ABSTRACT
Increasing evidence has reported that long non-coding RNA (lncRNA) plays a vital role in the development of pancreatic cancer (PC). However, the function and mechanism of LINC01133 in PC tumorigenesis are still unknown. Herein, we found that LINC01133 was highly expressed in PC tissues and cell lines, and LINC01133 knockdown could block the growth and metastasis of PC cells. Besides, upregulated LINC01133 in PC cells was induced by Yin Yang 1 (YY1). Furthermore, LINC01133 directly targeted miR-199b-5p and promoted cancer malignancy by suppressing miR-199b-5p. It was also discovered that myelin regulatory factor (MYRF) was targeted by miR-199b-5p and positively correlated with LINC01133 expression in PC, and LINC01133 modulated PC progression through miR-199b-5p/MYRF pathway. In conclusion, we demonstrated that YY1-mediated the upregulation of LINC01133 increased MYRF expression by sponging miR-199b-5p, resulting in the accelerated development of PC. These findings might offer a novel insight into the development of efficient therapeutics for PC patients.

Highlights
- LINC01133 was upregulated in PC
- YY1-induced LINC01133 upregulation promoted PC progression
- LINC01133 regulated PC malignancy through miR-199b-5p/MYRF axis

Introduction
Pancreatic cancer (PC) is a leading cause of cancer-related mortality worldwide [1]. In 2018 alone, 458,918 new PC cases were diagnosed, leading to approximately 432,242 deaths globally [2]. Despite significant progress in surgical and medical treat-
ments, such as neo-adjuvant chemo-radiotherapy and laparoscopic techniques, improvements in clinical outcomes have been insignificant [3], and the overall survival rate of PC patients is only 2% – 9% [4]. Multiple reasons account for the pessimistic prognosis, including late detection, fast progression, and resistance to therapeutic drugs [5–7]. Hence, it is important to explore molecular mechanisms underlying PC progression and identify novel biomarkers and therapeutic targets for PC.

Long non-coding RNA (lncRNA) is a type of functional transcript longer than 200 nucleotides in length [8]. It has been extensively studied that lncRNAs play oncogenic or tumor-suppressive roles in different types of cancers, such as lung adenocarcinoma [9], colorectal cancer [10], breast cancer [11], and prostate cancer [12]. In particular, lncRNAs have been reported to participate in the development of PC. For example, lncRNA PSMB8-AS1 promoted the development of PC by regulating the miR-382-3p/STAT1/PD-L1 pathway [13]. Hypoxia-induced lncRNA BX111 facilitated metastasis and progression of PC by regulating ZEB1 transcription [14]. RREB1 activated the transcription of lncRNA AGAP2-AS1 and the upregulated AGAP2-AS1 suppressed ANKRD1 and ANGPTL4 to promote the growth and metastasis of PC [15]. LINC01133, located in chromosome 1q23.2 [16], was identified to be upregulated in pancreatic ductal adenocarcinoma (PDAC) and associated with poor prognosis of PDAC patients [17]. However, the regulatory mechanisms of LINC01133 need to be further elucidated.

In the present study, we hypothesized that LINC01133 might act as an oncogene in PC, and aimed to explore the molecular mechanism and function of LINC01133 in PC. Our findings might provide new insight into PC development and identify potential targets for PC treatment in the future.

**Material and methods**

**Tissue specimen**

A total of 52 pairs of PC tissues and adjacent normal tissues were obtained from PC patients at the Affiliated Shuyang Hospital of Xuzhou Medical University. The clinicopathological features of PC patients are presented in **Table 1**. All samples were frozen in liquid nitrogen immediately after resection to be stored at −80°C for future use. Written consent forms were signed by all participants and this study was approved by the Affiliated Shuyang Hospital of Xuzhou Medical University.

**Cell culture**

Human PC cell lines (SW1990, PANC-1, AsPC-1, and BXPC-3), and normal pancreatic epithelial cells (HPDE) were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). These cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM, Gibco, USA, # 11–995–040) with 0.1 mg/ml streptomycin (Gibco, # 15,140–122), 100 U/ml penicillin (Gibco, # 15,140–122), and 10% FBS (Gibco, # 10099133C) at 37°C with 5% CO2.

