MALAT1 Promotes Epithelial-Mesenchymal Transition of Pancreatic Cancer Cells Through miR-141-5p/ TGFBR1 / TGFBR2 Axis

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Research

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Abstract

Background: Pancreatic cancer is one of the leading causes of cancer deaths, with high chance of metastasis associated with high mortality rates. Epithelial-to-mesenchymal transition (EMT) is one of the crucial steps in the initiation of metastasis, when cells begin to lose adhesion and gain invasive properties. Transforming growth factor beta (TGF- β) is known to promote EMT via binding to its receptors, TGFBR1 and TGFBR2, which are overexpressed in pancreatic cancer cells.

Methods: The expression of MALAT1 was detected in pancreatic cancer tissues. The dual-luciferase assay was performed to validate the binding between MALAT1 and miR-141-5p. The western blot was performed to detect the expression of both TGFBR1 and TGFBR2.

Results: The long noncoding RNA (lncRNA) MALAT1 is highly expressed in cancer cells and positively correlated with tumor growth and metastasis. Our study revealed that MALAT1 suppresses the expression of miR-141-5p by acting as a “miRNA sponge”. We validated the sequence-specific binding between MALAT1 and miR-141-5p by dual-luciferase experiment, and found that their expressions are negatively correlated in pancreatic cancer cells. Furthermore, miR-141-5p downregulates TGFBR1 and TGFBR2, and reduces cell migration and invasion that are caused by TGF- β -induced EMT. However, the overexpression of MALAT1 in pancreatic cancer cells suppresses the expression of miR-141-5p. Underexpression of miR-141-5p promotes the expression of TGFBR1 and TGFBR2, inducing EMT through the TGF- β pathway.

Conclusions: The overexpression of MALAT1 is a key component in initiating metastasis in pancreatic cancer, by inhibiting the effect of miR-141-5p and promoting TGF- β -induced EMT.

Background

Pancreatic cancer is one of the most lethal cancers across the world. Exocrine pancreatic cancer, also known as pancreatic adenocarcinoma, is a type of pancreatic cancer which originates from the pancreatic ductal epithelium within the pancreas for excreting secretions, and accounts for the majority of the cases [1–4]. Diagnosis of pancreatic cancer in the early stage is often difficult, due to the lack of obvious symptoms as well as its high tendency of metastasis. Neither chemotherapy nor radiotherapy provides significant effects on patients with pancreatic cancer. Moreover, curable treatments were no longer feasible at the time of diagnosis in most cases. Furthermore, prognosis of the disease is poor, with a five-year survival rate of only 5 percent and the mortality rate is almost the same as the incident rate [5–8]. In fact, there has been no significant improvement in either the incident rate or mortality rate for pancreatic cancer at the global level over the past decades. Therefore, it is important to further investigate the underlying mechanism of metastasis in pancreatic cancer, in order to discover effective treatments and prognosis for the disease.

TGF- β is a group of multifunctional polypeptides with four different isoforms (β 1, β 2, β 3, and β 4, with β 1 being the most abundant), and is known to regulate cell differentiation [9–11]. In normal epithelium cells and early-stage human carcinoma cells, TGF- β demonstrates tumor-suppression effects; while in later

stage of tumor development, it promotes cell proliferation and metastasis. EMT is the transition when epithelial cell loses cell-to-cell adhesion and starts to become mesenchymal stem cells, and is a crucial step for cell invasion during tumorigenesis. This process is known to be initiated by TGF- β by binding to the transmembrane protein TGF- β receptor (TGFR) [12–14]. Upon binding of TGF- β , TGFR1 activates TGFR2, which triggers autophosphorylation of Smad2 and Smad3 (R-Smads) [15, 16]. The phosphorylated R-Smads further form a complex with Smad4 and translocate into the nucleus. This complex then promotes the activation of transcription factors that induce EMT, such as Snail1, Snail2, and Snail3, as well as Smad-related proteins ZEB2, Twist1, and Twist [13]. TGFR1 and TGFR2 are found to be overexpressed in pancreatic cancer cells, and may be associated with disease progression [17].

