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LncRNA FGD5-AS1/miR-5590-3p axis facilitates the proliferation and metastasis of renal cell carcinoma through ERK/AKT signalling

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Abstract. – OBJECTIVE: Amongst noncoding RNAs, competing endogenous RNAs (ceRNAs) are popular and interesting regulatory mechanisms involved in oncogenesis and tumour progression. LncRNA FGD5-AS1, also known as miR-5590-3p, is involved in the regulatory role of ceRNA in many cancers. However, the roles of lncRNA FGD5-AS1 or miR-5590-3p in renal cell carcinoma (RCC) remain unclear. We investigated how FGD5-AS1 and miR-5590-3p regulated clear cell proliferation and metastasis in RCC.

PATIENTS AND METHODS: Real Time-quantitative PCR (RT-qPCR) was used to detect the expression of FGD5-AS1 in tumour issues and renal cancer cell lines. MTT, scratch test and transwell assay were performed to confirm the effect of FGD5-AS1 on the proliferation, migration or invasion of the above cell lines. RNA pull-down and Luciferase assays were used to detect the target site between FGD5-AS1 and miR-5590-3p. In addition, we examined the proteins related to ERK/AKT signalling related via Western blot analysis. Finally, we used the RT-qPCR method to detect the mRNA levels of E-cadherin and vimentin.

RESULTS: LncRNA FGD5-AS1 was highly expressed in renal cancer tissues, especially in patients with metastasis. This effect facilitated the proliferation, migration, epithelial-mesenchymal transition and invasion of renal cancer cells. Silencing the expression of FGD5-AS1 with FGD5-AS1 siRNA significantly inhibited the malignancy of tumour cells. RNA pull-down and Luciferase assays demonstrated that FGD5-AS1 targeted miR-5590-3p to interact with miR-5590-3p negatively. Furthermore, miR-5590-3p inhibitors could eliminate the FGD5-AS1 siRNA-induced upregulation of E-cadherin and downregulation of vimentin.

CONCLUSIONS: Mechanistically, lncRNA FGD5-AS1 can competitively interact with miR-5590-3p and regulate the downstream signalling of ErkAKT to enhance the malignancy of tumours. This lncRNA could become a potential target molecule for treating and diagnosing RCC.

Key Words:
LncRNA FGD5-AS1, MiR-5590-3p, RCC.

Introduction

Renal cell carcinoma (RCC) is one of the most common and malignant tumours of the urinary system, accounting for 80%-90% of renal malignant tumours. RCC is derived from renal tubular epithelial cells and includes more than 10 histological and molecular subtypes, amongst which clear cell RCC (ccRCC) is the most common and accounts for the majority of cancer-related deaths. Molecular histology revealed that other non-ccRCCs (nccRCCs) are involved in papillary, chromophobe and MiT family translocation in collecting duct, medullary and oncocytoma tumours[1,2]. The 5-year survival rate of RCC is more than 70% for patients without metastasis but is less than 10% for the patients with metastasis[1,3]. The number of estimated new cancer cases and deaths of RCC in the United States reached 73,750 and 14,830, respectively, in 2020[4]. RCC is also amongst the 10 most common cancers worldwide[1]. A series of genes, including VHL,

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SDHB-D, FH, MET, FLCN and TSC1-2, are involved in the occurrence of RCC. The oncogenesis of RCC has different pathological patterns that are distinct from each other. Discovering the detailed molecular mechanism underlying the carcinogenesis of RCC is imperative for developing diagnostic and therapeutic strategies with increased effectiveness.

Noncoding RNAs (ncRNAs) are a series of RNA molecules that are not translated into a protein. They mainly include miRNAs, tsRNAs, piRNAs, lncRNAs, pseudogenes, and circRNAs. They play important regulatory roles in tumorigenesis and participate in all hallmarks of the multistep development of human tumours, including breast cancer, lung cancer, liver cancer, lymphoma, glioblastoma, prostate cancer, ovarian cancer, and RCC[5,6]. LncRNAs can perform biological functions as oncogenic molecules and tumour suppressors and as novel diagnosis biomarkers or potential therapy molecules. Interestingly, lncRNAs, pseudogenes and circRNAs are involved with competing endogenous RNAs (ceRNAs), which regulate other RNA transcripts by competing for shared miRNAs[7,8].

Herein, we aimed to find lncRNA-miRNA pairs with a ceRNA mechanism for modulating the proliferation and metastasis of RCC. LncRNA FGD5-AS1 is highly expressed as an oncogene in many cancers, including oesophageal squamous cell carcinoma[9], non-small cell lung cancer (NSCLC)[10], oral cancer[11] and colorectal cancer[12]. It can promote cancer cell proliferation, migration and invasion by interacting with different miRNAs, such as miR-383, miR-107, miR-520b and miR-302e[9-12]. FGD5-AS1 also regulates the development of periodontitis and neuronal injury *via* competitively binding to miR-142-3p and miRNA-223[13,14], respectively. However, whether FGD5-AS1 plays a potential role in RCC by competitively combining with miRNA remains unknown.

In the study, we first found that lncRNA FGD5-AS1 was upregulated in renal cancer, especially in patients with the clinical metastasis of renal cancer. This effect facilitated the proliferation, migration, epithelial-mesenchymal transition (EMT) and invasion of renal cancer cells. Furthermore, it could competitively interact with miR-5590-3p and regulate downstream signalling ErkAKT to enhance the malignancy of tumours. Hence, this lncRNA may be a potential target molecule for the treatment and diagnosis of renal cancer.

