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ORIGINAL RESEARCH

LncRNA MIR194-2HG Promotes Cell Proliferation and Metastasis via Regulation of miR-1207-5p/TCF19/Wnt/β-Catenin Signaling in Liver Cancer

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Purpose: LncRNAs play an important role in tumorigenesis and cancer progression in liver cancer. Although many lncRNAs have been reported, the role of MIR194-2HG and the underlying mechanism mediated by it are still largely unknown in HCC. This study aimed to investigate the biological role and mechanism of MIR194-2HG in liver cancer.

Materials and Methods: The expression of MIR194-2HG was determined in liver cancer tissues and cells by RT-qPCR. The overall survival rate of MIR194-2HG was analyzed by Kaplan–Meier survival analysis. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), colony formation, and Transwell assays were carried out to detect cell migration and invasion. Western blotting was used to quantify the levels of all proteins. The regulatory mechanism of the MIR194-2HG/miR-1207-5p/TCF19 axis in liver cancer was investigated by dual-luciferase activity reporter assay, Kaplan-Meier survival analysis, and Western blotting.

Results: MIR194-2HG was upregulated in liver cancer tissues and cell lines. Liver cancer patients with higher expression of MIR194-2HG revealed poor overall survival compared with those who had lower expression of MIR194-2HG. MIR194-2HG promoted the proliferation, migration, and invasion of HepG2 and Huh7 cells by acting as a ceRNA mechanism for the miR-1207-5p/TCF19 axis to activate the Wnt/β-catenin signaling pathway.

Conclusion: MIR194-2HG acts in an oncogenic role and activates the Wnt/β-catenin signaling pathway via a miR-1207-5p/TCF19 axis-mediated mechanism, which provides a novel avenue for diagnostic or therapeutic interventions in liver cancer.

Keywords: MIR194-2HG, liver cancer, miR-1207-5p, TCF19, Wnt/β-catenin

Introduction

Liver cancer, the sixth most commonly diagnosed cancer and the fourth leading cause of cancer-related death worldwide, is a malignant neoplastic disease with an incidence of approximately 841,000 new cases and 782,000 deaths each year.[1,2] Due to the lack of outward signs at early stages, most patients with liver cancer are diagnosed when metastasis has already occurred.[3–5] To date, chemotherapy and targeted therapy are the main treatments for advanced patients.[6,7] However, the outcome of liver cancer treatment remains poor. Therefore, the discovery of novel diagnostic markers or therapeutic targets is of great importance.

Increasing evidence has shown that the majority of transcripts originating from the human genome are non-coding RNAs (ncRNAs) such as small ncRNAs and long ncRNAs (lncRNAs).[8,9] LncRNAs are stable and prevalent in mammalian cells

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and have been demonstrated to play an important role in tumorigenesis and cancer progression by regulating proliferation, apoptosis, metastasis, and glycolysis in cancer cells.[10–12] Current studies demonstrate that the majority of lncRNAs are involved in pathological processes through their actions as sponges for microRNAs (miRNAs) in cancers.[13,14] For example, lncRNA FAL1 promotes cell proliferation and migration by sponging miR-1236 in liver cancer.[15] Kong et al showed that lncRNA CDC6 elevated breast cancer progression by targeting CDC6 and sponging miR-215.[16] Recently, transcriptomic analysis of high-throughput sequencing revealed that the lncRNA MIR194-2HG was downregulated in bladder cancer.[17] However, the role of MIR194-2HG and the underlying mechanism mediated by it are still largely unknown in cancers, including liver cancer.

In this study, we evaluated the expression of MIR194-2HG, an infrequently investigated lncRNA, in liver cancer tissues and liver cancer cell lines, and investigated its role and molecular mechanism. We showed that MIR194-2HG played an oncogenic role in HepG2 and Huh7 cells by activating the Wnt/β-catenin signaling pathway. Moreover, we demonstrated that MIR194-2HG acted as a sponge for miR-1207-5p, thus removing the inhibition of TCF19 by miR-1207-5p. Our data suggested that MIR194-2HG could be a novel diagnostic marker or therapeutic target of liver cancer.

Materials and Methods

Clinical Samples

Thirty paired liver cancer samples and matched normal tissues were collected from The Second Hospital of Tianjin Medical University from July 2017 to July 2019. All tissues were immediately frozen in liquid nitrogen at −196°C after surgery. All the patients provided written informed consent for the collection and utilization of their tissue samples. This study was approved by the Research Ethics Committee of The Second Hospital of Tianjin Medical University.

Cell Culture

Liver cancer cell lines cell lines (Huh7, HepG2, HepG2.2.15, and SMMC-7721) and the normal human liver cell line HepaRG were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). Cells were cultivated in Dulbecco's modified Eagle's medium (DMEM, Thermo Fisher Scientific, CA, USA) that contained 10% fetal bovine serum (FBS Thermo Fisher Scientific, CA, USA) and 100 U/mL penicillin-streptomycin (Thermo Fisher Scientific, CA, USA) at 37°C under 5% CO_2 for cultivation.

Plasmid Construction

MIR194-2HG (2521 bp, NCBI Reference Sequence: NR_133638.1) and TCF19 (1038 bp, NCBI Reference Sequence: NM_007109.3) were synthesized by General Biosystems Co., Ltd. (Anhui, China) and cloned to pcDNA3, generation of pcDNA-MIR194-2HG (MIR194-2HG) and pcDNA-TCF19 (TCF19). pSilencer-MIR194-2HG (pshR-MIR194-2HG), pSilencer-TCF19 (pshR-TCF19), pGL/Luc-MIR194-2HG WT, pGL/Luc-MIR194-2HG Mut, pGL/Luc-TCF19 WT, and pGL/Luc-TCF19 Mut were constructed by General Biosystems Co., Ltd. (Anhui, China). miRNA negative control (miR-NC), miR-1207-5p mimics, antisense oligonucleotide negative control (ASO-NC), and antisense oligonucleotide against miR-1207-5p (ASO-miR-1207-5p) were synthesized by GenePharma Co., Ltd. (Shanghai, China).

Cell Transfection

Cells were transfected with Lipofectamine® 3000 (Thermo Fisher Scientific, Carlsbad, CA, USA) according to the supplier's instructions.

RT-qPCR

Total RNA in tissues or cells was isolated using TRIzol Regent (Invitrogen, Carlsbad, CA, USA) according to the supplier's instructions. The RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, Carlsbad, CA, USA) was used to synthesize first strand cDNA from RNA templates. Then, RT-qPCR was performed on a Bio-Rad iQ5 Real-Time PCR System using SYBR Green Real-Time PCR Master Mixes (Thermo Fisher Scientific, Carlsbad, CA, USA). The primers used in this work were as follows: MIR194-2HG: 5′-GAGGCCATCCTCATGAA ATCAGGCA-3′ (forward) and 5′-TCAACCCCAAATCG GCCTCATAAAG-3′ (reverse); TCF19, 5′-GTCCGAC TCCCAAGAGGTCA-3′ (forward) and 5′-GCAGCAAA GTCCTGAGGCT-3′ (reverse); U6, 5′-TGCGGGTGC TCGCTTCGGCAGC-3′ (forward) and 5′-CCAGTGCAG GGTCCGAGGT-3′ (reverse); GAPDH: 5′-CTGGGCTAC ACTGAGCACC-3′ (forward) and 5′-AAGTGGTCGTT GAGGGCAATG-3′ (reverse); miR-1207-5p: 5′-TGCG GTGGCTGGGTGGCTGGGT-3′ (forward) and 5′-CCAG TGCAGGGTCCGAGGT-3′ (reverse). MIR194-2HG or

TCF19 were normalized using GAPDH as the internal reference, and miR-1207-5p was normalized using U6 as the internal reference. The expression levels of MIR194-2HG, miR-1207-5p, and TCF19 were calculated using the $2^{-\Delta\Delta CT}$ method.

