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The lncRNA-MALAT1/miR-330-3p Axis Promotes Growth and Metastasis of Gastric Cancer Through Regulation of Vascular Endothelial Growth Factor A: A Genetic and Cell Evaluation Study

El Eje lncRNA-MALAT1/miR-330-3p Promueve el Crecimiento y la Metástasis del Cáncer Gástrico Mediante la Regulación del Factor de Crecimiento Endotelial Vascular A: Un Estudio de Evaluación Genética y Celular

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SUMMARY: Overexpression of metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) in various tumor tissues and cell lines was found to promote tumor cell proliferation, migration, and invasion. However, the role of MALAT1 in gastric cancer (GC) is still unclear. We aimed to investigate the correlation between long-chain non-coding RNAs (lncRNAs), MALAT1, MicroRNAs (miRNA) and vascular endothelial growth factor A (VEGFA) in gastric cancer and to disclose underlying mechanism. The correlation between MALAT1 levels and clinical features was analyzed by bioinformatics data and human samples. The expression of MALAT1 was down regulated in AGS cells to detect the cell proliferation, migration, and invasion characteristics, as well as the effects on signal pathways. Furthermore, we validated the role of MALAT1/miR-330-3p axis in GC by dual luciferase reporter gene assays. Expression of MALAT1 was higher in cancer tissues than in para-cancerous tissues. The high MALAT1 level predicted malignancy and worse prognosis. Down-regulation of MALAT1 expression in AGS cells inhibited cell proliferation, migration, and invasion by targeting VEGFA. By dual luciferase reporter gene assay and miR-330-3p inhibitor treatment, we demonstrate that MALAT1 sponged miR-330-3p in GC, leading to VEGFA upregulation and activation of the mTOR signaling pathway. The MALAT1/miR-330-3p axis regulates VEGFA through the mTOR signaling pathway and promotes the growth and metastasis of gastric cancer.

KEY WORDS: Gastric cancer; lncRNA MALAT1; miR-330-3p; VEGFA; mTOR signaling pathway.

INTRODUCTION

Gastric cancer is the most common malignancy of the digestive system and is one of the leading causes of cancer-related deaths worldwide. Despite advanced patient management and aggressive surgical techniques, the improvement in 5-year overall survival has been unsatisfactory (Russo & Strong, 2019). Furthermore, the molecular mechanisms underlying gastric cancer progression are unclear. Therefore, further studies are needed to elucidate the mechanisms of gastric cancer progression and to identify new therapeutic targets.

Long-chain non-coding RNAs play important roles in various biological processes such as immune response, angiogenesis, cell proliferation, apoptosis, autophagy, cell migration and invasion (Lee *et al*., 2012; Fang & Fullwood, 2016; Sun, 2018). Various studies have shown that lncRNA plays an important role in the development of various types of cancers, such as breast, colorectal, and liver cancers (Lee *et al*., 2012; Yang *et al*., 2012; Fang & Fullwood, 2016; Sun, 2018; Tsai *et al*., 2018; Zhang *et al*., 2019). In addition, various previous studies have reported the aberrant expression and function of lncRNA in cancer (Yang *et al*., 2012; Fang & Fullwood, 2016).

Metastasis-associated lung adenocarcinoma transcript 1 is a lncRNA that is strongly associated with lung

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cancer progression and poorer clinical outcomes (Ji *et al*., 2012). Currently, Xia *et al*., reported that MALAT1 can be served as a diagnostic marker for gastric cancer metastasis (Xia *et al*., 2016). Furthermore, MALAT1 has been reported to upregulate VEGFA and promote angiogenesis (Ren *et al*., 2019). However, the mechanism by which MALAT1 promotes GC progression is largely unclear.

We screened for miRNAs that could bind to MALAT1 or VEGFA respectively, in which miR-330-3p had opposite regulatory effects to MALAT1. In the present study, we investigated the effect of MALAT1 on GC and the correlation between MALAT1 and miR-330-3p using human GC in situ tissues and in vitro GC cell lines. We proved that MALAT1 expression was upregulated in gastric cancer tissues compared to normal gastric tissues and that MALAT1 was negatively correlated with miR-330-3p by targeting VEGFA and its downstream signaling molecules. Furthermore, our current evidence suggests that MALAT1 is an endogenous RNA (ceRNA) that competes with miR-330-3p.

MATERIAL AND METHOD

Patients and specimens. A total of 7 benign gastric tissue, 29 poor-differentiated and 48 well-differentiated cancerous tissue were collected under surgical resection in the Liuzhou People's Hospital during 2022 Jan to 2023 Nov. All of the samples were saved in liquid nitrogen until further analysis. Patients did not receive any chemotherapy or radiotherapy prior to the procedure. This research was permitted by Ethics Committee of the Liuzhou People's Hospital.

Bioinformatic analysis. The website of Gene Expression Profiling Interactive Analysis (http://gepia.cancer-pku.cn) was applied to inquire and analyze the expression differences of MALA1 between gastric cancer tissues (n=408) and para-cancer tissue (n=211). The gastric cancer data set from Kaplan-Meier Plotter online database (http:/kmplot.com/analysis/) was used for online data, and the gastric cancer samples were divided into the high-expression group and the low-expression group for survival analysis.