LncRNAs are RNA molecules longer than 200 nucleotides that do not code for any proteins, and are known to play important roles in regulating gene expression. One of the common functions of lncRNA is to attenuate the expression of miRNAs by acting as an endogenous sponge, which binds with miRNAs and interferes with their interaction with their target mRNAs [18]. Metastasis-associated lung adenocarcinoma transcript 1 (MALAT1), also known as noncoding nuclear-enriched abundant transcript 2 (NEAT2), is one of the most well-known lncRNA members [19]. MALAT1 is found to be highly expressed in multiple types of tumor tissues [19–21]. In addition, MALAT1 is also highly associated with the growth of pancreatic cancer cells [22–24]. Studies have indicated overexpression of MALAT1 in pancreatic cancer cells, whereas the expression level is positively correlated with the stage of tumor growth and metastasis.

The goal of this study is to understand the effect of MALAT1 on EMT in human pancreatic cancer cell line PANC-1. We aimed to investigate the “miRNA-sponging” effect of MALAT1 on TGF- β -induced EMT, and uncover the association between MALAT1 and TGF- β receptors. The purpose of this research is to discover unknown mechanisms in metastasis of pancreatic cancer, by understanding the regulatory role of lncRNA in gene expression, in order to provide new insights for therapeutic drug discovery for the disease.

Methods

Tumor tissues

Pancreatic cancer tissues were obtained from patients from West China Hospital between 2015 and 2017. Patients diagnosed with pancreatic cancer were selected from all age groups and genders, excluding patients that were diagnosed with other malignant tumors or benign tumors in the pancreas. Diagnoses were performed following the conventional diagnostic criteria of the World Health Organization (WHO). All tissue samples were obtained from untreated patients during surgery, froze immediately with liquid nitrogen, and stored at – 80 °C before use for the experiment. This study was approved by the ethical committee of West China Hospital after receiving consents from all participated patients.

Cell lines

Human embryonic kidney cells 293 (HEK293) and PANC-1 were purchased from Shanghai Institutes for Biological Science (Shanghai, China). Both HEK293 and PANC-1 cell lines were cultured in Dulbecco's Modified Eagle's Medium (DMEM) with 10% fetal bovine serum (FBS) (Gibco, Thermo Fisher Scientific Inc., Waltham, MA, USA). Both cell lines were incubated in 5% CO₂ and at 37 °C with saturated moisture.

RNA extraction

Cryogenic grinding was performed on tissue samples, followed by total RNA extraction by adding 1 ml of Trizol (Invitrogen, Carlsbad, CA, USA) according to manufacturer instructions. RNA purity of the extracts were examined by measuring their optical density (OD) value. Sample extracts with OD_{260/280} ratio between 1.8 and 2.0 were selected for use in experiments.

Quantitative real-time PCR (qRT-PCR)

Reverse transcription was carried out using High Capacity RNA-to-cDNA kit (Takara Bio Inc. Kusatsu, Japan), with no more than 500 ng RNA per 10 µL PCR system as the template. For the measurement of the expression of MALAT1 and miR-141-5p, real-time PCR was performed according to the standard SYBR Green protocol. cDNA was transcribed from total RNA using 9 random primers. Primers for miR-141-5p were purchased from Sigma (MIRAP00173-250RXN) (Sigma-Aldrich, St. Louis, MO, USA), and primers for others are as follows:

MALAT1 forward: 5'-AAAGCAAGGTCTCCCCACAAG-3'

MALAT1 reverse: 5'-GGTCT GTGCAGATCAAAGGCA-3'

U6 forward: 5'-CTCGCTTCGGCAGCACA-3'

U6 reverse 5'-AACGCTTCACGAATTTGCGT-3'

GAPDH forward: 5'-TTGGCCAGGGGTGCTAAG-3'

GAPDH reverse: 5'-AGCCAAAAGGGTCATCATCTC-3'

qPCR was performed at 95 °C for 10 seconds, then 60°C for 60 seconds, for 40 cycles, in 10 µl of reaction mix containing 3 µl of the cDNA, 2 µl of primers and 5 µl of SYBR Green master mix (Applied Biosystems, Foster City, CA, USA) using an ABI 7900 thermocycler. The expression of miR-141-5p was normalized to that of U6. Expression of MATA11 was normalized to that of GAPDH. Quantification of each gene was performed using the $\Delta\Delta C_t$ method.

