Am J Cancer Res 2023;13(6):2630-2643
www.ajcr.us /ISSN:2156-6976/ajcr0149792

Original Article

LINC01354 enhances the metastatic ability of gastric cancer cells by adjusting miR-153-5p/CADM2 expression

Fa Fang[1,2,3], Yin Gu[4], Jie Gao[5], Lili An[6], Junsheng Kang[3], Qingshuang Mu[7], Qin Luo[1]

[1]Department of Respiratory Neurology, Affiliated Tumor Hospital of Xinjiang Medical University, Urumqi 830000, Xinjiang, China; [2]Department of Minimally Invasive General Surgery, Shenzhen Samii Medical Center, Shenzhen 518038, Guangdong, China; [3]Department of General Surgery, Shenzhen Hospital of Southern Medical University, Shenzhen 518101, Guangdong, China; [4]Department of Anesthesiology, Hongkong University Shenzhen Hospital, Shenzhen 518040, Guangdong, China; [5]Department of Physical Examination, Longgang District Maternity & Child Healthcare Hospital of Shenzhen City (Longgang Maternity and Child Institute of Shantou University Medical College), Shenzhen 518100, Guangdong, China; [6]Department of Obstetrics, Zhuhai Center for Maternal and Child Health Care, Zhuhai 519000, Guangdong, China; [7]Department of Geriatrics, The Second Affiliated Hospital of Xinjiang Medical University, Urumqi 830000, Xinjiang, China

Received February 21, 2023; Accepted May 26, 2023; Epub June 15, 2023; Published June 30, 2023

Abstract: LINC01354 is a long non-coding RNA (lncRNA) highly expressed in gastric cancer (GC). However, studies have shown that it plays a critical role in the progression of other tumors. This study attempts to uncover the role of LINC01354 in GC. LINC01354 expression in GC tissues and cell lines was assessed using qRT-PCR. Subsequently, LINC01354 knockdown and overexpression were induced in GC cells, and epithelial-mesenchymal transition (EMT) progression was detected. A dual-luciferase reporter assay was used to assess the relation between LINC01354, miR-153-5p, and CADM2. Finally, the metastatic ability of GC cells was assessed by Transwell and wound healing assays. LINC01354 expression was abnormally elevated in cancerous tissues and GC cells, and LINC01354 knockdown suppressed EMT progression, migration, and invasion of GC cells. Transfection of miR-153-5p mimics inhibited the expression of CADM2 by banding to the 3'UTR region, while LINC01354 promoted CADM2 expression by blocking miR-153-5p. The fluorescence experiment indicated that CADM2 is directly regulated by LINC01354/miR-153-5p. Overexpression of LINC01354 promoted EMT progression, migration, and invasion of GC cells, which could be absolutely reversed by co-expression of miR-153-5p. Our research demonstrates that LINC01354 has an important function in the EMT progression of GC cells. LINC01354 promotes GC cell migration and invasion by adjusting miR-153-5p/CADM2 expression.

Keywords: Gastric cancer, epithelial-mesenchymal transition, LINC01354, cell adhesion molecule 2

Introduction

Gastric cancer (GC) is a common digestive tumor with malignant behavior and poor prognosis. The incidence rate of GC is rising rapidly in China due to helicobacter pylori infection and adverse dietary factors. Moreover, the mortality of GC remains among the highest of all cancers [1], which may be attributed to its low rate of early diagnosis. Traditional chemotherapy drugs for GC include carboplatin, cisplatin, paclitaxel, and 5-FU [2]. Despite the advances in therapeutic modalities, such as immunotherapy and monoclonal antibody therapy, most patients with advanced GC still exhibit disease progression [3, 4]. Hence, new biomarkers are required to improve the early diagnosis and new therapeutic targets should be explored to develop effective treatment strategies for GC.

Metastasis occurs in the advanced stages of most cancers, causing high mortality in GC. Mesenchymal-like tumor cells are associated with a poor prognosis due to their high transferability [5]. Another study found that the mesenchymal phenotype subtype is significantly correlated with poor prognosis and chemoresistance in GC [6]. In recent years, researchers

have found abundant genes that are associated with regulating epithelial-mesenchymal transition (EMT) in tumor cells, including non-coding RNA.

Long non-coding RNAs (lncRNAs) include more than 200 nucleotides and do not encode or express any protein. Current studies reported that lncRNAs were abnormally highly expressed in cancer and adjacent tissues of GC [7, 8], and some of them were markedly correlated with poor prognosis in GC patients. Researchers reported that lncRNAs may participate in regulating tumor growth [9], immune escape [10], EMT [11], chemoresistance [12], and angiogenesis [13] in GC. Details on the regulation of lncRNA expression and their functions in GC are discussed in later sections [14-16]. However, the function and mechanism of many differentially expressed lncRNAs still remain unknown in GC.

LINC01354 is an intergenic lncRNA with a total length of 944 nucleotides and is expressed on chromosome 1. Zhang *et al.* [17] reported that LINC01345 was differentially expressed in GC, as shown in both the GEO and TCGA databases. In addition, a study reported that LINC01354 interacts with hnRNP-D and activates the Wnt/β-catenin signaling pathway, which promotes the proliferation and metastasis of colorectal cancer [18]. Yang *et al.* found that LINC01354 enhances the proliferation and invasion of lung cancer cells by regulating the miR-340-5p/ATF1 signaling pathway [19]. Meanwhile, LINC01354 also functions as an oncogene in osteosarcoma and endometrial cancer [20, 21]. However, the role of LINC01354 in GC remains unclear. The present study aims to uncover the function and mechanism of LINC01354 in GC.

Materials and methods

Human participants

A total of 20 patients with GC were recruited from Shenzhen Hospital of Southern Medical University between September 2020 and July 2021. All participants were diagnosed with GC by pathological examination and an informed consent form was obtained from every patient before enrolment. This study was approved by the Ethics Committee of Shenzhen Hospital of Southern Medical University (No.: NYSZYYEC-20220027) and was in accordance with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Cell lines

Human embryonic gastric mucosal epithelial cell (GES1) was purchased from iCell Bioscience Inc. (Shanghai, China). HGC-27, NCI-N87, MKN-7, MKN-45, and SNU-1 cells were bought from Procell (Wuhan, China). All cells were cultured in RPMI-1640 medium supplemented with 10% FBS, 100 IU penicillin, and 100 mg/ml streptomycin. The culture was conducted in a humidified incubator at 37°C and 5% CO_2.

Transfection

To change the expression level of LINC01354 and cell adhesion molecule 2 (CADM2) in GC cells, their sequences were cloned and recombined into pcDNA3.0 plasmids. Small interfering RNA-specific targeting LINC01354 (5'-TGAATGTAAAATCCAAAAGCT-3') was synthesized by RiBobio Inc. (Guangzhou, China). The GC cell lines were transfected with miR-153-5p mimics (5'-UCAUUUUUGUGAUGUUGCAGCU-3') (GenePharma, Suzhou, China) to overexpress miR-153-5p. The transfection process was performed by using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA).

Fluorescence in situ hybridization (FISH)

Cyanine 3-labeled fluorescent probes specifically targeting LINC01354 were synthesized by RiBobio Inc. (Guangzhou, China) to explore the subcellular location of LINC01354 in GC cells.

HGC-27 and NCI-N87 cells attached to a glass slide were fixed with 4.0% neutral formaldehyde solution, followed by treatment with 0.5% Triton X-100 for 5 min at 4°C. The slides were then treated with the prepared hybridization buffer according to the Ribo™ Fluorescent In Situ Hybridization Kit instructions (RiBobio). Finally, the fluorescence signal of cyanine 3 was observed and photographed under a microscope (Olympus, Tokyo, Japan).

Cell activity

A CCK8 assay was applied to detect cell activity. Briefly, GC cells after transfection were collected and dissociated by trypsin. Then, nearly

1×10^4 cells per well were inoculated to a 96-well plate, and 1640 medium was added. After 24 h, the medium was replaced with one containing CCK-8 solution (10%), followed by 1 h incubation. Finally, the optical density value under 450 nm wavelength excitation light was measured by a Microplate Reader (Thermo Scientific, Waltham, MA, USA).