We screened the miRNA which can bind with MALAT1 or VEGFA by analysis of the public databases in starBase, miRTarbase and Lncbase, and then used the intersection to obtain miRNA that can predict the target with MALAT1 and VEGFA.

Cell culture. GES1, MKN45, MGC803, and AGS cells were purchased from the Model Culture Collection (ATCC, Manassas, VA, USA). All of the cells were cultured with RPMI1640 medium. All complete medium consisted of 10 % FBS (fetal bovine serum), 1 % penicillin (100 U/mL) and streptomycin (0.1 mg/mL). Total cells were cultivated at 37 ºC with 5 % CO_2. Reagents used in cell culture were bought from Invitrogen (Waltham, MA, USA).

Cell transfection. MALAT1 shRNA and shRNA negative control (NC) miR-330-3p inhibitor were bought from GenePharma (Shanghai, China). AGS cells were harvested at confluence of 70-80 % and 35nM shRNA were transfected into 10^5 cells in a 2 ml cell suspension. This experiment included untransfected cells as control group. The interval between transfection and the subsequent experiment was 24 h.

Total RNA and qPCR. Total RNA was isolated from human gastric cancer tissue samples using TRIzol® Reagent Invitrogen (Invitrogen, USA). Then RNA samples were reversely transcribed by PrimeScript RT Master Mix kit (Bestar, Shanghai, China). The expression levels of mRNA was examined by SYBR Premix Ex Taq™ (Bestar, Shanghai, China). GAPDH was considered as the internal reference gene. $2^{-\Delta\Delta Cq}$ was used to analyze the relative expression of each genes. Primers were designed as follows: GAPDH: sense, 5'- AGAAGGCTGGGGCTCATTTG -3' and antisense, 5'- AGGGGCCATCCACAGTCTTC -3; MALAT1: sense, 5'- TGGGATGGTCTTAACAGGGA -3' and antisense, 5'- CCTGAAGGTGTTCGTGCCAA -3; VEGFA: sense, 5'- CGCAGCTACTGCCATCAAT -3' and antisense, 5'- GTGAGGTTTGATCCGCATAATCT -3; AKT: sense, 5'- CCCAGGAGGTTTTTGGGCTT -3' and antisense, 5'- AAGGTGCGTTCGATGACAGT -3; mTOR: sense, 5'- AACCTCCTCCCCTCCAATGA -3' and antisense, 5'- AATCCCATGAGGCCTTGGTG -3; ERK1: sense, 5'- TGAAAACCACACGTCACATGG -3' and antisense, 5'- CCCTGCATTGATCCACCTGT -3; ERK2: sense, 5'- AGTTCTTGACCCCTGGTCCT -3' and antisense, 5'- CCTGGGACATCCCCAGAAAC -3.

CCK 8 assay. Cell proliferation was analyzed by using CCK 8 assay kit (Beyotime, China) according to the manufacturer instructions. Treated cells were incubated in 96-well plates with or without inhibitor for indicated times and followed by the addition of 10 μL CCK 8 reagent to each well. Samples were further incubated at 37 ºC for 1 h. OD values were measured at 450 nm.

Cell apoptosis and cell cycle assay. Cells were harvested after specific treatment, washed with ice-cold PBS for 3 times, and stained with annexin V-fluorescein isothiocyanate

apoptosis detection kits (KeyGEN Biotech, Nanjing, China). Cell apoptosis was analyzed in a flow cytometer (BD Biosciences).

After trypsinization, AGS cells were washed with cold PBS. Cold ethanol (75 %) was used to dissolve the cells, followed by incubation at 4 °C for 4 h. Cells were washed with cold PBS three times. After washing, cells were stained with BD Pharmingen™ PI/RNase for 30 min at 25 °C. The cell cycle was then analyzed by flow cytometry.

Western blot. After trypsinization, AGS cells were harvested and then lysed in RIPA buffer (RIBO-BIO) with protease inhibitors (Roche, Switzerland). The concentration of protein was examined by using the BCA Protein Assay kit (Genstar, China). Protein samples were separated by 10 % SDS-PAGE, and then transferred to PVDF membranes (Millipore, Boston, MA, USA). Next, the membranes were blocked with 5 % milk for 1 h at room temperature and following incubation of primary antibodies (anti-VEGFA, anti-AKT, anti-p-AKT, anti-mTOR, anti-p-mTOR, anti-p-ERK, anti-ERK, and anti-GADPH) at 4 °C overnight. The PVDF membranes were incubated for another 1 h at room temperature in secondary antibody after washing three times with TBST. Strips were exposed with StarSignal Plus Chemiluminescent Assay Kit (Genstar, China).

Transwell assay. Cells were cultured for 72 h, and then in serum-free medium for another 24 h. After detachment with 0.05 % trypsin- EDTA the cells were resuspended in a serum-free medium. Upper insert was filled with 100 µl of the cell suspension while reservoir chamber was filled with 600 µl of culture medium. Matrigel invasion assays were carried out in modified Boyden chambers with filter inserts with 8-mm pores in 24-well plates (Corning, NY, USA). The surfaces of the filters were coated with 50mg/L ice-cold Matrigel (Matrigel basement membrane matrix, BD Bioscience, NJ, USA). The migration experiment was treated the same as the invasion experiment except that no Boyden cavity was added. Migration or invasion of cells was monitored at 3, 6, and 12 h at 37 °C in 5 % CO_2. Crystal violet was used as the staining solution for staining. Afterwards, images were observed and captured using a microscope.