Patients and Methods

Tissue Samples

Renal cancer and adjacent nontumour tissue samples were collected from patients who underwent surgical operation at Juancheng County People's Hospital. A total of 28 patients with renal cancer, including 16 males and 12 females, were included in this study. The patients were aged 41-78 years old with an average age of 58 ± 11 years. Tumour pathological types were determined *via* HE staining after surgery. Amongst all cases, 28 were RCC. Patients with severe or unstable heart disease and incomplete data were excluded from this study. In accordance with the 2002 AJCC kidney cancer clinical staging criteria, specimens from 9, 5, 12 and 2 cases were classified as stage I, II, III and IV, respectively. All studies were approved by the Ethics Committee of Juancheng County People's Hospital and signed informed consent forms were provided by the participants. The tissue samples were snap-frozen in liquid nitrogen and stored at -80°C.

Cell Culture

The human proximal tubular cell line HK-2 and RCC cell lines (786-O, ACHN and SN12-PM6) were purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA). The HK-2 cell line was maintained in K-SFM supplemented with 0.05 mg/ml BPE and 5 ng/ml EGF and incubated at 37°C and 5% CO_2. Other RCC cell lines were cultured in Dulbecco's Modified Eagle's Medium (Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% foetal bovine serum (FBS; Gibco, Life Technologies, Rockville, MD, USA) and 1% penicillin/streptomycin (Hycult, Life Technologies, Rockville, MD, USA) and incubated at 37°C and 5% CO_2.

Cell Transfection

Si-FGD5-AS miR-5590-3p inhibitor, their negative control FGD5-AS1 overexpressed plasmid and blank plasmid were purchased from Ribobio (Guangzhou, China). Before transfection, cell lines were seeded in 12-well plates and cultured for 12 h. For transient overexpression, si-FGD5-AS, miR-5590-3p inhibitor and recombinant plasmid were transfected into the above-mentioned cells with the help of Lipofectamine 2000 (Life Technologies, Rockville, MD, USA). The samples were collected after 24 h to quantify the mRNA or protein expression of the target gene.

Quantitative Real-Time Polymerase Chain Reaction

TRIzol and microRNA isolation kits were purchased from Qiagen (Valencia, CA, USA). Tissue samples or cells were homogenised in TRIzol, and total RNA, including miRNAs, were extracted and reverse transcribed in accordance with the manufacturer's protocol. The expression levels of E-cadherin and vimentin mRNA (GAPDH as an internal reference) and lncRNA FGD5-AS1 and miR-5590-3p (U6 snRNA as internal reference) were detected *via* SYBRGreen quantitative PCR (qPCR). The PCR primer sequences were as follows: the upstream sequence of GAPDH was 5′-TGAAGGTCGGAGTCAACGGG-3′, and its downstream sequence was 5′-CCTGGAAGATGGTGATGGG-3′. The upstream sequence of U6 snRNA was 5′-CTCGCTTCGGCAGCACA-3′, and its downstream sequence was 5′-AACGCTTCACGAATTTGCGT-3′. The relative expression levels of the target genes were calculated via the $2^{-\Delta\Delta Ct}$ method.

Luciferase Reporter Assays

Dual-Luciferase reporter vectors were constructed to test the direct binding sequence of miR-5590-3p. HEK 293T cells were cotransfected with miR-5590-3p or control mimics and firefly Luciferase reporter vector containing wild-type (WT) or mutant (Mut) sequences of lncRNA FGD5-AS1 by using Lipofectamine 2000 reagent (Life Technologies, Rockville, MD, USA) and a Renilla Luciferase reporter vector to normalise transfection efficiency. Renilla and firefly Luciferase activities were measured at 24 h post-transfection by applying the Dual-Luciferase® Reporter Assay System.

Western Blot Analysis

Cells were homogenised and run on 12% sodium dodecyl sulphate-polyacrylamide gel electrophoresis gel. The proteins were transferred to a polyvinylidene difluoride membrane (PVDF, Roche Molecular Biochemicals, Quebec, Canada), and then, blocked with 5% skim milk powder at room temperature for 1 h. The polyvinylidene difluoride (PVDF) membrane (Millipore, Bedford, MA, USA) was incubated with primary antibodies at 4°C overnight, and then, incubated with the appropriate HRP-conjugated secondary antibodies. The films were developed with an ECL System (Millipore, Billerica, MA, USA). The antibodies for Phospho-Akt (#12178s, 1:2000), Akt (#4691s, 1:2000), Phospho-Erk (#4370p, 1:2000) and Erk (#3316s, 1:2000) and GAPDH (#3316s, 1:2000) were purchased from Cell Signalling Technology (Danvers, MA, USA).

MTT Assay

At 48 h after transfection, the cells were seeded into 24-well plates to a confluence of 60%. A total of 200 µl of diluted MTT solution was added to each well. The plates were then incubated for 2 h in accordance with the instructions of the MTT kit (Abcam, Cambridge, UK). Absorbance was recorded by using a microplate reader (MultiskanEX, Lab Systems, Helsinki, Finland).

Scratch Test

Cells were cultured for 48 h after transfection. When the cell confluence reached 95%, 200 µl pipette tips were used to create scratches. After changing the medium to serum-free medium for 24 h, the cells were observed under an inverted microscope. The relative migration capacity of the cells was determined. Three visual fields in each group were collected for analysis.

Transwell Assay

Cells were transfected with FGD5-AS1 and control siRNA or FGD5-AS1-overexpressing and blank plasmids in 12-well plates (10,000 cells/well) for 24 h. Then, 5000 cells per well were seeded in inserts that were pre-equilibrated in 8 µm-pore transwells (Corning, Costar, CA, USA) with media. After 24 h of incubation, the inserts were rinsed, fixed in 2% paraformaldehyde for 10 min, and then, stained with crystal violet (Beyotime, Shanghai, China). The numbers of cells that had migrated were counted under a microscope (ECLIPSE Ti, Nikon, Tokyo, Japan).

