGC7901 cells. The result showed that miR-873-5p prominently reduced the luciferase activity in DDX11-AS1-wt reporter, while this suppressive effect was abolished when miR-873-5p binding sites in DDX11-AS1 were mutated (Figure 3(G)). RIP assays revealed that DDX11-AS1 and miR-873-5p were preferentially enriched in Ago2-containing miRNPs relative to IgG immunoprecipitates (Figure 3(H)). Also, the abundance of DDX11-AS1 was found in miR-873-5p-wt pull-down pellets rather than miR-873-5p-mut (Figure 4(I)). Additionally, decreased expression of miR-873-5p was discovered in GC tumour tissues compared with adjacent non-cancerous tissues (Figure 3(I)). Furthermore, a negative correlation between miR-873-5p and DDX11-AS1 was observed in GC tumour tissues (Figure 3(K)). These data uncovered that DDX11-AS1 sponged miR-873-5p to suppress its expression.

**DDX11-AS1 positively regulated SPC18 expression through sponging miR-873-5p**

Ample evidence manifests that miRNAs could target 3’UTR of genes to suppress their expression. Based on starBase V3.0 project, we found lots of target genes that contain complementary sequences in the seed region of miR-873-5p. Among 13 candidate targets from preliminary screening, we focussed on SEC11A, GLI2 and DDR1 for their oncogenic roles in GC. Hence, we detected the mRNA levels of these genes in SGC7901 and BGC823 cells after transfection with miR-873-5p mimic or inhibitor. The result revealed that only SEC11A mRNA level was repressed by miR-873-5p overexpression, while was promoted following inhibition of miR-873-5p (Figure 4(A)). Subsequently, western blot was used to determine the effect of miR-873-5p on the protein level of SPC18, encoded by SEC11A gene. As a result, SPC18 protein expression was significantly impeded by introducing miR-873-5p mimic, while strikingly promoted by miR-873-5p inhibitor (Figure 4(B,C)). The complementary region between miR-873-5p and SEC11A was displayed in Figure 4(D). In the follow-up dual-luciferase reporter assay, we discovered that the relative luciferase activity was evidently blocked by miR-873-5p overexpression in wild-type group rather than in mutant group (Figure 4(E)). Additionally, TCGA Data Portal from starBase V3.0 showed an increase of SEC11A expression in GC tumour tissues compared with that in normal samples (Figure 4(F)). Correspondingly, high expression of SEC11A mRNA was also validated in GC tumour tissues from 72 patients (Figure 4(G)). On the basis of ceRNA hypothesis, we further explore whether DDX11-AS1 could regulate SPC18 expression through sponging miR-873-5p. SGC7901 and BGC823 cells were transfected with either miR-873-5p mimic or pcDNA-DDX11-AS1 alone, or co-transfected with miR-873-5p mimic and pcDNA-DDX11-AS1. The results revealed that miR-873-5p up-regulation suppressed SPC18 protein expression and DDX11-AS1 overexpression improved SPC18 protein level. Moreover, miR-873-5p-mediated decrease of SPC18 protein expression was effectively rescued by DDX11-AS1 overexpression (Figure 4(H,I)). Afterward, further analysis observed that SEC11A expression was passively correlated with miR-873-5p, but was positively related to DDX11-AS1 in GC patients (Figure 4(J,K)). In short, all these data manifested that DDX11-AS1 could serve as a ceRNA to facilitate SPC18 expression via sponging miR-873-5p.