Dual-luciferase reporter gene assay. The relationship between miR-330-3p and MALAT1 was identified using dual-luciferase reporter gene assay. MALAT1 and VEGFA untranslated region was artificially synthesized and inserted into pGL3-control vector (Promega, Madison) between XhoI and BamH sites. Using site-directed mutagenesis, MALAT1, MALAT1 mutant and VEGFA mutant sequence were conducted on the basis of wild-type sequence. Recombinant plasmids were co-transfected into HEK 293T cells (Shanghai Institute of Biological Sciences, Chinese Academy of Sciences, Shanghai, China) with miR-330-3p inhibitor and the negative control (NC) of miR-330-3p, respectively. After transfection for 48 h, the cells were lysed for determination of luciferase activity, which was measured on a Luminometer TD-20/20 (E5311; Promega, Madison, WI, USA) using a dual-Luciferase Reporter Assay System kit (Promega, Madison, WI, USA).

Statistical analysis. All assays were conducted in three separate experiments. All data were expressed as mean ± standard deviation (SD). Statistical analysis was performed with SPSS 22.0 software (SPSS, Chicago, IL, USA). Single factor analysis of variance is used to compare differences between multiple groups. Kaplan-Meier analysis was used to determine the effects on overall survival of GC patients. Differences were considered statistically significant when P values less than 0.05.

RESULTS

MALAT1 leads to poor clinical prognosis. According to the bioinformatics analysis of the database, we found that the expression of MALAT1 was higher in cancer tissues than in para-cancerous tissues (Fig. 1A).Kaplan-Meier curves suggested that elevated MALAT1 levels resulted in shorter overall survival (Fig. 1B). In addition, we analyzed MALAT1 levels in gastric cancer tissues with different degrees of malignancy and found that MALAT1 expression in cancer tissues was positively correlated with the malignancy of the tumor. MALAT1 expression was higher in low-differentiated carcinoma than in high-differentiated carcinoma, and MALAT1 expression was higher in gastric cancer than in benign gastric tissues (Fig. 1C).Kaplan-Meier curve was consistent with these results, and low-differentiated carcinoma with higher MALAT1 levels had a poorer prognosis (Fig. 1D).

MALAT1 promotes cell proliferation, migration and invasion by targeting VEGFA. Since we have found a positive correlation between MALAT1 levels and tumor characteristics, we will next attempt to demonstrate the role of MALAT1 in gastric cancer cells. We tested four different types of gastric cells and found that MALAT1 was the most highly expressed in AGS cells (Fig. 2A). PCR analysis showed that all three Lnc-shRNAs were well downregulated, with Lnc-sh1 being the most downregulated (Fig. 2B).