**Cell transfection**

The short hairpin RNAs (shRNAs) against LINC01133 (shLINC01133) and YY1 (shYY1) with the negative control (shNC), miR-199b-5p mimics/inhibitor and the respective controls (NC mimics/inhibitor), and overexpression plasmid of LINC01133 (pcDNA3.1/LINC01133), YY1 (pcDNA3.1/YY1) and MYRF (pcDNA3.1/MYRF) with their negative control (pcDNA3.1) were all purchased from Shanghai GenePharma. Transfection of the aforementioned vectors into PC cells was performed using Lipofectamine 2000 reagent (Invitrogen, # 11–668–500).

**Table 1. The relationship between LINC01133 expression and clinicopathological characteristics of PC patients.**

| LINC01133 | Age | Gender | TNM stage | Lymph node metastasis |
|-----------|------|--------|------------|-----------------------|
|           | N    | High = 30 | Low = 22 | P value              |
| ≥60 years | 31   | 18      | 13         | 0.687                 |
| <60 years | 21   | 12      | 9          | 0.943                 |
| Male      | 28   | 17      | 11         | 0.943                 |
| Female    | 24   | 13      | 11         | 0.943                 |
| I–II      | 23   | 10      | 13         | 0.022                 |
| III–IV    | 29   | 20      | 9          | 0.022                 |
| Negative  | 27   | 9       | 18         | <0.001                |
| Positive  | 25   | 21      | 4          |                       |
**RT-qPCR**

Total RNA was isolated from tissues and cells with TRizol reagent (Invitrogen, # 15,596–026) and PrimeScript™ RT-PCR Kit (TaKaRa, Japan, # 2680A) was used to reversely transcribe the RNAs into cDNA. Next, qPCR was conducted on ABI 7300 Thermocycler (Thermo Fisher Scientific) by using SYBR Premix Ex Taq kit (Thermo Fisher Scientific, # A46109). The relative expressions were obtained with the 2−ΔΔCT method and GAPDH and U6 were applied as internal controls. The primer sequences were as follows: LINC01133, forward 5'- GGCAAG GTGAACCTCAAAAA-3' and reverse 5'-TTGCTG CAAGAGGAGAAAGC-3'; YY1, forward 5'-ACG GCTTCGAGGATCAGATTC-3' and reverse 5'-TGACCCCTTTTCCAATGT-3'; miR-199b-5p, forward 5'-GCCGCCAGGCACTGGTT AGACTAT-3', and reverse 5'-GTGCAGGGTCCGAGGT -3'; MYRF, forward 5'- CCAGATCTCAGAGCGT ATCATTG-3' and reverse 5'-TGCCACAGGCAC GTCAGTGT-3'; GAPDH, forward 5'-GCACCACACA CACTGCTTAGCA-3' and reverse 5'-GTCTTC TTGGTGCCAGTGT-3'; U6, forward 5'-CT CGCTTCCGAGCACA-3' and reverse 5'-AAGCG TTCACGAATTTCG-3'.

**Western blotting**

Total proteins were isolated using radioimmuno-precipitation lysis buffer (Beyotime) and loaded onto SDS-PAGE gel for separation and then transferred to PVDF membranes (BioRad, 1,620,177). After that, the membranes were blocked with skim milk for 2 h, incubated with primary antibodies against MYRF (1:1000; Abcam, ab227721), cleaved-Caspase-3 (1:1000; Abcam, ab32499), Bax (1:1000; Abcam, ab182733), BCL2 (1:1000; Abcam, ab182858), and GAPDH (1:1000; Abcam, ab9485) at 4°C overnight. Then an enhanced chemiluminescence kit (Santa Cruz, sc-2048) was employed to detect the protein signals following another 2 h incubation with HPR-conjugated secondary antibody. Protein levels were quantified using Image-Pro® Plus software (Media Cybernetics) [18].