MTT Assay

Cells were transfected with the indicated plasmids or miRNAs. At 24 h, the transfected HepG2 or Huh7 cells were seeded into 96-well plates at a density of about 3×10^3 cells per well. At 48 h, 72 h, and 96 h post-transfection, 10 μL MTT solution (Thermo Fisher Scientific, Carlsbad, CA, USA) was then added into each well, and the cells were cultured with the MTT solution for another 4 h. Subsequently, the medium was discarded carefully, 100 μL dimethyl sulfoxide added to each well, and the MTT formazan crystals dissolved by shaking for 5 min protected from light. Finally, the optical density (OD) value was measured using a Spectra Max M5 Microplate reader (Molecular Devices, Sunnyvale, CA, USA) at a wavelength of 570 nm. Cell viability was calculated from the ratio of the average OD value of wells from the experimental group to those of the control group.

Colony Formation Assay

Cells were transfected with the indicated plasmids or miRNAs. At 24 h, the transfected HCC cells (300 cells per well) were seeded in 12-well plates and cultured at 37°C for 2 weeks. Then, cells were stained using crystal violet (Solarbio Science & Technology Co., Ltd., Beijing, China) for 20 mins, and the colonies were counted under an inverted microscope and photographed.

Transwell Assay

The Transwell assay was used to detect cell migration and invasion. The upper chambers (8 μm pore size, BD Biosciences, CA, USA) were coated with Matrigel (Solarbio Science & Technology Co., Ltd., Beijing, China) for the invasion assay but not for the migration assay. Briefly, liver cancer cells were transfected with the indicated plasmids or miRNAs. At 24 h, liver cancer cells were dispersed using 0.25% trypsin and resuspended using serum-free DMEM medium. After, cells were counted, and 100 μL cell suspension (6×10^4 cells for migration and 1×10^5 cells for invasion) was inoculated into the upper chambers. Then, 600 μL DMEM with 20% FBS was added to the lower chambers and cultured for approximately 36 h. After that, the cells on the upper surface of the membrane were gently wiped off using a cotton swab, and the invaded cells were fixed using 4% paraformaldehyde. Finally, the cells were stained using 0.5% crystal violet solution and observed under an inverted microscope and counted.

Western Blot

Cells were lysed using RIPA (Solarbio, China) lysis buffer, and total protein was quantified using the BCA method. The proteins were separated by SDS-PAGE and then transferred to PVDF membranes (EMD, Millipore Corp., Billerica, MA, USA). After that, the membranes were blocked in 5% skim milk and then incubated with primary antibodies at 4°C overnight. After washing, the membranes were incubated with horseradish peroxidase-conjugated Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody (Thermo Fisher Scientific, AB_2534046, 1:2000) at room temperature for 2 h. Finally, the protein bands were visualized using enhanced chemiluminescence (ECL) reagent. The grey value of each protein band was analyzed using the ImageJ analysis Tool. The primary antibodies included β-catenin (1:2000, ab6302, Abcam), TCF19 (1:2000, ab230005, Abcam), GAPDH (1:5000, ab181602, Abcam), and H2A. X (1:500, ab188819, Abcam).

Dual-Luciferase Reporter Gene Assay

To determine the target relationship between MIR194-2HG and miR-1207-5p, either MIR194-2HG-WT or MIR194-2HG-Mut vector was co-transfected with miR-NC, miR-1207-5p mimics, ASO-NC, and ASO-miR-1207-5p in HCC cells. To determine the target relationship between TCF19 and miR-1207-5p, either TCF19-WT or TCF19-Mut vector was co-transfected with miR-NC, miR-1207-5p mimics, ASO-NC and ASO- miR-1207-5p in HCC cells. After 48 h, the luciferase activity was detected using a Dual-Luciferase Reporter assay system (Promega Corporation, Madison, WI, USA).

Statistical Analysis

GraphPad Prism 8 statistical software was used for data analysis. All the experiments were performed at least three times, and the data were presented as mean ± standard deviation (SD). Student's *t*-test was used to compare the differences between two groups, and for multivariate comparisons, one-way analysis of variance were used to analyse the differences among multiple groups. Differences were considered statistically significant at $P < 0.05$.

Results

MIR194-2HG Was Upregulated in HCC Tissues and Liver Cancer Cell Lines

To investigate the role of MIR194-2HG in HCC, RT-qPCR was first used to analyze the expression of MIR194-2HG in HCC tissues and adjacent normal tissues. As shown in Figure 1A, the expression of MIR194-2HG was markedly higher in HCC tissues than that in adjacent normal tissues. Next, Kaplan-Meier survival analysis results indicated that HCC patients with higher expression of MIR194-2HG revealed poor overall survival compared with HCC patients with lower expression of MIR194-2HG (Figure 1B). Additionally, the expression of MIR194-2HG in liver cancer cell lines (Huh7, HepG2, HepG2.2.15, and SMMC-7721) were significantly increased compared with the human normal liver cell line HepaRG (Figure 1C).

MIR194-2HG Played an Oncogenic Role in Liver Cancer Cells

To explore the role of MIR194-2HG in liver cancer cells, we first transfected MIR194-2HG overexpression or knockdown plasmids into liver cancer cells, and the results showed that MIR194-2HG was significantly increased after transfection with MIR194-2HG overexpression plasmids, while the expression of MIR194-2HG was markedly reduced in MIR194-2HG-silencing cells (Figure 2A). MTT results showed that MIR194-2HG overexpression increased the viability of liver cancer cells, whereas MIR194-2HG knockdown significantly reduced cell viability (Figure 2B). A colony formation assay was performed to investigate the cell proliferation ability of HCC cells. The results showed that MIR194-2HG elevation led to a marked increase of the colony number in liver cancer cells, whereas downregulation of MIR194-2HG showed the opposite effect (Figure 2C). In addition, we detected migration and invasion in liver cancer cells by altering the expression of MIR194-2HG. The Transwell assay showed that the migration ability was markedly increased when MIR194-2HG was overexpressed in HepG2 and Huh7 cells; while the migration ability was significantly inhibited by silencing of MIR194-2HG (Figure 2D, left). Additionally, MIR194-2HG elevation markedly enhanced the invasion ability of HepG2 and Huh7 cells, whereas MIR194-2HG knockdown prominently inhibited their invasion ability (Figure 2D, right). These results suggested an oncogenic role for MIR194-2HG in liver cancer cells.