Wound healing assay

Twenty-four hours after transfection, nearly 1×10^5 cells per well were seeded into 6-well plates, then cultured for 24 h. The next day, the cells were scratched with the head of pipette tips, and photos were taken. After 24 h of culture, the wound healing was recorded under a microscope (Olympus).

Transwell assay

Cell migration was assessed in a 24-well Transwell chamber. Briefly, about 1×10^5 cells after grouping treatment were incubated with FBS-free medium for 4 h and transferred to the upper chamber. Then, 700 μl medium was added to 10% FBS into the lower chamber. After 48 h of culture, the cells that migrated into the lower chamber were fixed with 75% ethanol, stained with 0.5% crystal violet, and counted in 6 random fields under a light microscope (BD Biosciences, Heidelberg, Germany). For detecting cell invasion, the chamber was precoated with 1 mg/ml Matrigel (Millipore, MA, USA), and other processes are consistent with the above description.

Real-time PCR

TRIzol (Millipore) was used to extract the total RNA of cells and tissues. Then, cDNA was synthesized with a Reverse transcription Kit (TaKaRa, Tokyo, Japan), followed by quantification with SYBR Green/real-time fluorescence quantitative PCR premix (Thermo Scientific) on a Stratagene Mx3000P Real-time PCR system (Agilent, Santa Clara, CA, USA). The primer sequences used for PCR are listed in Table S1. The reaction procedure was as follows: 95°C for 2 min; 95°C for 20 s; annealing and extending at 58°C for 30 s, 72°C for 20 s in each cycle, for a total of 40 cycles. Finally, the relative expressions of LINC01354, miR-153-5p, and CADM2 were calculated using the standard method $2^{-\Delta\Delta Ct}$.

Western blotting

Total protein was separated from cells and tissues with radioimmunoprecipitation assay (RIPA) buffer (Solarbio, Beijing, China). The protein samples were separated by electrophoresis with 10% SDS-PAGE and transferred to polyvinylidene fluoride films (Millipore). The samples were incubated with primary antibodies against CADM2 (1:1000 diluted, Abcam, Cambridge, MA, USA), E-cadherin (1:2000 diluted, Abcam), N-cadherin (1:2000 diluted, Abcam), Vimentin (1:5000 diluted, Abcam), GAPDH (1:5000 diluted, Abcam). Subsequently, HRP-conjugated secondary antibodies were used. Finally, the horseradish peroxidase substrate was added and the luminous bands were observed.

Statistical analysis

All data were collected from three independent experiments and analyzed with GraphPad Prism 6. Paired Student's t-test or One-way ANOVA followed by Dunnett's test was used to analyze the differences between two groups and among multiple groups, respectively. In this study, $P < 0.05$ was considered statistically significant.

Results

LINC01354 expression is elevated in GC and negatively correlated with miR-153-5p

First, the differences in LINC01354 expression between GC cancer tissue (Ct) and paracancerous tissue (Pt) were assessed. The results revealed a significantly higher level of LINC01354 in Ct than in Pt (**Figure 1A**). Furthermore, LINC01354 was described to function as a ceRNA according to the LncBase v.2 database (http://dibresources.jcbose.ac.in/zhumur/lncrbase2/start2.php); and miR-153-5p was found to possess 4 reverse complementary binding sites in LINC01541 (**Figure 1B**). Subsequently, the results indicated a significantly lower level of miR-153-5p in Ct than that in Pt (**Figure 1C**). Notably, the expression of LINC01354 showed a negative association with the level of miR-153-5p (**Figure 1D**). Subsequently, the expressions of LINC01354 and miR-153-5p were detected in GC cells and GES1 cells. Moreover, the expression of LINC01354 was significantly higher in HGC-27, NCI-N87, MKN-7, and MKN-45 cells compared to GES1 (**Figure**

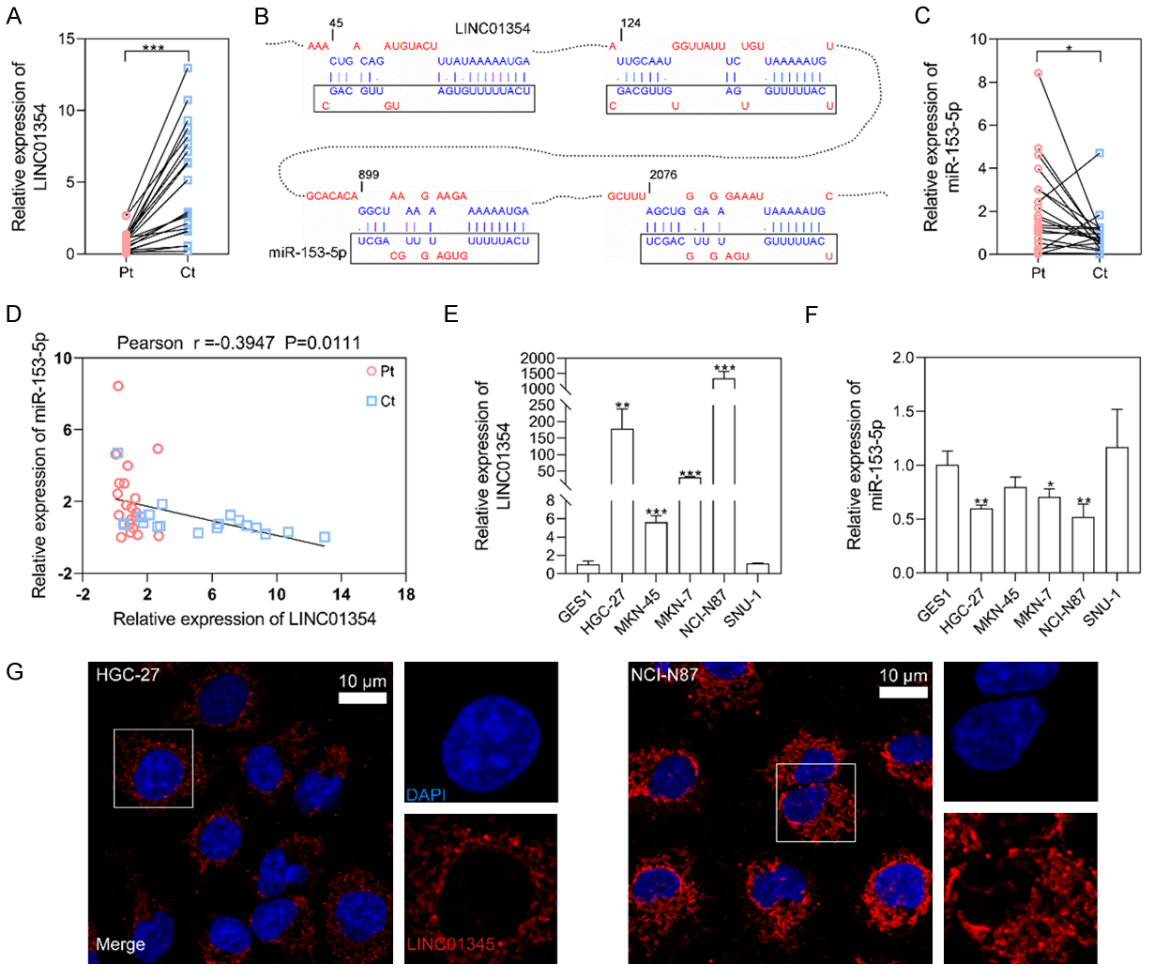

Figure 1. Higher expression of LINC01354 and lower expression of miR-153-5p was observed in GC tissues and cell lines. A. LINC01354 expression in GC cancer tissue (Ct) and paracancerous tissue (Pt) was detected by qRT-PCR. B. The predicted targeting sites of LINC01354 binding to miR-153-5p were shown in database LncBase v.2. C. miR-153-5p expression was detected by qRT-PCR. D. Correlation between the expression level of LINC01354 and miR-153-5p. E, F. qRT-PCR was performed to determine the expression of LINC01354 and miR-153-5p in GC cell lines. G. The subcellular location of LINC01354 in HGC-27 and NCI-N87 cells was examined by fluorescence in situ hybridization assay. **P < 0.01, ***P < 0.001.

