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Original Article

Long non-coding RNA LINC00520 promotes malignant progression of gastric adenocarcinoma through miR-519b-3p/HIF1A axis

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Abstract

Gastric cancer is a prevalent malignant tumor, characterized by high morbidity and mortality rates globally. Long non-coding RNAs (lncRNAs), a class of transcripts exceeding 200 nucleotides in length, are non-protein-coding molecules that exert crucial regulatory functions in cellular biology. Investigating the regulatory mechanisms of lncRNAs in gastric cancer is essential. This study aimed to elucidate the functional role and molecular mechanisms of LINC00520 in gastric cancer. Initially, the GEO database was screened for differentially expressed genes associated with the malignant progression of gastric cancer. Quantitative reverse transcription polymerase chain reaction (qRT-PCR) was utilized to ascertain the LINC00520 expression in gastric cancer tissues. Subsequently, cellular functional assays were conducted to investigate the potential effects of LINC00520 on cellular behavior. The interaction between LINC00520, miR-519b-3p, and HIF1A was examined through bioinformatics analysis, and their binding interactions were confirmed using dual-luciferase reporter gene assays and RNA immunoprecipitation (RIP) assays. Our findings revealed a marked increase in the LINC00520 expression in gastric cancer tissues. Overexpression of LINC00520 was observed to enhance the malignant progression of gastric cancer cells. Through bioinformatics analysis, dual-luciferase reporter assays, and RIP assays, we demonstrated that LINC00520 upregulated HIF1A expression by competitively binding to miR-519b-3p, thereby acting as a molecular sponge. In conclusion, this study indicates that LINC00520, which is highly expressed in gastric cancer, exerts its effects by targeting the miR-519b-3p/HIF1A axis. These insights provide a foundation for developing diagnostic and therapeutic strategies for gastric cancer.

Keywords: Gastric cancer, LINC00520, miR-519b-3p, HIF1A, ceRNA.

1. Introduction

Gastric cancer, a prevalent malignant tumor of the gastrointestinal tract, is diagnosed in over one million individuals annually [1]. Notably, China stands as a region of high incidence, contributing to roughly 40% of global new cases and gastric cancer-related mortality [2]. Early clinical symptoms of gastric cancer are not clear, which often leads to delayed diagnosis, and most of the patients are in the advanced stage. At this time, the disease is highly invasive, and the prognosis is poor, with a five-year survival rate of about 20% [3]. The primary contributors to patient mortality are the invasion and metastasis of gastric cancer [4]. Therefore, the identification and evaluation of critical molecules associated with the malignant progression of gastric cancer are essential for both treatment and prognosis.

Long non-coding RNAs (lncRNAs), a class of transcripts exceeding 200 nucleotides in length, cannot encode proteins yet exert pivotal regulatory roles in cellular metabolism and proliferation [5-6]. They are also implicated in the modulation of apoptosis and tumour cells' invasive and metastatic capabilities [7]. Evidence suggests that lncRNAs may be candidate biomarkers for cancer diagnosis. The lncRNA LINC00520, identified from the Gene Expression Omnibus (GEO) database, is associated with the oncogenic process of various malignant tumors. Despite this, the function and mechanisms of LINC00520 in gastric cancer remain elusive, necessitating further research to elucidate its contribution.

Cytoplasmic lncRNAs are known to regulate the expression of downstream target genes through competitive binding to miRNAs. Yuan et al. [8] demonstrated that the lncRNA CAT104 is overexpressed in gastric cancer and significantly correlates with tumor dimensions, TNM staging, lymphatic metastasis, and an unfavourable prognosis. The inhibition of CAT104 resulted in diminished cell viability, reduced migratory and invasive capacities of NCI-N87 cells, and enhanced apoptosis. Furthermore, CAT104 was found to facilitate gastric cancer progression by competitively binding to miR-381, thereby inducing the

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overexpression of zinc finger E-box binding homologous box 1 (ZEB1). These discoveries underscore the pivotal role of lncRNAs in gastric cancer development. This study is intended to deeply analyze the potential biological functions and regulatory mechanisms of LINC00520 in gastric cancer. These research efforts provide a novel theoretical basis for the prognosis and treatment of gastric cancer, thus improving our understanding of the disease.

2. Materials and methods

2.1. Tissue samples

In this investigation, we collected surgical specimens and corresponding normal tissues from 67 gastric cancer patients at Bethune International Peace Hospital from January 2019 to December 2023. Patients had not received any chemotherapy or radiotherapy before the surgical procedure. The samples were divided into two portions: one portion was promptly cryopreserved in liquid nitrogen and stored within a -80℃ freezer for subsequent RNA extraction and qRT-PCR analysis; the other was prepared into sections for immunohistochemical experiments. The research team adhered to stringent ethical guidelines and obtained informed consent from all participants. The study protocol was approved by the Ethics Committee of Bethune International Peace Hospital (approval number 2021-KY-56).