RNA Pull-Down Assay

Biotin-labelled miR-5590-3p and negative control were designated as biotin-miR-5590-3p and biotin-NC, respectively, and incubated with cell lysates. Then, streptavidin-coated magnetic beads (Life Technologies, Rockville, MD, USA) were added. The pull-down assay was performed with the biotin-coupled RNA complex, and the quantity of FGD5-AS1 was measured via qPCR.

Statistical Analysis

Each experiment was performed at least three times, and data were analysed with GraphPad Prism 5.0 software (GraphPad Software, Inc., San Diego, CA, USA). Comparison between two groups was performed via *t*-test. The com-

parison of means amongst multiple groups was performed *via* one-way analysis of variance, and the Bonferroni test was selected as the post hoc test. $p<0.05$ was considered statistically significant.

Results

LncRNA FGD5-AS1 Was Upregulated in Renal Cancer Tissues and Cells and in Patients with Renal Cancer and Clinical Metastasis

We firstly examined the expression of FGD5-AS1 in renal tumour tissues and adjacent normal tissues in patients with renal cancer and clinical metastasis to understand the expression of lncRNA FGD5-AS1 in renal cancer. We collected 14 paired renal cancer and adjacent normal tissues and detected the expression of FGD5-AS1 *via* real-time PCR. qPCR results showed that FGD5-AS1 expression in renal tumour samples was significantly increased compared with that in adjacent non tumour tissue samples (Figure 1A). In addition, we determined that the expression of FGD5-AS1 in RCC lines was higher than that in normal renal cell lines (Figure 1B). Furthermore, FGD5-AS1 expression in 14 patients with renal cancer and clinical metastasis was higher than that in 14 patients with renal cancer but without clinical metastasis (Figure 1C). These data indicated that lncRNA FGD5-AS1, which was highly expressed in renal cancer tissues, was related specifically and substantially with the malignant degree of renal cancer.

FGD5-AS1 Facilitated the Proliferation, Migration and Invasion of Renal Cancer Cells

We treated SN12-PM6 and 786-O cell lines with si-FGD5-AS1 to investigate the function of LncRNA FGD5-AS1 in the development of RCC and found that the treatment significantly silenced the expression of FGD5-AS1 (Figure 2A) with a considerable reduction in cell viability (Figure 2B). We also successfully enhanced the expression of FGD5-AS1 with FGD5-AS1-overexpressing plasmid in the above cells (Figure 2A). This effect resulted in a remarkable increase in cell viability (Figure 2B). Cell migra-

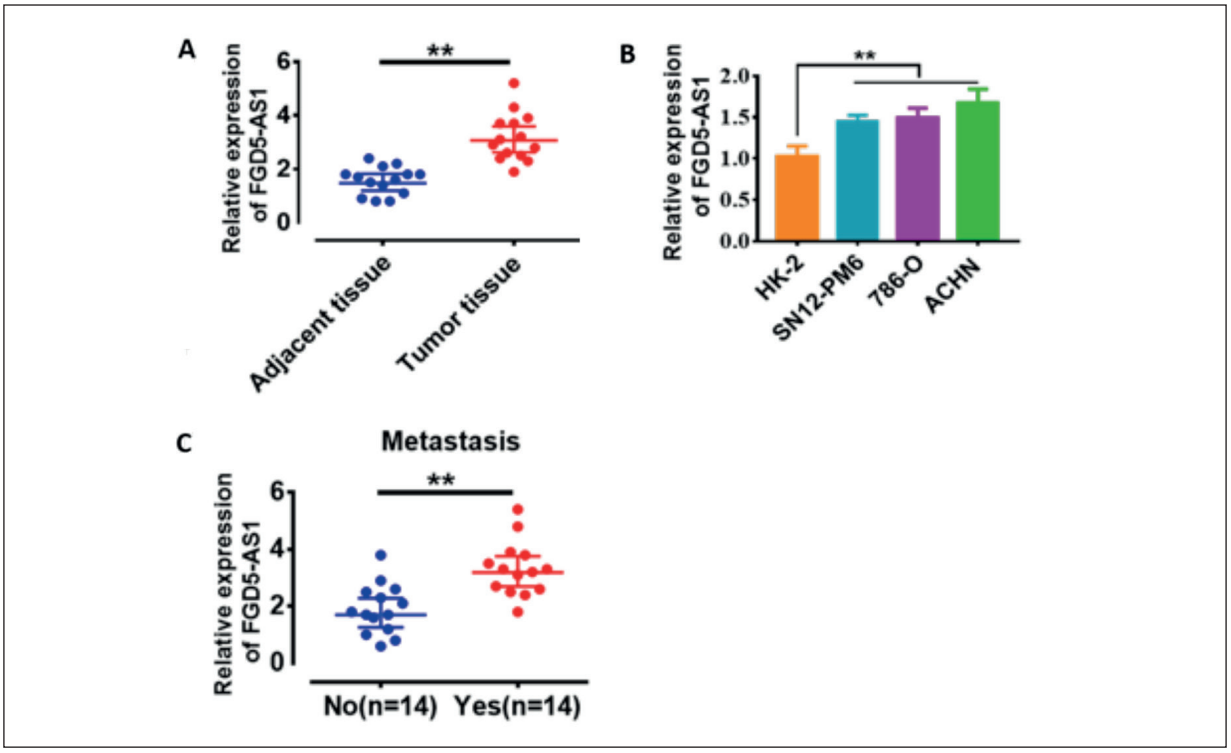

Figure 1. Expression of FGD5-AS1 in renal tumour tissues and cells and its correlation with the clinical metastasis of patients with renal cancer. **A,** qPCR analysis of the expression of FGD5-AS1 in tumour and adjacent tissue from patients with kidney tumours. **B,** qPCR analysis of the expression of FGD5-AS1 in normal renal and kidney cancer cell lines. **C,** qPCR analysis of the expression of FGD5-AS1 in tumour tissue from patients with metastasis and without metastasis. $^{**}p < 0.01$.

