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Downregulation of circular RNA circ-LDLRAD3 suppresses pancreatic cancer progression through miR-137-3p/PTN axis

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ABSTRACT

Emerging evidence suggests that dysregulation of circular RNAs (circRNAs) closely associated with cancer progression. In this paper, we focus on exploring the functional role of circ-LDLRAD3 in pancreatic cancer. Gene expression was determined using quantitative reverse transcriptase polymerase chain reaction and Western blot. Cell count kit-8 and 5-ethynyl-2′-deoxyuridine assay were applied to evaluate the proliferation of PANC-1 and SW1990 cells. The migration and invasion of PANC-1 and SW1990 cells were assessed using wound healing assay and transwell invasion assay. Luciferase reporter assay was performed for target validation. The results showed that circ-LDLRAD3 was overexpressed in pancreatic cancer tissues and cell lines. Increased expression of circ-LDLRAD3 was indicative of a poor prognosis in patients with pancreatic cancer. Knockdown of circ-LDLRAD3 repressed the growth of pancreatic cancer *in vitro* and *in vivo*. miR-137-3p was identified as a direct target of circ-LDLRAD3. More importantly, upregulation of circ-LDLRAD3 could mitigate the inhibitory effect of miR-137-3p on the proliferation, migration and invasion of pancreatic cancer cells. Besides, circ-LDLRAD3 could regulate the expression of pleiotrophin (PTN) through miR-137-3p. Taken together, knockdown of circ-LDLRAD3 repressed the proliferation, migration and invasion of pancreatic cancer cells through miR-137-3p/PTN axis, providing a new mechanism for pancreatic cancer progression.

1. Introduction

Pancreatic cancer is one of the most malignant digestive tract tumors, for which mortality closely parallels incidence [1]. Pancreatic cancer is the fourth cause of cancer-related death throughout the world [2]. Despite innovations have significantly increased survival, the 5-year survival rate of pancreatic cancer is about 6% [3]. As common cancer with symptoms hidden, most patients with pancreatic cancer are diagnosed at an advanced stage, which is an important contributor to the low survival rate [4]. Pancreatic cancer is hallmarked by the presence of perineural invasion, which is a chief factor causing post-operative recurrence [5]. However, the pathological and molecular mechanism of pancreatic cancer remain uncharacterized, and the understanding of which will help in the development of novel targeted therapies for pancreatic cancer.

Circular RNAs (circRNAs) are a class of non-coding RNA (ncRNA) without the 3′ and 5′ ends [6]. The 3′ and 5’ ends of circRNAs join together to form a covalently closed loop through backsplicing [7]. Such structure makes circRNAs show profound resistance to exonuclease-mediated degradation and more stability [8]. In the last few years, circRNAs have been widely discussed in the development of human diseases, including human cancers [9]. A growing body of evidence shows that circRNAs serve important regulators in tumorigenesis, and may be potential tumor targets [10]. Nevertheless, the biological functions of circRNAs are just beginning to be elucidated. circ-LDLRAD3 (hsa_circ_0006988) is of interest as a recent study showed their implication in pancreatic cancer [11]. However, the contribution of circ-LDLRAD3 to the progression of pancreatic cancer is still uncharacterized.

In comparison to circRNAs, microRNAs (miRNAs), 21-nucleotide-long ncRNAs, are the most extensively studied ncRNAs in human cancers [12]. miRNAs have been proposed to function as tumor suppressors or oncogenes in human cancers [13]. miRNAs are able to bind to the 3’ untranslated regions of messenger RNA (mRNA) and then inhibit the