After transfection of Lnc-sh1 in AGS cells, AGS

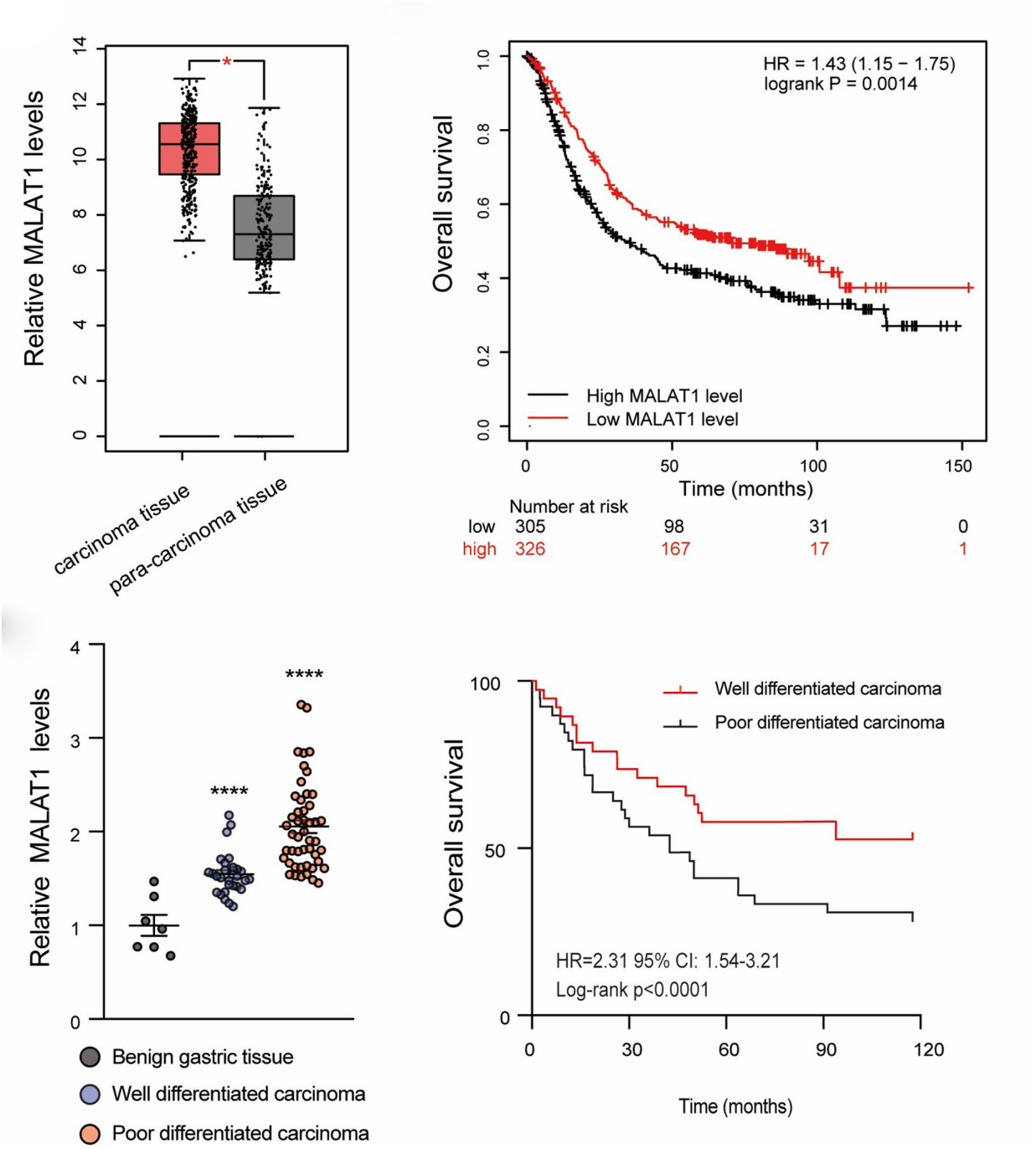

Fig. 1. MALAT1 is positively associated with clinical outcomes of patients with gastric cancer. (A) Relative MALAT1 expression in carcinoma tissue and para-carcinoma tissue of gastric cancer. (B) Kaplan-Meier analysis of patients with high or low MALAT1 level gastric cancer. (C) Relative MALAT1 expression in benign gastric tissue, well or poor differentiated carcinoma of gastric cancer. (D) Kaplan-Meier analysis of patients with well or poor differentiated gastric cancer.

cell survival was significantly decreased at 48 h and 72 h compared with normal AGS cells and NC-transfected AGS cells (Fig. 2C). After Lnc-sh1 transfection in AGS cells, most of the cells remained in the G0/G1 phase, which was the result of reduced cell viability (Fig. 2D). Furthermore, based on the apoptosis assay, we found that reducing MALAT1 levels in AGS cells could significantly induce apoptosis (Fig. 2E). Moreover, down-regulation of MALAT1 expression significantly inhibited cell migration and invasion capacity (Figs. 2F and 2G).