RNA oligoribonucleotides and cell transfection

The MALAT1 nucleotide was designed and subcloned into the pcDNA3.1 vector (pcDNA3.1- MALAT-1) to overexpress MALAT1. The small interfering RNA (siRNA) targeting human MALAT1 (si-MALAT1), the

negative control (siControl), miR-141-5p, and the miRNA negative control (miRNA-NC), were purchased from RiboBio (Guangzhou, China). The negative control for the miRNA mimic or siRNA were not homologous to any human genome sequences. Transfection of the RNA oligoribonucleotides (100 nmol/L) and plasmids was performed using Lipofectamine 2000 (Life Technologies, Carlsbad, CA, USA), following manufacturer instructions. Cells were evaluated 48 hours after transfection under an inverted fluorescence microscope to assess transfection efficiency. Cells were then harvested for the subsequent experiments.

Dual-luciferase reporter assay

The psiCHECK2 dual luciferase vector (Promega, Madison, WI, USA) was used to generate a series of constructs. A fragment of MALAT1 consisting of the predicted target sites for miR-141-5p (wt) or a mutant variants (mut) were directly synthesized (Shenggong, Shanghai, China) and then subcloned into the psiCHECK-2 vector to create the constructs. Each construct was then co-transfected with the miR-141-5p mimic or the negative control into HEK293 cells. In a separate experiment, the wild-type and mutant MALAT1 construct was each co-transfected with a miR-141-5p inhibitor or the negative control into HEK293 cells for comparison. To analyze the binding of miR-141-5p on TGFBR1 and TGFBR2, the 3'-UTR of TGFBR1 and TGFBR2 containing the putative binding sites for miR-141-5p (wt) or the mutant variants (mut) were each synthesized and subcloned into the psiCHECK-2 vector. Each construct was co-transfected with either the miR-141-5p mimic or the miRNA NC into HEK293 cells. All transient transfections were carried out using Lipofectamine 2000 (Life Technologies, Carlsbad, CA, USA) following manufacturer instructions. After 48 hours, cells were harvested and luciferase activities were determined using the Dual-Luciferase Reporter Assay Kit (Promega, Madison, WI, USA). Each experiment was done in triplicates. Results are presented as the relative Renilla luciferase activities, which were normalized to Firefly luciferase activities.

Western blot

Cell samples were lysed, and total proteins were extracted. Total protein concentration was determined using BCA Protein Assay kit (Beyotime, Shanghai, China), following manufacturer instructions. 30 µl of each protein sample was loaded for separation via SDS-PAGE. The gel was then transferred onto a PVDF transfer membrane. After that, the membrane was blocked with 5% fat-free dry milk for 2 hours, followed by overnight incubation at 4 °C with the primary antibody anti-TGFBR1, TGFBR2, N-cadherin, vimentin, E-Cadherin, and GAPDH (Cell Signaling Technology, Danvers, MA, USA). The membrane was then washed, followed by one hour of incubation with the secondary antibody, HRP-conjugated goat anti-rabbit IgG (Santa Cruz Biotechnology, Dallas, TX, USA). The gel was then washed and evaluated by enhanced chemiluminescence (ECL) using test kits (KeyGEN BioTech, Jiangsu, China).

Transwell migration and invasion assay

Cell samples were suspended in FBS-free DMEM and adjusted density to 5×10^5 cells/ ml. For testing the effect of miR-141-5p on TGF- β -mediated EMT, PANC-1 cells were transfected with miR-141-5p mimic, miRNA NC, or a mock vector, followed by a treatment with 5 ng/mL TGF- β or blank solvent for 24 hours.

