The role of long noncoding RNA AL161431.1 in the development and progression of pancreatic cancer

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Research

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Abstract

Background: Pancreatic cancer is known for its notorious fast progression and poor prognosis. Various long noncoding RNAs (lncRNAs) have been shown to be involved in the pathogenesis processes of pancreatic cancer.

Methods: We first identified lncRNA AL161431.1 through bioinformatic analysis. Then, we explored the role of lncRNA AL161431.1 in the development and progression of pancreatic cancer by *in vitro* and *in vivo* experiments, including qRT-PCR, Western blot, immunofluorescence and immunohistochemistry assays, and flow cytometry, in BxPC-3 and SW1990 cells, as well as clinical samples.

Results: We found that lncRNA AL161431.1 was highly expressed in patients with pancreatic cancer. Knock down of lncRNA AL161431.1 led to increased cancer cell death and cell cycle arrest. Xenograft growth of SW1990 cells with stable knockdown of lncRNA AL161431.1 in mice was significantly slower than that of SW1990 cells with scrambled control shRNA. Finally, we showed the involvement of lncRNA AL161431.1 in pancreatic cancer was related to its promotion of the epithelial mesenchymal transition pathway.

Conclusions: LncRNA AL161431.1 is involved in the progression of pancreatic cancer through its promotion of the epithelial mesenchymal transition pathway.

Background

According to the American Cancer Society's estimates for pancreatic cancer in the United States for 2020, about 57,600 people were predicted to be diagnosed with pancreatic cancer, with 47,050 deaths due to pancreatic cancer. In the US, pancreatic cancer represents about 3% of cancers and 7% of cancer deaths [1]. Based on people diagnosed with pancreatic cancer between 2009 and 2015, the 5-year relative survival rate of all stage combined pancreatic cancer was only 9% [2]. Furthermore, pancreatic cancer often presents at late stage, and only 20% of patients showed surgically resectable lesions at diagnosis [3]. Therefore, understanding the mechanisms of tumorigenesis and development of pancreatic cancer is pivotal to its early diagnosis and improved prognosis.

Long noncoding RNAs (lncRNAs) are mRNA transcripts longer than 200 nucleotides but are not destined to be translated into protein [4]. Although originally determined to be non-functional for decades, more and more evidence has shown that lncRNAs are critical regulators of pathogenesis, progression, and metastasis of a broad scope of cancers [5]. Many of those lncRNAs are promising diagnostic and prognostic markers for pancreatic cancer [6].

In the present study, we explored the role of lncRNA AL161431.1 in the development and progression of pancreatic cancer by employing bioinformatic analysis and *in vitro* and *in vivo* experiments in two different cell lines of pancreatic origin as well as clinical samples. We showed that lncRNA AL161431.1 was highly expressed in pancreatic cancer tissues. Knocking down of lncRNA AL161431.1 led to increased cancer cell death and cell cycle arrest. The involvement of lncRNA AL161431.1 in pancreatic cancer was related to its promotion of the epithelial mesenchymal transition (EMT) pathway.

Methods

Bioinformatic Analysis

Transcriptome sequencing data of 181 samples were generated by R package in TCGA-PAAD dataset obtained from TCGAbiolinks (https://bioconductor.org/packages/release/data/experiment/vignettes/TCGAbiolinksGUI.data/inst/doc/vignettes.html). Differential mRNA abundance analyses were carried out using DESeq2 (http://www.r-project.org/). Genes with reads < 5

in any sample were filtered out from the final quantitative analysis. Heatmap and volcano plot were constructed by normalized gene expression via R package. The normalized gene expressions were subjected to Gene Set Variation Analysis (GSVA, a non-parametric, unsupervised method for estimating variation of gene set enrichment through the samples of an expression dataset). R package survival was used for overall survival analysis. Cox proportional hazard (PH) model was executed by the functions of survival and survminer in R package. The best-scanned cutoff points are defined as the one with the most significant (log-rank test) split. R package survival ROC was used for Receiver Operating Characteristic (ROC) curve and Area Under Curve (AUC) plotted for different durations of survival analysis.

Patients

Ethical approval was obtained from the Ethical Committee of the First Affiliated Hospital of China Medical University (a tertiary hospital and regional cancer center in Shenyang, China). Written informed consent was obtained from all patients at admission. The diagnosis of pancreatic cancer was based on the National Comprehensive Cancer Network Guidelines (NCCN 2018), and no patient received preoperative chemotherapy. Pancreatic cancer tissues and adjacent normal tissue from the same patients were obtained during pancreaticoduodenectomy at the First Affiliated Hospital of China Medical University from June 1, 2018 to May 31, 2019. Tissue samples were cryopreserved in liquid nitrogen immediately after surgical resection until further experiments.

Cell culture

All experiments were performed with mycoplasma-free cells. Human pancreatic adenocarcinoma cell lines PANC-1 (RRID:CVCL_0480), BxPC-3 (RRID:CVCL_0186), and SW1990 (RRID:CVCL_1723) were purchased from Shanghai Zhong Qiao Xin Zhou Biothechnology Co., Ltd (Shanghai, China). Cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco, Thermo Fisher Scientific, Inc.) supplemented with 10% fetal bovine serum (FBS, Gibco), 10 mM HEPES (Gibco), 2 mM L-glutamine (Gibco), 1 mM pyruvate sodium (Gibco), 100 U/ml penicillin and 100 μg/ml streptomycin (Gibco) at 37°C with 5% CO_2.

Stable lncRNA-knockdown cell line construction

A specific SW1990 cell line (SW1990-LNC-KD) with stable knockdown of lncRNA AL161431.1 or control SW1990 cell line was constructed using a lnc-shRNA sequence targeting 5'- GCAGTATTCCTGCACTTCT -3' or scramble control sequence 5'- TTCTCCGAACGTGTCACGT -3' cloned into the LV3(H1/GFP&Puro) vector (Figure S1), respectively, and packaged with lentivirus (Shanghai GenePharma, China). Cells containing the lnc-shRNA were selected by media containing 5 μg/ml puromycin (Sigma, St. Louis, MO).