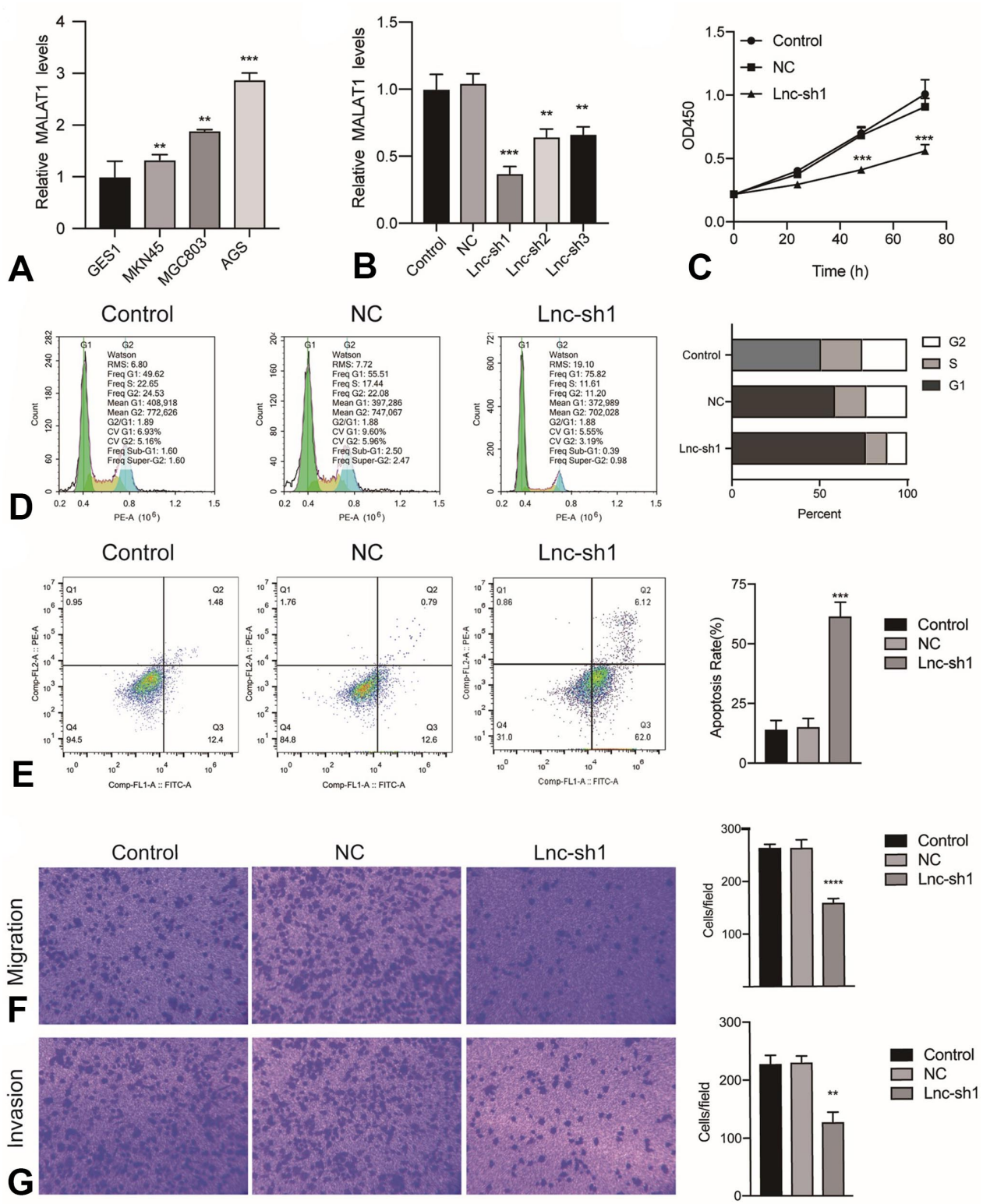

Fig. 2. MALAT1 promotes cell proliferation, migration, and invasion. (A) Relative MALAT1 expression in GES1, MKN45, MGC803, and AGS cell lines. (B) Relative MALAT1 expression in AGS cell after transfection with NC, Lnc-sh1, Lnc-sh2, and Lnc-sh3. (C) Cell viability of AGC cells after transfection with NC, Lnc-sh1. Representative and quantification of (D) cell cycle and (E) cell apoptosis of AGC cells after transfection with NC, Lnc-sh1.Representative and quantification of (F) cell migration and (G) cell invasion assays of AGC cells after transfection with NC, Lnc-sh1. NC: Negative control, Lnc-sh1: shRNA1 Lnc-MALAT1.

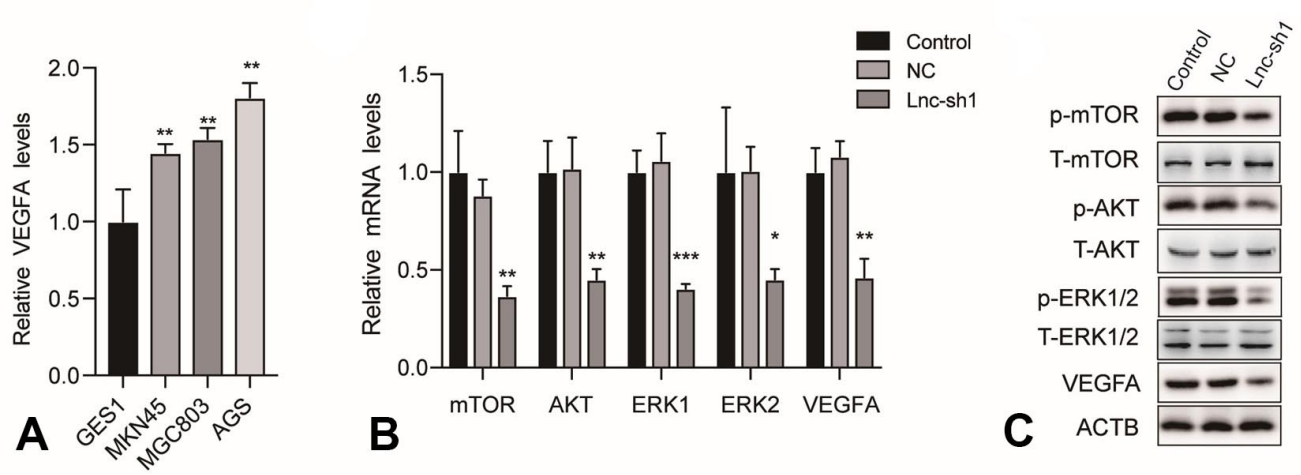

Fig. 3. MALAT1 regulates VEGFA and activates mTOR signal pathway. (A) Relative VEGFA expression in GES1, MKN45, MGC803, and AGS cell lines. (B) Relative mRNA levels of mTOR, AKT, ERK1, ERK2, and VEGFA in AGS cells after transfection with NC, Lnc-sh1. (C) Western blot analysis of activation of mTOR signal pathway and VEGFA in AGS cells after transfection with NC, Lnc-sh1. NC: Negative control, Lnc-sh1: shRNA1 Lnc-MALAT1.

Due to the critical role of VEGFA in tumor progression, we detected the expression of VEGFA in four different gastric cancers, and we found that AGS cells expressed more VEGFA than other cells, which was consistent with the level of MALAT1 (Fig. 3A). By qPCR and western blot analysis, knockdown of MALAT1 expression significantly inhibited the activation of ERK and mTOR signaling pathways and VEGFA expression (Figs. 3B and 3C).

miR-330-3p has binding sites to both of MALAT1 and VEGFA. Based on bioinformatics analysis, we screened 16 microRNAs for the potential to bind MALAT1 and VEGFA, and dual luciferase assay showed that the inhibitor of mir-330-3p in AGS, the ability to express luciferase was elevated in cells with dual luciferase vectors for either the MALAT1 target or the VEGFA target. Whereas the luciferase expression capacity was not significantly different from miRNA-NC transfected cells after mutation of the target gene. The above results indicated that miR-330-3p had binding sites for both MALAT1 and VEGFA (Figs. 4A and 4B). In addition, the miR-330-3p level in Lnc-sh1-transfected AGS cells was higher than that in the control group, indicating a strong negative correlation between MALAT1 and miRNA-330-3p (Fig. 4C).