To analyze the effect of MALAT1 together with the effect of miR-141-5p, PANC-1 cells were co-transfected with either pcDNA3.1-MALAT1 or a mock vector, each with or without miR-141-5p mimic, followed by a treatment with 5 ng/ml TGF- β or blank solvent for 24 hours. Cell migration test was performed by adding 200 μ l of the suspended cells to the upper chamber and adding 500 μ l of DMEM with 10% FBS to the lower chamber. For cell invasion assay, Matrigel was first thawed overnight at 2–8 °C and diluted in 1:4 ratio with pre-chilled FBS-free DMEM. Prior to adding the suspended cells, 50 μ l of the diluted Matrigel was first added from the upper chamber to cover the membrane. Next, the chamber was incubated overnight at 37 °C, followed by addition of 500 μ l DMEM with 10% FBS to the lower chamber. For both migration assay and invasion assay, the suspended cells were incubated for 48 hours. Medium from the upper chamber was discarded. A swab was used to remove excess cells on the upper surface of the membrane. 4% paraformaldehyde was then added to the chamber to fix for 10 min, followed by washing three times with phosphate buffered saline (PBS). Then, the membrane was stained for 20 min using crystal violet staining solution and then washed. For each membrane, 5 randomized views under the inverted microscope were used for cell counting to evaluate the migration and invasion.

Statistical analysis

All statistical data in this study are presented as mean $\pm$ standard deviation, using SPSS 19.0 statistics software (SPSS Inc., Chicago, IL, UA). T-test was used for comparisons between two groups. Two Way ANOVA was used for comparisons between multiple groups. All pairwise multiple comparison procedures performed using Holm-Sidak method. $p < 0.05$ was considered statistically significant, whereas $p < 0.01$ indicated exceptional significance.

Results

Expression of MALAT1 and miR-141-5p are negatively correlated in pancreatic cancer tissues

MALAT1 is found to be overexpressed in various types of tumors, and is associated with the expression of miRNAs. MiR-141 is a member of the miR-200 family, and is underexpressed in pancreatic cancer [25]. We identified a putative binding site between MALAT1 and miR-141-5p using Blast sequence analysis (Fig. 1A), and it was worthwhile to further investigate whether there is association between the two. We first measured the expression of MALAT1 and miR-141-5p in both pancreatic cancer (PC) tissues and pericarcinomatous tissues, and analyzed the relationship between them. Results showed that expression of miR-141-5p was lower in PC tissue (Fig. 1B), while expression of MALAT1 was higher in PC tissue (Fig. 1C), compared to those expressed in pericarcinomatous tissue ($p < 0.05$). The different patterns in the expression of miR-141-5p and MALAT1 indicated negative correlation between the two in tumor tissues (Fig. 1D) ($p < 0.001$).

MiR-141-5p directly binds to MALAT1 in pancreatic cancer cells

To find out whether there is direct biological activity between MALAT1 and miR-141-5p, we performed the dual-luciferase assay to validate their binding within HEK293 cells. MiR-141-5p significantly reduced luciferase activity when co-transfected with the wild type MALAT1 ($p < 0.05$) (Fig. 2B). In addition, co-transfecting the wild type MALAT1 with miR-141-5p inhibitor into HEK293 cells showed increased luciferase activity compared to transfection with the miRNA negative control ($p < 0.05$). These results indicate direct binding of miR-141-5p on MALAT1. For further confirmation of the relationship between miR-11-5p and MALAT1, we transfected miR-141-5p mimic to PANC-1 cells and monitored the change in expression of MALAT1. Results indicated that transfection with miR-141-5p mimic increased the expression of miR-141-5p (Fig. 2C) and suppressed the expression of MALAT1 ($p < 0.05$) (Fig. 2D). On the other hand, knockdown of MALAT1 in PANC-1 cells using siRNA (Fig. 2E) significantly increased the expression of miR-141-5p ($p < 0.05$) (Fig. 2F). The above results suggested that the lncRNA MALAT1 acts as a competing endogenous RNA (ceRNA) in pancreatic cancer cells, and is a direct target of miR-141-5p.