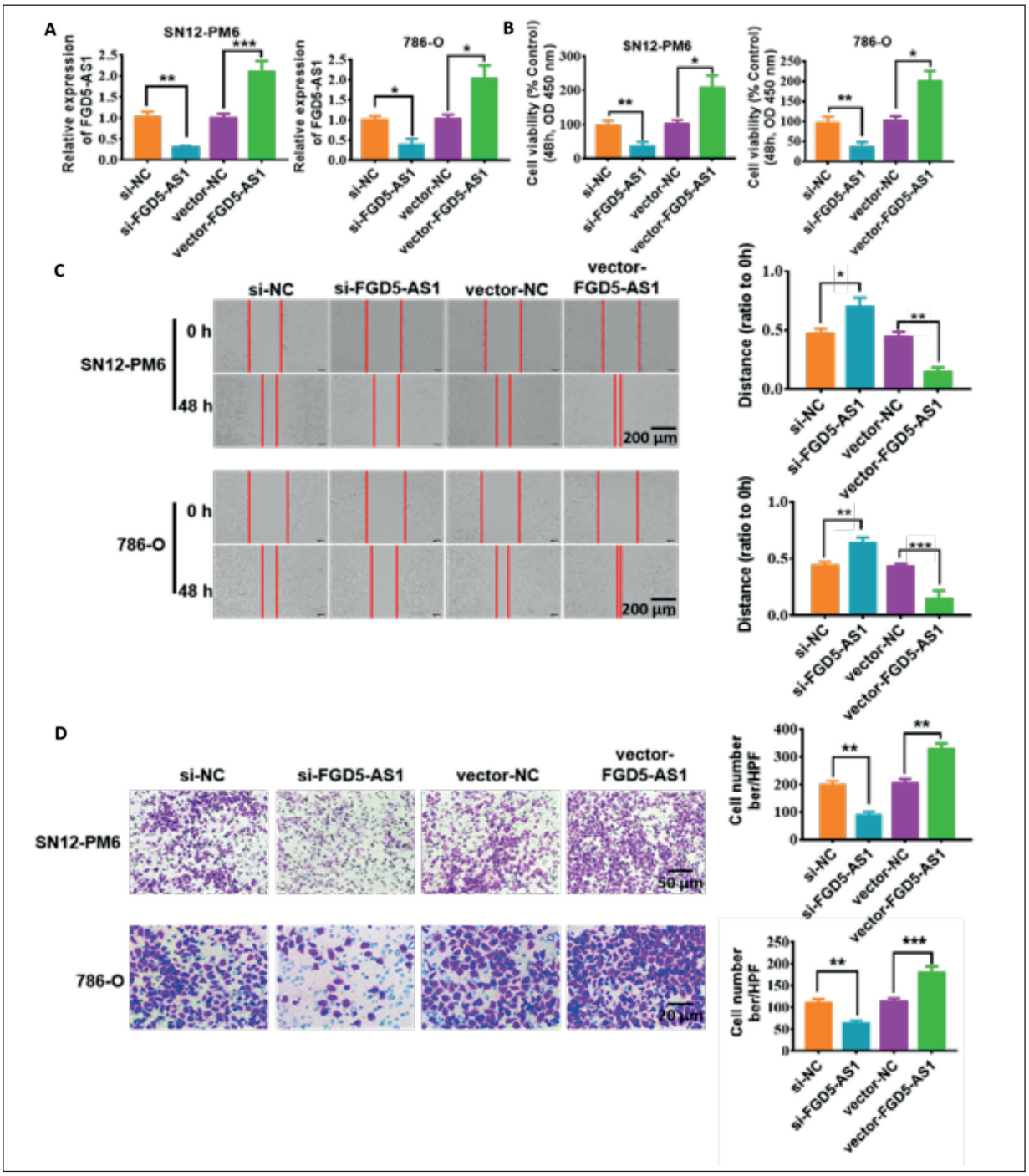

Figure 2. FGD5-AS1 facilitated the proliferation, migration and invasion of renal cancer cells *in vitro*. **A,** qPCR examination of the expression of FGD5-AS1 in SN12-PM6 and 786-O cell lines with FGD5-AS1 siRNA or its recombinant plasmid. **B,** MTT assay of the proliferation of SN12-PM6 and 786-O cell lines with FGD5-AS1 siRNA or its recombinant plasmid. **C,** Wound scratch test of the cell migration capability of SN12-PM6 and 786-O cell lines with FGD5-AS1 siRNA or its recombinant plasmid. **D,** Transwell test of the invasion capability of SN12-PM6 and 786-O cell lines with FGD5-AS1 siRNA or its recombinant plasmid. Data are presented as mean ± SD (n = 3). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

tion capacity was determined *via* the wound scratch test. The scratch in SN12-PM6 or 786-O cells in the si-FGD5-AS1 group was wider than that in the siRNA negative control group (Figure 2C). The wound width in SN12-PM6 or 786-O cells treated with FGD5-AS1-overex-

pressing plasmid was lower than that in the cells treated with the siRNA negative control (Figure 2C). We further tested the effect of FGD5-AS1 on cell invasion *via* transwell assay and found that the number of SN12-PM6 or 786-O cells in the si-FGD5-AS1 group was less than that in the siRNA negative control group (Figure 2D). By contrast, the number of SN12-PM6 or 786-O cells in the FGD5-AS1 recombinant plasmid-treated group was higher than that in the blank plasmid-treated group (Figure 2D). These findings indicated that LncRNA FGD5-AS1 facilitated the proliferation, migration and invasion of renal cancer cells.