**Subcellular fractionation**

Nuclear and cytoplasmic fractions were obtained from PANC-1 and SW1990 cells using nucleoplastic fractionation buffer (140 mmol/l NaCl, 1.5 mmol/l MgCl2, 10 mmol/l Tris-HCl pH 8.5, 0.5% NP-40). First, the cell pellet was resuspended through nucleoplastic fractionation buffer and incubated for 5 min on ice. After centrifugation, the cytoplasmic and nuclear fractions were collected, respectively. RNA was extracted from nuclear/cytoplasmic fractions, and RT-qPCR was then employed to determine the expressions of LINC01133, GAPDH, and U6. GAPDH and U6 served as the cytoplasmic endogenous control, and nuclear endogenous control, respectively [19].

**ChIP**

ChIP assay was conducted by using EZ ChIP™ Chromatin Immunoprecipitation Kit (Millipore, USA). The transfected PANC-1 and SW1990 cells were subjected to 20-min crosslink with PFA. Next, the cells were lysed with lysis buffer for a 30-min sonication to fragment DNA to 1000 bp in length. After that, DNA fragments were immunoprecipitated with YY1 or IgG antibody overnight at 4°C. Finally, RT-qPCR was used to analyze the precipitated DNA.

**Luciferase reporter assay**

To examine the binding sites between LINC01133 and YY1, PC cells were co-transfected with pGL3-LINC01133 promoter (site 1+ site 2 or site 1) and pcDNA3.1-YY1. To examine the relationship between miR-199b-5p and LINC01133 or MYRF, PC cells were co-transfected with miR-199b-5p mimics and pmirGLO-LINC01133 (wildtype or mutant) or pmirGLO-MYRF (wildtype or mutant). After incubation for 48 h, luciferase reporter assay was performed by Dual-Luciferase® Reporter Assay System (Promega) [20].

**CCK-8 assay**

Cell Counting Kit-8 (CCK-8, Abcam, # ab228554) was used to detect the cell viability of transfected
PANC-1 and SW1990 cells. Briefly, PC cells were cultured in a 96-well plate at 37°C for 24, 48, and 72 h. Then 10 µl CCK-8 solution was added to each well. After that, plates were incubated at 37°C for 2 h. The cell viability was determined by measuring the absorbance at 450 nm.

**Transwell assay**

The invasive and migrative abilities of PC cells were evaluated using Transwell chambers (EMD Millipore). For the evaluation of cell invasion ability, the cells were seeded in the upper Transwell chambers in serum-free medium which were pre-coated with Matrigel (Becton Dickinson). In the lower chambers, 600 µl DMEM supplemented with 10% FBS was added. In 48 h incubation, the cells invaded to the lower chamber were fixed in 4% formaldehyde, stained with 0.1% crystal violet both for 20 min at room temperature. Finally, cells were counted with a light microscope. To evaluate cell migration ability, the abovementioned method was repeated, except for that the upper chamber was not coated with Matrigel.

**TUNEL assay**

PC cells (PANC-1 and SW1990) were washed with PBS and fixed in 4% paraformaldehyde solution for 1 h at 4°C. The cells were permeabilized with 0.25% Triton-X 100 (Sigma-Aldrich), and incubated in TUNEL reaction mixture (Roche) for 1 h at 37°C. Subsequently, the TUNEL-stained cells were counterstained with DAPI at room temperature. Images were captured from ≥5 fields of view under a fluorescence microscope.

**In vivo assay**

Six Male BALB/c nude mice were randomly divided into 2 groups and maintained under pathogen-free conditions. Then, mice were inoculated with SW1990 cells (1 × 10^6) with transfection of shLINC01133 or shNC by subcutaneous injection. Every 7 days, tumor volume was recorded, and mice were sacrificed after 4 weeks. The animal experiments were approved by the Affiliated Shuyang Hospital of Xuzhou Medical University.