miR-141-5p directly binds to the 3'-UTR of TGFBR1 and TGFBR2 and suppresses their expressions

TargetScan analysis revealed possible binding sites of miR-141-5p in the 3'-UTR of the TGF- β receptors TGFBR1 and TGFBR2 (Fig. 3A). Dual-luciferase experiment further confirmed binding between miR-141-5p and TGFBR1 or between miR-141-5p and TGFBR2. Results showed that miR-141-5p mimic significantly suppressed luciferase activity with the wild type TGFBR1 or TGFBR2 in HEK293 cells ($p < 0.05$) (Fig. 3B-3C). Also, western blot result revealed that miR-141-5p mimics significantly reduced the expression of both TGFBR1 and TGFBR2 (Fig. 3D). These results suggested that miR-141-5p suppressed the expression of TGFBR1 and TGFBR2 via binding to their 3'UTR.

Overexpression of miR-141-5p inhibits TGF- β -induced EMT

Both TGFBR1 and TGFBR2 are important mediators for TGF- β , in order to induce EMT and other biological functions. Results from our experiments have confirmed that the expression of TGFBR1 and TGFBR2 are suppressed by miR-141-5p. We further investigated whether miR-141-5p is associated with cell migration and invasion during TGF- β -induced EMT. We first treated TGF- β 1 on cell samples transfected with either miR-141-5p mimic or miR-NC. Then, expressions of a series of EMT-related markers, including mesenchymal markers N-cadherin and vimentin, and epithelial marker E-cadherin, were analyzed and detected for changes. When transfected with miR-NC, TGF- β 1 significantly downregulated E-cadherin and upregulated N-cadherin and vimentin; contrary, these effects were inhibited by transfecting with miR-141-5p mimic ($p < 0.05$) (Fig. 4A). Furthermore, we analyzed the effect of miR-141-5p on cell migration and invasion induced by TGF- β 1. In cells transfected with miR-NC, we found that TGF- β 1 significantly promoted cell migration and invasion, while overexpression of miR-141-5p significantly reduced these effect ($p < 0.05$) (Fig. 4B-4C).

Overexpression of MALAT1 reduces inhibitory effect of miR-141-5p on TGF- β -induced EMT, cell migration, and cell

invasion

Our experiment has confirmed that overexpression of miR-141-5p inhibits TGF- β -induced EMT, cell migration, and cell invasion. Since MALAT1 acts as a molecular sponge for miR-141-5p, it was worthwhile to investigate whether MALAT1 further inhibits this effect. We co-transfected either the pcDNA3.1-MALAT1 or a mock vector with miR-141-5p mimics on PANC-1 cells, then treated cells with TGF- β 1 or blank solvent. Western blot results revealed that, compared to the mock vector, overexpression of MALAT1 significantly upregulated N-cadherin and vimentin, while E-cadherin was downregulated ($p < 0.05$) (Fig. 5A). These changes inferred that MALAT1 suppresses the inhibitory effect of miR-141-5p on TGF- β -induced EMT. Experiments on cell migration and invasion both also showed that miR-141-5p reduced cell migration and invasion caused by TGF- β , while MALAT1 reduced these anti-tumorigenic effects ($p < 0.05$) (Fig. 5B-5C). Besides, there are significant difference for the effect of 141-5p between Vehicle and TGF- β 1 treated groups ($p < 0.05$).

Discussion

The role of lncRNA in gene regulation has been a popular topic in disease-related research, especially in oncology. Numerous researches have found that lncRNAs have important roles and are crucial in the development of pancreatic cancer [22–24]. There are studies indicating that overexpression of MALAT1 in pancreatic cancer is associated with its poor prognosis. It was found that MALAT1 binds with the RNA-binding protein HuR, and promotes autophagy via the HuR-TIA-1 pathway [22]. Moreover, MALAT1 is known to regulate the expression of Kras via competitive binding with miR-217, which promotes tumorigenesis. The present study focused on analyzing the sequence-specific binding between MALAT1 and miR-141-5p, based on the hypothesis that MALAT1 plays regulatory role in miRNA expressions via endogenous sponging. We initially revealed the correlation between MALAT1 and miR-141-5p in clinical tissue samples (Fig. 1B-1D), followed by further confirmation for binding between the two in HEK293 cells (Fig. 2B), and finally validated their negative correlation in expressions within PANC-1 cells (Fig. 2C-2D).