FGD5-AS1 Aactivated AKT/ERK Signalling in Renal Cancer Cells

We treated SN12-PM6 cells with si-FGD5-AS1or FGD5-AS1-overexpressing plasmid, and then, determined the expression patterns of phosphorylated AKT and ERK *via* Western blot analysis to study the signal pathway involved in FGD5-AS1. We found that FGD5-AS1 siRNA inhibited the phosphorylation of Akt and ERK, whereas the FGD5-AS1-overexpressing plasmid promoted the phosphorylation of Akt and ERK compared with the negative control (Figure 3A). Moreover, we subjected 786-O cells to the same detection method and found that the results of Ln-

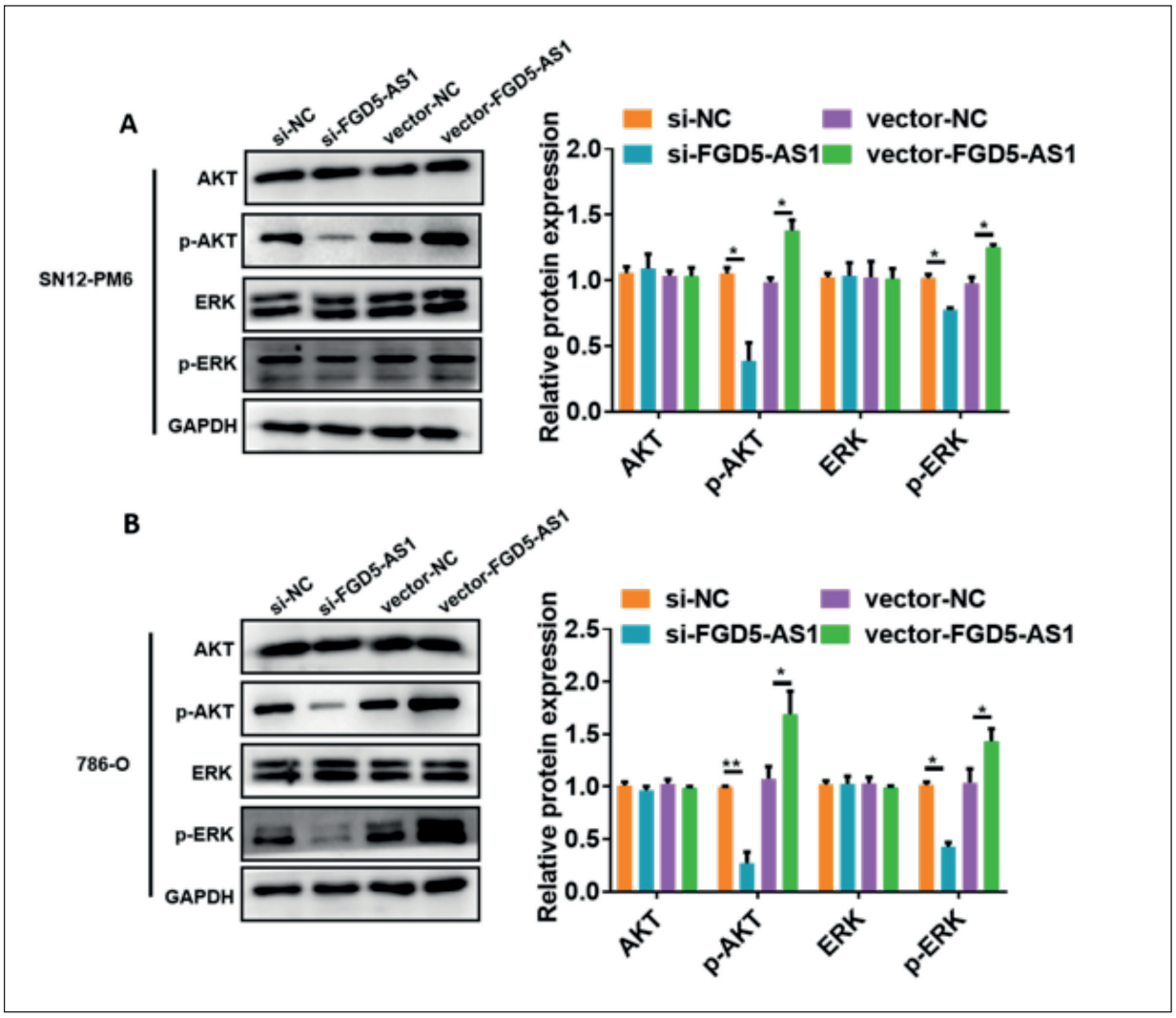

Figure 3. FGD5-AS1 activated AKT/ERK signalling in SN12-PM6 and 786-O cells. **A,** Western blot analysis (left) and statistical analysis (right) showing the expression levels of p-Akt and p-ERK in si-NC-, si-FGD5-AS1-, vector-NC- or vector-FGD5-AS1-treated SN12-PM6 cells at 48 h post-transfection. **B,** Western blot analysis (left) and statistical analysis (right) of the expression levels of p-Akt and p-ERK in si-NC-, si-FGD5-AS1-, vector-NC- or vector-FGD5-AS1-treated 786-O cells at 48 h post-transfection. Data are presented as mean ± SD (n = 3). *$p < 0.05$, **$p < 0.01$.

cRNA FGD5-AS1 were similar to those of SN12-PM6 cells (Figure 3B). These results demonstrated that LncRNA FGD5-AS1 might activate AKT/ERK signalling in renal cancer cells.