1E). Conversely, miR-153-5p was lowly expressed in HGC-27, NCI-N87 and MKN-7 cells (**Figure 1F**). The FISH results showed that LINC01354 was located in the cytoplasm in HGC-27 and NCI-N87 cells (**Figure 1G**). These results suggested an association between LINC01354 and miR-153-5p in GC.

LINC01354/miR-153-5p directly targets CADM2

A dual luciferase reporter assay was performed to validate the sponging role of LINC01354 on miR-153-5p. The results found that the fluorescence ratio was significantly decreased by miR-153-5p mimics in all 4 band sites, and the reduction disappeared when the sequence of the binding site was mutated (**Figure 2A**). Furthermore, the target gene of miR-153-5p was predicted in the database TargetScan Human 7.2 to investigate the functions of LINC01354/miR-153-5p in GC. As displayed in **Figure 2B**, a reverse complementary site between miR-153-5p and CADM2 was found. Similarly, the direct correlation between miR-153-5p and its target gene CADM2 was confirmed (**Figure 2C**). In addition, CADM2 was highly expressed in all 4 stages of tumor tissues in the TCGA database (**Figure 2D**), and its elevated expression was significantly related to poor survival of GC patients (**Figure 2E**).

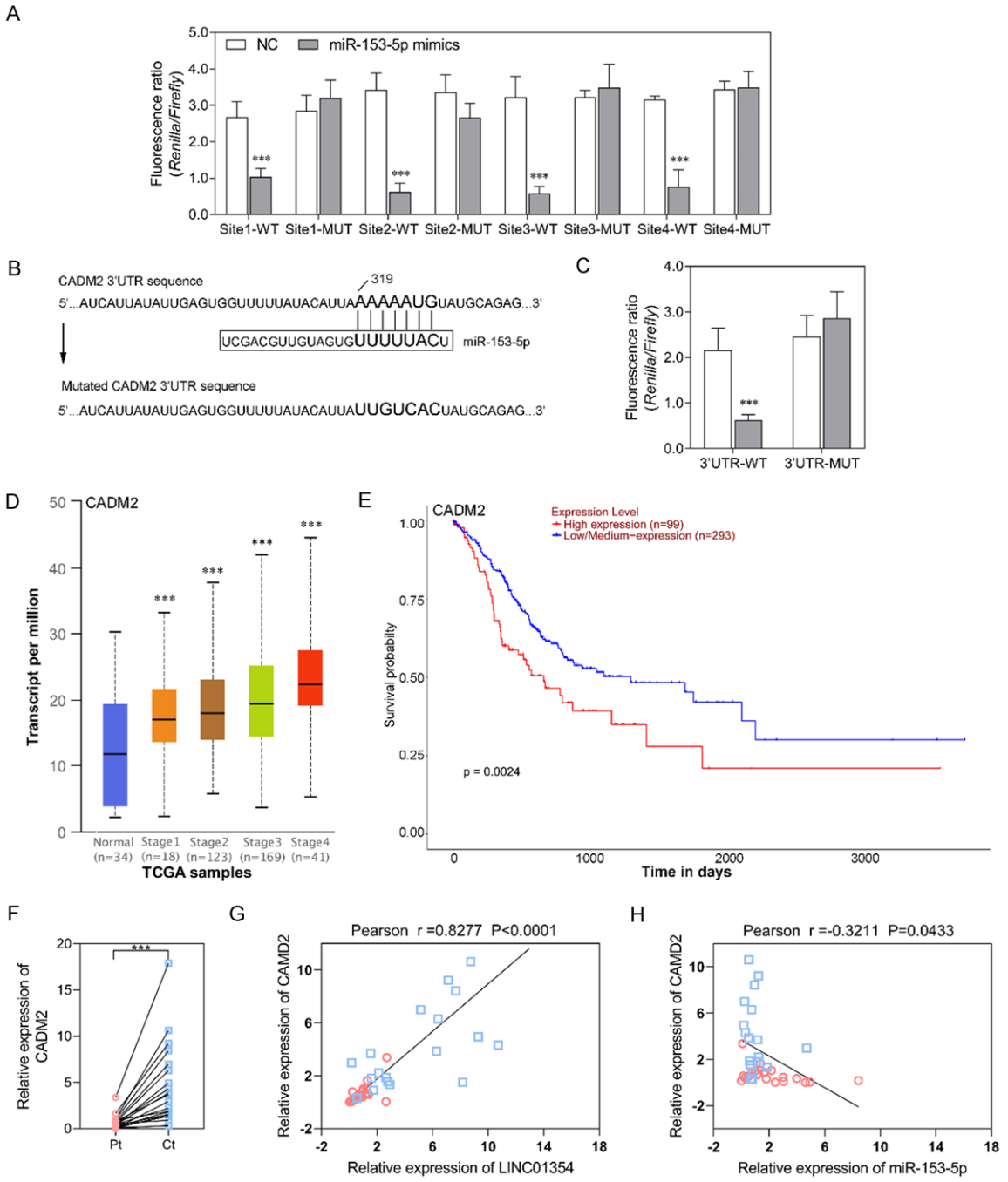

Figure 2. Abnormally high expression of CADM2 in GC tissues. A. Wild-type LINC01354 site (Site1-4 WT) or mutant-type LINC01354 (Site1-4 MUT) reporters and NC mimic or miR-153-5p were co-transfected into HEK-293T cells. Firefly/Renilla luciferase activity was measured. B. The binding site of miR-153-5p to CADM2 3'UTR was shown in the database TargetScanHuman 7.2. C. A dual-luciferase reporter assay was used to assess the relation between miR-153-5p and CADM2. D, E. CADM2 expression and its relation to the survival of GC patients were shown in the TCGA Database. F. CADM2 expression in GC cancer tissue (Ct) and paracancerous tissue (Pt) was measured by qRT-PCR. G. Association between the levels of LINC01354 and CADM2 mRNA. H. Association between the levels of miR-153-5p and CADM2 mRNA. ***P < 0.001.

Moreover, CADM2 was highly expressed in Ct compared with Pt (**Figure 2F**). The level of CADM2 mRNA was positively associated with the level of LINC01354 (**Figure 2G**), while nega-

tively associated with the level of miR-153-5p (**Figure 2H**). Furthermore, the protein level of CAMD2 was similar to its mRNA (**Figure 3A**). In addition, the results of qPCR, western blotting, and immunofluorescence assays showed increased mRNA and protein levels of CADM2 in GC cells (**Figure 3B-D**). These findings suggest that the expression of CADM2 is elevated in GC and it may serve as a target gene of LINC01354/miR-153-5p.

Knockdown of LINC01354 inhibits the metastatic ability of GC cells

To uncover the role of LINC01354 in GC cells, LINC01354 expression was knocked down using its specific targeting siRNA in HGC-27 and NCI-N87 cells. QPCR detection found that the knockdown of LINC01354 significantly promoted miR-153-5p expression, while significantly inhibiting CADM2 expression (**Figure 4A**). Western blotting and immunofluorescence assays showed that the levels of CADM2, N-cadherin, and vimentin were decreased in the siLINC01354 group, while elevated levels of E-cadherin were observed (**Figure 4B-D**). The cell activity was measured after transfection, which showed no significant change (**Figure 4E**). In addition, cell migration and invasion were significantly promoted by the knockdown of LINC01354 (**Figure 4F-H**). Therefore, LINC01354 may exert essential functions in GC cell metastasis.