EMT is one of the crucial steps in metastasis of tumor cells. In order to initiate EMT, TGF- β must induces downstream signaling through the tetramer unit formed by its two receptor proteins TGFBR1 and TGFBR2, and activates the Smad signaling pathway [15, 16]. Through our experiments, we showed that miR-141-5p is able to target the TGFBR1 and TGFBR2 gene, and inhibits their expressions (Fig. 3D). By suppressing the TGF- β pathway, miR-141-5p also demonstrated significant effects in regulating the expressions of EMT-related proteins (Fig. 4A), and suppressed TGF- β -induced cell migration (Fig. 4B) and invasion (Fig. 4C) in PANC-1 cells.

lncRNA is known to bind with miRNA and attenuate the activity of the miRNA, by preventing it from binding to its target mRNA, which indirectly promotes the expression of the mRNA. Our results have shown that overexpression of MALAT1 reduces free miR-141-5p via endogenous sponging, which in turn upregulates TGFBR1 and TGFBR2 for the TGF- β pathway. The overall effect promotes TGF- β -induced EMT, and increases cell migration and invasion (Fig. 5).

Conclusions

In summary, our study revealed that the lncRNA MALAT1 downregulates miR-141-5p and promotes the TGF- β pathway that induces EMT. Thus, we suggest that overexpression of MALAT1 in pancreatic cancer cells is responsible for the initiation of metastasis during development of the disease. These findings may provide useful insights in the discovery of potential therapeutic treatments for pancreatic cancer.

Abbreviations

EMT

Epithelial-to-mesenchymal transition

TGF- β

Transforming growth factor beta

lncRNA

long noncoding RNA

MALAT1

Metastasis-associated lung adenocarcinoma transcript 1

NEAT2

nuclear-enriched abundant transcript 2

WHO

World Health Organization

HEK293

Human embryonic kidney cells 293

DMEM

Dulbecco's Modified Eagle's Medium

FBS

fetal bovine serum

OD

optical density

qRT-PCR

Quantitative real-time PCR

siRNA

small interfering RNA

miRNA-NC

miRNA negative control

ECL

enhanced chemiluminescence

PBS

phosphate buffered saline

PC

pancreatic cancer
ceRNA
competing endogenous RNA

Declarations

Ethics approval and consent to participate

Ethical approval was obtained from the Ethics Committee of West China Hospital. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Consent for publication

The study followed the tenets of the Declaration of Helsinki, and informed written consent was obtained from all patients and controls after we explained the nature and possible consequences of the study.

Availability of data and materials

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

Competing interests

The authors declare that they have no competing interests.

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No funding was received.

Authors' contributions

HL did all the experiments, analyzed all data and was a major contributor in writing the manuscript. ML, JY, LZ and SL did part of experiment work. All authors read and approved the final manuscript.