Transfection of siRNAs

Short interfering RNAs (siRNAs) and scrambled negative control for lncRNA were provided by Shanghai GenePharma (Shanghai, China). The siRNAs (sequence listed in Table S1) were transfected with X-tremeGENE siRNA transfection reagent (Roche Applied Science, Shanghai, China) according to the manufacturer's manual.

Total mRNA extraction and qRT-PCR

Fresh cells or frozen tissues were homogenized in TRIzol reagent (Thermo Fisher Scientific, Inc.) for total mRNA extraction following the manual's instructions. The purified mRNAs were quantified by a NanoDrop 2000 Spectrophotometers (Thermo Fisher Scientific, Inc.), reverse transcribed using an RT reagent Kit (Nachuan Bio-Tech Co., Binzhou, China) based on the manufacturer's instructions. qRT-PCR assays were performed using SYBR Green Master Mix (Nachuan Bio-Tech Co.) in an Exicycle 96 Real-Time Quantitative Thermal Block (Bioneer) according to the manufacturer's protocol (sequence of primers listed in Table S1). The average of triplicate qRT-PCR results of target

lncRNA expression from each sample was normalized by β-actin of the same sample, and the relative expression was calculated using $2^{-\Delta\Delta Ct}$.

Cell count kit-8 assay for cell proliferation

Cell proliferation was detected using a CCK-8 assay kit (DOJINDO, Japan). Cells were seeded into 96-well plates (1×10^4 /well). At 10 am each day, 10 μl CCK-8 reagent in 100 μl medium was added to each well. The absorbance at 450 nm of each well was measured after 2 hour incubation with a microplate spectrophotometer.

Flow cytometry

Forty-eight hours after siRNA transfection, cell death/apoptosis or cell cycle were analyzed by flow cytometry (LSR, BD Biosciences) using Annexin V-FITC/PI Apoptosis Detection Kit (Beyotime Institute of Biotechnology, Shanghai, China) or Propidium Iodide (PI; Solarbio Biotech, China), respectively, according to the manufacturer's instructions. For cell death/apoptosis, cells were collected, washed three times with cold PBS, stained in 500 μl staining buffer (Annexin V-FITC/PI in PBS) at room temperature for 30 min in dark. For cell cycle analysis, cells were collected, washed three times with cold PBS, fixed in precooled anhydrous ethanol at 4°C for 30 min, stained with 50 ug/mL PI in 500 μl PBS. All experiments were triplicated.

Immunofluorescence staining

Cells were grown on sterile glass slides, fixed with 4% paraformaldehyde for 30 min, blocked with 1% BSA for 30 min, incubated with primary antibodies against CDH1 (E-cadherin, AF0131, 1:500; Affinity Biosciences LTD.), CDH2 (N-cadherin AF4039, 1:200; Affinity Biosciences LTD.), and VIM (Vimentin AF7013, 1:250; Affinity Biosciences LTD.) at 4°C overnight, followed by incubation with Alexa Fluor® 594 conjugated Affinipure Goat Anti-Rabbit IgG (H + L) secondary antibodies (Jackson ImmunoResearch Laboratories, PA) for 1 hour. Nuclei were counterstained with DAPI. Images were captured with Olympus IX81 inverted fluorescence microscope (Olympus, Beijing, China).

Western blotting

Anti-beta actin (AF7018, 1:3000), anti-E-cadherin (AF0131, 1:10000), anti-Vimentin (AF7013, 1:1000), anti-N-cadherin (AF4039, 1:1000) were obtained from Affinity Biosciences LTD., and the secondary antibody concentration was 1:5000 (S0001, Affinity Biosciences LTD.). Forty-eight hours after siRNA transfection, cells were lysed in RIPA lysis buffer (Merck Group, Germany) for 30 min on ice. Sample protein concentrations were quantified using a BCA assay kit (Solarbio, China). Equal amounts of proteins were separated by SDS-polyacrylamide gel electrophoresis, transferred to polyvinylidene difluoride membranes, and probed with the antibodies of interest. The intensity of bands was quantified using ImageJ, using β-actin as internal loading control.

Xenograft tumor growth in nude mice

Animals were properly treated in accordance with the institutional ethical requirements of experimental animals. Male nude mice were kept in a temperature-controlled specific-pathogen-free animal laboratory, with a 12h light/12h dark cycle. All animals had free access to food and water. SW1990-LNC-KD cells (1.5×10^6 cells in 0.1 ml sterile PBS, with stable knockdown of lncRNA AL161431.1), or SW1990-LNC-NC cells (with scrambled shRNA) were subcutaneously injected into the left flank of mice at 8 weeks of age. The volume of tumor was measured every morning by length x width x depth in mm. The mice were euthanized 2 weeks after injection, and the growth of subcutaneous tumors were compared (n = 7 in each group).

Immunohistochemistry

PCNA immunohistochemistry staining were performed on 4 mm sections of paraffin-embedded tissue samples. In brief, the slides were incubated in PCNA antibody (AF0239, Affinity Biosciences LTD.; at 1:100 dilution) at 4°C overnight followed by the secondary antibody (S0001, Affinity Biosciences LTD.; at 1:200 dilution) at 37°C for 1 hours. Nuclei were counterstained with hematoxylin. PCNA positive cells were quantified using IHC Profiler [7].

Transwell assay

Twenty-four hours after transfection, SW1990 ($1x10^5$) or BxPC-3 ($1x10^5$) cells were resuspended in 100 µl serum-free medium and seeded into the upper chamber with 12 µm pore polycarbonate membranes pre-coated with Matrigel Basement Membrane Matrix (BD Biosciences, Bedford, MA). The lower chamber was filled with 600 µl of 1640 medium supplemented with 20% FBS. After 24 hours of incubation at 37°C with 5%CO_2, cells remaining in the upper membrane surface were removed with a cotton swab, whereas invaded cells were fixed and stained with 0.5% crystal violet [8].

Wound-healing assay

Twenty-four hours after transfection, cells were seeded in 24-well plates at $1x10^5$ cells/well. Cells were cultured in medium containing 5% FBS with 5% CO_2 for 24 h. A 1-mm wide scratch was made in the confluent cultures with a pipette tip, followed by wash twice with PBS to remove debris. The area of the scratch was measured using images taken by a phase-contrast microscope [9].