Overexpression of LINC01354 promotes EMT of GC cells

LINC01354 and miR-153-5p were co-expressed in MKN-45 and SNU-1 cells. The qPCR revealed that the levels of miR-153-5p were markedly higher in the co-expression group than in the LINC01354 overexpression group, while the levels of CADM2 mRNA were significantly decreased (**Figure 5A**). Similarly, the protein levels of CADM2 were inhibited by overexpression of miR-153-5p, as well as N-cadherin and Vimentin (**Figure 5B**). Meanwhile, the E-cadherin levels were elevated in the co-expression group. Protein levels of CADM2, N-cadherin, and E-cadherin were verified by immunofluorescence (**Figure 5C** and **5D**). Furthermore, the wound healing and transwell assays revealed that overexpressing LINC01354 induced cell migration, and invasion was significantly suppressed by miR-153-5p (**Figure 5E-G**). In addition, LINC01354 overexpression plasmid and siRNA-targeted CADM2 were co-transfected in MKN-45 and SNU-1 cells. Results showed that the elevated protein levels of CADM2, N-cadherin, and Vimentin induced by LINC01354 overexpression were reversed by CADM2 knockdown, while that of E-cadherin was inhibited by CADM2 knockdown (**Figure 6A**). Wound healing and transwell assays also proved that overexpressing LINC01354 induced cell migration and invasion was reversed by CADM2 knockdown (**Figure 6B-D**). Therefore, it can be speculated that LINC01354 promotes EMT by regulating the expression of CADM2, and miR-153-5p attenuates the impact of LINC01354 on GC cells.

Overexpressing CADM2 enhances the metastatic ability of GC cells

miR-153-5p and CADM2 were co-expressed in HGC-27 and NCI-N87 cells to further investigate the regulatory relationship of miR-153-5p with CADM2. Marked increases in mRNA and protein levels of CADM2 were observed after overexpressing CADM2 (**Figure 7A** and **7B**). Overexpressing miR-153-5p alone promoted the increase of E-cadherin, and reduced N-cadherin and Vimentin, which was reversed by overexpressing CADM2 (**Figure 7C** and **7D**). In addition, wound healing and transwell assays indicated that miR-153-5p mimics markedly promoted cell migration and invasion, while overexpressing CADM2 significantly reversed the influences of miR-153-5p on GC cells (**Figure 7E-G**). The results verified the role of miR-153-5p in regulating CADM2.

Discussion

In recent years, a considerable amount of literature has been published on lncRNAs in cancers. Some lncRNAs, such as H19 [22], MEG3 [23], HOTAIR [24, 25], and DANCR [26], have been reported to be differentially expressed in GC and exert important functions. In this research, abnormally high LINC01354 expression was found in GC, and the results indicated that it functions as an oncogene. The role of LINC01354 in promoting tumor development has also been reported by Jiang *et al.* [20] and Zhang *et al.* [21]. In addition, overexpressing LINC01354 promotes the EMT of GC cells, which was consistent with the study by Li *et al.*

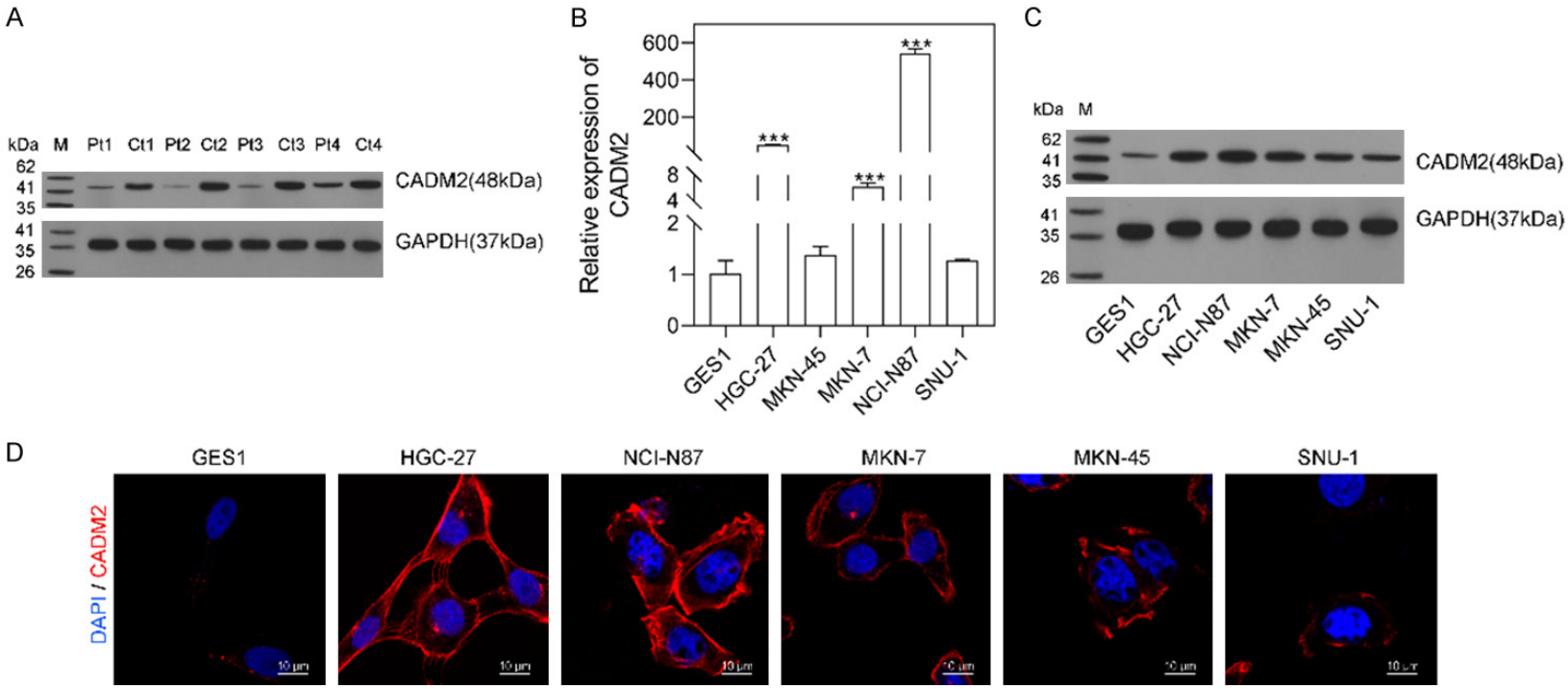

Figure 3. CADM2 expression is elevated in GC cells. (A) The protein levels of CADM2 in GC cancer tissue (Ct) and paracancerous tissue (Pt) were detected by western blotting. (B) CADM2 expression in GC cells was measured by qRT-PCR. The protein levels of CADM2 in GC cells were evaluated by western blotting (C) and immunofluorescence assay (D). *P < 0.05, ***P < 0.001.