2.2. Cell culture

Gastric cancer cell lines (SGC7901, HGC27, MGC803, BGC823) were purchased from the Typical Cultures Conservation Center, USA. The cells were routinely cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) at 37℃ in a 5% CO_2 atmosphere. The cell lines SGC7901 and BGC823 were chosen for further experimentation based on their markedly distinct expression profiles.

2.3. RNA isolation and qRT-PCR

Cell samples were collected, and total RNA was extracted using TRIzol reagent (Invitrogen). The Transcriptor First Strand cDNA Synthesis Kit (Roche) was utilized to transcribe RNA into cDNA. According to the manufacturer's instructions for the GoTaq® qPCR Master Mix (Yisheng), the expression of LINC00520, HIF1A, and miR-519b-3p were assessed using qRT-PCR. The qPCR reaction (10 μl total volume) contained 0.2 μl each of forward and reverse primers, 5 μl of master mix, 3.6 μl of H_2O, and 1 μl of cDNA. Cycling parameters were: denaturation at 95℃ for 10 min, then 50 cycles of 95℃ for 15 s, 60℃ for 15 s, and 70℃ for 30 s. The $2^{-\Delta\Delta CT}$ method was employed to calculate the relative gene expression, and primer details are provided in Table 1.

2.4. Cell transfection

GenePharma synthesized short hairpin RNAs (shRNAs) to silence LINC00520 expression, and a sh-NC plasmid served as a negative control. miR-519b-3p mimics and inhibitors were also obtained from GenePharma. Gastric cancer cells in the logarithmic growth phase were seeded into 6-well plates. Once the cell confluence reached 80~90%, the plasmids were introduced into BGC823 and SGC7901 cells using Lipofectamine 2000 reagent (Invitrogen) and cultured continuously for 48 h.

2.5. Subcellular isolation localization

Nuclear and cytoplasmic RNA was isolated using a Nuclear/Cytoplasmic Isolation Kit (Biovision). qRT-PCR was conducted to quantify LINC00520 expression levels in the cytoplasmic and nuclear fractions. GAPDH and U6 served as endogenous controls for normalization across these fractions.

2.6. MTS assay

Cell density was adjusted to 1000 cells per well and seeded into 96-well plates. Following incubation at 0, 12, 24, 48, 72, and 96 h, 20 μL of MTS reagent were added to each well. Subsequently, after 2-h incubation, the absorbance was measured at 492 nm using a Spark® multimode microplate reader (Tecan Group Ltd.). This experiment assesses changes in cell growth and metabolic activity.

2.7. Clone formation assay

BGC823 and SGC7901 cells were seeded into six-well plates at a density of 3000 cells per well. Following seeding, the cells were incubated for 7 days. The cells were fixed with 10% formaldehyde for 20 min. Then, the cells were washed thrice with phosphate-buffered saline (PBS) for 5 min each. Cells were stained with 0.1% crystal violet for 20 min. The number of cell colonies was visualized and manually counted using a light microscope.

2.8. Transwell assay

The abilities for cell migration and invasion were assessed using a 24-well transwell chamber with an 8 μm pore-sized polycarbonate membrane (Corning, USA) in the absence (for migration) or presence (for invasion) of Matrigel. 5×10^4 cells were seeded into the upper chamber, and 500 μL of medium supplemented with 10% FBS in the lower chamber. To assess invasive capacity, 50 μL of Matrigel (BD Biosciences) was applied to coat the membrane surface. Following a 24-h incubation at 37℃, cells that had invaded were fixed with 10% formaldehyde and stained with 0.1% crystal violet for 20 min. The number of invaded cells was quantified using an inverted microscope.

Table 1. qPCR primer sequence and reaction conditions

gene	Primer sequence	Annealing temperature (℃)	Product size (bp)
LINC00520	F:5'-GCAGTTGAGCTTCTTGTGCC-3' R:5'-GTGGTCAGAGGTGGCCATAC-3'	60	250
HIF1A	F:5'-GCCCTAACGTGTTATCTGTCG-3' R:5'-CTTTTGCTCCATTCCATTCTG-3'	58	248
GAPDH	F:5'-AGGTGAAGGTCGGAGTCAACG-3' R:5'-AGGGGTCATTGATGGCAACA-3'	58	102

Note: Forward primer (F); Reverse primer (R)

2.9. Immunohistochemical staining

Gastric tissue samples were immersed in 4% paraformaldehyde for fixation, dehydrated, and then embedded in paraffin. Sections were cut to a thickness of 4 μm, deparaffinized using a xylene-based clearing agent, and rehydrated through a graded series of ethanol washes. Subsequently, the sections were treated with a specific primary antibody against HIF1A (Proteintech) at 4℃, followed by incubation with a corresponding secondary antibody for 30 min at room temperature, and counterstained with hematoxylin. Ultimately, the stained sections were examined and photographed using a microscope.