MIR194-2HG Activated the Wnt/β-Catenin Signaling Pathway in Liver Cancer Cells

As we know, Wnt/β-catenin signaling pathway plays a crucial role in carcinogenesis by regulating cell proliferation and metastasis in several cancers. To further investigate the molecular mechanism of MIR194-2HG in liver cancer, we then assessed whether the Wnt/β-catenin signaling pathway was affected by the MIR194-2HG

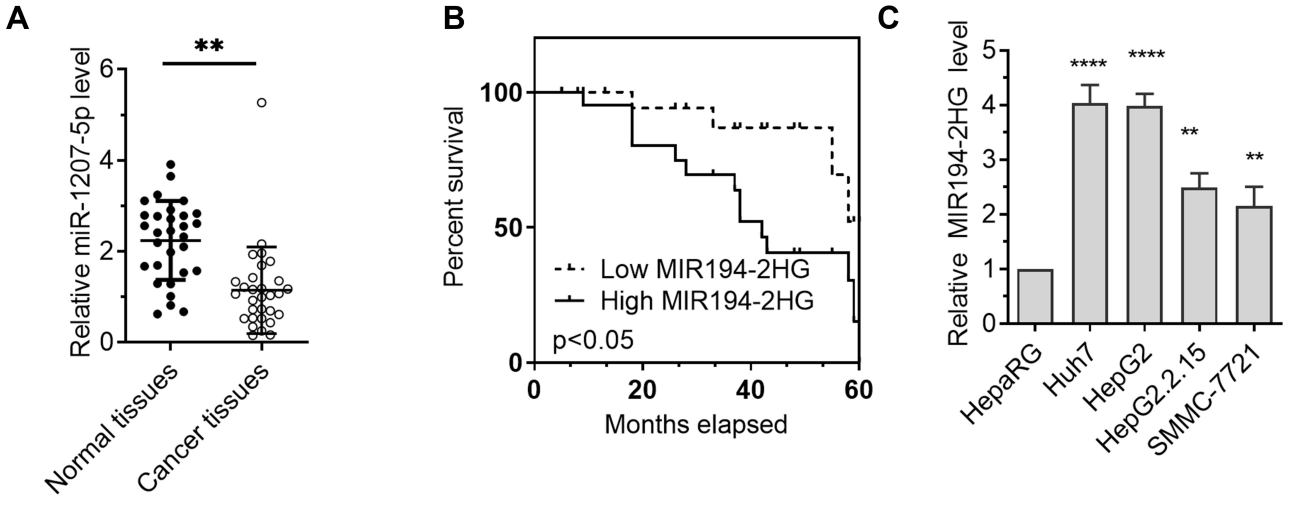

Figure 1 MIR194-2HG was upregulated in live cancer. (**A**) The expression levels of MIR194-2HG in 30 pairs of live cancer tissues and adjacent normal tissues were determined by RT-qPCR. (**B**) Kaplan-Meier survival analysis showed that high expression of MIR194-2HG predicts poor overall survival from thirty-liver cancer samples. Cutoff-High (%): 50; Cutoff-Low (%): 50. (**C**) The expression levels of MIR194-2HG in four live cancer cell lines (Huh7, HepG2, HepG2.2.15, and SMMC-7721) and the human normal liver cell line HepaRG were determined by RT-qPCR. (**A**) Student's *t*-test; (**C**) ANOVA followed by Bonferroni's post hoc test. Data were expressed as mean ± SD, **P < 0.01; ****P < 0.0001.

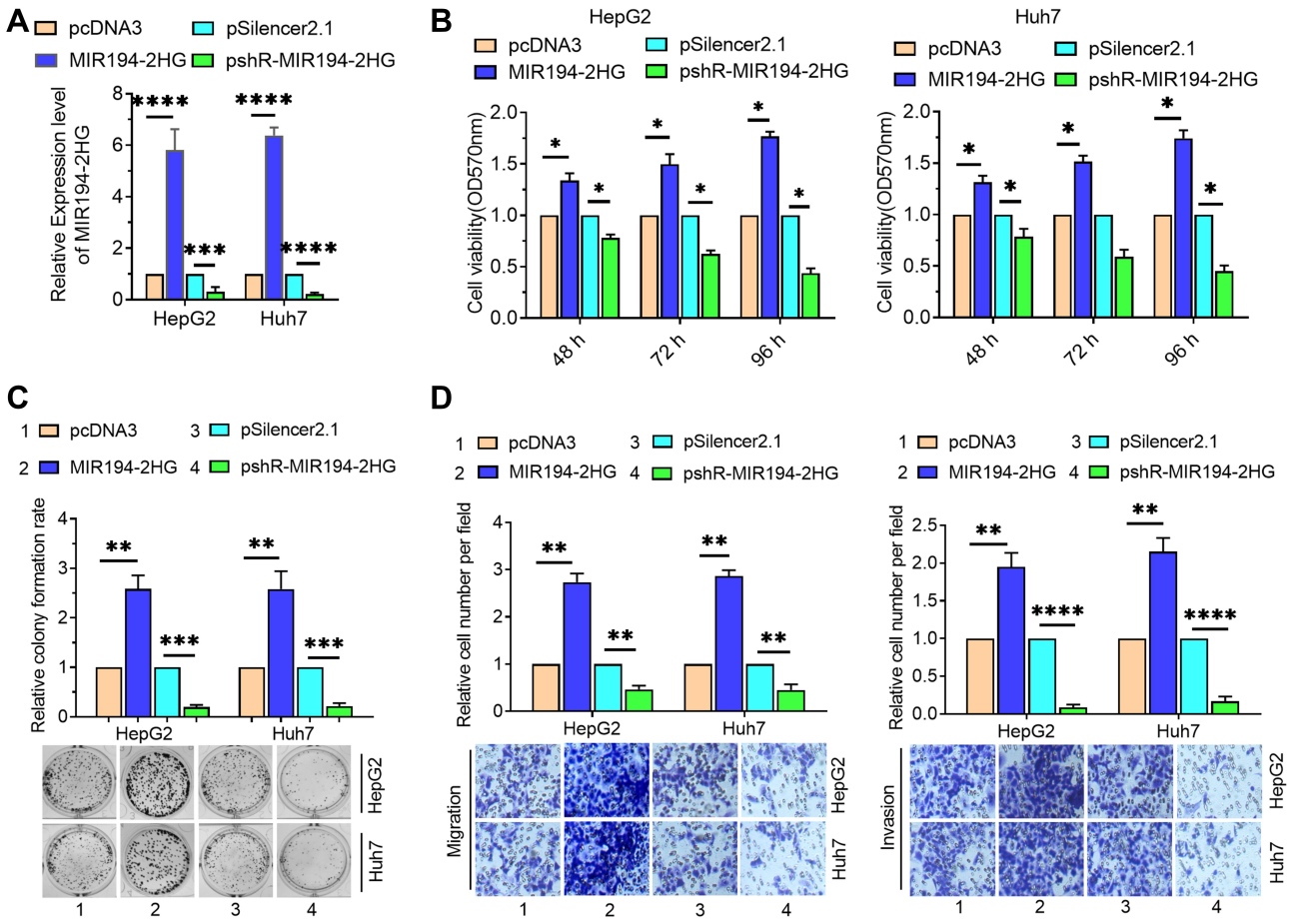

Figure 2 MIR194-2HG promoted the proliferation, migration, and invasion of live cancer cells. (**A**) HepG2 and Huh7 cells were transfected using MIR194-2HG overexpression or silencing plasmids, and the transfection efficiency was verified by RT-qPCR. (**B**) The MTT method was utilized to determine the cell viabilities of live cancer cells. (**C**) The colony formation ability of cells was investigated by colony formation assay. (**D**) The Transwell assay was utilized to determine the migration and invasion of HCC cells. (**A–D**) ANOVA followed by Bonferroni's post hoc test. Data are expressed as mean ± SD, **P < 0.01; *P < 0.05; ***P < 0.001; ****P < 0.0001.