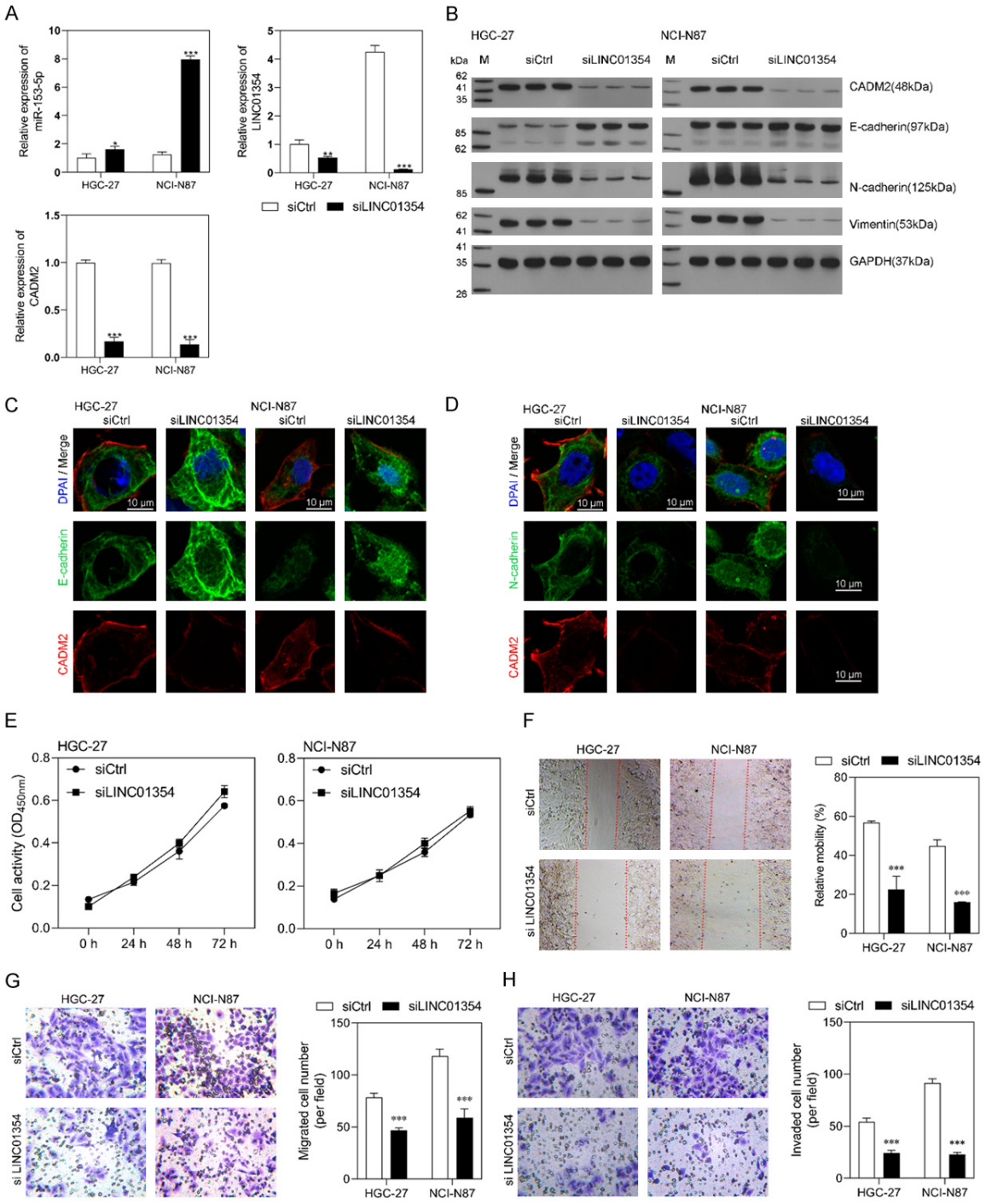

Figure 4. LINC01354 knockdown inhibited EMT progress by decreasing CADM2 expression. LINC01354 knockdown was performed in NCI-N87 and HGC-27 cells. A. Levels of LINC01354, miR-153-5p, and CADM2 mRNA were measured by qRT-PCR. B. Proteins of CADM2, E-cadherin, N-cadherin, and Vimentin were determined by western blotting. C, D. Proteins of CADM2, E-cadherin, and N-cadherin were assessed by immunofluorescence assay. E. Cell activity was assessed by a CCK8 assay. F. A wound-healing assay was performed to evaluate cell migration. G, H. Cell migration and invasion were detected by using a Transwell chamber. ***P < 0.001.

[18]. Furthermore, database prediction suggested that LINC01354 may interact with miR-153-5p, and subsequent experiments proved that LINC01354 enhances the metastatic abil-

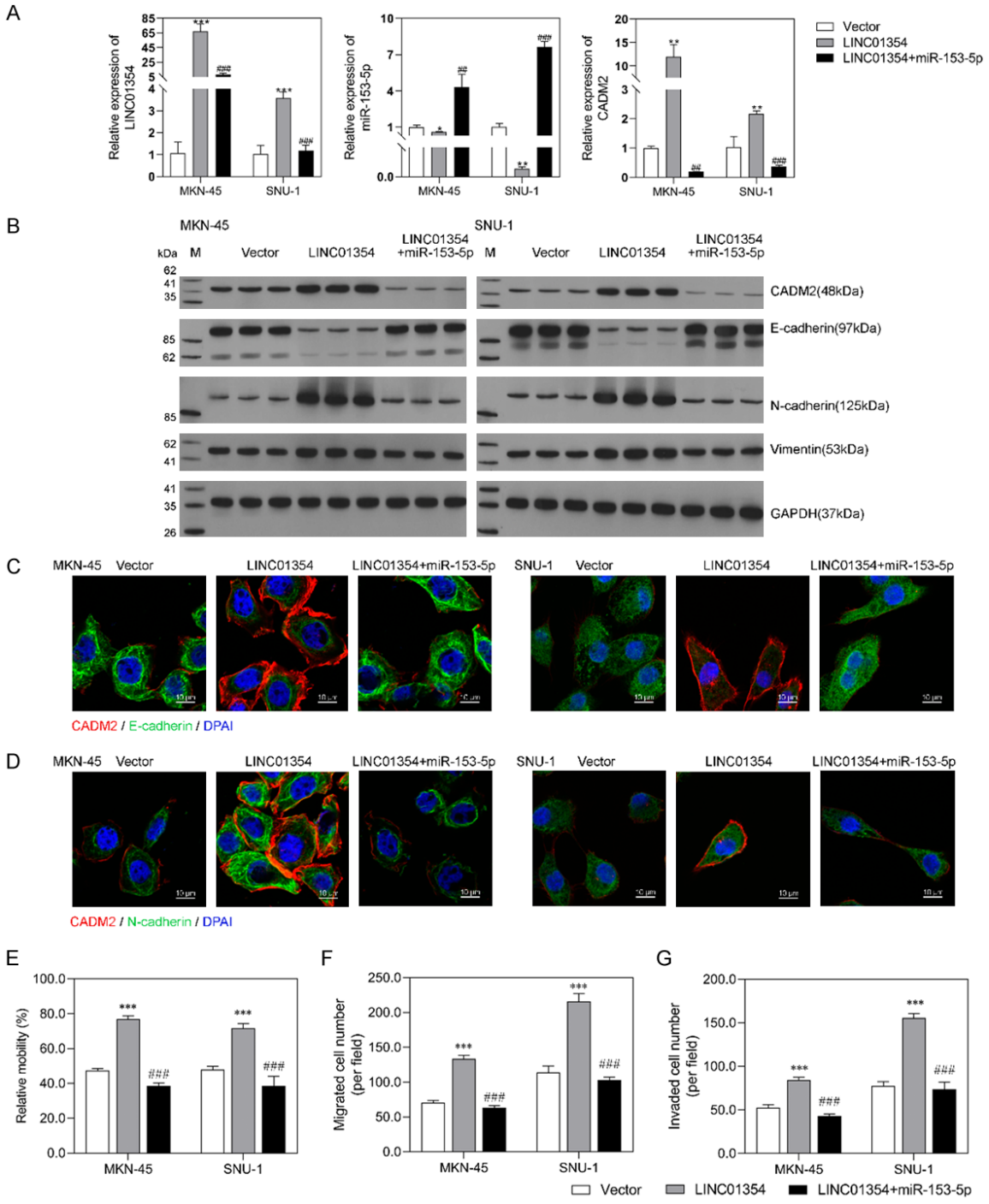

Figure 5. miR-153-5p overexpression attenuated the influence of LINC01354 on GC cells. LINC01354 and miR-153-5p were co-expressed in MKN-45 and SNU-1 cells. A. The levels of LINC01354, miR-153-5p, and CADM2 mRNA were measured via qRT-PCR. B. Proteins of CADM2, E-cadherin, N-cadherin, and Vimentin were determined by western blotting. C, D. Proteins of CADM2, E-cadherin, and N-cadherin were explored by immunofluorescence assay. E. A wound-healing assay was performed to assess cell migration. F, G. Cell migration and invasion were detected by using a Transwell chamber. ***P < 0.001, *vs*. Vector group; ###P < 0.001, *vs*. LINC01354 group.

ity of GC cells by sponging miR-153-5p. However, previous studies found that LINC01354 not only serves as a competing endogenous RNA (ceRNA) [19], but also regulates the stabil-