2.10. Bioinformatics analysis

The gastric cancer chip GSE79973 from the GEO database (https://www.ncbi.nlm.nih.gov/geo/) was adopted to determine the variably expressed genes among gastric cancer and normal tissues ($\log|\text{fold change}| > 1$, $P < 0.05$). Bioinformatics analysis predicted the interactions among LINC00520, miR-519b-3p, and HIF1A. Starbase v2.0 (http://starbase.sysu.edu.cn) identified the binding sites of LINC00520 and miR-519b-3p. TargetScan 7.2 (http://www.targetscan.org) and miRWalk 3.0 (http://zmf.umm.uni-heidelberg.de/apps/zmf/mirwalk2) predicted the target genes of miR-519b-3p.

2.11. Dual luciferase activity assay

All luciferase reporter plasmids (LINC00520-MT, LINC00520-WT, HIF1A-MT, HIF1A-WT) were synthesized by GenePharma. SGC7901 cells were seeded in 6-well plates and cultured to reach 80% confluence. The respective luciferase reporter plasmids were co-transfected with miR-519b-3p minics or miR-NC into SGC7901 cells using Lipofectamine 2000. At 48 h post-transfection, luciferase activities were measured following the manufacturer's protocol for the Dual-Luciferase Reporter Assay (Promega).

2.12. RNA Immunoprecipitation (RIP) assay

MS2-RIP assay was performed using Magna RIP™ RNA Binding Protein Immunoprecipitation Kit (Millipore). Transfected gastric cancer cells were harvested and lysed with RIP Lysis Buffer. Magnetic beads (Invitrogen) were added to the RIP lysis buffer, and then the beads were ligated with GFP antibody (Roche) or IgG (overnight, 4℃). Immunoprecipitated RNA was obtained after digestion with proteinase K (Solarbio) and then quantified by qRT-PCR.

2.13. RNA pull-down assay

The SGC7901 cells underwent transfection with bio-NC and bio-miR-519b-3p (GenePharma). Subsequently, after a 48 h incubation period, the cells were harvested and exposed to a lysis buffer (Thermo) for 10 min at 4 ℃. The lysed cells were processed with streptavidin beads (Millipore) overnight at 4 ℃. Following sequential washes using pre-cooled pyrolysis buffer, low salt buffer, and high salt buffer, the RNA bound to the beads was isolated and subjected to qRT-PCR analysis.

2.14. Western blot analysis

The cells were harvested and lysed using RIPA buffer containing PMSF (Solarbio). Subsequently, the total protein lysates were fractionated by 10% SDS-PAGE and transferred onto PVDF membranes (Millipore). The membrane was immersed in 5% nonfat milk for 2 h at 37°C, followed by overnight incubation with a primary antibody (rabbit anti-human, diluted 1:1000) at 4°C. After washing three times with TBST buffer, the membrane was incubated with an HRP-conjugated secondary antibody (sheep anti-rabbit, diluted 1:5000) for 1 h. Finally, enhanced chemiluminescence (ECL) was used to visualize the results.

2.15. Animal xenograft experiments

Six male mice were acclimated in animal rooms for three days, housed singly under standard conditions, and provided with food and water ad libitum. Subsequently, the mice were randomly assigned to two groups, each consisting of three animals. The mice in the experimental groups received a subcutaneous injection of gastric cancer cells stably transfected with sh-LINC00520 into their right dorsal region (2×10^6 cells per mouse), while the control group received sh-NC transfected cells. Tumor xenograft volumes were measured every four days after the establishment of the xenograft model using digital callipers, with the volume calculated using the formula: volume = 1/2 (length × width^2). After 24 days, the mice were euthanized, and the xenograft tumors were excised and weighed.

2.16. Statistical analysis

SPSS 20.0 and GraphPad Prism 6.0 were utilized to analyze the experimental data, and results were expressed as the mean ± standard deviation. Differences between two or more groups were assessed using Student's t-test or one-way ANOVA. Spearman or Pearson correlation analysis was performed to evaluate the correlation between LINC00520, miR-519b-3p, and HIF1A. *P*-values below 0.05 were considered to indicate statistical significance.

3. Results

3.1. LINC00520 was significantly upregulated in gastric cancer

The microarray data of gastric cancer GSE79973 was found in the GEO database, and the differential gene expression analysis was performed with the criteria of $\log|\text{fold change}| > 2$ and $P < 0.05$. LINC00520 was upregulated 2.25-fold in gastric cancer tissues relative to normal tissues (Fig. 1A and B). GEPIA database demonstrated that LINC00520 was significantly elevated in gastric cancer tissues(Fig. 1C). To investigate the potential role of LINC00520 in gastric cancer, qRT-PCR analysis was conducted to quantify LINC00520 expression in gastric cancer tissues. The results indicated that LINC00520 expression was significantly higher in gastric cancer tissues (Fig. 1D) and was also associated with lymph node metastasis (Fig. 1E). LINC00520 was significantly elevated in gastric cancer cells (Fig. 1F). Receiver operating characteristic (ROC) curve analysis demonstrated that the area under the curve (AUC) of LINC00520 was 0.8297 (0.7521-0.9073), with a sensitivity of 76.12% and specificity of 85.07%(Fig. 1G). These findings imply that LINC00520 may serve an oncogenic role in gastric cancer tumorigenesis and hold potential as a biomarker for diagnosing and predicting the prognosis of gastric cancer.