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References

1. Frelove R, Walling AD. Pancreatic cancer: diagnosis and management. Am Fam Physician. 2006;73:485-92.

2. Lowenfels AB, Maisonneuve P, DiMagno EP, Elitsur Y, Gates LK Jr, Perrault J, et al. Hereditary pancreatitis and the risk of pancreatic cancer. International Hereditary Pancreatitis Study Group. *J Natl Cancer Inst.* 1997;89(6):442-6.
3. Lynch HT, Smyrk T, Kern SE, Hruban RH, Lightdale CJ, Lemon SJ, et al. Familial pancreatic cancer: a review. *Semin Oncol.* 1996;23(2):251-75.
4. Michaud DS. Epidemiology of pancreatic cancer. *Minerva Chir.* 2004;59:99-111.
5. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Murray T, et al. Cancer statistics, 2008. *CA Cancer J Clin.* 2008;58(2):71-96.
6. Li D, Xie K, Wolff R, Abbruzzese JL. Pancreatic cancer. *Lancet.* 2004;363:1049-57.
7. Vincent A, Herman J, Schulick R, Hruban RH, Goggins M. Pancreatic cancer. *Lancet.* 2011;378:607-20.
8. Ilic M, Ilic I. Epidemiology of pancreatic cancer. *World J Gastroenterol.* 2016;22:9694-9705.
9. Derynck R, Akhurst RJ. Differentiation plasticity regulated by TGF-beta family proteins in development and disease. *Nat Cell Biol.* 2007;9:1000-4.
10. Bieri B, Moses HL. Tumour microenvironment: TGFbeta: the molecular Jekyll and Hyde of cancer. *Nat Rev Cancer.* 2006;6:506-20.
11. Hata A, Shi Y, Massague J. TGF-beta signaling and cancer: structural and functional consequences of mutations in Smads. *Mol Med Today.* 1998;4:257-62.
12. Neuzillet C, de Gramont A, Tijeras-Raballand A, de Mestier L, Cros J, Faivre S, et al. Perspectives of TGF- β inhibition in pancreatic and hepatocellular carcinomas. *Oncotarget.* 2014;5(1):78-94.
13. Xu J, Lamouille S, Derynck R. TGF-beta-induced epithelial to mesenchymal transition. *Cell Res.* 2009;19:156-72.
14. Truty MJ, Urrutia R. Basics of TGF-beta and pancreatic cancer. *Pancreatol.* 2007;7:423-35.
15. Shi Y, Massague J. Mechanisms of TGF-beta signaling from cell membrane to the nucleus. *Cell.* 2003;113:685-700.
16. Feng XH, Derynck R. Specificity and versatility in tgf-beta signaling through Smads. *Annu Rev Cell Dev Biol.* 2005;21:659-93.
17. Lu Z, Friess H, Graber HU, Guo X, Schilling M, Zimmermann A, et al. Presence of two signaling TGF-beta receptors in human pancreatic cancer correlates with advanced tumor stage. *Dig Dis Sci.* 1997;42(10):2054-63.
18. Salmena L, Poliseno L, Tay Y, Kats L, Pandolfi PP. A ceRNA hypothesis: the Rosetta Stone of a hidden RNA language? *Cell.* 2011;146:353-8.
19. Hirata H, Hinoda Y, Shahryari V, Deng G, Nakajima K, Tabatabai ZL, et al. Long Noncoding RNA MALAT1 Promotes Aggressive Renal Cell Carcinoma through Ezh2 and Interacts with miR-205. *Cancer Res.* 2015;75(7):1322-31.
20. Michalik KM, You X, Manavski Y, Doddaballapur A, Zörnig M, Braun T, et al. Long noncoding RNA MALAT1 regulates endothelial cell function and vessel growth. *Circ Res.* 2014;114(9):1389-97.

21. Gutschner T, Hämmerle M, Eissmann M, Hsu J, Kim Y, Hung G, et al. The noncoding RNA MALAT1 is a critical regulator of the metastasis phenotype of lung cancer cells. *Cancer Res.* 2013;73(3):1180-9.
22. Jiao F, Hu H, Yuan C, Wang L, Jiang W, Jin Z, et al. Elevated expression level of long noncoding RNA MALAT-1 facilitates cell growth, migration and invasion in pancreatic cancer. *Oncol Rep.* 2014;32(6):2485-92.
23. Li L, Chen H, Gao Y, Wang YW, Zhang GQ, Pan SH, et al. Long Noncoding RNA MALAT1 Promotes Aggressive Pancreatic Cancer Proliferation and Metastasis via the Stimulation of Autophagy. *Mol Cancer Ther.* 2016;15(9):2232-43.
24. Liu JH, Chen G, Dang YW, Li CJ, Luo DZ. Expression and prognostic significance of lncRNA MALAT1 in pancreatic cancer tissues. *Asian Pac J Cancer Prev.* 2014;15(7):2971-7.
25. Zhao G, Wang B, Liu Y, Zhang JG, Deng SC, Qin Q, et al. miRNA-141, downregulated in pancreatic cancer, inhibits cell proliferation and invasion by directly targeting MAP4K4. *Mol Cancer Ther.* 2013;12(11):2569-80.