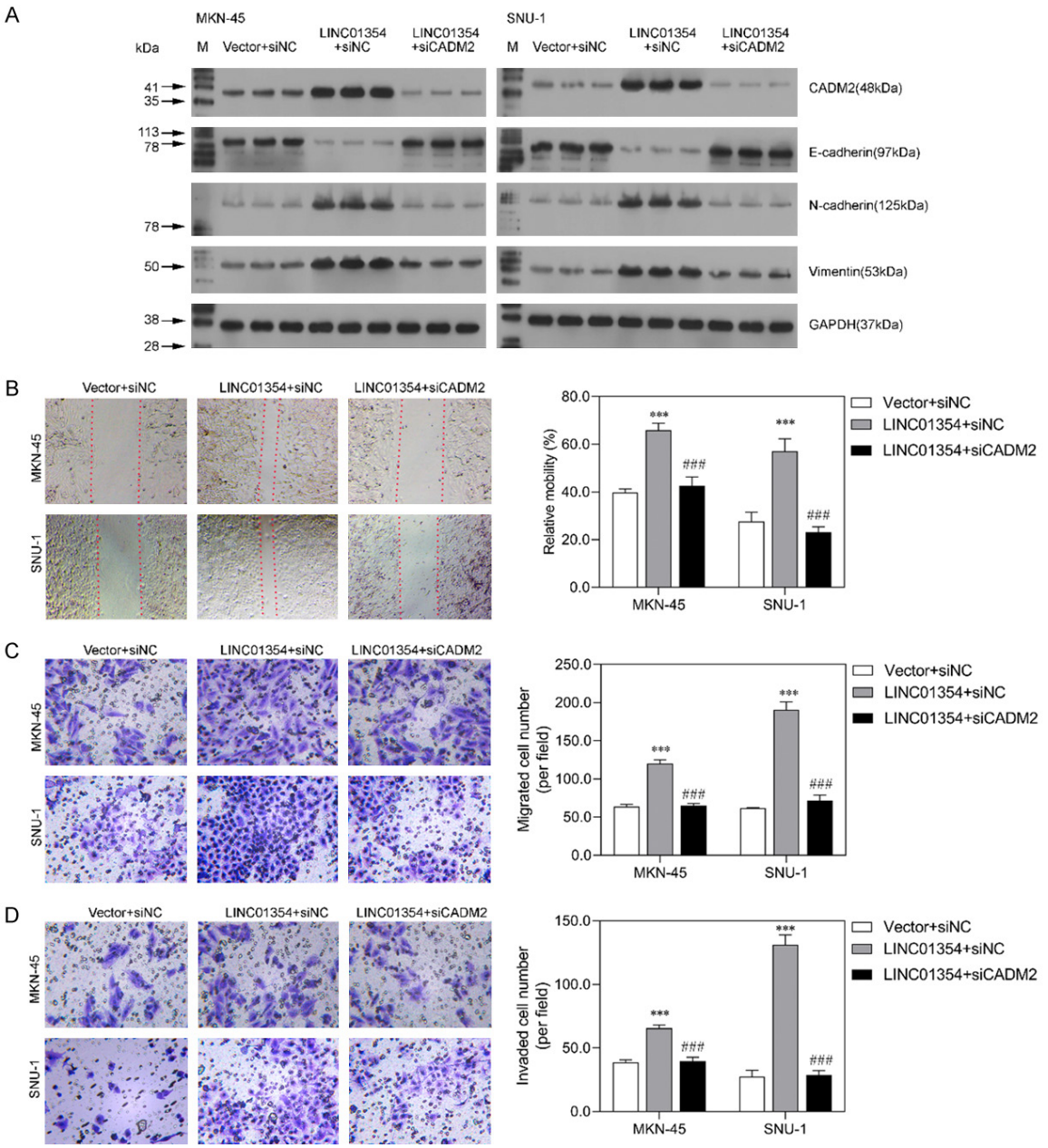

Figure 6. CADM2 knockdown antagonized the effects of LINC01354 overexpression. LINC01354 overexpression plasmid and siRNA-targeted CADM2 were co-transfected in MKN-45 and SNU-1 cells. A. Proteins of CADM2, E-cadherin, N-cadherin, and Vimentin were determined through western blotting. B. A wound-healing assay was performed to assess cell migration. C, D. Cell migration and invasion were detected by a Transwell chamber. ***P < 0.001, *vs*. Vector+siNC group; ###P < 0.001, *vs*. LINC01354+siNC group.

ity of target gene mRNA by binding protein [18]. Another difference is that LINC01354 knockdown has no effect on proliferation in GC cells, while Yang *et al.* found that LINC01354 promotes lung cancer cell proliferation by adjusting miR-340-5p/ATF1 expression [19]. LINC01354 may have multiple target genes and possibly targets different miRNAs, leading to different functions. The miR-153-5p/CADM2 axis was confirmed to be regulated by LINC01354 in the present study.

The role of miR-153-5p remains controversial in a variety of tumors. In renal cell carcinoma,

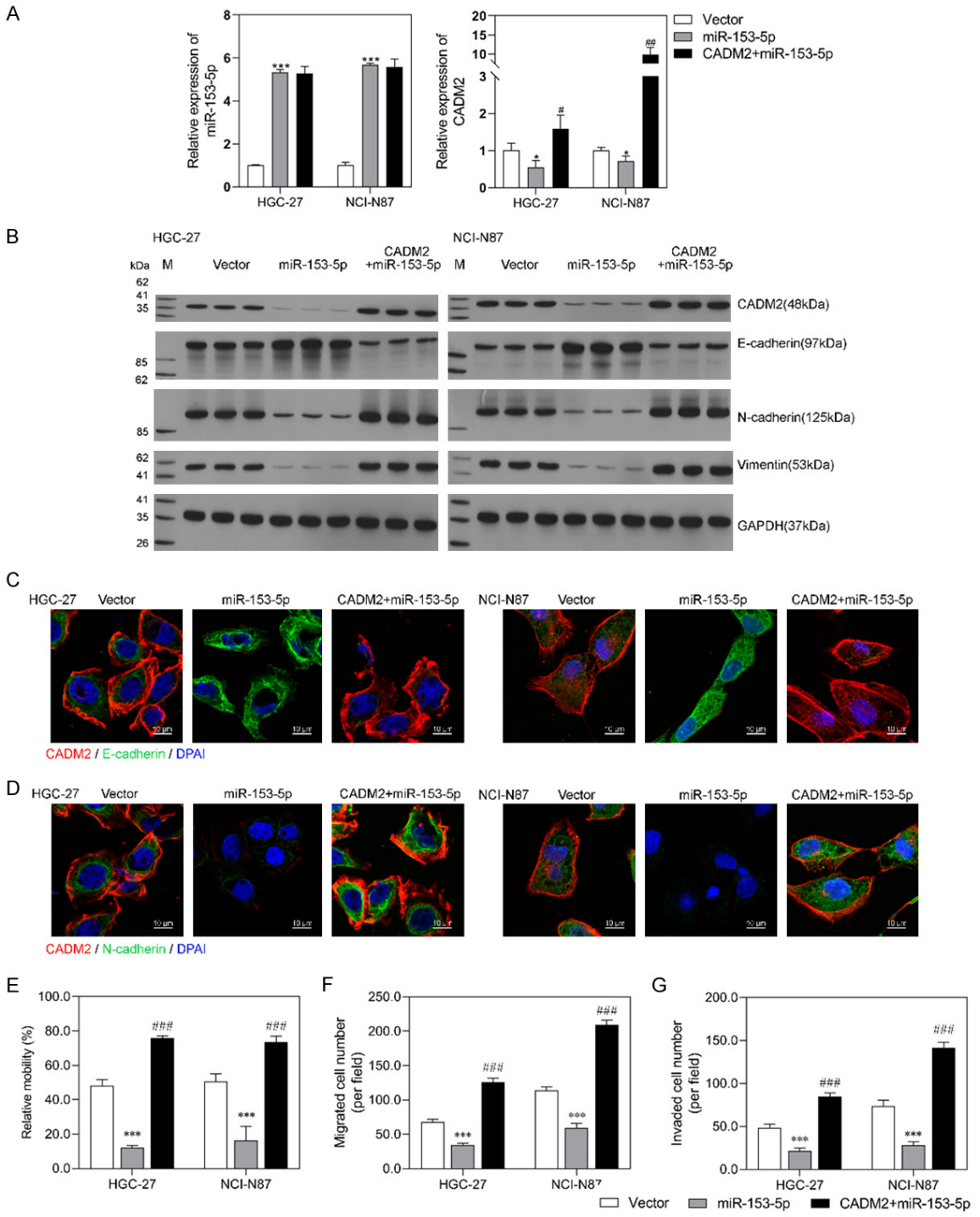

Figure 7. CADM2 overexpression inhibited the influence of miR-153-5p on GC cells. CADM2 and miR-153-5p were co-expressed in NCI-N87 and HGC-27 cells. A. Levels of LINC01354, miR-153-5p, and CADM2 mRNA were measured by qRT-PCR. B. Proteins of CADM2, E-cadherin, N-cadherin, and Vimentin were determined by western blotting. C, D. Proteins of CADM2, E-cadherin, and N-cadherin were evaluated by immunofluorescence assay. E. A wound-healing assay was performed to assess cell migration. F, G. Cell migration and invasion were detected by a Transwell chamber. ***P < 0.001, *vs*. Vector group; ###P < 0.001, *vs*. miR-153-5p group.