expression level. We observed that MIR194-2HG overexpression markedly increased the activity of the TCF-dependent luciferase reporter pTop-flash/pFop-flash, whereas downregulation of MIR194-2HG decreased the luciferase activity, suggesting that the Wnt/β-catenin signaling pathway might be activated by MIR194-2HG overexpression (Figure 3A). Furthermore, we detected the protein levels of total β-catenin and nuclear β-catenin by Western blotting in liver cancer cells. The results showed that MIR194-2HG overexpression increased the total β-catenin expression and nuclear translocation of β-catenin compared with the empty vector group (Figure 3B and C). Conversely, transfection of MIR194-2HG knockdown plasmid into liver cancer cells inhibited total β-catenin expression and nuclear translocation of β-catenin (Figure 3B and C). These results suggest that MIR194-2HG could activate Wnt/β-catenin signaling in liver cancer cells.

MIR194-2HG Acted as a Sponge for miR-1207-5p in Liver Cancer Cells

Next, RegRNA2.0 (http://regrna2.mbc.nctu.edu.tw/) was utilized to predicted miRNA target sites.[18] The results showed that MIR194-2HG contained several miRNA targeting sites (Figure 4A). Accordingly, we detected the expression of these six miRNAs by transfection of MIR194-2HG overexpression plasmid in HCC cells and found that the expression level of miR-1207-5p was significantly lower than that of other miRNAs (Supplementary Figure S1). Moreover, recent studies demonstrated that miR-1207-5p was downregulated and acted as a tumor suppressor in several cancers, including HCC.[19–21] Therefore, miR-1207-5p was selected as a candidate in the following study. We then carried out Dual-Luciferase Reporter assays in liver cancer cells and observed that transfection of miR-1207-5p mimics prominently reduced the luciferase intensity of MIR194-2HG-WT (Figure 4B, left). Conversely, transfection of ASO-miR-

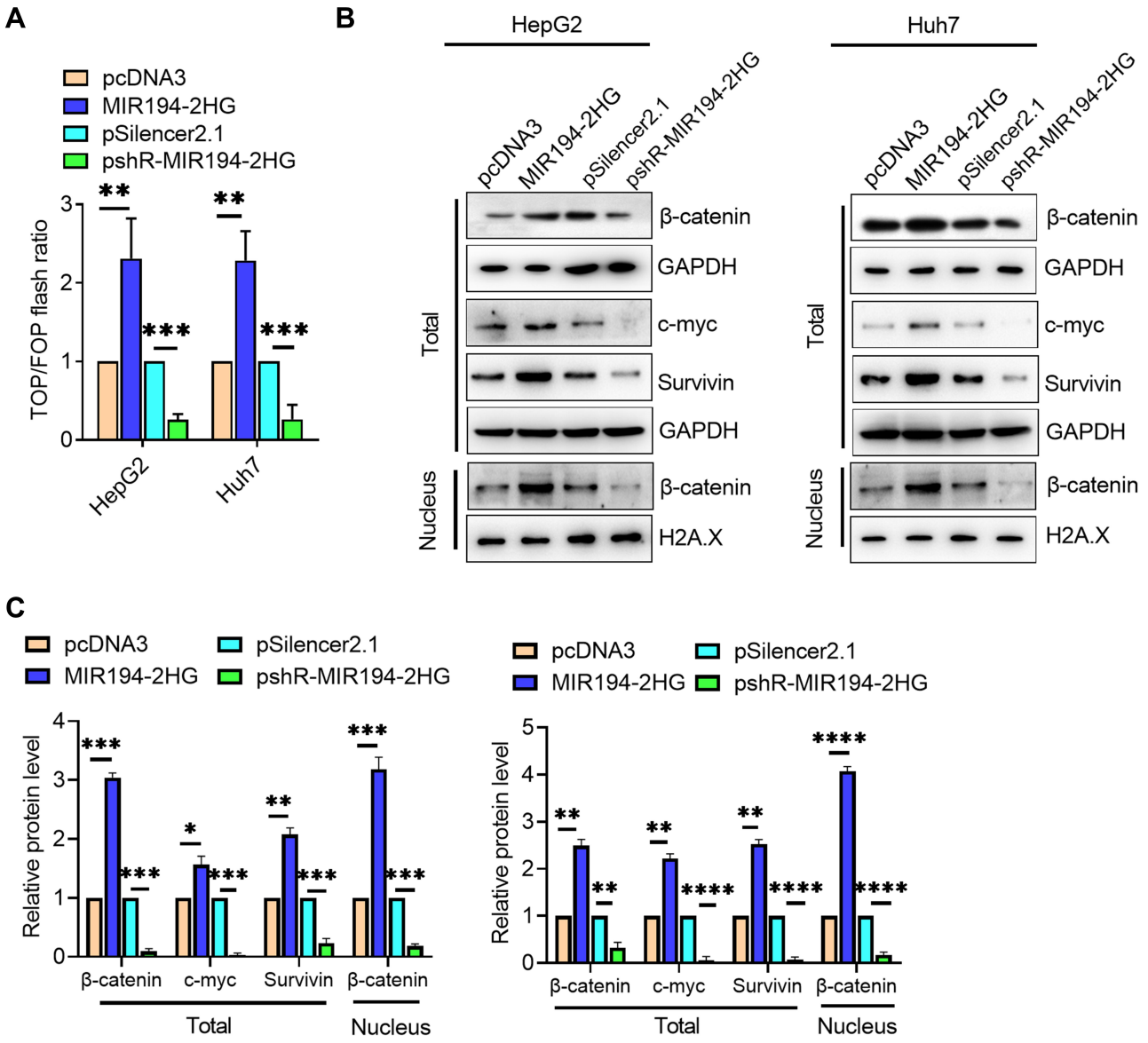

Figure 3 MIR194-2HG could regulate Wnt/β-catenin signaling pathway. (**A**) pTop-flash/pFop-flash reporter system was utilized to determine the activation of the Wnt/β-catenin pathway after transfection with MIR194-2HG overexpression or silencing plasmids. (**B**) The protein levels of total β-catenin and nuclear β-catenin were determined by Western blotting in HCC cells. (**C**) Quantification of the protein levels of total β-catenin and nuclear β-catenin in (**B**). (**A–C**) ANOVA followed by Bonferroni's post hoc test. Data are expressed as mean ± SD, *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001.