Figures

Figure 1

Expressions of MALAT1 and miR-141-5p in pancreatic cancer tissues and their correlation. A. Blast analysis of the putative binding site between MALAT1 and miR-141-5p; B. Expression of MALAT1 in pancreatic tissues, compared to that in pericarcinomatous tissues; C. Expression of miR-141-5p in pancreatic tissues, compared to that in pericarcinomatous tissues; D. Correlation between the expression of MALAT1 and miR-141-5p in pancreatic tissues.

Figure 2

Direct sequence-specific binding between MALAT1 and miR-141-5p, and their effects on each other's expression. A. The fragment containing the wild type (wt) putative binding sequence of MALAT1 and the mutant (mut), and the sequence for miR-141-5p, for subcloning into the psiCHECK-2 luciferase vector; B. Luciferase by co-transfecting the wild type or mutant MALAT1 with miR-141-5p mimics or inhibitor, compared with co-transfecting with the miRNA NC, to the HEK293 cells; C-D. qTR-PCR results showing the relative expression of miR-114-5p and MALAT1 when transfected with miR-141-5p mimic or miRNA-NC to PANC-1 cells; E-F. qTR-PCR results showing the relative expression of miR-114-5p and MALAT1 when

transfected with siMALAT1 or siControl to PANC-1 cells. Each experiment was performed in triplicates, **p<0.01.

Figure 3

Direct binding of miR-141-5p on the 3'-UTR of TGFBR1 and TGFBR2 inhibits their protein expression. A. Fragments containing the wild type (wt) putative binding site of TGFBR1 and TGFBR2 and their mutant (mt) variant, as well as the sequence for miR-141-5p mimic, for subcloning to the luciferase vector; B. Luciferase activity by co-transfecting the miRNA-NC or the miR-141-5p mimic, with the wild type or mutant TGFBR1; C. Luciferase activity by co-transfecting the miRNA-NC or the miR-141-5p mimic, with the wild type or mutant TGFBR2; D. western blot result of both TGFBR1 and TGFBR2. Each experiment was performed in triplicates, **p<0.001.

Figure 4

Overexpression of miR-141-5p inhibited TGF-β-induced EMT, cell migration, and cell invasion. A. Western blot analysis showing the expressions of EMT-related proteins in PANC-1 cells, after transfecting with miR-141-5p mimic, miRNA-NC, or the mock, followed by TGF-β1 treatment; B. Results from Transwell migration assay using PANC-1 cells transfected with miR-141-5p mimic, miRNA-NC, or the mock, followed by treatment with TGF-β1 or solvent blank for; C. Results from invasion assay with Matrigel, using PANC-

1 cells transfected with miR-141-5p mimic, miRNA-NC, or the mock, followed by treatment with TGF-β1 or solvent. Each experiment was performed in triplicates, **p<0.01.

Figure 5

MALAT1 suppressed the inhibitory effect of miR-141-5p on EMT, cell migration, and cell invasion induced by TGF-β. A. Western blot results showing the expression of EMT-related proteins in PANC-1 cells, which are co-transfected with either pcDNA3.1-MALAT1 or a mock vector, with or without miR-141-5p mimic,

followed by treatment with TGF- β 1 or solvent blank; B. Results from Transwell migration assay using PANC-1 cells transfected with either pcDNA3.1-MALAT1 or a mock vector, with or without miR-141-5p mimic, followed by treatment with TGF- β 1 or solvent blank; C. Results from invasion assay with Matrigel, using PANC-1 cells transfected with either pcDNA3.1-MALAT1 or a mock vector, with or without miR-141-5p mimic, followed by treatment with TGF- β 1 or solvent blank. Each experiment was performed in triplicates.