miR-153-5p enhances the growth and metastatic ability by direct targeting of AGO1 [27]. On the contrary, Zhang *et al.* [28] discovered that miR-153-5p inhibits the growth and meta-

static ability in NSCLC, and Sun *et al.* [29] reported that LINC00511 accelerates colorectal cancer proliferation by reducing miR-153-5p expression. Meanwhile, another report showed that miR-153-5p inhibits metastasis by regulating ARHGAP18 signaling in hepatocellular carcinoma [30]. The current study revealed that overexpressing miR-153-5p inhibits the EMT progress by decreasing the expression of CADM2. The findings indicate that miR-153-5p functions as a tumor suppressor in GC. Moreover, studies indicated that miR-153-5p enhances the sensitivity of acute myeloid leukemia [31], colorectal cancer [32], and triple-negative breast cancer [33] to chemotherapeutic drugs. Also as a target for LINC01354, miR-340-5p, inhibits GC cell proliferation by targeting CDK1 [34]. It can be inferred that miR-340-5p and miR-153-5p have synergistic effects on the expression of LINC01354 in GC cells.

CADM2 is a synaptic cell adhesion molecule 1 (SynCAM) family protein. Similar to miR-153-5p, CADM2 plays different roles in different tumor cells. Papers showed that CADM2 inhibits proliferation, migration, and invasion in human glioma [35], lung squamous cell carcinoma [36], and kidney cancer [37]. However, researchers found that CADM2 promotes the EMT of cancer cells in breast cancer [38], liver cancer [39], retinoblastoma [40], and pancreatic cancer [41]. In this study, CADM2 was overexpressed in HGC-27 and NCI-N87 cells, with the results showing that CADM2 inhibits E-cadherin protein levels and promotes N-cadherin and Vimentin protein accumulation, which means CADM2 promotes EMT in GC cells. Nevertheless, our study did not indicate the specific mechanism of CADM2 in regulating EMT, which will be further explored in future research.

In conclusion, this experiment revealed that miR-153-5p inhibits the level of CADM2 mRNA by banding to the 3'UTR region, while LINC-01354 promotes CADM2 expression by sponging miR-153-5p. LINC01354 enhances the metastatic ability of GC cells by influencing miR-153-5p/CADM2 expression.

Acknowledgements

This work was found by grant from Basic research project of Shenzhen Science and Technology Innovation Commission (No. JCYJ-20190814110605528).

Disclosure of conflict of interest

None.

Address correspondence to: Qin Luo, Department of Respiratory Neurology, Affiliated Tumor Hospital of Xinjiang Medical University, No. 789, Suzhou Street, Xinshi District, Urumqi 830000, Xinjiang, China. E-mail: luoqin07052022@126.com

References

[1] Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A and Bray F. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2021; 71: 209-249.

[2] Eom SS, Choi W, Eom BW, Park SH, Kim SJ, Kim YI, Yoon HM, Lee JY, Kim CG, Kim HK, Kook MC, Choi IJ, Kim YW, Park YI and Ryu KW. A comprehensive and comparative review of global gastric cancer treatment guidelines. J Gastric Cancer 2022; 22: 3-23.

[3] Kole C, Charalampakis N, Vailas M, Tolia M, Sotiropoulou M, Tsakatikas S, Kouris NI, Tsoli M, Koumarianou A, Karamouzis MV and Schizas D. Immunotherapy for gastroenteropancreatic neuroendocrine neoplasms (GEP-NENs): a 2021 update. Cancer Immunol Immunother 2022; 71: 761-768.

[4] Basile D, Simionato F, Cappetta A, Garattini SK, Roviello G and Aprile G. State-of-the-art of monoclonal antibodies for the treatment of gastric cancer. Biologics 2021; 15: 451-462.

[5] Cristescu R, Lee J, Nebozhyn M, Kim KM, Ting JC, Wong SS, Liu J, Yue YG, Wang J, Yu K, Ye XS, Do IG, Liu S, Gong L, Fu J, Jin JG, Choi MG, Sohn TS, Lee JH, Bae JM, Kim ST, Park SH, Sohn I, Jung SH, Tan P, Chen R, Hardwick J, Kang WK, Ayers M, Hongyue D, Reinhard C, Loboda A, Kim S and Aggarwal A. Molecular analysis of gastric cancer identifies subtypes associated with distinct clinical outcomes. Nat Med 2015; 21: 449-456.

[6] Oh SC, Sohn BH, Cheong JH, Kim SB, Lee JE, Park KC, Lee SH, Park JL, Park YY, Lee HS, Jang HJ, Park ES, Kim SC, Heo J, Chu IS, Jang YJ, Mok YJ, Jung W, Kim BH, Kim A, Cho JY, Lim JY, Hayashi Y, Song S, Elimova E, Estralla JS, Lee JH, Bhutani MS, Lu Y, Liu W, Lee J, Kang WK, Kim S, Noh SH, Mills GB, Kim SY, Ajani JA and Lee J. Clinical and genomic landscape of gastric cancer with a mesenchymal phenotype. Nat Commun 2018; 9: 1777.