**Suppressive effects of DDX11-AS1 knockdown were abolished by either miR-873-5p inhibition or SEC11A overexpression**

To shed light on whether DDX11-AS1 contributed to GC progression through modulating miR-873-5p/SPC18 signalling, SGC7901 and BGC823 cells were transfected with si-DDX11-AS1, or together with anti-miR-873-5p or pcDNA-SEC11A. Western blot assay results showed that DDX11-AS1 knockdown dramatically repressed SPC18 expression, while this inhibitory effect was relieved by either miR-873-5p inhibitor or SEC11A up-regulation (Figure 5(A,B)). Functionally, miR-873-5p inhibitor and SEC11A overexpression could evidently
Figure 3. DDX11-AS1 interacted with miR-873-5p to inhibit its expression. (A) Database IncLocator (http://www.csbio.sjtu.edu.cn/bioinf/IncLocator/) was used to predict the cellular location of DDX11-AS1. (B) The percentage of DDX11-AS1 in cytoplasm and nuclear fractions of SGC7901 and BGC823 cells, with GAPDH and U6 as cytoplasmic and nuclear localisation control, respectively. (C) Luciferase reporter analysis was performed in 293 T cells co-transfected with DDX-AS1-wt reporter and six different candidate miRNAs. (D) StarBase V3.0 algorithm predicted the binding sequence DDX11-AS1 and miR-873-5p. (E and F) qRT-PCR assay was applied to analyse miR-873-5p level in both SGC7901 and BGC823 cells with DDX11-AS1 overexpression or knockdown. (G) Effect of miR-873-5p on the luciferase activity of DDX11-AS1-wt or DDX11-AS1-mut reporter in SGC7901 cells. (H) qRT-PCR assay was used to measure the relative levels of DDX11-AS1 and miR-873-5p in immunoprecipitates with Ago2 or IgG antibody. (I) RNA pull-down assay was conducted in SGC7901 cells transfected with Bio-miR-NC, Bio-miR-873-5p-wt, or Bio-miR-873-5p-mut. (J) Expression difference of miR-873-5p in tumour tissues (n = 72) or corresponding non-tumour tissues (n = 72) was measured by qRT-PCR. (K) qRT-PCR was carried out to analyse the correlation between DDX11-AS1 and miR-873-5p expression in GC patients. *p < .05, **p < .01, ***p < .001.
**Figure 4.** DDX11-AS1 promoted SPC18 expression by functioning as a molecular sponge of miR-873-5p. (A) SGC7901 and BGC823 cells transfected with miR-873-5p mimic or inhibitor were subjected to qRT-PCR for determining the mRNA levels of SEC11A, GLI2 and DDR1. (B and C) Western blot analysis was carried out to assess the effect of miR-873-5p mimic or inhibitor on SPC18 protein level. (D) Prediction of the binding sites between miR-873-5p and SEC11A by starBase V3.0 software. (E) Dual-luciferase reporter assay was carried out to determine the interaction between miR-873-5p and SEC11A. (F) SEC11A mRNA expression of gastric cancer tissues (n = 375) and normal controls (n = 32) in the TCGA data portal from Starbase V3.0. Pan-cancer analysis. (G) SEC11A mRNA levels in tumour and adjacent non-tumour tissues of 72 GC patients were identified by qRT-PCR. (H and I) The effect of miR-873-5p or DDX11-AS1 on SPC18 protein expression was detected via western blot assay. (J and K) qRT-PCR was conducted to analyse the expression correlation between SEC11A mRNA and miR-873-5p or DDX11-AS1. *p < .05, **p < .001.
reverse the inhibiting effect of DDX11-AS1 silencing on cell proliferation (Figure 5(C,D)) and clonality (Figure 5(E,F)). Moreover, the pro-apoptotic effect induced by DDX11-AS1 down-regulation was attenuated by either suppression of miR-873-5p or increase of SEC11A (Figure 5(G,H)). These above data provide strong evidence that DDX11-AS1 facilitated GC development through derepressing SPC18 by competitively binding to miR-873-5p in vitro.

Knockdown of DDX11-AS1 repressed GC tumour growth in vivo

Synchronously, we investigated the biological significance of DDX11-AS1 in GC tumorigenesis in vivo. Results showed that the tumour volumes and weights were both lowered by DDX11-AS1 knockdown when compared with sh-NC group (Figure 6(A,B)). DDX11-AS1 and SPC18 expression was
decreased (Figure 6(C,E)), while miR-873-5p expression was increased (Figure 6(D)) in tumours formed from sh-DDX11-AS1-transfected cells when compared to the control group. From the above, we concluded that silencing of DDX11-AS1 repressed GC cell proliferation in vivo.

Discussion

In recent years, increasing attention has been paid to lncRNAs for their important involvement in a serial of biological functions in carcinogenesis [22]. Nevertheless, the biological significance of most lncRNAs is still elusive. A previous report elucidated the oncogenicity of DDX11-AS1 in GC [16]. This work was designed to further investigate the underlying mechanisms of DDX11-AS1 in GC pathogenesis.