**Statistical analysis**

Student’s t-tests and one-way ANOVA were used for statistical analysis between two groups or among no less than three groups respectively. Correlation between LINC01133 expression and the clinical features of PC patients was assessed using the Chi-square test. The correlation between gene expressions was analyzed using Pearson’s analysis. Statistics were analyzed with GraphPad Prism 7. All results were presented as the mean ± standard deviation (SD) of at least three independent experiments. P < 0.05 was considered statistically significant.

**Results**

The current research aimed to explore the biological function of LINC01133 in the tumorigenesis of PC and the underlying mechanism involved. Through a series of experiments, we found that YY1-mediated the upregulation of LINC01133 facilitated the development of PC by suppressing miR-199b-5p to upregulate MYRF.

**LINC01133 is highly expressed in PC tissues and cell lines**

To investigate the role of LINC01133 in PC, its expression pattern was first searched in TCGA database. Figure 1(a) showed obviously abundant expression of LINC01133 in pancreatic adenocarcinoma (PAAD) tissues. Besides, TCGA database indicated PC patients with high LINC01133 expression exhibited a lower survival rate compared with those with low LINC01133 expression (Figure 1(b)). Next, RT-qPCR assay indicated that LINC01133 expression was markedly higher in PC tissues and cell lines (SW1990, PANC-1, AsPC-1, and BXPC-3) in comparison with non-tumor tissues and normal pancreatic epithelial cell line (HPDE) (Figure 1(c,d)). Furthermore, higher LINC01133 levels were associated with worse overall survival of PC patients in comparison with lower LINC01133 expression (Figure 1(e)). Additionally, LINC01133 was related to TNM stage and lymph node metastasis but irrelevant with age and gender (Table 1). In sum,
LINC01133 was upregulated in PC and correlated with unfavorable clinicopathological features.

**LINC01133 knockdown blocks the growth and metastasis of PC cells**

Subsequently, the effect of LINC01133 on the activities of PC cells was investigated. PANC-1 and SW1990 cells were transfected with shLINC01133 and RT-qPCR confirmed that the transfection was successful (Figure 2(a)). Next, CCK-8 assay showed that LINC01133 knockdown remarkably retarded cell proliferation (Figure 2(b)). Transwell assays observed that depletion of LINC01133 greatly reduced the number of migrated and invaded PC cells (Figure 2(c,d)). TUNEL and flow cytometry assays showed that the apoptosis of PC cells was enhanced by LINC01133 silencing (Figure 1(e,f)). Besides, the knockdown of LINC01133 upregulated the protein levels of cleaved-caspase 3 and Bax, but downregulated the protein levels of Bcl-2 (Figure 1(g)). Furthermore, in vivo experiments were performed to validate the effect of LINC01133 silence on PC tumor growth. The results showed that the volume and weight of tumors were reduced in shLINC01133 group compared to the control group (Figure 1(h,i)). Moreover, IHC results demonstrated that the level of Ki-67 was decreased in the LINC01133 knockdown group (Figure 1(j)). All in all, LINC01133 deficiency inhibited PC progression both in vitro and in vivo.

**YY1 activates LINC01133 transcription in PC cells**

As LINC01133 dysregulation was related to the development of PC, the underlying mechanism leading to upregulated LINC01133 was explored. By using PROMO website (http://alggen.lsi.upc.es), YY1 was predicted as a potent transcription factor of LINC01133. TCGA database also showed a significantly elevated level of YY1 in PAAD tissues (Figure 3(a)). Then, the DNA motif of LINC01133 was obtained from JASPAR and two possible YY1 binding sites in the LINC01133 promoter were found (Figure 3(b)). A ChIP assay indicated that YY1 could bind to site 2 in LINC01133 promoter (Figure 3(c)). To re-verify, full LINC01133 promoter (LINC01133-pGL3-F) or site 2-deleted LINC01133 promoter (LINC01133-pGL3-D) and pcDNA3.1/YY1 were co-transfected into PANC-1 and SW1990 cells to perform luciferase reporter assay. The results indicated that YY1 abundance specifically enhanced the relative luciferase activity in LINC01133-pGL3-F group while that in LINC01133-pGL3-D group barely fluctuated (Figure 3(d)). Moreover, YY1 depletion remarkably decreased the expression of LINC01133 (Figure 3(e)). In addition, RT-qPCR and IHC indicated that YY1 was upregulated in PC tissues (Figure 3(f,g)), and YY1 expression was positively correlated with LINC01133 expression in PC tissues (Figure 3(h)). Taken together, LINC01133 was mediated by YY1 in PC.
Figure 2. LINC01133 promotes PC malignancy.