Statistics

Statistical analysis was performed using SPSS software (version 24, Armonk, NY: IBM Corp.). Quantitative data are presented as mean ± SD. Differences in the mean of two samples were analyzed by Student's t-test. All 2-tailed statistical tests were considered significant when $p < 0.05$.

Data availability

Data will be available by contacting the corresponding author.

Results

LncRNA AL161431.1 in pancreatic adenocarcinoma

In order to identify lncRNAs that were most relevant to pancreas cancer, we obtained data on tumor (PAAD) transcriptome-level 3 (including 181 pancreas cancer cases) from the TCGA database [10]. Using DESeq2 software package [11], the expression of lncRNA AL161431.1 (also named RP11-54H7.4) in pancreatic cancer tissues was found to be significantly higher than that of normal pancreas tissues (Figure 1A-C.). Furthermore, using GEPIA online tool [12] and R language survival analysis, patients in the high lncRNA AL161431.1 expression group had a shorter survival period (Figure 1D, E). After grouping according to the expression of lncRNA AL161431.1 level, lncRNA AL161431.1 was shown to be positively correlated with the prognosis of pancreatic adenocarcinoma (Figure 1F). Pancreatic cancer tissues (n=26) and paired adjacent normal tissue from the same patients (n=26) were obtained from 14 male and 12 female patients aged between 43 and 63 (mean age 53.8 ± 6.0 years), analyzed by qRT-PCR). The level of lncRNA AL161431.1 was significantly higher in cancer tissues than that of the normal tissues ($p<0.05$, Figure 1H). The above results suggested that lncRNA AL161431. 1 was highly expressed in pancreatic cancer tissues, and as a carcinogenic factor, could indicate a poor prognosis.

LncRNA AL161431.1 in the growth, infiltration and migration of pancreatic adenocarcinoma

The expression of lncRNA AL161431.1 in pancreatic cancer cell lines was detect by qRT-PCR, and was found to be significantly higher in SW1990 and BxPC-3 cells (Figure 2A). In order to study the function of AL161431.1, we tested specific siRNAs to knock down the expression of lncRNA AL161431.1, and found both siRNA1 and siRNA2 had significant knockdown effect ($p<0.05$ for both, Figure 2B). siRNA1 was picked for the downstream knockdown experiments. In both SW1990 and BxPC-3 cell lines, the cell activity in the knockdown group was significantly lower than that in the control group (by CCK-8 assay, Figure 2C). Wound healing and Transwell experiments showed that after knocking down lncRNA AL161431.1, cell migration and infiltration were significantly inhibited (Figure 2D, E). Compared with the scrambled control group, siRNA knock down of AL161431.1 significantly promoted cell death (Figure 2F), and the knockdown group of cells accumulated in G1/S phase (Figure 2G). The above results suggested that knocking down LncRNA AL161431.1 can inhibit the growth, infiltration and migration of pancreatic adenocarcinoma cells, and promote apoptosis.

LncRNA AL161431.1 in nude mice xenografts

The above experiments have demonstrated that knockdown of lncRNA AL161431.1 can inhibit cell growth *in vitro*. In order to further study the role of AL161431.1 *in vivo*, we constructed lncRNA AL161431.1 stable knockdown cell line (SW1990-LNC-KD cells) using specific shRNA. The 8th passage was found to have minimum residual level of lncRNA AL161431.1 compared with the 3rd and 5th passages by qRT-PCR analysis (data not shown), and was injected into nude mice for xenograft tumor growth analysis. The volume of xenograft tumor in the stable knockdown group was significantly smaller at 2 weeks post injection (Figure 3A). The knockdown group also showed slower growth of xenograft (Figure 3B). The expression of lncRNA AL161431.1 in the tumor tissue was lower in the knockdown group (Figure 3C). HE staining and PCNA (cell proliferation index) immunohistochemistry experiments showed reduced cell atypia and fewer PCNA-positive cells in the knockdown group (Figure 3D-E). There was significant lower number of PCNA-positive cells in xenograft tumor of SW1990-LNC-KD cells ($p<0.01$; Figure 3F). The above results suggested that knocking down lncRNA AL161431.1 can inhibit the growth of xenografts.

Prediction of the function of lncRNA AL161431.1 in cells

The above i*n vivo* and *in vitro* experiments suggested that lncRNA AL161431.1 promoted cell growth. We reanalyzed the data of TCGA pancreatic cancer by dividing the samples into a high lncRNA AL161431.1 expression group and a low lncRNA AL161431.1 expression group using the median as the threshold. DEseq2 was used to analyze the expression of differential genes (Figure 4A-B). The GSEA software was used to analyze the enrichment degree of differentially expressed genes and the known gene set, and the AL161431.1 high expression group was found to be highly enriched in pancreatic cancer up-regulated gene set. Through GO enrichment analysis [13], AL161431.1 was found to play roles in various cell functions and processes such as cell junction, extracellular space, and extracellular region (Figure 4C-D), which was consistent with the above experimental results already obtained.

The mechanism of lncRNA AL161431.1 in regulating cell function

The above bioinformatics analysis indicated that lncRNA AL161431.1 mainly regulated cell migration and infiltration, and we further analyzed the role of lncRNA AL161431.1 in regulating cell junction, extracellular space, and extracellular region. We found that there was an interaction network centered by lncRNA AL161431.1 (Figure 5A-B). Moreover, through GSEA analysis, we found that the differential genes of the high and low lncRNA AL161431.1 expression groups were highly enriched in the WU_CELL_MIGRATION gene set (Figure 5C), which was highly correlated with the EMT pathway (Figure 5D-F) involved in cell migration and infiltration. The above bioinformatic analysis suggested that lncRNA AL161431.1 could potentially regulate the migration and infiltration of pancreatic cancer cells through the EMT pathway.

In order to further verify the above bioinformatic findings about the role of lncRNA AL161431.1 in cell migration and infiltration, we knocked down the expression of lncRNA AL161431.1 with siRNA1 in both SW1990 and BxPC-3 cells, which was confirmed by qRT-PCR (Figure 6A). Using qRT-PCR (Figure 6A), Western blot (Figure 6B) and immunofluorescence (Figure 6C) analyses, we found significant increase in the expression of CDH1 (E-cadherin), and decrease in the expression CDH2 (N-cadherin) and VIM (Vimentin) in both RNA level and protein level after knocking down of lncRNA AL161431.1. The above results suggested that lncRNA AL161431. 1 can promote cell migration and infiltration by promoting the EMT pathway.