FGD5-AS1 Targeted MiR-5590-3p to Negatively Interact With MiR-5590-3p

The interaction of lncRNA and miRNA is a crucial method of gene regulation. We searched an online database to identify which miRNAs have complementary sequences with FGD5-AS1 to determine whether lncRNA FGD5-AS1 performed a gene regulatory function *via* underlying lncRNA-miRNA interactions. Bioinformatics assays showed that miR-5590-3p could strongly interact with FGD5-AS1 sequences (Figure 4A). We constructed WT and Mut FGD5-AS1 reporters with a Dual-Luciferase reporter system to confirm the interaction between FGD5-AS1 and miR-5590-3p. MiR-5590-3p mimics, together with a WT- or Mut-FGD5-AS1 plasmid, were cotransfected into SN12-PM6 cells, and then, luciferase assays were performed. We found that miR-5590-3p mimics significantly downregulated the Luciferase activity of WT-FGD5-AS1 plasmids but did not dramatically change that of Mut-FGD5-AS1 plasmids (Figure 4B). We also examined the expression of miR-5590-3p in SN12-PM6 cells treated with FGD5-AS1 siRNA and dis-

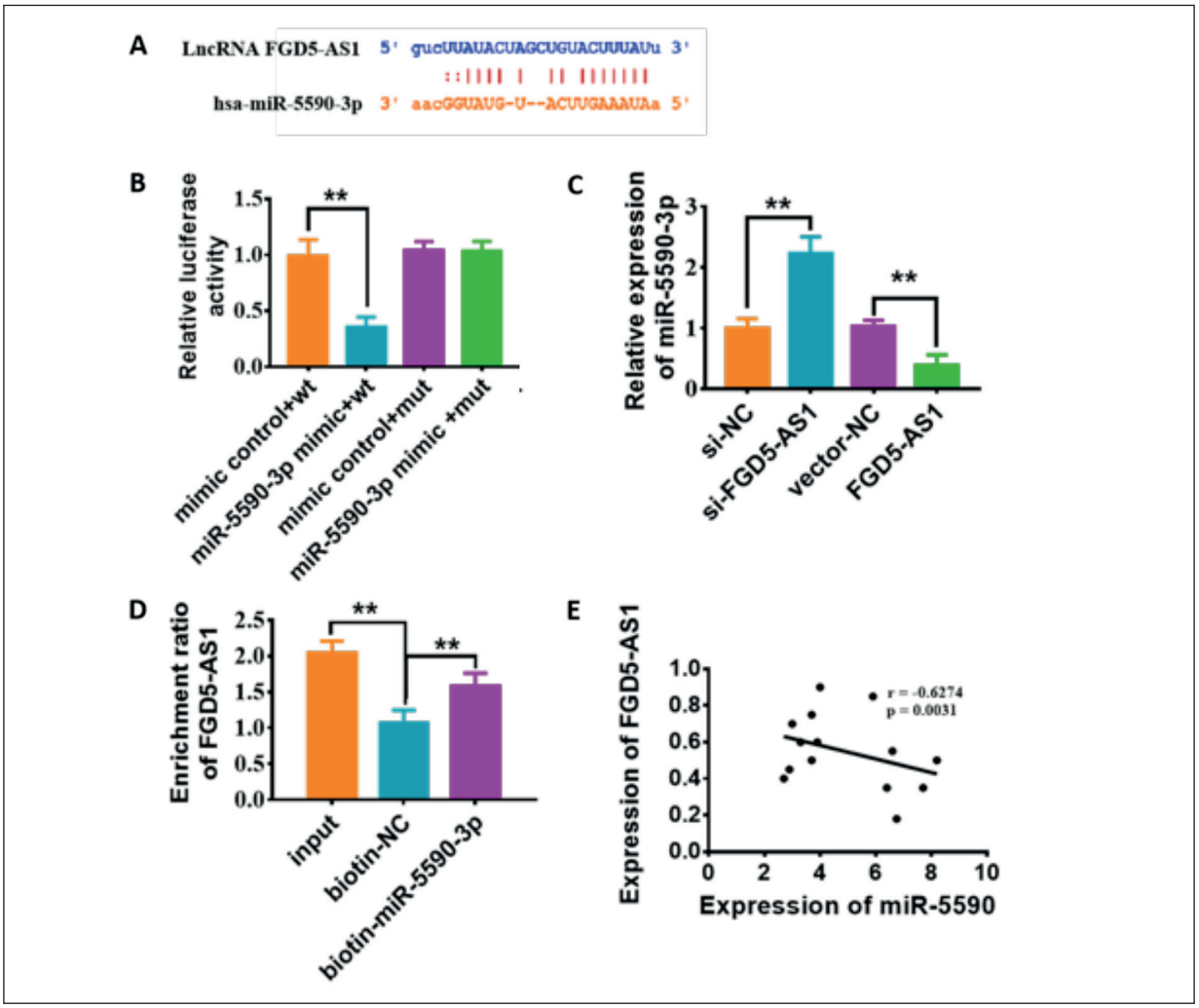

Figure 4. FGD5-AS1 targeted miR-5590-3p to negatively interact with miR-5590-3p. **A,** Sequences of miR-5590-3p are in orange and those of the target lncRNA FGD5-AS1 are in blue. **B,** Luciferase reporter gene assays for confirming the direct targeting of FGD5-AS1 to miR-5590-3p. **C,** qPCR analysis of the expression of miR-5590-3p in SN12-PM6 cells with FGD5-AS1 siRNA or its recombinant plasmid. **D,** RNA pull down assay of the binding of miR-5590-3p and lncRNA FGD5-AS1 by the miR-5590-3p biotin probe. **E,** Negative correlation between miR-5590-3p and FGD5-AS1. $^{**}p < 0.01$.

covered that FGD5-AS1 siRNA could upregulate miR-5590-3p expression (Figure 4C). By contrast, miR-5590-3p expression was considerably downregulated in the group treated with FGD5-AS1-overepressing plasmids (Figure 4C). Furthermore, we performed the RNA pull-down assay to detect the enrichment of LncRNA FGD5-AS1 by the miR-5590-3p biotin probe. As shown in Figure 4D, FGD5-AS1 enrichment by the miR-5590-3p biotin probe was remarkably more than that by the negative control probe. In addition, correlation analysis demonstrated that LncRNA FGD5-AS1 expression was negatively correlated with miR-5590-3p (Figure 4E). These findings indicated that FGD5-AS1 played a role in tumour progression by targeting miR-5590-3p to interact with miR-5590-3p negatively.