3.2. Knockdown of LINC00520 inhibited malignant progression in gastric cancer cells

To evaluate the biological function of LINC00520 in

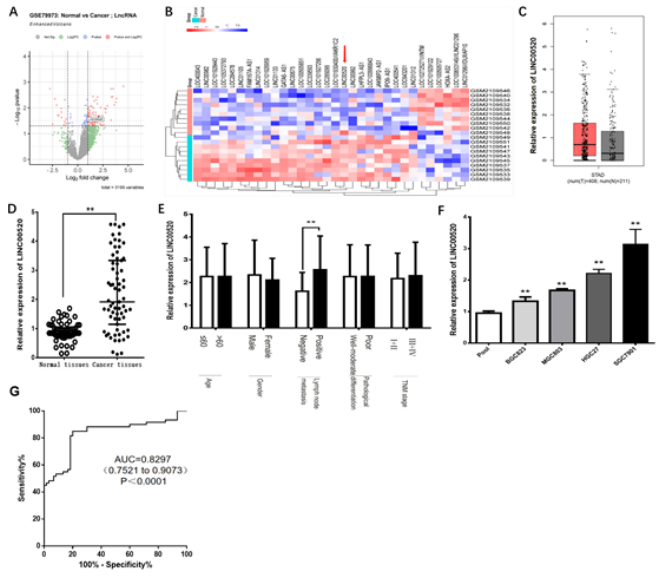

Fig. 1. LINC00520 is overexpressed in gastric cancer. GEO database identified differentially expressed genes in gastric cancer (A: volcano map, B: heat map). (C) GEPIA database showed that LINC00520 was highly expressed in gastric cancer tissues. (D) qRT-PCR was performed to detect the LINC00520 expression in 67 pairs of gastric cancer and normal samples. (E) Relative expression of LINC00520 in different subgroups. (F) qRT-PCR detection of LINC00520 expression in gastric cancer cell lines. (G) Receiver operating characteristic curve analysis showing an AUC of 0.8297 to distinguish gastric cancer from adjacent normal tissues. **P<0.01.

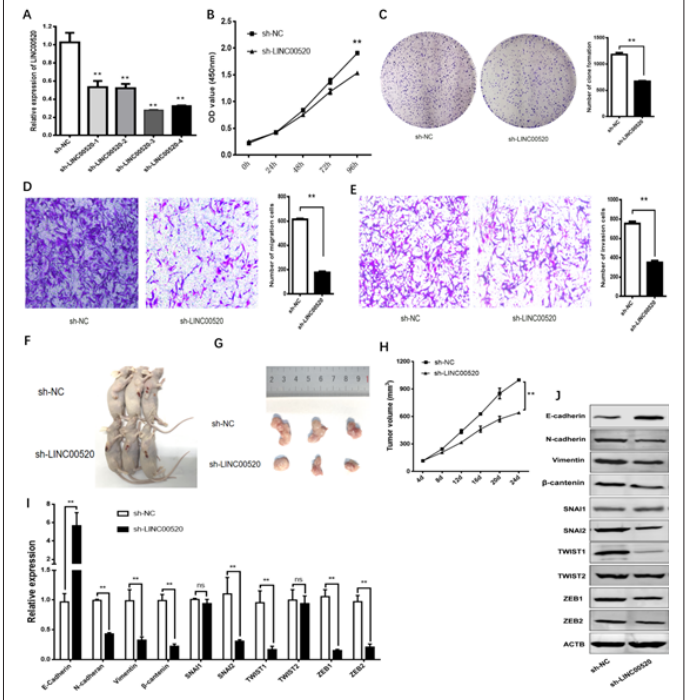

Fig. 2. LINC00520 promotes gastric cancer cell proliferation, migration and invasion ability. (A) qRT-PCR to detect the LINC00520 expression in gastric cancer cells transfected with sh-LINC00520. (B) MTS detection of the proliferation ability of gastric cancer cells transfected with sh-LINC00520. (C) The clone formation assay was performed to detect the number of clones of sh-LINC00520-transfected gastric cancer cells. Transwell assay was performed to verify the migratory ability (D) and invasive ability (E) of gastric cancer cells transfected with sh-LINC00520. (F, G, H) sh-LINC00520 inhibits the growth of gastric cancer cells in vivo. The mRNA (I) and protein (J) levels of EMT-related genes (E-cadherin, β-catenin and vimentin) in transfected gastric cancer cells with sh-LINC00520 as determined by qRT-PCR or Western blot. **P<0.01.