Discussion

Various lncRNAs have been shown to be involved in the development and progression of pancreatic cancer. A search using key words of (lncRNA or (long noncoding RNA)) and (pancreatic cancer)" in PubMed on July 28, 2020 yielded 589 publications, with the most relevant one dating back in 2011 [14]. Through different pathways, most lncRNAs have been reported to promote the proliferation of pancreatic cancer [15, 16].

The employment of bioinformatics analyses were beneficial in identifying both candidate lncRNAs and lncRNAs-involving pathways for mechanistic and therapeutic studies. Based on the clues from the analysis of TCGA-PAAD dataset, we found an increase of lncRNA AL161431.1 in clinical pancreatic cancer tissues, as well as in SW1990 and BxPC-3 cells of pancreatic origin. To further test if those phenomena were coincident, siRNA or shRNA were used to knock down lncRNA AL161431.1 in those cells, with the finding of significant more cell death and cell cycle arrest, which showed lncRNA AL161431.1 is truly involved in pancreatic cancer growth and progression.

EMT is crucial in tumorigenesis by enhancing metastasis and tumor stemness. The signature of EMT is the upregulation of N-cadherin accompanied by the downregulation of E-cadherin, the process of which is regulated by a complicated network of pathways and transcription factors, including the TGF- β Pathway, the MAPK Pathway, the JAK/STAT Pathway, the Hedgehog Pathway, the Wnt Pathway, the Hippo-YAP/TAZ Pathway, etc. [17]. E-cadherin plays an important role in the initiation and maintenance of EMT, where cleavage of E-cadherin leads to the destabilization of cell junctions as well as the release of β-catenin as a transcriptional activator for cell proliferation [18]. In lung cancer cells, there was increased level of vimentin and decreased level of E-cadherin [19]. Vimentin has been reported as the main intermediate filament protein of normal mesenchymal tissue [20], with a major role of sustaining cellular integrity [21]. Furthermore, vimentin expression was a potential independent adverse prognostic molecular marker in patients with pancreatic ductal adenocarcinoma [22]. Our finding of increased E-cadherin accompanied by decreased N-cadherin and vimentin in lncRNA AL161431.1 knockdown pancreatic cell lines clearly indicated that lncRNA AL161431.1 is critical in the EMT pathway.

A recent study has shown lncRNA AL161431.1 can target and bind to miR-1252-5p, and results in the de-repression of MAPK signaling in endometrial carcinoma cells [23]. Another study found lncRNA AL161431.1 is a hypoxia-associated lncRNA in hypoxic TCGA BRCA tumors [24]. lncRNA AL161431.1 has also been shown to have a significant prognostic value in lung squamous cell carcinoma [25].

In summary, we showed by bioinformatics, *in vivo* and *in vitro* experiments that lncRNA AL161431.1 was involved in the pathogenesis of pancreatic cancer by promotion of the EMT pathway. LncRNA AL161431.1 might also be a potential prognostic predictor and treatment target in pancreatic cancer.

Declarations

Ethics approval and consent to participate:

Ethical approval for enrolled patients was obtained from the Ethical Committee of the First Affiliated Hospital of China Medical University (No. [2015]100). Written informed consent was obtained from all patients at admission. Animals were properly treated in accordance with the institutional ethical requirements of experimental animals

Consent for publication:

Not applicable

Availability of data and material:

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests:

None

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Authors' contributions:

GM and ZL designed the study, analyzed and interpreted patient data, and were major contributors in writing the manuscript. GL, WF performed the cell culture, qRT-PCR and Western blot experiments, YX and SS performed flow cytometry, IF, and IHC experiments, KG performed xenografts experiments. All authors read, revised, and approved the final manuscript

Acknowledgements:

Not applicable.