MALAT1/miR-330-3p axis regulate VEGFA and activating mTOR signal pathway. After downregulation of MALAT1 expression, cell viability was significantly inhibited, which was reversed by miR-330-3p inhibitor (Fig. 4D). The cell cycle results showed that compared with the Lnc-NC group, the proportion of cells with G0/G1 phase block was reduced after miR-330-3p inhibitor treatment (Fig. 4E). Moreover, compared with the Lnc-NC group, miR-330-3p inhibitor treatment significantly inhibited apoptosis (Fig. 4F). In addition, miR-330-3p inhibitor had a strong promoting effect on cell migration and invasion, whereas the inhibitory effect of MALAT1 could be completely reversed (Figs. 4G and 4H).

After we demonstrated the ability of MALAT1 to regulate miR-330-3p and that VEGFA is downstream of the MALAT1/miR-330-3p axis, we sought to demonstrate the mechanism. As shown in Figure 5, qPCR and western blot data indicated that inhibition of MALAT1 expression in AGS cells inhibited VEGFA expression and activation of the mTOR signaling pathway. Negative control cells treated with miR-330-3p inhibitor showed a significant increase in VEGFA levels and activation of mTOR signaling, whereas knockdown of MALAT1 reversed these promotive effects, indicating that MALAT1 sponging miR-330-3p to regulates VEGFA and activation of signaling pathways.

DISCUSSION

Various studies have shown that lncRNA plays a crucial role in the development of various types of cancers. The function of lncRNA in GC has been less studied. The interaction of tumor-associated lncRNA and miRNAs is crucial for exploring their roles in tumor progression and developing new therapeutic strategies. This study explored the role of lncRNA MALAT1 and its related miRNAs in GC and their potential mechanisms. This study explored the role of lncRNA MALAT1 in GC and its mechanism.