gastric cancer cells, a LINC00520 knockdown plasmid was transfected into SGC7901 cells, with sh-LINC00520-3 selected for subsequent experiments due to its superior knockdown efficiency (Fig. 2A). The MTS assay results demonstrated that the proliferative capacity of SGC7901 cells was significantly diminished following LINC00520 knockdown (Fig. 2B). Clone formation assays indicated that knockdown of LINC00520 significantly suppressed the proliferative capacity of SGC7901 cells (Fig. 2C). The effects of LINC00520 on the migration and invasion of gastric cancer cells were assessed using a transwell assay. The results revealed that LINC00520 knockdown significantly decreased the number of migrated and invaded cells (Fig. 2D and E). We established a nude mouse model to verify the role of LINC00520 in vivo. Nude mice were injected with gastric cancer cells stably expressing sh-LINC00520, and tumor volumes were measured every 4 days for 24 days. At 24 days after injection, mice were sacrificed, and tumors were excised. As shown in (Fig. 2F, G, H), sh-LINC00520 reduced the tumor size compared to the sh-NC group. These results suggest that sh-LINC00520 inhibited the growth of gastric cancer cells in vivo. Moreover, epithelial-mesenchymal transition (EMT) is an essential element that mediates gastric cancer metastasis. EMT markers include E-cadherin, vimentin and β-catenin. Our results record that E-cadherin expression was elevated after sh-LINC00520, while vimentin and β-catenin expression were downregulated (Fig. 2I, J). Consequently, it is inferred that LINC00520 is highly expressed in gastric cancer, and its knockdown significantly inhibits gastric cancer cells' proliferative, migratory, and invasive capacities.

3.3. miR-519b-3p as a downstream target gene of LINC00520

Nucleoplasmic isolation experiments indicated that LINC00520 was predominantly localized within the cytoplasm (Fig. 3A and B). Numerous reports have demonstrated that cytoplasmic lncRNAs can function as ceRNAs, counteracting the endogenous inhibitory effects of miRNAs on target mRNAs. The miRNAs targeted by LINC00520 were identified using the bioinformatics tool starbase v2.0, revealing a complementary binding site between LINC00520 and miR-519b-3p (Fig. 3C). qRT-PCR analysis revealed that miR-519b-3p expression was significantly downregulated in gastric cancer tissues (Fig. 3D and E) and was significantly inversely correlated with LINC00520 expression (Fig. 3F). To characterize this interaction further, RIP assays demonstrated that overexpression of LINC00520 led to an increased enrichment of miR-519b-3p (Fig. 3G). Luciferase assays indicated that in gastric cancer cells treated with miR-519b-3p mimics, luciferase activity was significantly diminished in the pmirGLO-LINC00520-WT group. In contrast, no significant change was observed in the mutant group (Fig. 3H). The results of the RNA pull-down experiment showed that LINC00520 in the Bio-miR-519b-3p-WT transfer group was significantly enriched compared with the negative control Bio-miR-NC (Fig. 3I). Based on these findings,

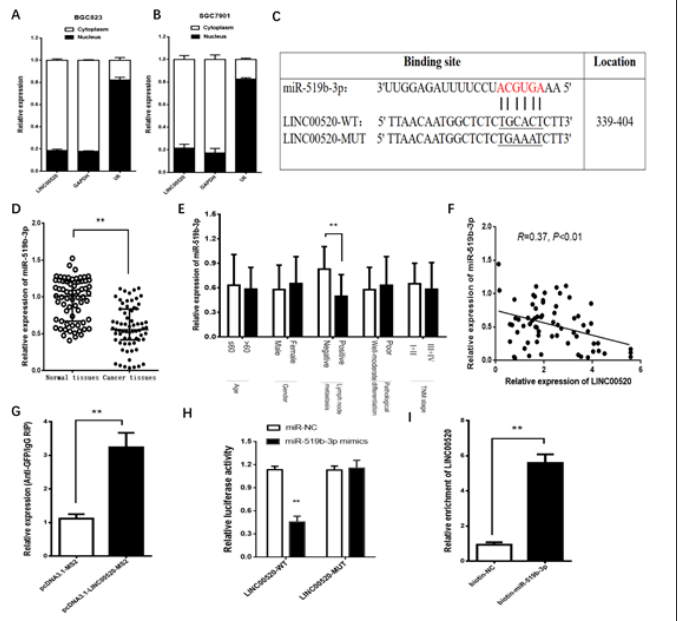

Fig. 3. LINC00520 acts on miR-519b-3p in gastric cancer. (A, B) Relative amounts of LINC00520 in the nucleus and cytoplasm of BGC823 and SGC7901 cells. (C) Bioinformatics analysis of the binding sites between miR-519b-3p and LINC00520 sequences. (D) qRT-PCR detection of miR-519b-3p expression in gastric cancer. (E) Relative expression levels of miR-519b-3p in different subgroups. (F) qRT-PCR analysis of the correlation between LINC00520 and miR-519b-3p. (G) RIP assay showing the enrichment of miR-519b-3p in LINC00520 complexes. (H) Dual luciferase reporter assay to verify the binding activity between LINC00520 and miR-519b-3p. (I) The enrichment of LINC00520 was detected by RNA pull-down assay after biotin labelling miR-519b-3p. **P<0.01.

we conclude that LINC00520 has a target-binding relationship with miR-519b-3p and negatively modulates its expression.