(a) LINC01133 expression in PC cells after LINC01133 knockdown was evaluated by RT-qPCR. (b-f) Following LINC01133 depletion, cell proliferation was evaluated by CCK-8 (b), cell migration and invasion were detected by Transwell assays (C and D), cell apoptosis was assessed by TUNEL and flow cytometry assays (e and f). (g) The protein levels of apoptosis-related proteins (cleaved-Caspase-3, Bax, and Bcl-2) after silencing LINC01133 were detected by Western blot. (h and i) The impact of LINC01133 knockdown on xenograft tumor growth. (j) Change of Ki-67 level after LINC01133 depletion was evaluated by IHC. *** P < 0.001.
LINC01133 directly targets miR-199b-5p

Next, nuclear-cytoplasmic fractionation assays were performed and the results indicated that LINC01133 was mainly distributed in the cytoplasm of PC cells (Figure 4(a)). Next, Starbase website was used to screen the potential target genes of LINC01133 and a total of 15 miRNAs were predicted. Among the predicted genes, only miR-199b-5p was downregulated in both PC tissues and cell lines (Figure 4(b,c)). Therefore, miR-199b-5p was selected for subsequent experiments. The potential binding sites between LINC01133 and miR-199b-5p were presented in Figure 4(d). Luciferase reporter assays verified the binding ability as miR-199b-5p mimics significantly reduced the luciferase activity of LINC01133-WT, but not that of LINC01133-Mut (Figure 4(e)). Furthermore, miR-199b-5p levels in PC cells could be elevated and decreased by LINC01133 depletion and overexpression, respectively (Figure 4(f)). Besides, Pearson analysis found out that miR-199b-5p expression was negatively correlated with LINC01133 expression in PC tissues (Figure 4(g)). In addition, low expression of miR-199b-5p was found to be associated with TNM stage and lymph node metastasis of PC patients (Table 2). To summarize, LINC01133 was a sponge for miR-199b-5p in PC.

LINC01133 inhibits miR-199b-5p in PC to promote tumor malignancy

To investigate the functional roles of miR-199b-5p in LINC01133-regulated PC cell activities, rescue experiments were conducted. shLINC01133 and miR-199b-
5p inhibitor were co-transfected into PANC-1 and SW1990 cells. Figure 5(a) showed a clear elevation of miR-199b-5p expression following LINC01133 silencing, but the introduction of miR-199b-5p inhibitor abolished the elevation. Furthermore, the impacts of shLINC01133 on attenuated PC cell proliferation, migration, and invasion, and enhanced cell apoptosis were all reversed by miR-199b-5p inhibitor (Figure 5(b-e)). These results indicated that LINC01133 accelerated PC cell growth and metastasis by suppressing miR-199b-5p expression.

LINC01133 regulates MYRF expression via miR-199b-5p

The downstream target of miR-199b-5p was predicted via multiple online programs (microT, miRanda, miRmap, PITA, and RNA22) and two mRNAs (MYRF and TXLNB) were identified (Figure 6(a)). Additionally, the level of MYRF was shown to be significantly elevated in PAAD samples in TCGA database (Figure 6(b)). The binding sequence of miR-199b-5p and MYRF was presented in Figure 6(c). Luciferase reporter assays indicated that miR-199b-5p overexpression significantly reduced the luciferase activity of MYRF-WT while luciferase activity of mutant MYRF was not affected (Figure 6(d)). Moreover, mRNA and protein levels of MYRF were reduced by silencing LINC01133 but the introduction of miR-199b-5p inhibitor re-elevated MYRF levels in PC cells.