1207-5p, an inhibitor of miR-1207-5p, markedly increased the luciferase intensity (Figure 4B, left). In addition, we observed that neither miR-1207-5p mimics nor ASO-miR-1207-5p exerted a distinct effect on the luciferase activity of MIR194-2HG-Mut (Figure 4B, right). Subsequently, RT-qPCR results confirmed that, compared with miR-NC control group, MIR194-2HG expression was reduced after transfection of miR-1207-5p mimics (Figure 4C). On the contrary, transfection of ASO-miR-1207-5p could increase the level of endogenous MIR194-2HG in liver cancer cells (Figure 4C). We also demonstrated that MIR194-2HG could adsorb endogenic miR-1207-5p in liver cancer cells (Figure 4D). Besides, miR-1207-5p expression was downregulated in liver cancer tissues (Figure 4E), and its expression was inversely related to that of MIR194-2HG (Figure 4F). Taken together, these results suggested that MIR194-2HG acted as a sponge for miR-1207-5p in liver cancer cells.

TCF19 Was a Direct Target of miR-1207-5p

To explore the downstream target of miR-1207-5p, we used the online tool TargetScan Human 7.0 to predict the potential targets of miR-1207-5p, and TCF19 was

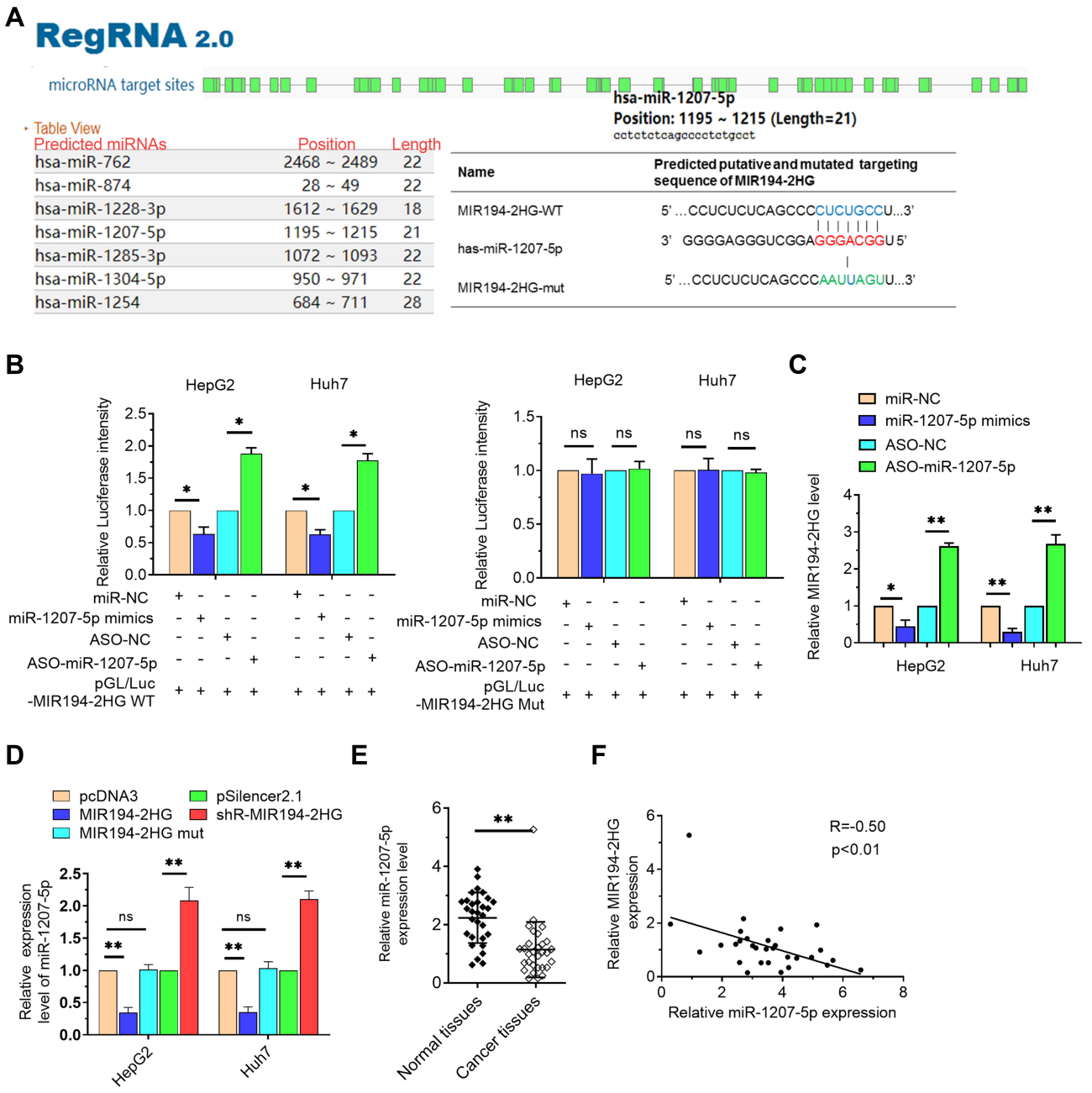

Figure 4 MIR194-2HG adsorbed miR-1207-5p in live cancer cells. (**A**) The RegRNA2.0 online tool was utilized to forecast miRNA binding to MIR194-2HG. The sequences of MIR194-2HG containing the wild-type or mutant miR-1207-5p binding sites are shown. (**B**) The MIR194-2HG-WT or MIR194-2HG-mut vector was co-transfected with miR-NC, miR-1207-5p mimics, ASO-NC, and ASO- miR-1207-5p in live cancer cells. The luciferase activity was then measured. (**C**) The expression levels of MIR194-2HG were determined by RT-qPCR after transfection with miR-1207-5p mimics or inhibitor. (**D**) The expression levels of miR-1207-5p were determined by RT-qPCR after transfection with MIR194-2HG WT, MIR194-2HG mut, and MIR194-2HG knockdown plasmids. (**E**) The expression of miR-1207-5p in live cancer tissues and adjacent normal tissues were measured by RT-qPCR. (**F**) Pearson's correlation analysis was utilized to determine the correlation between MIR194-2HG and miR-1207-5p expression in live cancer tissues. (**B–D**) ANOVA followed by Bonferroni's post hoc test; (**E** and **F**) Student's *t*-test. Data were expressed as mean ± SD, *P < 0.05; **P < 0.01; ns, not significant.

selected as a candidate for further investigation (Figure 5A). The Dual-Luciferase Reporter assay showed that transfection of miR-1207-5p mimics inhibited the luciferase activity of the pGL/Luc-TCF19 WT reporter vector, and downregulation of miR-1207-5p increased the luciferase activity (Figure 5B). However, miR-1207-5p did not affect the luciferase activity of the pGL/Luc-TCF19 WT reporter vector, which indicated the targeting relationship between miR-1207-5p and the 3′UTR of TCF19 (Figure 5C). In addition, we showed that the TCF19 mRNA level was reduced after transfection of miR-1207-5p mimics (Figure 5D). Conversely,

transfection of ASO-miR-1207-5p could increase the TCF19 mRNA level in HCC cells (Figure 5D). Accordingly, the TCF19 mRNA level was upregulated in HCC tissues (Figure 5E), which was consistent with the demonstrations in the previous report.[22] We also observed that the expression of miR-1207-5p was inversely related to the TCF19 mRNA level (Figure 5F). These results suggest that TCF19 is a direct target of miR-1207-5p, and there is a high probability that it is positively regulated by MIR194-2HG.