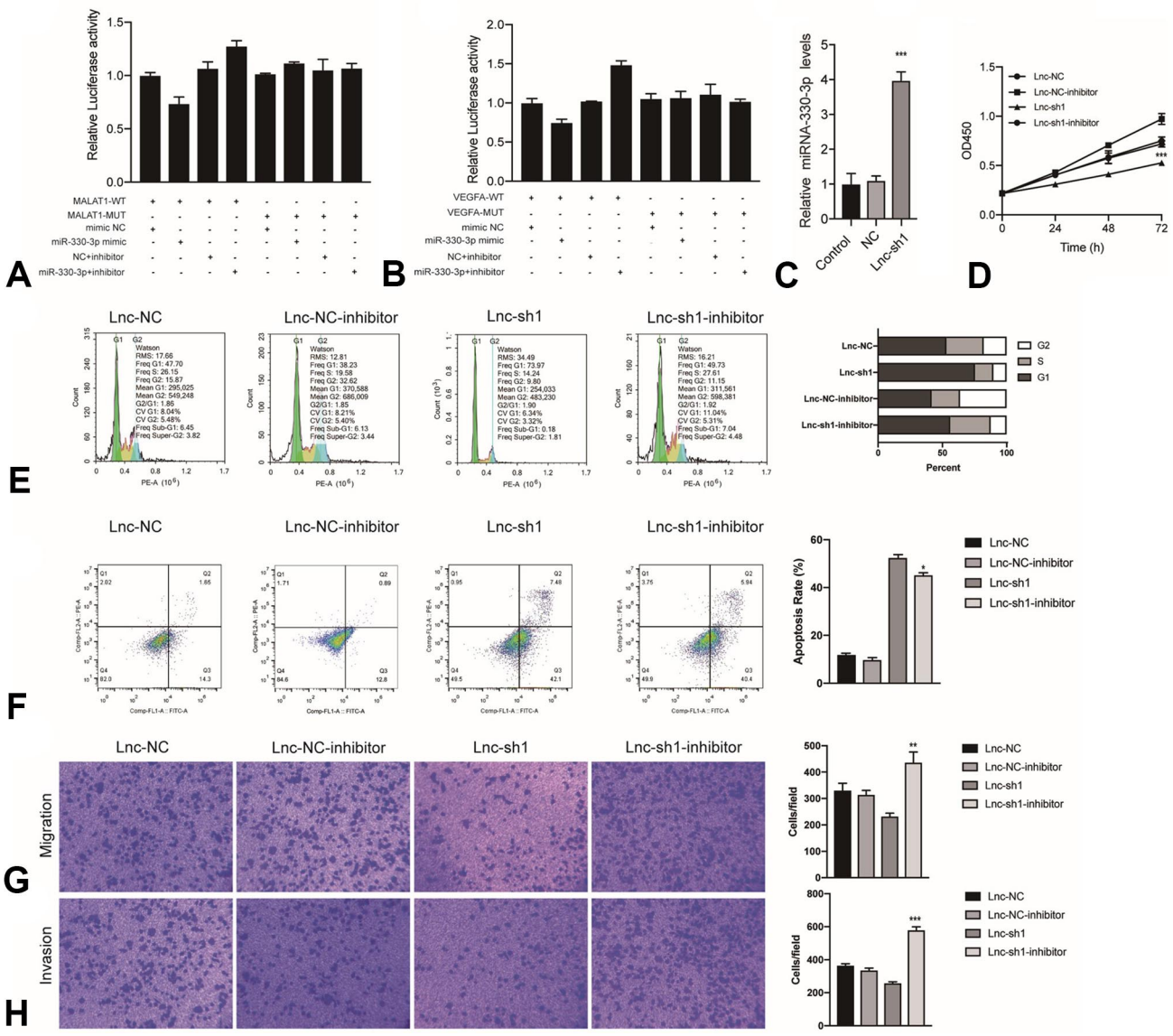

Fig. 4. MALAT1 exerts promoting function by down-regulation of miR-330-3p. (A-B) Double luciferase experiment. (C) Relative miRNA-330-3p levels in AGS cells after transfection of NC, Lnc-sh1. (D) Cell viability of AGS cells after transfection of NC, Lnc-sh1 with or without treatment of miR-330-3p inhibitor. Representation and quantification of (E) cell cycle and (F) cell apoptosis of AGS cells after transfection of NC, Lnc-sh1 with or without treatment of miR-330-3p inhibitor. Representation and quantification of (G) cell migration and (H) cell invasion of AGS cells after transfection of NC, Lnc-sh1 with or without treatment of miR-330-3p inhibitor. Lnc-sh1: shRNA1 Lnc-MALAT1, Lnc-NC: LncRNA Negative control, Lnc-NC-inhibitor: LncRNA Negative control+miR-330-3p inhibitor, Lnc-sh1-inhibitor: shRNA1 Lnc-MALAT1+miR-330-3p inhibitor

Firstly, lncRNA MALAT1 was found to be highly expressed in non-small cell lung cancer, and later confirmed to be expressed in a variety of tumors. previous studies have shown that MALAT1 promotes GC cell proliferation, migration, and invasion, and can be used as a GC marker in the clinic (Wang *et al*., 2014; Xia *et al*., 2016). In addition, MALAT1 plays an important role in angiogenesis of gastric cancer and promotes its growth and metastasis (Li *et al*., 2017a). In line with our findings, we found that MALAT1 expression leads to poor prognosis and promotes gastric cancer growth and metastasis by targeting VEGFA.

MicroRNAs are non-coding regulatory RNA of 20 - 25 bases in length that target specific mRNAs to interact

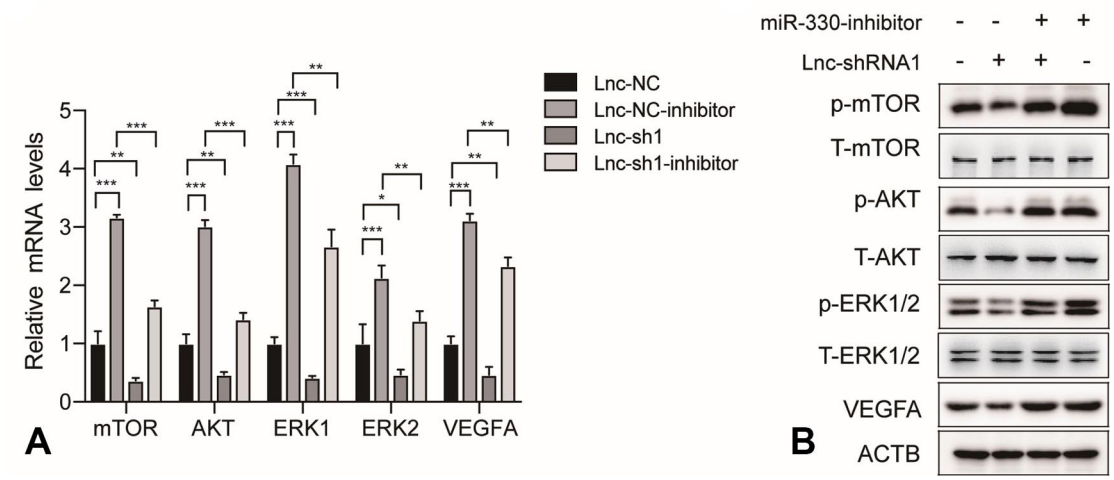

Fig. 5. MALAT1/miR-330-3p axis regulate VEGFA and mTOR signal pathway. (A) Relative mRNA levels of mTOR, AKT, ERK1, ERK2, and VEGFA in AGS cells after transfection of NC, Lnc-sh1 with or without treatment of miR-330-3p inhibitor. (B) Western blot analysis of activation of mTOR signal pathway and VEGFA in AGS cells after transfection of NC, Lnc-sh1 with or without treatment of miR-330-3p inhibitor. NC: Negative control, Lnc-sh1: shRNA1 Lnc-MALAT1, Lnc-NC: LncRNA Negative control, Lnc-NC-inhibitor: LncRNA Negative control+miR-330-3p inhibitor, Lnc-sh1-inhibitor: shRNA1 Lnc-MALAT1+miR-330-3p inhibitor.