3.4. miR-519b-3p inhibits gastric cancer progression

To further validate the role of miR-519b-3p in gastric cancer, inhibitors and mimics of miR-519b-3p were transfected into BGC823 cells, with significant transfection efficiency observed (Fig. 4A). MTS and clone formation assays were conducted to assess cell proliferation. The results showed that the proliferation level of BGC823 cells was down-regulated in the miR-519b-3p mimics group, whereas miR-519b-3p inhibitor enhanced cell proliferation (Fig. 4B and C). Transwell assays demonstrated that miR-519b-3p overexpression significantly inhibited the migratory and invasive capabilities of BGC823 cells (Fig. 4D and E). Based on these results, it is hypothesized that miR-519b-3p plays a role in gastric cancer progression, potentially functioning as a tumor suppressor gene.

3.5. HIF1A is a downstream target of miR-519b-3p

Molecular targets of miR-519b-3p were identified using TargetScan and Starbase to discover potential candidate genes. Bioinformatics analysis indicated that miR-519b-3p is bound to HIF1A (Fig. 5A). qPCR and IHC analysis revealed significantly elevated HIF1A expression in gastric cancers compared to normal tissues (Fig. 5B, C, D) and an inverse correlation with miR-519b-3p expression (Fig. 5E). Conversely, HIF1A expression positively correlated with LINC00520 levels (Fig. 5F). To further subs-

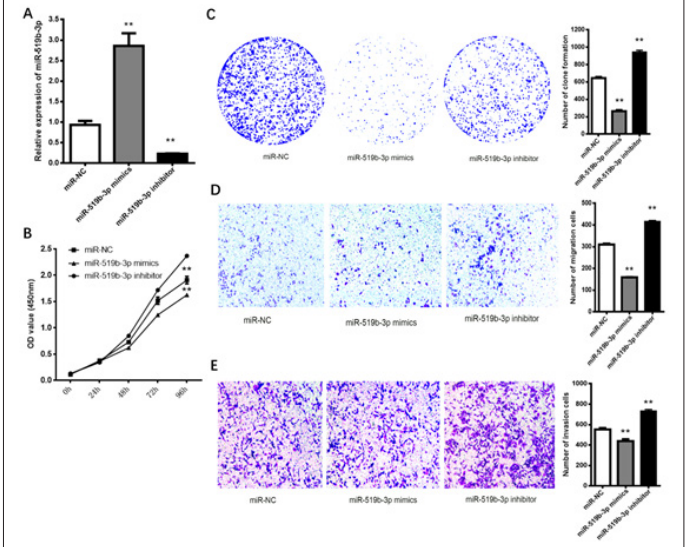

Fig. 4. Effects of miR-519b-3p on the biological behavior of gastric cancer cells. (A) qRT-PCR was performed to detect the transfection efficiency after transfection with miR-519b-3p mimics or inhibitors. MTS (B) and clone formation assay (C) experiments showed the effect of miR-519b-3p on proliferation ability. (D) Transwell migration assay shows the effect of miR-519b-3p on migration ability. (E) Transwell invasion assay shows the effect of miR-519b-3p on invasion ability. **P<0.01.