Table 2. The relationship between miR-199b-5p expression and clinicopathological characteristics of PC patients.

| miR-199b-5p | N | High = 24 | Low = 28 | P value |
|-------------|---|----------|----------|---------|
| Age         |   |          |          |         |
| ≥60 years   | 31| 14       | 17       | 0.646   |
| <60 years   | 21| 10       | 11       |         |
| Gender      |   |          |          |         |
| Male        | 28| 10       | 18       | 0.611   |
| Female      | 24| 14       | 10       |         |
| TNM stage   |   |          |          |         |
| I–II        | 23| 16       | 7        | 0.017   |
| III–IV      | 29| 8        | 21       |         |
| Lymph node  |   |          |          |         |
| metastasis  |   |          |          |         |
| Negative    | 27| 15       | 12       | <0.001  |
| Positive    | 25| 9        | 16       |         |

Figure 4. LINC01133 functions as a ceRNA for miR-199b-5p.
(a) Subcellular localization of LINC01133 in PC cells. (b and c) Expression of miR-199b-5p in tumor and non-tumor tissues and cell lines was detected by RT-qPCR. (d) Starbase prediction of the binding sites between LINC01133 and miR-199b-5p by luciferase reporter assay. (e) RT-qPCR evaluated the change of miR-199b-5p expression in PANC-1 and SW1990 cells following transfection with shLINC01133 or pcDNA3.1/LINC01133. (g) Correlation between levels of LINC01133 and miR-199b-5p in PC tissues. *P < 0.05; ** P < 0.01; *** P < 0.001.
Figure 5. LINC01133 regulates PC progression through miR-199b-5p.
(a) RT-qPCR results of miR-199b-5p expression in PANC-1 and SW1990 cells following depletion of LINC01133 and miR-199b-5p. (b-e) Cell proliferation (b), cell migration (c), cell invasion (d) and cell apoptosis (e) following co-transfection of shLINC01133 with miR-199b-5p inhibitor were evaluated by CCK-8, Transwell and TUNEL assays. ** P < 0.01; *** P < 0.001.
Besides, Pearson’s analysis indicated that MYRF expression was negatively correlated with that of miR-199b-5p, but positively correlated with that of LINC01133 in PC tissues (Figure 6(g,h)). All in all, LINC01133 elevated the expression of MYRF in PC by absorbing miR-199b-5p.

**LINC01133 modulates PC progression by regulating MYRF expression**

Finally, the regulatory role of the LINC01133/miR-199b-5p/MYRF axis was investigated by depleting LINC01133 or overexpressing MYRF in PC cells. RT-qPCR and Western blot indicated that transfection of MYRF overexpression
plasmid partially reversed the inhibitory effects of LINC01133 knockdown on the mRNA and protein levels of MYRF (Figure 7(a,b)). In functional assays, cell proliferation was retarded by LINC01133 depletion, but the elevation of MYRF level could revive the growth of PC cells (Figure 7(c)). The inhibitory effects of LINC01133 silencing on PC cell migration and invasion were reversed by pcDNA3.1/MYRF (Figure 7(d,e)). By depleting LINC01133, apoptosis of PC cells was significantly increased, which was overturned by MYRF overexpression (Figure 7(f)). To conclude, LINC01133 promoted PC cell proliferation and mobility via upregulating MYRF expression.
Discussion

Despite advances in the understanding of the molecular biology of PC, tailored and efficient treatment options for individual patients remain largely unmet [21]. The majority of PC patients are diagnosed in advanced stages [22], and not sensitive to most existing chemotherapeutic drugs [23]. Therefore, it is extremely important to investigate the biological mechanisms and identify new biomarkers contributing to early diagnosis and targeted treatments.