FGD5-AS1 Enhanced the EMT of Renal Cancer Cells by Negatively Interacting With MiR-5590-3p

Given that EMT has been shown to be a critical process for the migration, invasion and metastasis of cancer cells[16], we treated renal cancer cells with si-NC, si-FGD5-AS1, inhibitor-NC or miR-5590-3p inhibitor and analysed the expression of EMT markers, such as E-cadherin and vimentin, *via* real-time PCR assays. The expression of miR-5590-3p showed that transfection was effective (Figure 5A). We found that compared with the negative control, si-FGD5-AS1 could significantly enhance the expression of E-cadherin in SN12-PM6 cells. Compared with si-FGD5-AS1 alone, si-FGD5-AS1 together with the miR-5590-3p inhibitor remarkably restrained

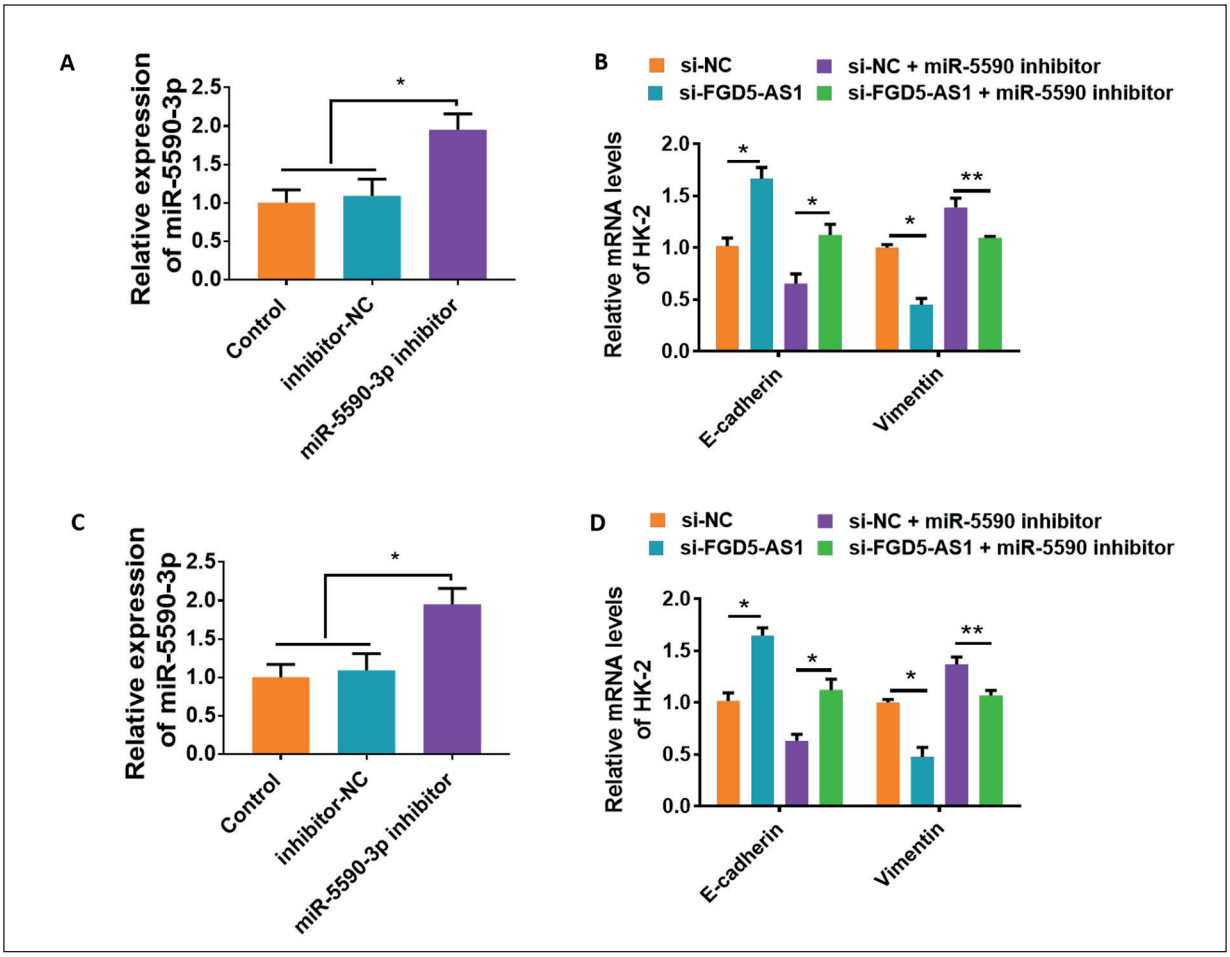

Figure 5. FGD5-AS1 enhanced the EMT of renal cancer cell lines SN12-PM6 and 786-O by negatively interacting with miR-5590-3p. **A,** MiR-5590-3p expression was verified via RT-qPCR analysis. **B,** qPCR analysis of the expression of E-cadherin and Vimentin in si-NC-, si-FGD5-AS1-, si-NC + miR-5590-3p inhibitor- and si-FGD5-AS1 + miR-5590-3p inhibitor-treated SN12-PM6 cells. **C,** MiR-5590-3p expression was verified via RT-qPCR analysis. **D,** qPCR analysis of the expression of E-cadherin and vimentin in 786-O cells treated with si-NC, si-FGD5-AS1, si-NC + miR-5590-3p inhibitor and si-FGD5-AS1 + miR-5590-3p inhibitor. *p<0.05, **p<0.01.

the expression of E-cadherin (Figure 5B). The expression of vimentin contradicted the above results. The mRNA level of E-cadherin and vimentin in 786-O cells was consistent with that in SN12-PM6 cells (Figure 5C, D). These findings indicated that FGD5-AS1 enhanced the EMT of renal cancer cell by negatively interacting with miR-5590-3p.