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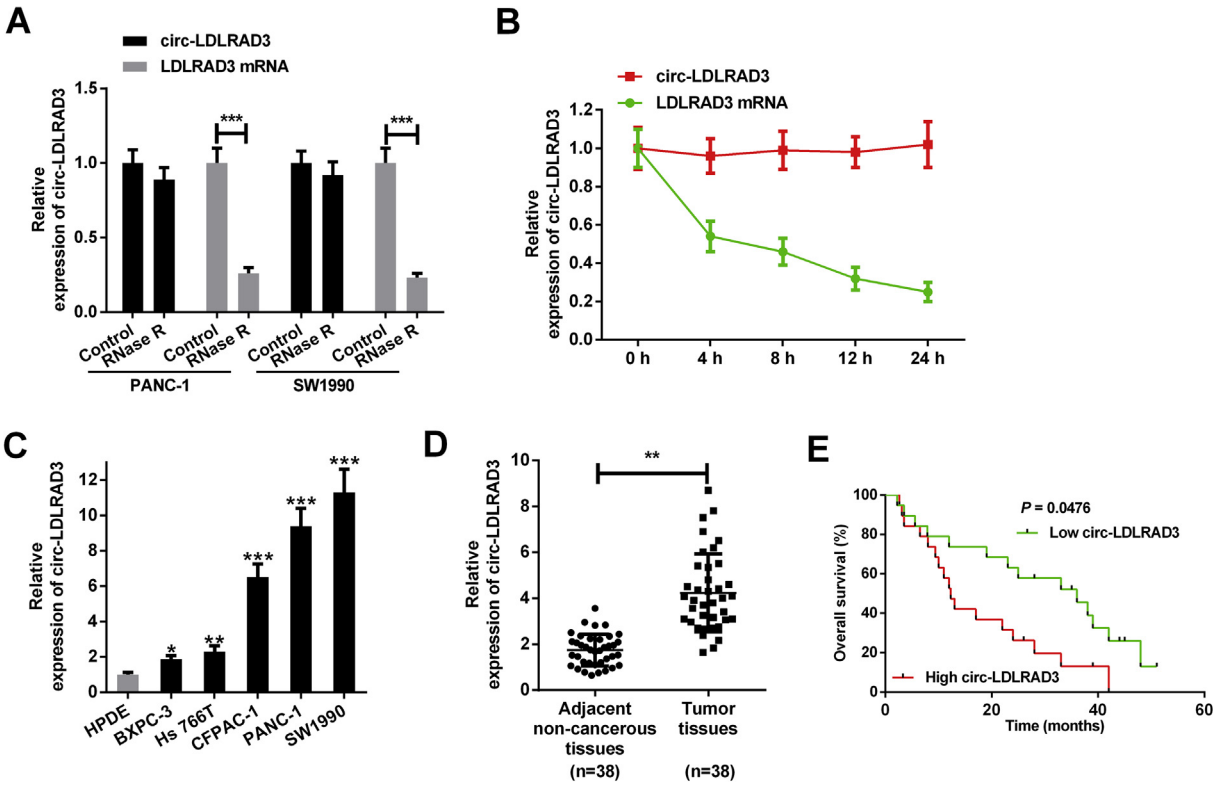

Fig. 1. Characterization of circ-LDLRAD3 in pancreatic cancer. (A) Evaluation of circ-LDLRAD3 and LDLRAD3 expression by RT-qPCR in PANC-1 and SW1990 cells following treatment with RNase R. (B) Evaluation of circ-LDLRAD3 and LDLRAD3 expression by RT-qPCR in SW1990 cells following treatment with actinomycin D. (C) RT-qPCR analysis of circ-LDLRAD3 expression in pancreatic cancer cell lines (BXPC-3, Hs 766T, CFPAC-1, PANC-1 and SW1990) and normal pancreatic duct epithelial cell line (HPDE). (D) RT-qPCR analysis of circ-LDLRAD3 expression in pancreatic cancer tissues and their adjacent non-cancerous tissues. (E) Kaplan-Meier survival analysis revealed that increased expression of circ-LDLRAD3 was indicative of poor prognosis in patients with pancreatic cancer. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

translation or promote the degradation of mRNA, finally repressing the expression of mRNA in a diverse range of biological functions [14]. Notably, a recently discovered mechanism for mRNA escape suggests that based on target sites acting as competing endogenous RNA (ceRNA) or miRNA sponge, circRNA with miRNA binding sites might sequester away the miRNA from its target mRNAs [15]. However, whether circ-LDLRAD3 functions as a miRNA sponge to regulate the progression of pancreatic cancer have yet to be explored.

In this study, we predicted that circ-LDLRAD3 regulates pancreatic cancer progression as miRNA sponges. We further explored the molecular mechanism of circ-LDLRAD3 in pancreatic cancer, and identified that circ-LDLRAD3 promoted cell proliferation and metastases as a ceRNA to target pleiotrophin (PTN) by sponging miR-137-3p in pancreatic cancer.