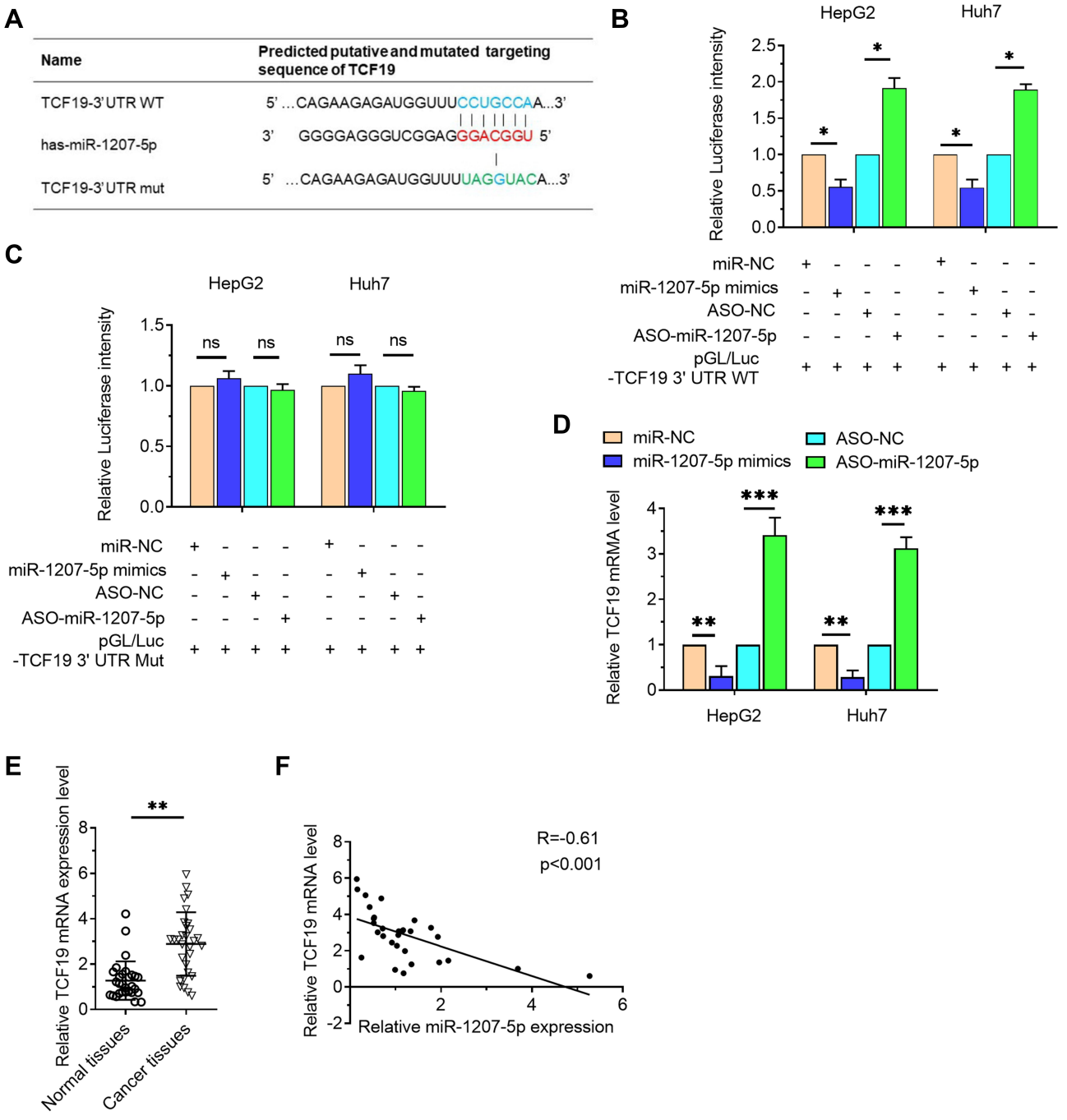

Figure 5 miR-1207-5p targeted TCF19 in live cancer cells. (**A**) The TargetScan Human 7.0 online tool was utilized to predicted microRNA targets. The 3′UTR sequences of TCF19 containing the wild-type or mutant miR-1207-5p binding sites were shown. (**B** and **C**) TCF19-WT or TCF19-mut vector were co-transfected with miR-NC, miR-1207-5p mimics, ASO-NC, and ASO- miR-1207-5p in HCC cells. The luciferase activity was then measured. (**D**) The expression levels of TCF19 were determined by RT-qPCR after transfection with miR-1207-5p mimics or inhibitor. (**E**) The expression of TCF19 in live cancer tissues and adjacent normal tissues were measured by RT-qPCR. (**F**) Pearson's correlation analysis was utilized to determine the correlation between TCF19 and miR-1207-5p expression in live cancer tissues. (**B–D**) ANOVA followed by Bonferroni's post hoc test; (**E** and **F**) Student's *t*-test. Data were expressed as mean ± SD, *P < 0.05; **P < 0.01; ***P < 0.001; ns, not significant.

TCF19 Promoted the Proliferation and Metastasis of Liver Cancer Cells

Several studies have reported that TCF19 played an oncogenic role in cancers.[22–24] Herein, we also demonstrated that TCF19 promoted cell proliferation in liver cancer by MTT assay and colony formation assay (Figure 6A–C), which was consistent with the demonstrations in the previous report.[22] However, whether TCF19 also affected the migration and invasion abilities of liver cancer cells was unreported. Therefore, we measured the migration and invasion of liver cancer cells by altering the expression of TCF19. The results showed TCF19 elevation markedly increased the migration ability of HepG2 and Huh7 cells; while MIR194-2HG knockdown significantly inhibited the migration ability (Figure 6D, left). In addition, upregulation of TCF19 markedly increased the invasion ability of HepG2 and Huh7 cells, whereas downregulation of TCF19 prominently inhibited their invasion ability (Figure 6D, right). These results suggested that TCF19 promoted cell proliferation and metastasis in liver cancer.