Discussion

Noncoding RNAs are widely involved in the multistep development of tumours[5,17,18]. LncRNAs are the most popular and important noncoding RNAs and have been widely researched in various cancers, including RCC[19,20]. LncRNAs with aberrant expression exhibit biological functions as an oncogene or tumour suppressor. HOTAIR, MALAT1, H19 and SPRY4-IT1 are highly expressed as oncogenes, whereas CADM1-AS1, TRIM52-AS1, MEG3 and BX357664 are expressed at low levels as tumour suppressors involved in the recurrence, metastasis and prognosis of RCC[21,22]. To our knowledge, lncRNA FGD5-AS1 has not been discovered in RCC. Our investigation showed that this lncRNA was highly expressed as an oncogene in RCC. In RCC, its artificial overexpression enhanced the proliferation, migration and invasion of renal cancer cells.

The purpose of this study was to describe the regulatory role of the FGD5-AS1/miR-5590-3p/ERK/AKT axis in RCC and the cellular/molecular mechanisms underlying the function of this axis. Our experiment yielded several new discoveries. Our study is the first to demonstrate that lncRNA FGD5-AS1 could competitively interact with miR-5590-3p and activate ERK/AKT signalling to facilitate the proliferation, migration, EMT and invasion of renal cancer cells. In addition, FGD5-AS1 was negatively correlated with miR-5590-3p expression, with the former being upregulated and the latter being downregulated in RCC. Silencing the expression of FGD5-AS1 with FGD5-AS1 siRNA significantly inhibited the malignancy of tumour cells. This effect showed the potential roles of FGD5-AS1 in the diagnosis and treatment of RCC.

MicroRNAs are small noncoding RNAs[23] and include miR-22, miR-185 and miR-210, which are downregulated and involved in the tumorigenesis of RCC[24,25]. Human miR-5590-3p has been demonstrated to target the 3′-UTR of TGFβ-R1, TGFβ-R2, SMAD3, SMAD4 and DDX5 genes to suppress the proliferation of colorectal cancer[26] and gastric cancer cells[27]. However, until now, evidence showing that miR-5590-3p has been investigated in RCC does not exist.

The mechanism of ceRNA is the Rosetta stone of the hidden RNA language[8]. LncRNA FGD5-AS1 or miR-5590-3P is involved in ceRNA regulation in several kinds of cancers except for RCC. FGD5-AS1 upregulates FGFRL1 *via* sponging miR-107 to promote lung cancer cell proliferation[10]. FGD5-AS1 also acts as a ceRNA on miR-383 to promote the malignancy of oesophageal cancer by enhancing SP1 expression[9]. In addition, miR-302e and miR-520b are competitively bound by FGD5-AS1 to promote CDCA7 and USP21 expression in colorectal cancer[12] and oral cancer[11], respectively. However, miR-5590-3p interacts competitively with LncRNAs SOX9-AS1 and SNHG14 to enhance SOX9 and ZEB1 expression in hepatocellular carcinoma[28] and lymphoma[29], respectively. Interestingly, RNA pull-down and Luciferase assays demonstrated that FGD5-AS1 targeted miR-5590-3p to negatively interact with miR-5590-3p. Furthermore, miR-5590-3p inhibitors could eliminate the FGD5-AS1-siRNA-induced the upregulation of E-cadherin and the down-regulation of vimentin. Our study found that the lncRNA FGD5-AS1/miR-5590-3p axis facilitated the cell proliferation and metastasis of RCC through the ceRNA mechanism of lncRNA and miRNA. ERK/AKT, as the target gene of miR-5590-3P, might mediate the RCC-promoting effect of the FGD5-AS1/miR-5590-3p axis.

In addition, the abnormal activation of signals, such as Erk and AKT, is usually found in many cancers. Inhibiting ERK and AKT phosphorylation in lung cancer[30] and renal cancer cells[31] suppresses cell proliferation, whereas activating ERK and AKT signals enhances the invasion and metastasis of renal cancer cells[32]. Our findings are consistent with the view that FGD5-AS1 overexpression promotes ERK and AKT phosphorylation, whereas FGD5-AS1 silencing inhibits ERK and AKT phosphorylation.

EMT is a biological process wherein epithelial cells are transformed into mesenchymal phenotypic cells through a specific process. Through EMT, epithelial cells lose polarity and connection to the basement membrane and other epithelial phenotypes. At the same time, cells exhibit strong interstitial phenotypes, such as the capability for migration and invasion, antiapoptosis, and extracellular matrix degradation. In this study, the expression of the epithelial marker E-cadherin

decreased after the overexpression of FGD5-AS1 in RCC cells, whereas the expression of the mesenchymal marker vimentin was upregulated. These results further confirmed that FGD5-AS1 might induce EMT in RCC cells, thereby promoting the invasion and metastasis of RCC.

Conclusions

We first demonstrated that lncRNA FGD5-AS1 could competitively interact with miR-5590-3p and regulate downstream ERK/AKT signalling to facilitate the proliferation, migration, EMT and invasion of renal cancer cells. Our study revealed new aspects of the cellular function and pathophysiological role of FGD5-AS1 and mir-5590-3p, both of which can be considered as potential molecular targets for the treatment of RCC.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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