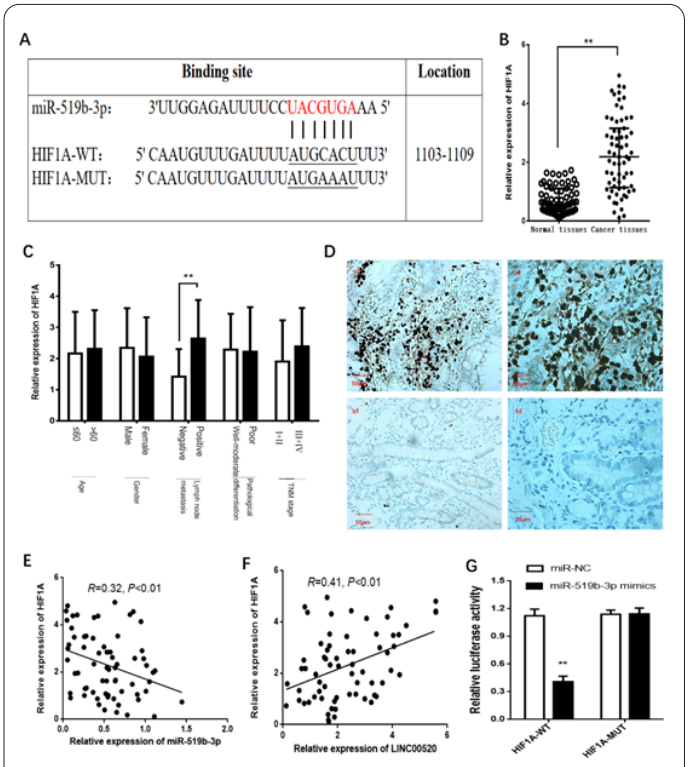

Fig. 5. HIF1A, a target gene of miR-519b-3p, is regulated by LINC00520. A bioinformatics approach to predict the binding site of miR-519b-3p on HIF1A 3'UTR and the corresponding mutant sequence. (B) qRT-PCR was used to detect the expression of HIF1A in gastric cancer tissues. (C) Expression levels of HIF1A in different subgroups. (D) Immunohistochemical staining (left: ×200, right: ×400) of HIF1A in gastric cancer tissues (a) and normal tissues (b). (E) Correlation analysis between miR-519b-3p and HIF1A expression in gastric cancer samples. (F) Correlation analysis between LINC00520 and HIF1A expression in gastric cancer samples. (G) Luciferase reporter assay showing the binding activity of miR-519b-3p with HIF1A. **P<0.01.

tantiate the interaction between miR-519b-3p and HIF1A, wild-type and mutant pmirGLO-HIF1A luciferase vectors were constructed and co-transfected with miR-519b-3p mimics or a non-targeting control (NC) into SGC7901 cells. Cells co-transfected with pmirGLO-HIF1A-WT and miR-519b-3p mimics exhibited significantly decreased luciferase activity, whereas cells with the mutant vector showed no significant change (Fig. 5G). These results suggest that HIF1A is a target of miR-519b-3p and is potentially regulated by the LINC00520/miR-519b-3p axis, implicating a ceRNA network that may modulate gastric cancer progression.

3.6. LINC00520 and miR-519b-3p co-regulate gastric cancer biological behavior

To elucidate the shared role of LINC00520 and miR-519b-3p in the regulation of gastric cancer progression, sh-LINC00520 and miR-519b-3p inhibitor plasmids were transfected into SGC7901 cells, and HIF1A expression was assessed using qRT-PCR or Western blot analysis. Knockdown of LINC00520 led to a significant reduction in HIF1A expression. Moreover, co-transfection with a miR-519b-3p inhibitor partially restored HIF1A expression (Fig. 6A and B). MTS and clone formation assays demonstrated that LINC00520 knockdown significantly inhibited the proliferative capacity of gastric cancer cells, and co-transfection partially restored this proliferative capacity (Fig. 6 C and D). The impact of co-transfection on the migration and invasion of gastric cancer cells was assessed using a transwell assay. The results indicated that LINC00520 knockdown significantly decreased cell migration and invasion. In contrast, co-transfection yielded opposing results (Fig. 6 E and F). Under hypoxic conditions, the stabilization and activity of HIF-1α is increased, leading to increased expression of VEGF. Subsequently, VEGF binds to VEGFR, further promoting angiogenesis and tumor cell adaptation to hypoxia. We observed that transfection of sh-LINC00520 inhibited VEGF and VEGFR expression, while miR-519b-3p reversed the promotion of VEGF and VEGFR expression by LINC00520 (Fig. 6 G and H). These findings indicate that LINC00520 and miR-519b-3p jointly regulate HIF1A expression and gastric cancer cells' proliferative, migratory, and invasive behaviors.

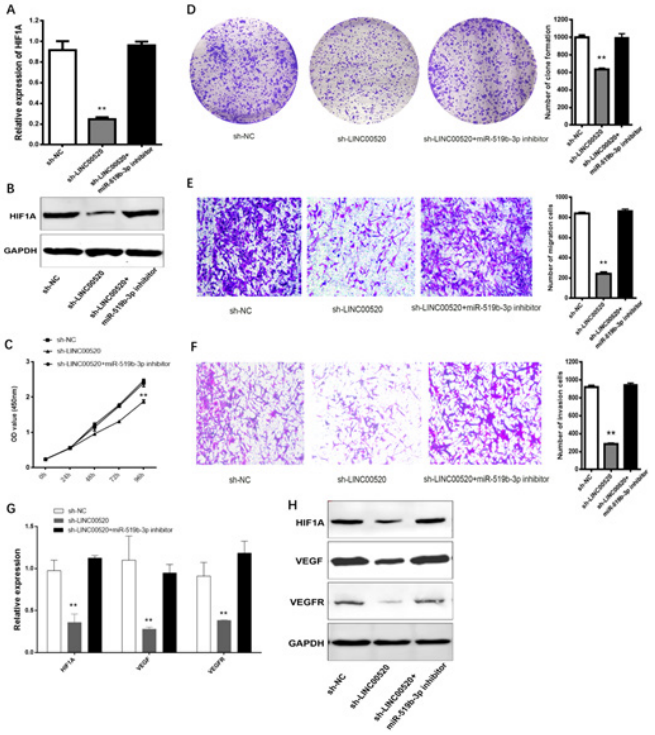

Fig. 6. Co-regulation of HIF1A expression and gastric cancer progression by LINC00520 and miR-519b-3p. (A) qRT-PCR or (B) Western blot was used to determine the effect of transfection with LINC00520 and miR-519b-3p on HIF1A levels. (C) MTS assay showing the effect of co-transfection on the proliferation of gastric cancer cells. (D) Clone formation assay showing the effect of co-transfection on the proliferation of gastric cancer cells. Transwell migration assay (E) shows co-transfection's effect on gastric cancer cells' migration ability. Transwell invasion assay (F) shows co-transfection's effect on gastric cancer cells' invasive ability. (G) qRT-PCR or (H) Western blot was used to determine the effect of transfection with LINC00520 and miR-519b-3p on VEGF and VEGFR levels. **P<0.01.