The functions of dysregulated lncRNAs in malignant cancers have been extensively reported [24–26]. So far, LINC01133 has been reported to be dysregulated in various cancers and participate in the tumorigenesis of cancers. For instance, LINC01133 suppressed the development of gastric cancer through miR-106a-3p/APC/Wnt/β-catenin axis [27]. LINC01133 upregulated miR-199b-5p to upregulate TP52 and promoted the metastasis of epithelial ovarian cancer [28]. LINC01133 was downregulated in breast cancer and inhibited the metastasis of cancer cells by suppressing the expression of SOX4 via EZH2 [29]. Herein, we demonstrated that the level of LINC01133 was significantly elevated in PC and the high LINC01133 expression was associated with a poor prognosis. In addition, silencing of LINC01133 inhibited the proliferation, migration, and invasion in vitro, and tumor growth in vivo, indicating the oncogenic role of LINC01133 in PC.

YY1 is a zinc-finger transcription factor belonging to the GLI-Kruppel protein family which interferes with various biological processes, particularly tumorigenesis [30]. So far, the regulation of YY1 on the expression of lncRNAs in cancers has been extensively reported. For example, YY1-activated upregulation of LINC00673 accelerated the viability of breast cancer cells through miR-515-5p/MARK4/Hippo pathway [31]. ZFPM2-AS1 could be activated by YY1 and facilitated small cell lung cancer progression by increasing TRAF4 expression [32]. YY1-regulated lncRNA PCA16 promoted the development of glioblastoma through miR-513/IGF2BP1 pathway [33]. In our study, YY1 was confirmed as a transcriptional factor of LINC01133. Besides, YY1 was highly expressed in PC tissues, and knockdown of YY1 significantly decreased LINC01133 expression, indicating the regulatory effect of YY1 on LINC01133.

Accumulating studies revealed that lncRNAs served as ceRNAs for miRNAs to participate in the progression of different types of cancers [34,35], we hypothesized that LINC01133 could sponge miRNAs to modulate the development of PC. MiR-199b-5p has been reported to serve as either a tumor inhibitor or promoter in various cancers [36–38]. Moreover, miR-199b-5p was confirmed to be targeted by lncRNAs to regulate the progression of human cancers. For instance, Du et al. demonstrated that IncRNA DLX6-AS1 sponged miR-199b-5p to upregulate PXN to facilitate EMT and cisplatin resistance in triple-negative breast cancer [39]. Chen et al. discovered that IncRNA LINC01783 facilitated the development of cervical cancer via the miR-199b-5p/GBP1 axis [40]. Pang et al. demonstrated that LRRCA75A-AS1 suppressed multiple myeloma by modulating miR-199b-5p/PDCD4 pathway [41]. Our study revealed that miR-199b-5p was targeted by LINC01133. Additionally, knockdown of LINC01133 inhibited the malignant behaviors of PC cells, while inhibition of miR-199b-5p partially reversed these effects, indicating that LINC01133 promoted PC progression via miR-199b-5p.

MYRF is a highly conserved gene in eukaryotic organisms ranging from fungi to mammals and a transcriptional regulator that is necessary for oligodendrocyte differentiation and myelin maintenance [42]. A previous study identified that MYRF was overexpressed in pancreatic ductal adenocarcinomas (PDACs) [43]. In our study, the binding ability between miR-199b-5p and MYRF was predicted by bioinformatic analysis and confirmed by luciferase reporter assays. Furthermore, we found that knockdown of LINC01133 increased MYRF expression by sponging miR-199b-5p. Functional assays revealed that overexpressed MYRF could reverse the inhibitory effect of LINC01133 knockdown on proliferation, migration, and invasion of PC cells.

Conclusion

Our study demonstrated for the first time that LINC01133 was activated by YY1 and the upregulated LINC01133 further accelerated the development of PC via miR-199b-5p/MYRF pathway.
These findings suggested LINC01133 might be a new therapeutic target for PC treatment.

**Disclosure statement**

No potential conflict of interest was reported by the author(s).

**Funding**

The author(s) reported there is no funding associated with the work featured in this article.

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