MIR194-2HG Activated Wnt/β-Catenin Signaling Through the miR-1207-5p/TCF19 Axis

To investigate whether MIR194-2HG affected the proliferation and metastasis of HCC cells and activated Wnt/β-catenin signaling by regulating the miR-1207-5p/TCF19 axis, we performed rescue experiments by co-transfecting ASO-miR-1207-5p and MIR194-2HG knockdown plasmids into liver cancer cells. MTT assay, colony formation assay, and Transwell assay results demonstrated that downregulation of MIR194-2HG markedly impeded the proliferation, migration, and invasion of liver cancer cells, and the proliferation, migration, and invasion abilities were significantly increased by co-transfection with

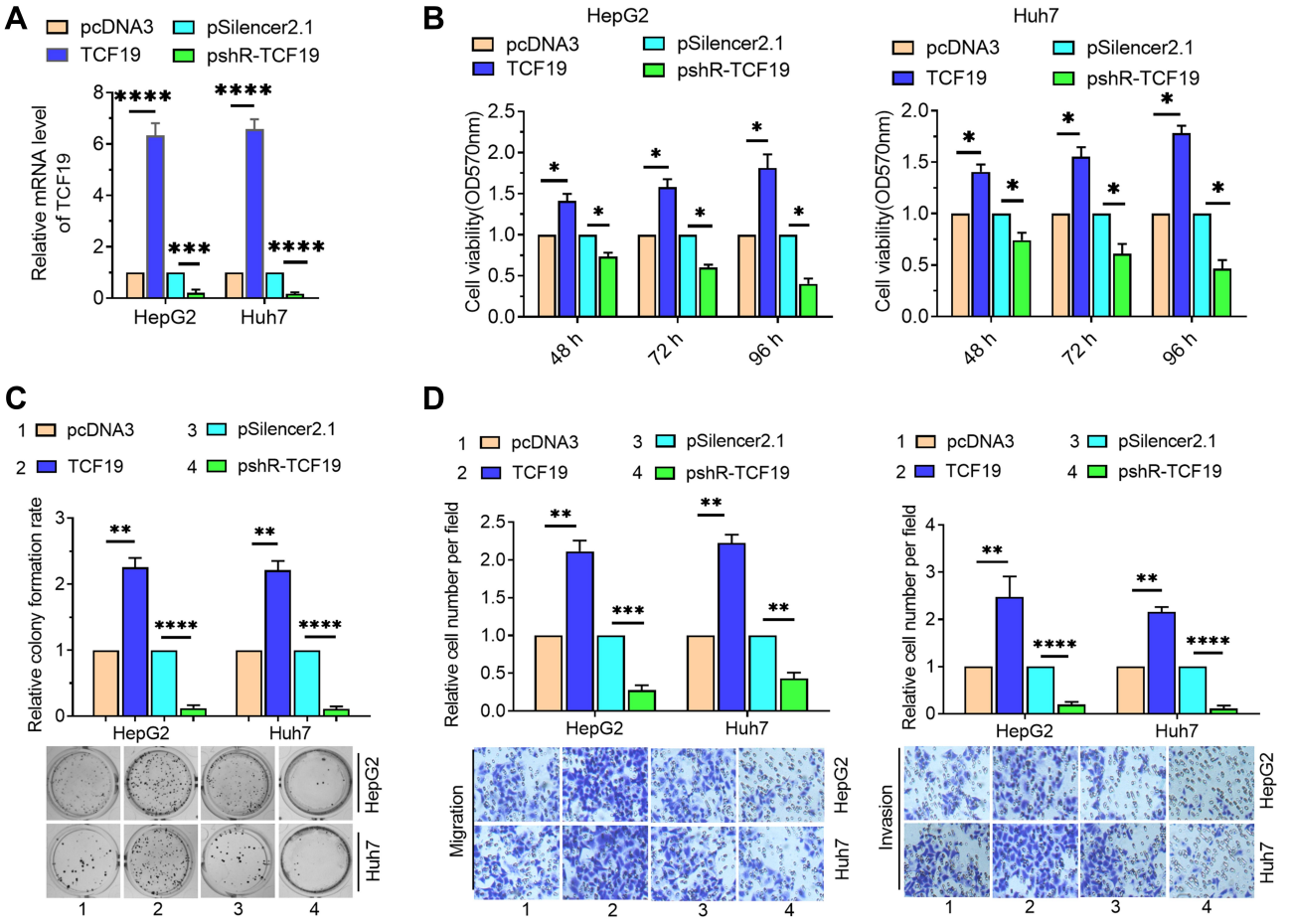

Figure 6 TCF19 promoted the proliferation, migration, and invasion of live cancer cells. (**A**) HepG2 and Huh7 cells were transfected with TCF19 overexpression or silencing plasmids, and the transfection efficiency was verified by RT-qPCR. (**B**) MTT method was utilized to determine the cell viabilities of live cancer cells. (**C**) The colony formation ability of cells was investigated by colony formation assay. (**D**) Transwell assay was utilized to determine the migration and invasion of live cancer cells. (**A–D**) ANOVA followed by Bonferroni's post hoc test. Data were expressed as mean ± SD, **P < 0.01; *P < 0.05; ***P < 0.001; ****P < 0.0001.

ASO-miR-1207-5p (Figure 7A–C). Furthermore, we showed that downregulation of MIR194-2HG markedly inhibited the activity of the TCF-dependent luciferase reporter pTop-flash/pFop-flash, whereas con-transfection with ASO-miR-1207-5p MIR194-2HG significantly increased the luciferase activity (Figure 7D). Western blot results showed that the protein levels of total TCF19 and β-catenin were downregulated in liver cancer cells after MIR194-2HG knockdown, and their protein levels were significantly increased after the co-transfection of ASO-miR-1207-5p (Figure 7E and F). Accordingly, we also demonstrated that MIR194-2HG knockdown alone reduced the nuclear β-catenin protein level, which could be significantly increased by inhibiting the expression of miR-1207-5p (Figure 7E and F). Taken together, these results suggested that MIR194-2HG could promote the proliferation, migration, and invasion of liver cancer cells by regulating miR-1207-5p/TCF19/Wnt/β-catenin axis (Figure 7G).

Discussion

Accumulating evidence has shown that lncRNAs act as important regulators related to tumorigenesis and cancer progression.[25–27] However, the function of lncRNAs, including MIR194-2HG, in HCC cells has not been totally elucidated.

MIR194-2HG was first reported to be upregulated in bladder cancer,[17] and its dysregulation might be related to cancer progression. Herein, MIR194-2HG was demonstrated to be upregulated in liver cancer tissues and cells, and the overall survival rate of liver cancer patients with higher expression of MIR194-2HG was poorer than that of liver cancer patients with lower expression of MIR194-2HG. A series of functional experiments, such as the MTT assay, colony formation assay, and Transwell assay, were performed to demonstrated that MIR194-2HG functioned in an oncogenic role and could promote cell proliferation, migration, and invasion in liver cancer. It is well known that the Wnt/β-catenin signaling pathway exhibited a critical role in human diseases, including cancers. Accumulating evidence has shown that activation of the Wnt/β-catenin signaling pathway is frequently demonstrated in several cancers.[28–30] For example, it was reported that activation of Wnt/β-catenin signaling by linc00210 promoted liver tumor progression in a CTNNBIP1-dependent manner.[31] Liu et al illustrated that lncRNA DSCR8 could activate the Wnt/β-catenin signaling pathway by adsorbing miR-485-5p in liver cancer.[32] Wang et al showed that lncRNA CASC15 acted in an oncogenic role by activating the SOX4/Wnt/β-catenin signaling pathway in liver cancer.[33] Our study demonstrated that MIR194-2HG could activate Wnt/β-catenin signaling to promote the proliferation, migration, and invasion of liver cancer cells.