4. Discussion

Gastric cancer is a prevalent gastrointestinal malignancy with a progressively increasing global incidence and generally poor prognosis [9-10]. Apart from genetic predispositions, its aetiology is primarily linked to modifiable environmental factors, such as tobacco use, alcohol consumption, and the intake of high-calorie foods [11-12]. Identifying molecular targets for diagnosing and treating gastric cancer is crucial for improving patient outcomes [13-14]. An emerging body of experimental research underscores the significant role of long non-coding RNAs (lncRNAs) in gastric cancer development [15]. This study aimed to explore the LINC00520 expression profile in gastric cancer and elucidate its underlying mechanisms. Our findings indicate that LINC00520 is upregulated in gastric cancer and enhances gastric cancer cell proliferation, migration, and invasive capabilities, affirming its oncogenic role in disease progression.

MicroRNAs (miRNAs), a class of non-coding RNAs approximately 20-22 nucleotides long, are integral regulators of various biological processes, including cell differentiation, cellular homeostasis maintenance, and tumour cell malignancy modulation [16-18]. Cytoplasmic lncRNAs can serve as miRNA "sponges" post-transcriptionally regulating miRNA target genes through competitive binding, thereby influencing miRNA activity and cellular functions [19-23].

LINC00520 is predominantly located in the cytoplasm of gastric cancer cells. Bioinformatics analysis has revealed several miRNAs with potential interactions with LINC00520, with miR-519b-3p being significantly downregulated in gastric cancer and inversely correlated with LINC00520 expression. The interaction between LINC00520 and miR-519b-3p was validated through dual-luciferase reporter assays, RNA pull-down and RNA immunoprecipitation (RIP) assays. Moreover, miR-519b-3p has been shown to inhibit gastric cancer cell proliferation, migration, and invasion.

The mechanism by which miR-519b-3p influences gastric cancer can be explained by the canonical ceRNA (lncRNA-miRNA-mRNA) model. Bioinformatics and luciferase assays have identified that miR-519b-3p targets the 3' untranslated region (UTR) of the HIF1A gene, repressing its expression. HIF1A, a transcription factor implicated in angiogenesis, glycolipid metabolism, cell growth, apopto-

sis, migration, inflammation, and other physiological processes, is closely associated with oncogenesis and tumor progression [24-26]. Elevated HIF1A expression has been observed in various cancers, including gastric cancer [27-31]. qRT-PCR results demonstrate that HIF1A is significantly upregulated in gastric cancer tissues, positively correlated with LINC00520 expression, and inversely correlated with miR-519b-3p levels. Furthermore, overexpression of HIF1A promotes the malignant behavior of gastric cancer cells, reinforcing its role as an oncogenic factor. Our study suggests that LINC00520 promotes HIF1A expression by modulating miR-519b-3p during gastric cancer development, thereby facilitating the proliferation and metastasis of cancer cells. HIF-1α is a transcription factor that plays a key role in cellular adaptation to hypoxia. Under hypoxic conditions, the stabilization and activity of HIF-1α is increased, leading to increased expression of VEGF. Subsequently, VEGF binds to VEGFR, further promoting angiogenesis and cellular adaptation to hypoxia. This interaction forms a complex regulatory network, allowing cells to cope better with the hypoxic environment. This regulatory axis implicates LINC00520 as a potential therapeutic target for gastric cancer.

In conclusion, elevated LINC00520 expression significantly contributes to the malignant behavior of gastric cancer, enhancing cell proliferation, migration, and invasiveness. This lncRNA achieves this by modulating the expression of HIF1A by targeting miR-519b-3p within the cytoplasm, thereby facilitating tumor progression. Given these results, LINC00520 emerges as a promising therapeutic target for gastric cancer. Our findings offer a novel perspective for further research and lay the groundwork for potential therapeutic strategies against this devastating disease.

Abbreviations

LINC00520: Long intergenic non-protein coding RNA520; LncRNAs: Long non-coding RNAs;

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Availability of data and materials

The main paper included all the data and materials supporting the conclusions.

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Ethics approval and consent to participate

The study was conducted according to the Declaration of Helsinki principles and was approved by the Ethics Committee of Bethune International Peace Hospital.

Consent for publication

All authors have reviewed and approved the manuscript for publication.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Jie An is responsible for the literature review, discussion, and introduction of the article. Yunfeng Niu and Wei Liu are responsible for the manuscript's bioinformatics analysis, Materials and Methods, and Results sections.

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