with oncogenes and tumor suppressors (O'Donnell *et al*., 2005). It has been shown that more than 50 % of genes are located in cancer-associated genomic regions, suggesting that miRNAs may play a key role in tumor formation and progression (Calin *et al*., 2004). Based on bioinformatics analysis, we screened for miR-330-3p, which is an endothelium-specific microRNA that regulates vascular integrity and angiogenesis and is involved in the expression of a wide range of biological functions. Abnormal expression of miR-330-3p can be observed in a variety of tumors. Through targeting CCBE1, miR330-3p can accelerate the metastasis of breast cancer (Wei *et al*., 2017). In addition, recently, it has been demonstrated that miR-330-3p can affect the invasion and migration of GC cells via Wnt/b-catenin pathway, which may be an effective therapeutic target for diagnosis and treatment of GC (Ma *et al*., 2020).

In our study, we examined the levels of miR-330-3p in AGS cells with or without MALAT1 knockdown and found that the expression of miR-330-3p was significantly increased after downregulation of MALAT1. Several studies have shown that MALAT1 is involved in tumor progression and development, playing the role of ceRNA (Wang *et al*., 2016; Li *et al*., 2017b; Chang & Hu, 2018). On the basis of dual luciferase reporter gene experiments, we demonstrated the binding of MALAT1 and miR-330-3p. Furthermore, we demonstrated that MALAT1 expression down-regulates miR-330-3p levels, whereas knockdown of MALAT1 had the opposite effect. To further elucidate this point, we down-regulated MALAT1 expression in AGS cells by lentiviral vectors and combined with miR-330-3p inhibitors to maintain low expression of miR-330-3p, promote increased VEGFA and activation of mTOR signaling, and enhance cell proliferation, migration, and invasion.

In our study, we found that VEGFA could bind to miR-330-3p in GC cells by luciferase reporter gene experiments. Previous studies have shown that VEGFA can activate ERK and mTOR, two known kinases, which play a role in ovarian and hepatocellular carcinoma (Park *et al*., 2014; Siveen *et al*., 2014). Thus, the expression of miR-330-3p down-regulated VEGFA levels and inhibited the expression of downstream signaling molecules (p-AKT, p-mTOR, p-ERK). mTOR signaling pathway was not directly regulated by miR-330-3p but was significantly activated with the restoration of miR-330-3p levels. This suggests that the mTOR and ERK signaling pathways are involved in the miR-330-3p/VEGFA axis in GC. This molecular function experiment fully confirmed that MALAT1 weakened the regulation of miR-330-3p on VEGFA and downstream signaling pathways through the adsorption of miR-330-3p by sponge, which was the ceRNA regulatory mechanism between miRNA and lncRNA.

CONCLUSION

MALAT1 elevation plays an important role in promoting tumor growth and metastasis, and MALAT1 can significantly reduce miR-330-3p levels via ceRNA and

enhance the expression of VEGFA and activate signaling pathways in vitro, thereby inhibiting GC cell proliferation and invasion. Understanding how MALAT1 is involved in GC will help to develop potential therapeutic targets in clinical practice.

Ethics approval and consent to participate

Liuzhou People's Hospital Ethics Committee approved this study. All procedures performed in studies involving human participants were in accordance with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Written informed consent was obtained from all individual participants included in the study.

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RESUMEN: Se descubrió que la sobreexpresión del transcrito 1 de adenocarcinoma de pulmón asociado a metástasis (MALAT1) en varios tejidos tumorales y líneas celulares promueve la proliferación, migración e invasión de células tumorales. Sin embargo, el papel de MALAT1 en el cáncer gástrico (CG) aún no está claro. Nuestro objetivo fue investigar la correlación entre los ARN no codificantes de cadena larga (lncRNA), MALAT1, los microARN (miARN) y el factor de crecimiento endotelial vascular A (VEGFA) en el cáncer gástrico y revelar el mecanismo subyacente. La correlación entre los niveles de MALAT1 y las características clínicas se analizó mediante datos bioinformáticos y muestras humanas. La expresión de MALAT1 se reguló negativamente en las células AGS para detectar las características de proliferación, migración e invasión celular, así como los efectos sobre las vías de señales. Además, validamos el papel del eje MALAT1/miR-330-3p en GC mediante ensayos de genes indicadores de luciferasa dual. La expresión de MALAT1 fue mayor en tejidos cancerosos que en tejidos paracancerosos. El alto nivel de MALAT1 predijo malignidad y peor pronóstico. La regulación negativa de la expresión de MALAT1 en células AGS inhibió la proliferación, migración e invasión celular al apuntar a VEGFA. Mediante un ensayo de gen indicador de luciferasa dual y un tratamiento con inhibidor de miR-330-3p, demostramos que MALAT1 esponjaba miR-330-3p en GC, lo que lleva a la regulación positiva de VEGFA y la activación de la vía de señalización mTOR. El eje MALAT1/miR-330-3p regula VEGFA a través de la vía de señalización mTOR y promueve el crecimiento y la metástasis del cáncer gástrico.

PALABRAS CLAVE: Cáncer gástrico; ARNnc MALAT1; miR-330-3p; VEGFA; Vía de señalización mTOR.

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