[7] Xiao N, Hu Y and Juan L. Comprehensive analysis of differentially expressed lncRNAs in gastric cancer. Front Cell Dev Biol 2020; 30: 557.
[8] Qi M, Yu B, Yu H and Li F. Integrated analysis of a ceRNA network reveals potential prognostic lncRNAs in gastric cancer. Cancer Med 2020; 9: 1798-1817.
[9] Cui Y, Pu R, Ye J, Huang H, Liao D, Yang Y, Chen W, Yao Y and He Y. LncRNA FAM230B promotes gastric cancer growth and metastasis by regulating the miR-27a-5p/TOP2A axis. Dig Dis Sci 2021; 66: 2637-2650.
[10] Liang X, Zha L, Yu G, Guo X, Qin C, Cheng A and Wang Z. Construction and comprehensive prognostic analysis of a novel immune-related lncRNA signature and immune landscape in gastric cancer. Int J Genomics 2022; 2022: 4105280.
[11] Wu Q, Ma J, Wei J, Meng W, Wang Y and Shi M. lncRNA SNHG11 promotes gastric cancer progression by activating the Wnt/β-catenin pathway and oncogenic autophagy. Mol Ther 2021; 29: 1258-1278.
[12] Luo Y, Zheng S, Wu Q, Wu J, Zhou R, Wang C, Wu Z, Rong X, Huang N, Sun L, Bin J, Liao Y, Shi M and Liao W. Long noncoding RNA (lncRNA) EIF3J-DT induces chemoresistance of gastric cancer via autophagy activation. Autophagy 2021; 17: 4083-4101.
[13] Zhao J, Du P, Cui P, Qin Y, Hu C, Wu J, Zhou Z, Zhang W, Qin L and Huang G. LncRNA PVT1 promotes angiogenesis via activating the STAT3/VEGFA axis in gastric cancer. Oncogene 2018; 37: 4094-4109.
[14] Ghafouri-Fard S and Taheri M. Long non-coding RNA signature in gastric cancer. Exp Mol Pathol 2019; 113: 104365.
[15] Yuan L, Xu ZY, Ruan SM, Mo S, Qin J and Cheng XD. Long non-coding RNAs towards precision medicine in gastric cancer: early diagnosis, treatment, and drug resistance. Mol Cancer 2020; 19: 96.
[16] Li Y, Lu L, Wu X, Li Q, Zhao Y, Du F, Chen Y, Shen J, Xiao Z, Wu Z, Hu W, Cho CH and Li M. The multifaceted role of long non-coding RNA in gastric cancer: current status and future perspectives. Int J Biol Sci 2021; 17: 2737-2755.
[17] Zhang X, Zhang W, Jiang Y, Liu K, Ran L and Song F. Identification of functional lncRNAs in gastric cancer by integrative analysis of GEO and TCGA data. J Cell Biochem 2019; 120: 17898-17911.
[18] Li J, He M, Xu W and Huang S. LINC01354 interacting with hnRNP-D contributes to the proliferation and metastasis in colorectal cancer through activating Wnt/β-catenin signaling pathway. J Exp Clin Cancer Res 2019; 38: 161.
[19] Yang G, Yang C, She Y, Shen Z and Gao P. LINC01354 enhances the proliferation and invasion of lung cancer cells by regulating miR-340-5p/ATF1 signaling pathway. Artif Cells Nanomed Biotechnol 2019; 47: 3737-3744.
[20] Jiang Y and Luo Y. LINC01354 promotes osteosarcoma cell invasion by up-regulating integrin β1. Arch Med Res 2020; 51: 115-123.
[21] Zhang Y, Zhao W, Na F, Li M and Tong S. LINC01354/microRNA-216b/KRAS axis promotes the occurrence and metastasis of endometrial cancer. Nanoscale Res Lett 2022; 17: 21.
[22] Li T, Zhang X, Cheng L, Li C, Wu Z, Luo Y, Zhou K, Li Y, Zhao Q and Huang Y. Modulation of lncRNA H19 enhances resveratrol-inhibited cancer cell proliferation and migration by regulating endoplasmic reticulum stress. J Cell Mol Med 2022; 26: 2205-2217.
[23] Dan J, Wang J, Wang Y, Zhu M, Yang X, Peng Z, Jiang H and Chen L. LncRNA-MEG3 inhibits proliferation and metastasis by regulating miRNA-21 in gastric cancer. Biomed Pharmacother 2018; 99: 931-938.
[24] Zhang J, Qiu WQ, Zhu H, Liu H, Sun JH, Chen Y, Shen H, Qian CL and Shen ZY. HOTAIR contributes to the carcinogenesis of gastric cancer via modulating cellular and exosomal miRNAs level. Cell Death Dis 2020; 11: 780.
[25] Wei Z, Chen L, Meng L, Han W, Huang L and Xu A. LncRNA HOTAIR promotes the growth and metastasis of gastric cancer by sponging miR-1277-5p and upregulating COL5A1. Gastric Cancer 2020; 23: 1018-1032.
[26] Wang M, Gu J, Zhang X, Yang J, Zhang X and Fang X. Long non-coding RNA DANCR in cancer: roles, mechanisms, and implications. Front Cell Dev Biol 2021; 9: 753706.
[27] Li Z, Zhao S, Zhu S and Fan Y. MicroRNA-153-5p promotes the proliferation and metastasis of renal cell carcinoma via direct targeting of AG01. Cell Death Dis 2021; 12: 33.
[28] Zhang W, Liu K, Pei Y, Tan J, Ma J and Zhao J. Long noncoding RNA HIF1A-AS2 promotes non-small cell lung cancer progression by the miR-153-5p/S100A14 axis. Onco Targets Ther 2020; 13: 8715-8722.
[29] Sun S, Xia C and Xu Y. HIF-1α induced lncRNA LINC00511 accelerates the colorectal cancer proliferation through positive feedback loop. Biomed Pharmacother 2020; 125: 110014.
[30] Chen J, Huang X, Wang W, Xie H, Li J, Hu Z, Zheng Z, Li H and Teng L. LncRNA CDKN2BAS predicts poor prognosis in patients with hepatocellular carcinoma and promotes metastasis via the miR-153-5p/ARHGAP18 signaling axis. Aging (Albany NY) 2018; 10: 3371-3381.
[31] Shang J, Chen WM, Wang ZH, Wei TN, Chen ZZ and Wu WB. CircPAN3 mediates drug resistance in acute myeloid leukemia through the

miR-153-5p/miR-183-5p-XIAP axis. Exp Hematol 2019; 70: 42-54, e43.
[32] He Y, Zhang L, Tan F, Wang LF, Liu DH, Wang RJ and Yin XZ. MiR-153-5p promotes sensibility of colorectal cancer cells to oxaliplatin via targeting Bcl-2-mediated autophagy pathway. Biosci Biotechnol Biochem 2020; 84: 1645-1651.
[33] Wang Y, Wu N, Zhang J, Wang H and Men X. MiR-153-5p enhances the sensitivity of triple-negative breast cancer cells to paclitaxel by inducing G2M phase arrest. Onco Targets Ther 2020; 13: 4089-4097.
[34] Zhang L, Kang W, Lu X, Ma S, Dong L and Zou B. LncRNA CASC11 promoted gastric cancer cell proliferation, migration and invasion in vitro by regulating cell cycle pathway. Cell Cycle 2018; 17: 1886-1900.
[35] Jinyang M, Bojuan L, Lixin X, Wan D and Sun T. Long non-coding RNA HCG11 inhibits glioma cells proliferation and migration through decoying miR-590-3p and up-regulating CADM2. Pathobiology 2022; 89: 233-244.
[36] He J, Zhou X, Li L and Han Z. Long noncoding MAGI2-AS3 suppresses several cellular processes of lung squamous cell carcinoma cells by regulating miR-374a/b-5p/CADM2 axis. Cancer Manag Res 2020; 12: 289-302.
[37] He W, Li X, Xu S, Ai J, Gong Y, Gregg JL, Guan R, Qiu W, Xin D, Gingrich JR, Guo Y and Chang G. Aberrant methylation and loss of CADM2 tumor suppressor expression is associated with human renal cell carcinoma tumor progression. Biochem Biophys Res Commun 2013; 435: 526-532.
[38] Jin L, Zhang J, Fu HQ, Zhang X and Pan YL. FOXO3a inhibits the EMT and metastasis of breast cancer by regulating TWIST-1 mediated miR-10b/CADM2 axis. Transl Oncol 2021; 14: 101096.
[39] Li D, Zhang Y, Zhang H, Zhan C, Li X, Ba T, Qiu Z, E F, Lv G, Zou C, Wang C, Si L, Zou C, Li Q and Gao X. CADM2, as a new target of MIR-10b, promotes tumor metastasis through FAK/AKT pathway in hepatocellular carcinoma. J Exp Clin Cancer Res 2018; 37: 46.
[40] Huang YX, Nie XG, Li GD, Fan DS, Song LL and Zhang XL. Downregulation of microRNA-182 inhibits cell viability, invasion and angiogenesis in retinoblastoma through inhibition of the PI3K/AKT pathway and CADM2 upregulation. Int J Oncol 2018; 53: 2615-2626.
[41] Wang L, Bi R, Li L, Zhou K and Liu H. Functional characteristics of autophagy in pancreatic cancer induced by glutamate metabolism in pancreatic stellate cells. J Int Med Res 2020; 48: 300060519865368.

Table S1. Primers used in RT-qPCR

Name	Sequence (5'-3')
GAPDH forward	TGTTCGTCATGGGTGTGAAC
GAPDH reverse	ATGGCATGGACTGTGGTCAT
LINC01354 forward	TCAATCACGAGAGCACACAG
LINC01354 reverse	CATCTCCTTAGCAATCCGGG
CADM2 forward	GCAGAGTAGATCACGAATCCCT
CADM2 reverse	AATTCTCCGCCATCCTTTGTC
U6 forward	CTCGCTTCGGCAGCACA
U6 reverse	AACGCTTCACGAATTTGCGT
hsa-miR-153-5p RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGAGCTGCA
hsa-miR-153-5p forward	ACACTCCAGCTGGGTCATTTTTGTGATGTTGC
hsa-miR-153-5p reverse	CTCAACTGGTGTCGTGGA