2. Materials and methods

2.1. Pancreatic cancer samples

A total of 38 pairs of tumor samples and corresponding adjacent non-cancerous tissues (> 2 cm away from the edge of tumor) were obtained from pancreatic cancer patients who underwent surgical resection of primary tumor at Cancer Institute, The First Affiliated Hospital, and College of Clinical Medicine of Henan University of Science and Technology. Fifteen tumors located in the head of pancreas. Twenty-three tumors located in the body and tail of pancreas. There were 11 tumors larger than 4 cm in diameter. There were 27 tumors less than 4 cm in diameter. All patients had not received neoadjuvant treatment, and their permissions were acquired before surgery. All procedures in the patients were approved by the ethics committee of The First Affiliated Hospital, and College of Clinical Medicine of Henan University of Science and Technology.

2.2. Cell culture

Human pancreatic cancer cell lines (SW1990, BXPC-3, Hs 766T, CFPAC-1 and PANC-1) and normal pancreatic duct epithelial cell line (HPDE) were proceeded from American Type Culture Collection (ATCC, Manassas, VA, USA) and grown in DMEM medium (Solarbio, Beijing, China) with 10% fetal bovine serum (FBS; Solarbio) and 1% streptomycin/penicillin (Solarbio) under 5% CO_2/95% air 37 °C.

2.3. Actinomycin D treatment

SW1990 cells were seeded in a 24-well plate and exposed to actinomycin D (2 μg/ml; Sigma-Aldrich, St Louis, MO) for 0, 4, 8, 12 and 24 h. After treatment, SW1990 cells were collected and tested for circ-LDLRAD3 and LDLRAD3 expression using quantitative reverse transcriptase polymerase chain reaction (RT-qPCR).

2.4. Cell transfection

sh-circ-LDLRAD3, sh-NC, miR-137-3p mimic and mimic NC were designed and synthesized by GenePharma (Shanghai, China). To overexpress circ-LDLRAD3, we introduced the full sequence of circ-LDLRAD3 into pcDNA-3.1 vectors to generate the recombinant plasmid of pcDNA-circ-LDLRAD3. These plasmids were transfected into PANC-1 and SW1990 cells using Lipofectamine™ 2000 (Invitrogen, Carlsbad, CA) per manufacturer's instructions.