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Figures

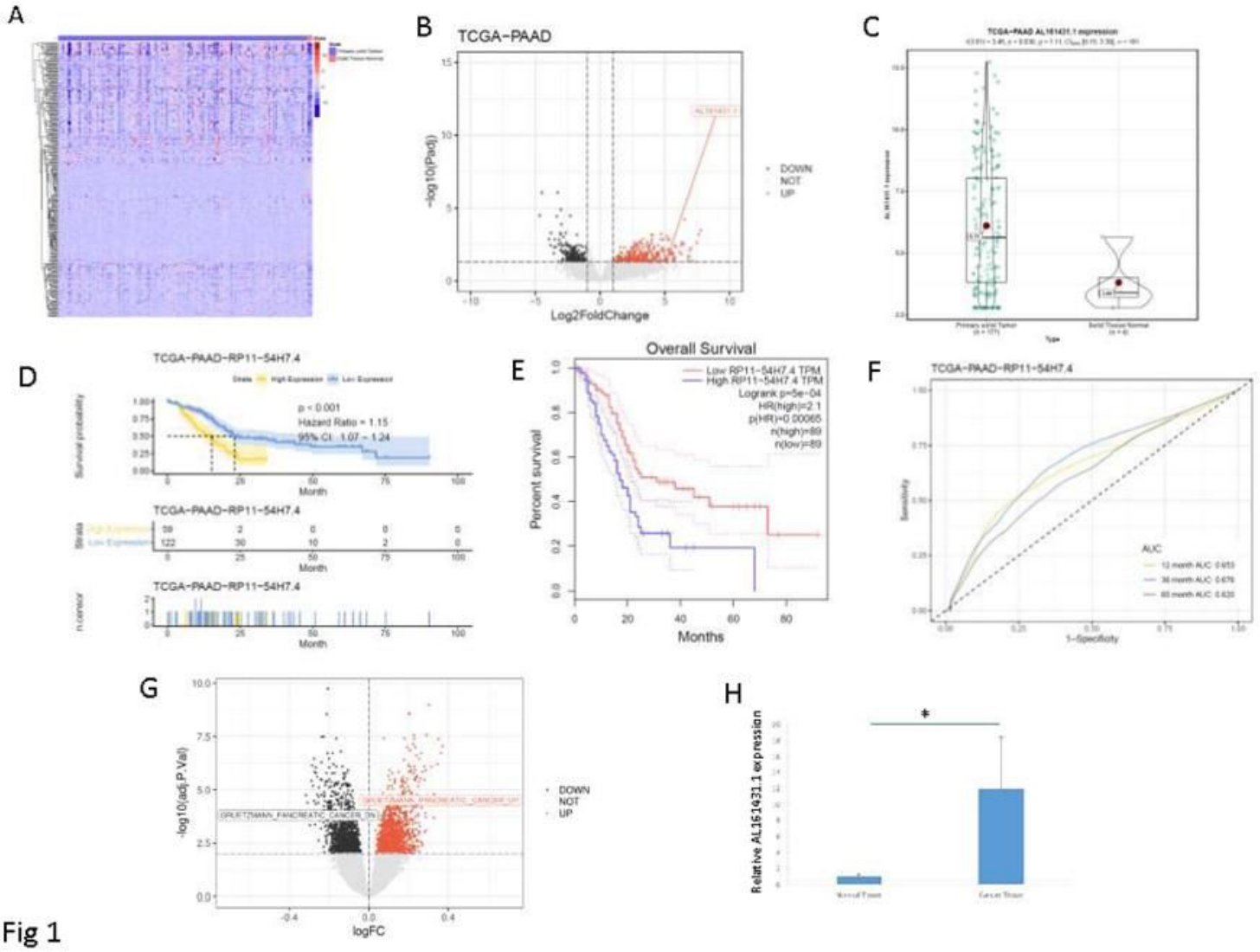

Fig 1

Figure 1

LncRNA AL161431.1 was highly expressed in pancreatic adenocarcinoma. (A) Heatmap of TCGA-PAAD transcriptome; (B) Volcano plot of different gene expressions in TCGA-PAAD; (C) LncRNA AL161431.1 expression in TCGA-PAAD cohort; (D,E,F) Kaplan-Meier curves of the overall survival (D,E) and ROC curves (F) of patients in the TCGA cohort; (G) GSVA analysis of lncRNA AL161431.1 effect on Pancreatic adenomas in TCGA cohort; (H) Relative AL161431.1 expression in pancreatic cancer and normal tissues from clinical samples (qRT-PCR). * p<0.05, **p<0.01, ***p<0.001.