In this study, we firstly confirmed that DDX11-AS1 was overexpressed in GC tumour tissues and cell lines. Simultaneously, DDX11-AS1 level was found to be increased with the development of TNM stage and lymph node metastasis. Functionally, knockdown of DDX11-AS1 inhibited cell proliferation, clone formation and cell cycle progression, and enhanced apoptosis in vitro, while DDX11-AS1 overexpression exerted the contrary effect. Consistently, DDX11-AS1 silencing repressed GC tumogenesis in vivo. Previous to our research, DDX11-AS1 promoted hepatocellular carcinoma progression by suppressing LATS2 expression via interacting with EZH2 and DNMT1 [23]. A recent research highlighted the oncogenic property of DDX11-AS1 in colorectal cancer through targeting miR-873/CLDN7 axis [24].

It is acknowledged that subcellular localisation of lncRNA is responsible for their specialised functions [25]. On the basis of lncLocator prediction and nucleus-cytoplasm fractionation assay, DDX11-AS1 was found to mainly exist in the cytoplasm. LncRNAs in cytoplasm can serve as miRNA sponges, thus lowering their modulatory effects on mRNAs [26]. By using bioinformatic tools, luciferase reporter, RIP and RNA pull-down assays, miR-873-5p was identified as a target of DDX11-AS1. Moreover, DDX11-AS1 could act as an endogenous sponge to repress miR-873-5p expression. miR-873-5p was demonstrated as a tumour-suppressor in kinds of malignancies, such as colon cancer [27], thyroid cancer [28], endometrial cancer [29], glioma [30], and osteosarcoma [31]. qRT-PCR further verified the increase of miR-873-5p expression in GC tumour tissues, which was in accordance with a previous research [19].

The lncRNA-miRNA-mRNA ceRNA network has been implicated in the carcinogenesis of diverse cancers, including GC [32]. Thus, we speculated that SNHG1 may promote GC progression through the similar regulatory mechanism. SEC11A was confirmed as a direct target by using web-based softwares and luciferase reporter assays. Moreover, DDX11-AS1-induced increase of SPC18 protein was prominently abated by miR-873-5p overexpression. Furthermore, SEC11A mRNA expression was inversely correlated with miR-873-5p, while was positively related to DDX11-AS1. That is to say, DDX11-AS1 could serve as a ceRNA to antagonise the suppressive effect of miR-873-5p on SEC11A. Subsequent restoration experiments showed that si-DDX11-AS1-mediated
suppression of cell proliferation and enhancement of apoptosis were greatly mitigated by the decrease of miR-873-5p and up-regulation of SEC11A, validating the DDX11-AS1/miR-873-5p/SPC18 regulatory axis in GC.

SPC18 protein, encoded by SEC11A, is one of the subunits of the signal peptidase complex and has catalytic activity of signal peptidase [33]. Several reports elucidated the association of SPC18 protein with tumorigenesis. For example, overexpression of SEC11A promoted cell growth and invasiveness in bladder cancer [34]. Down-regulation of SPC18 inhibited cell proliferation and invasive activity in colorectal cancer through inactivation of EGFR signalling [35]. Also, SPC18 expression was up-regulated in GC tissues, and SPC18 overexpression facilitated GC malignant progression via inducing TGF-α secretion [36].

In summary, DDX11-AS1 expression was increased in GC tumour tissues and cells. High DDX11-AS1 expression was associated with advanced TNM stage and lymph node metastasis. DDX11-AS1 facilitated GC progression through acting as a ceRNA for miR-873-5p to up-regulate SPC18 expression. Our findings provide a novel insight into the post-transcriptional regulation mechanism of DDX11-AS1 involved in GC pathogenesis and highlight a potential therapy target for GC. However, more regulatory networks of DDX11-AS1 in human cancer need to be further clarified for the multilayered complexity of ceRNA crosstalk and competition.

Ethical approval

The procedures involving human participants were approved by the Ethics Committee of Huaihe Hospital of Henan University and performed in compliance with the Helsinki declaration. The signed written informed consents were gained from each patient. All animal procedures were carried out with approval of Animal Ethics Committee of Huaihe Hospital of Henan University in accordance with the guide for the Care and Use of Laboratory Animals of National Institutes of Health.

Author contributions

Zheng Ren and Xiaochun Liu designed the concept and conducted the experiments. Yaoran Si performed the relevant data analysis. Zheng Ren wrote the draft. Desheng Yang reviewed and approved the manuscript.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Data availability

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

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