Typically, lncRNAs serve as ceRNAs to adsorb their binding miRNAs, thereby regulating downstream target mRNA expression.[34] In this study, we demonstrated that MIR194-2HG could sponge miR-1207-5p to repress its expression level. The expression levels of miR-1207-5p and MIR194-2HG were negatively correlated in live cancer tissues. The functions of miR-1207-5p have been reported previously in several cancers, and its role could be cancer specific. In colorectal cancer, lung cancer, gastric cancer, and HCC, it acted as a tumor suppressor that could inhibit cancer cell proliferation and metastasis.[19–21,35,36] However, a lncRNA PVT1-induced microRNA, miR-1207-5p, has been reported to promote growth by directly suppressing STAT6 in breast cancer cells.[37] Subsequently, we demonstrated that miR-1207-5p directly inhibited the expression of TCF19, a newly identified target of miR-1207-5p, that could play an oncogenic role in several cancers, such as colorectal cancer,[23] non-small cell lung cancer,[24] and liver cancer.[22,38] Consistent with the findings of previous studies,[22,38] we demonstrated that TCF19 promoted cell proliferation in HCC. Besides, we further showed the migratory capacities of liver cancer cells regulated by TCF19. Finally, our findings demonstrated that downregulation of MIR194-2HG markedly impeded the proliferation, migration, and invasion of liver cancer cells, and these effects were abated by miR-1207-5p inhibition. Accordingly, we demonstrated that downregulation of MIR194-2HG markedly inhibited the expression of TCF19 and β-catenin, which could be rescued by miR-1207-5p inhibition. These results suggested that MIR194-2HG played an oncogenic role by affecting the miR-1207-5p/TCF19/Wnt/β-catenin axis in liver cancer cells.

Conclusion

In conclusion, our study revealed that MIR194-2HG was upregulated in liver cancer and functioned in an oncogenic role by activating the Wnt/β-catenin signaling pathway. In addition, MIR194-2HG could act as a molecular sponge for miR-1207-5p to modulate the expression of its downstream target TCF19, which has an accelerating effect on the proliferation and invasion of liver cancer cells. Our study finally demonstrated that MIR194-2HG plays an

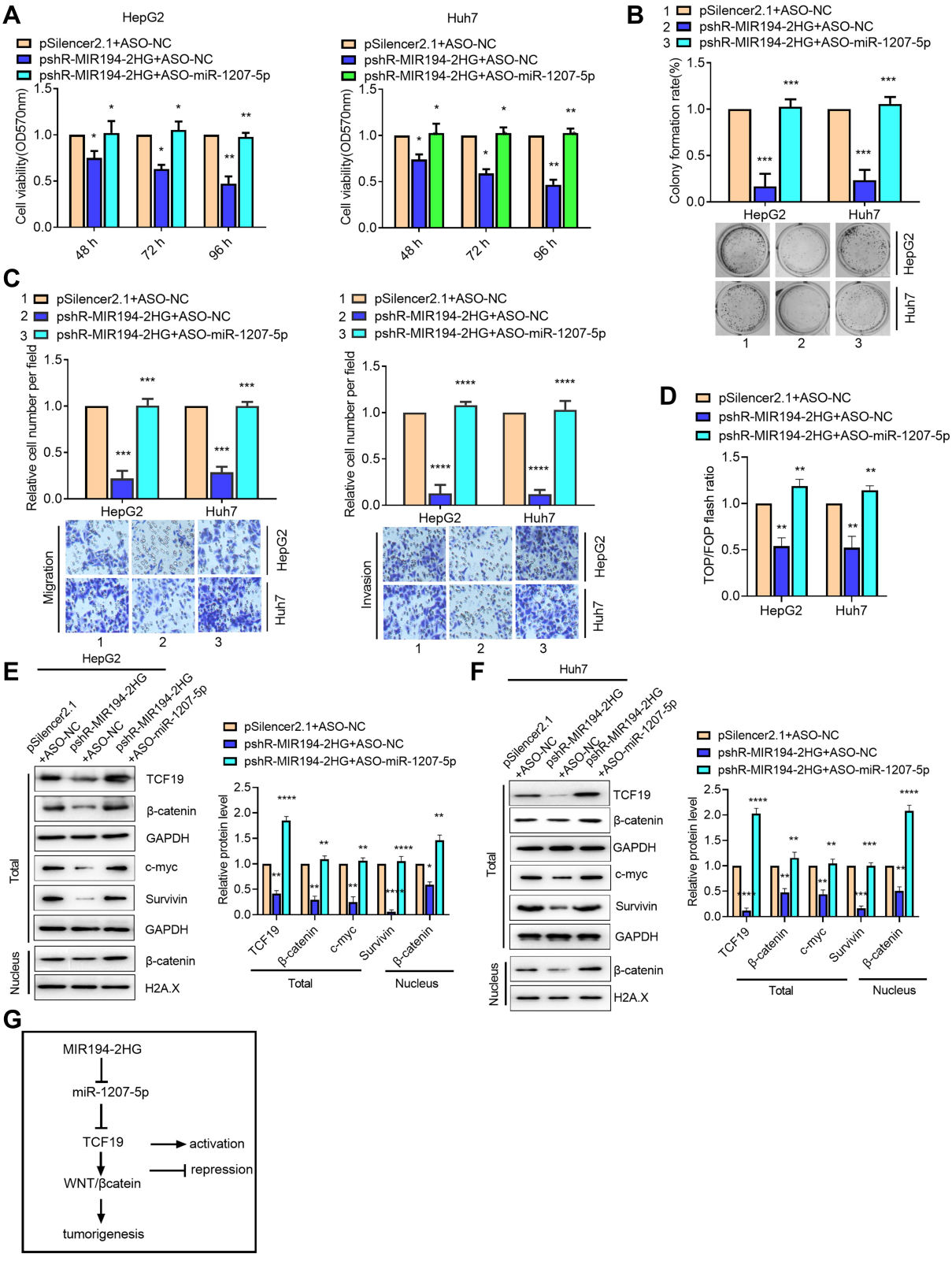

Figure 7 MIR194-2HG activation of Wnt/β-catenin signaling in live cancer cells was dependent on the miR-1207-5p/TCF19 axis. miR-1207-5p inhibitors were transfected into live cancer cells with MIR194-2HG knockdown plasmids. (**A–C**) The MTT, colony formation, and Transwell assays were performed to detect cell viability, colony formation ability, migration, and invasion of live cancer cells, respectively. (**D**) the pTop-flash/pFop-flash reporter system was utilized to determine the activation of the Wnt/β-catenin pathway. (**E** and **F**) The protein levels of TCF19 and β-catenin as well as nuclear β-catenin were determined by Western blotting in live cancer cells. (**G**) The proposed molecular mechanism of MIR194-2HG. MIR194-2HG acted as a sponge for miR-1207-5p, thus releasing the inhibition of TCF19 by miR-1207-5p, and activated Wnt/β-catenin signaling in live cancer cells. (**A–F**) ANOVA followed by Bonferroni's post hoc test. Data were expressed as mean ± SD, *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001.

oncogenic role and activates the Wnt/β-catenin signaling pathway via a mechanism mediated by the miR-1207-5p/TCF19 axis, which provides a novel avenue for diagnostic or therapeutic interventions in liver cancer.

Data Sharing Statement

The datasets used in this study are available from the corresponding author upon reasonable request.

Ethics and Consent Statement

This study was approved by the Research Ethics Committee of The Second Hospital of Tianjin Medical University.

Author Contributions

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the manuscript.

Disclosure

The authors report no conflicts of interest in this work.

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