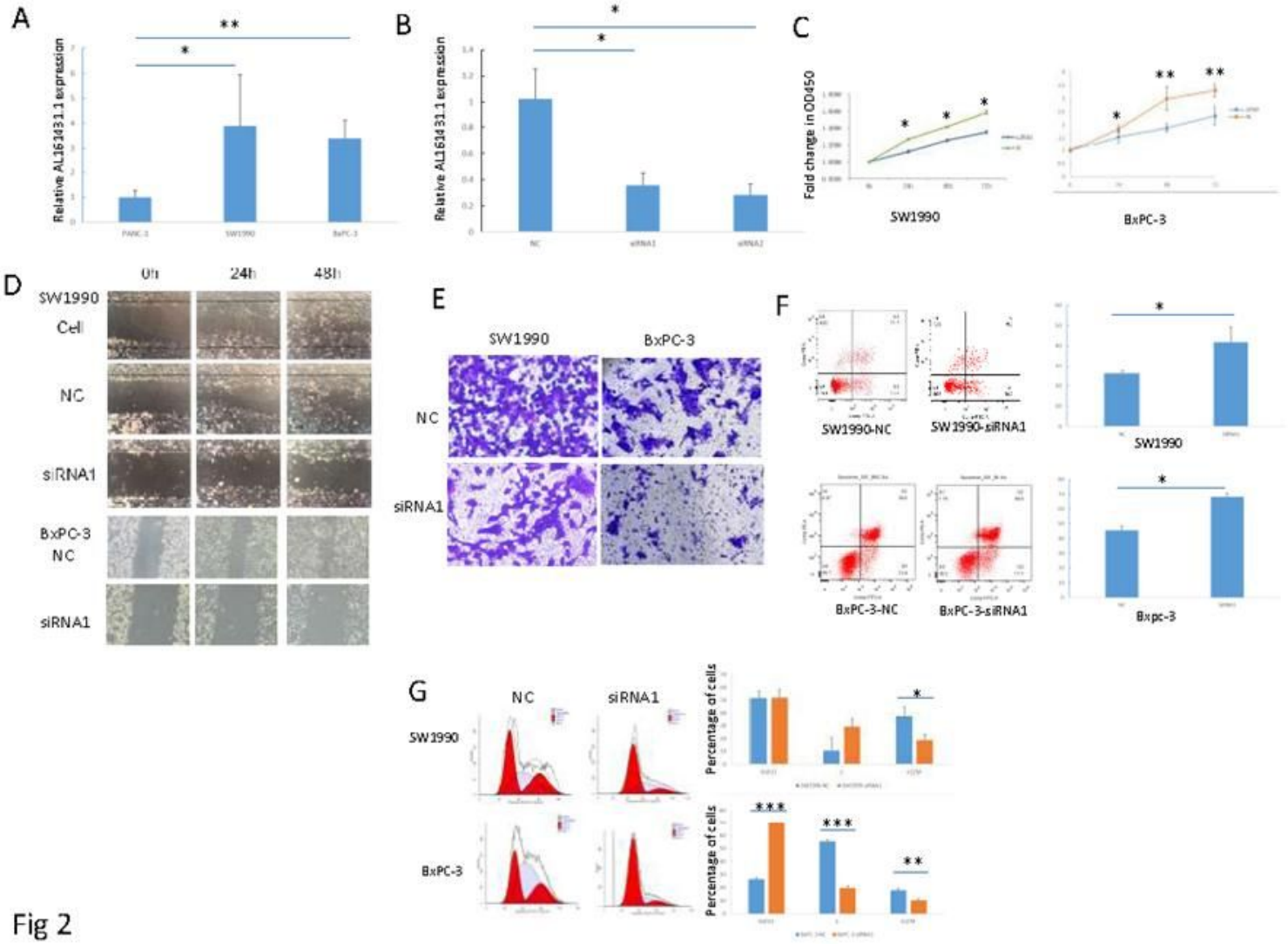

Figure 2

Knockdown of lncRNA AL161431.1 inhibited the growth, infiltration and migration of pancreatic adenocarcinoma cells and promote apoptosis. (A) Relative AL161431.1 expression in PANC-1, SW1990, and BxPC-3 cells analyzed by qRT-PCR; (B) Relative AL161431.1 expression in SW1990 cells after siRNA transfection analyzed by qRT-PCR; (C) CCK-8 assay showing fold change at OD450, representing growth curve of SW1990 and BxPC-3 cells at 24, 48, and 72 hours after siRNA1 transfection; (D) Wound healing assay in naïve, scramble siRNA, or siRNA1 transfected SW1990 or BxPC-3 cells at 0, 24, and 48 hours post scratching (field of view: 100x); (E) Transwell assay in scrambled control siRNA or siRNA1 transfected SW-1990 or BxPC-3 cells (field of view: 100x); (F) Cell death (Q2+Q3) in scrambled control siRNA or siRNA1 transfected SW1990 and BxPC-3 cells analyzed by flow cytometry; (G) Cell cycle analysis of SW1990 and BxPC-3 cells after transfection of scrambled control siRNA or siRNA1 by flow cytometery. NC: scrambled control siRNA or shRNA.

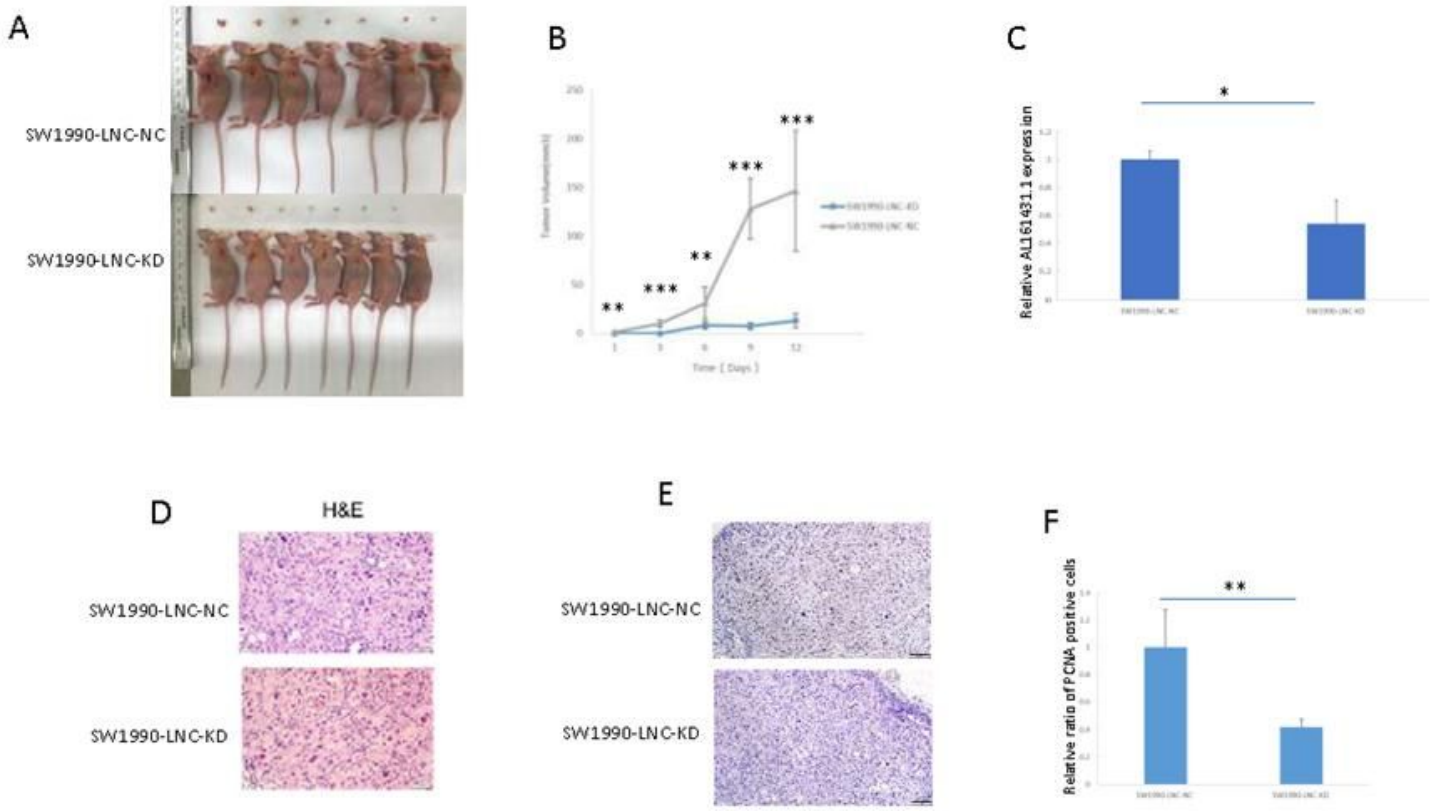

Fig 3

Figure 3

Knockdown of lncRNA AL161431.1 in nude mice xenografts inhibited the growth of xenografts. (A) A. Size of SW1990 cell xenografts with SW1990-LNC-KD or SW1990-LNC-NC cells at 14 days after injection; (B) Time course of in vivo xenografts growth measured in mm3; (C) Relative AL161431.1 expression in engrafted tumors with SW1990-LNC-NC cell or SW1990-LNC-KD cell; (D) HE staining of xenograft slides; (E) PCNA immunohistochemistry xenograft slides; (F) Relative ratio of PCNA positive cells in the engrafted tumors with SW1990-LNC-NC cell or SW1990-LNC-KD cell.

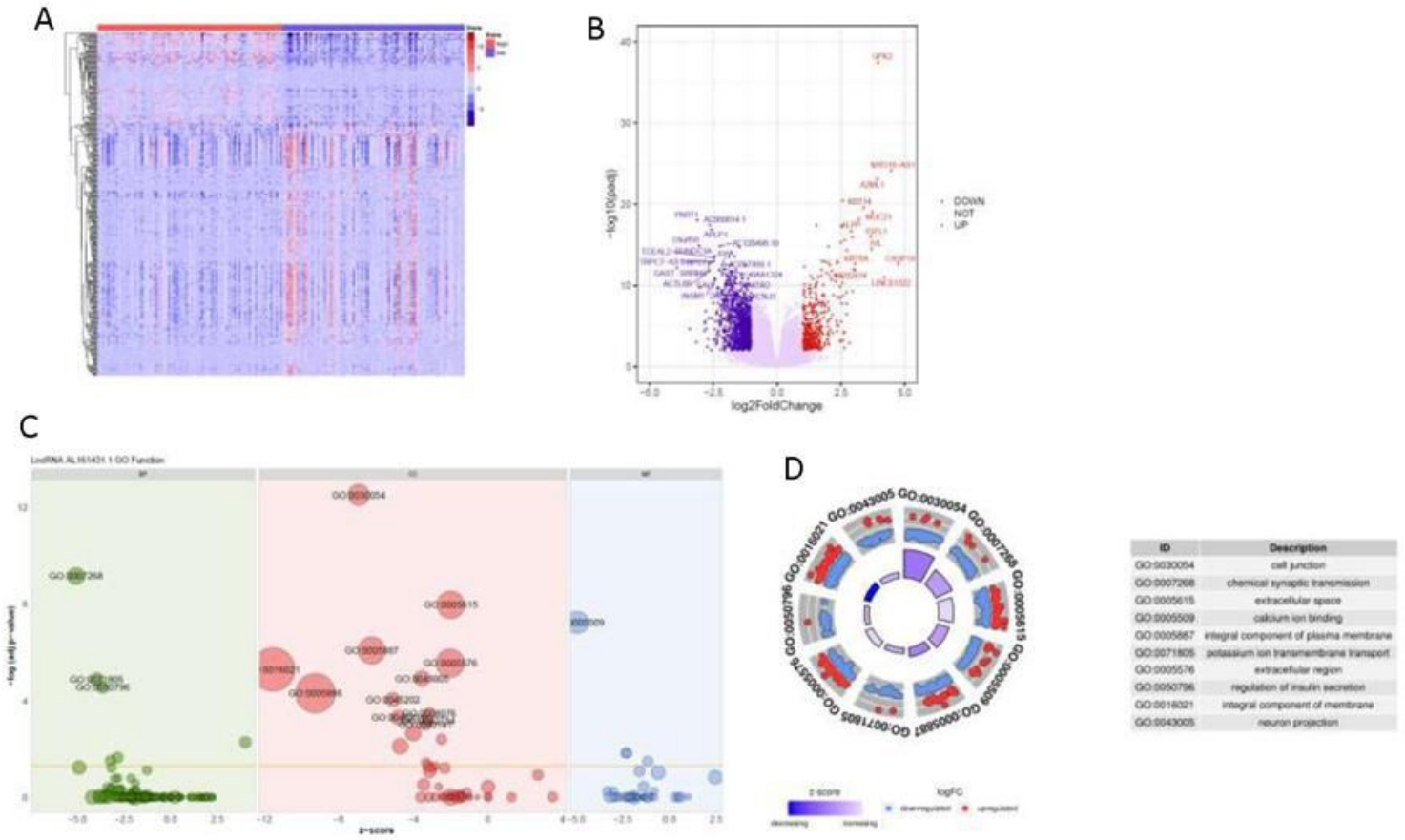

Fig 4

Figure 4

Prediction of the function of lncRNA AL161431.1 in cells. Heatmap (A) and Volcano plot (B) of different gene expressions between with higher AL161431.1 expression and lower AL161431.1 expression patients in TCGA-PAAD; (C, D) GO function analysis of AL161431.1.

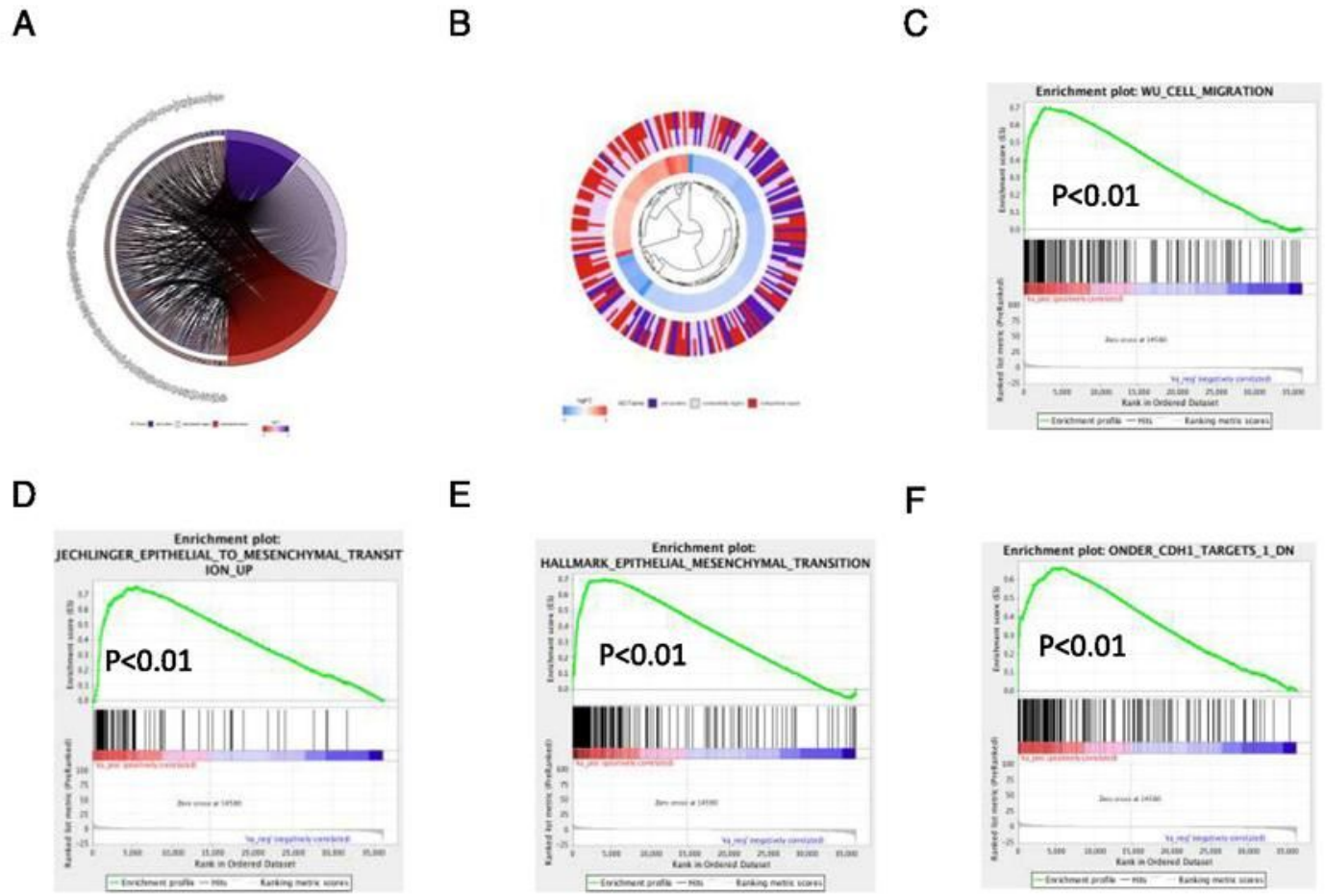

Fig 5

Figure 5

The mechanism of lncRNA AL161431.1 in regulating cell function. GO function relative genes (A) and fold changes (B); (C-F) GSEA analysis for different gene expressions between patients with higher AL161431.1 expression and lower AL161431.1 expression in TCGA-PAAD.

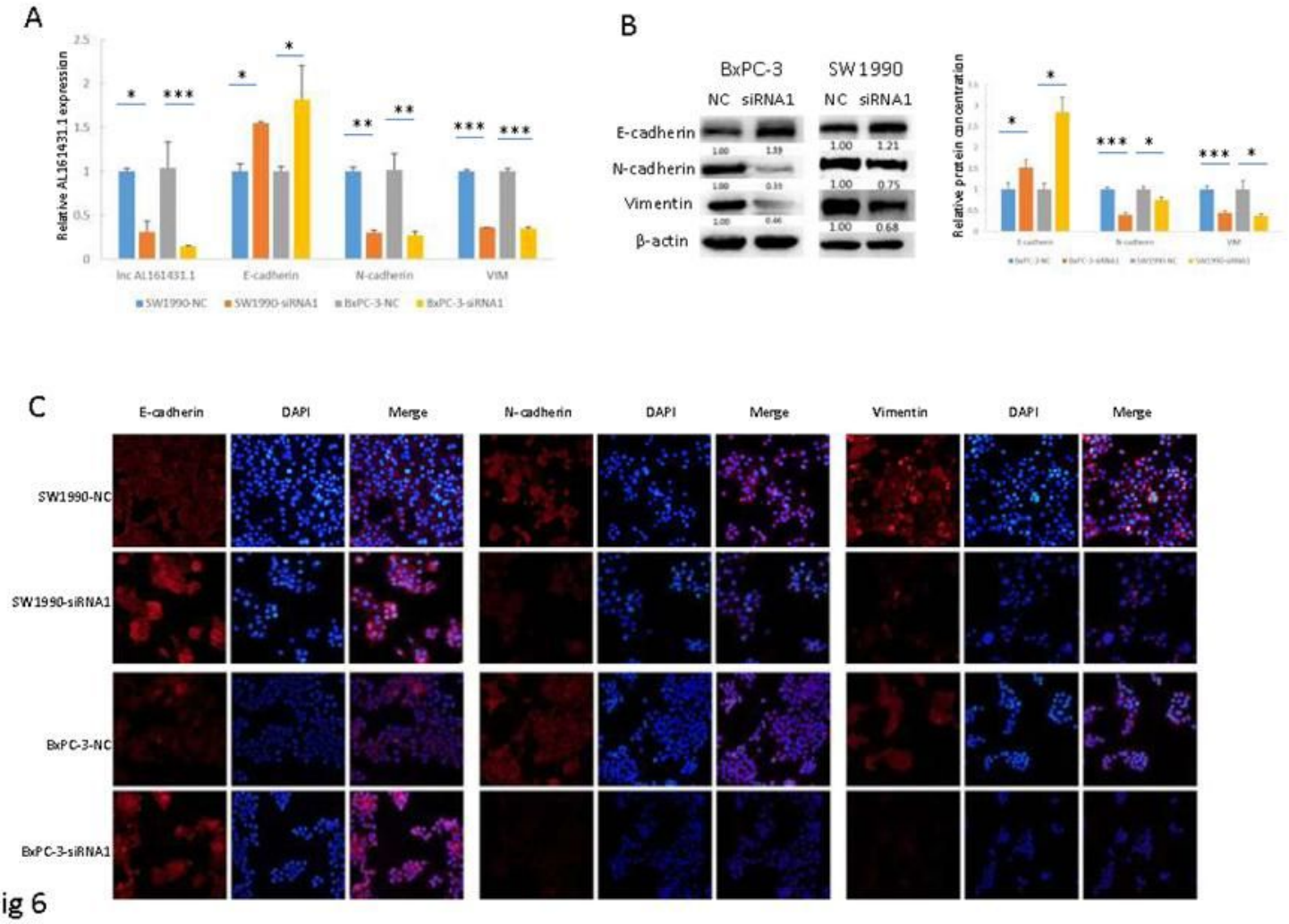

Figure 6

Knockdown of lncRNA AL161431.1 inhibited EMT pathway. (A) qRT-PCR results showing the knock down of lncRNA AL161431.1, and the corresponding changes in E-cadherin, N-cadherin, and vimentin in SW1990 and BxPC-3 cells; (B) Western blots showing the changes in protein level in E-cadherin, N-cadherin, and vimentin in SW1990 and BxPC-3 cells after transfection of scrambled siRNA or siRNA1; (C) Immunofluorescence analysis showing the changes in protein level in E-cadherin, N-cadherin, and vimentin in SW1990 and BxPC-3 cells after transfection of scrambled siRNA or siRNA1 (field of view: 200x).