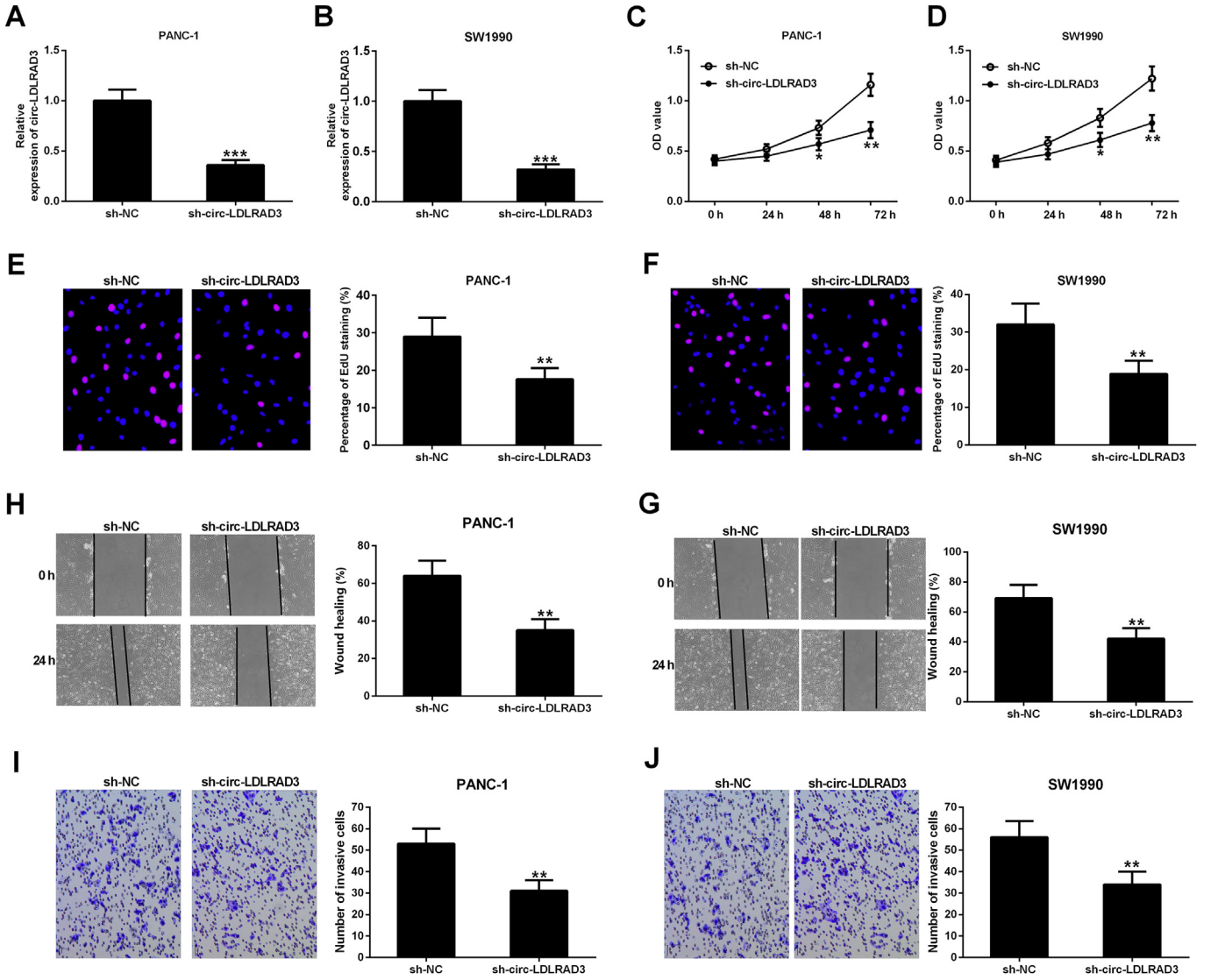

Fig. 2. Knockdown of circ-LDLRAD3 inhibits proliferation, migration and invasion of pancreatic cancer *in vitro*. PANC-1 and SW1990 cells were transfected with sh-circ-LDLRAD3A or sh-NC. (A and B) RT-qPCR showing circ-LDLRAD3 downregulation in PANC-1 and SW1990 cells after sh-circ-LDLRAD3A transfection. (C and D) CCK-8 assay showing inhibition of cell viability in PANC-1 and SW1990 cells after sh-circ-LDLRAD3A transfection. (E and F) EdU assay showing that suppression of cell proliferation in PANC-1 and SW1990 cells after sh-circ-LDLRAD3A transfection. (H and G) Wound-healing assay showing inhibition of cell migration in PANC-1 and SW1990 cells after sh-circ-LDLRAD3A transfection. (I and J) Transwell invasion assay showing inhibition of cell invasion in PANC-1 and SW1990 cells after sh-circ-LDLRAD3A transfection. $^{**}P < 0.01$; $^{***}P < 0.001$.

2.5. Detection of cell proliferation

The proliferation of PANC-1 and SW1990 cells was evaluated using cell count kit-8 (CCK-8; Solarbio) assay and 5-ethynyl-2′-deoxyuridine (EdU; Beyotime, Shanghai, China) assay.

For CCK-8 assay, PANC-1 and SW1990 cells were digested and seeded into 96-well plates after transfection. After incubation for indicated times (24 h, 48 h or 72 h), the cells were treated with CCK-8 reagent for 2 h. The absorbance of each well at 450 nm was determined using a microplate reader.

For EdU assay, PANC-1 and SW1990 cells were subjected to transfection and then cultured for 48 h. After 2 h of incubation with EdU, the cells were fixed with 4% formaldehyde, washed with PBS, and permeated with P0097 solution. After staining with Hoechst 33342, the number of EdU staining cells were counted under a fluorescence microscopy.

2.6. Wound-healing assay

PANC-1 and SW1990 cells were seeded into 6-well plates. After transfection, confluent cells were scraped with a 20 μl pipette tip to create an artificial wound, and washed with PBS to remove the cell debris. After 24 h of incubation in serum-free medium, the wound widths were measured using a phase-contrast microscope (Olympus Corporation, Tokyo, Japan) integrated with Image J software (NIH Image, Bethesda, MD, USA).

2.7. Transwell invasion assay

After transfection, PANC-1 and SW1990 cells were incubated in serum-free medium overnight, while the underside of the transwell chamber (Corning, Steuben County, New York, USA) was coated with Matrigel (Franklin Lakes, NJ, USA). DMEM medium with 10% FBS was added into the lower chamber, and cells in serum-free medium were placed into the upside of the transwell membrane. After 24 h of

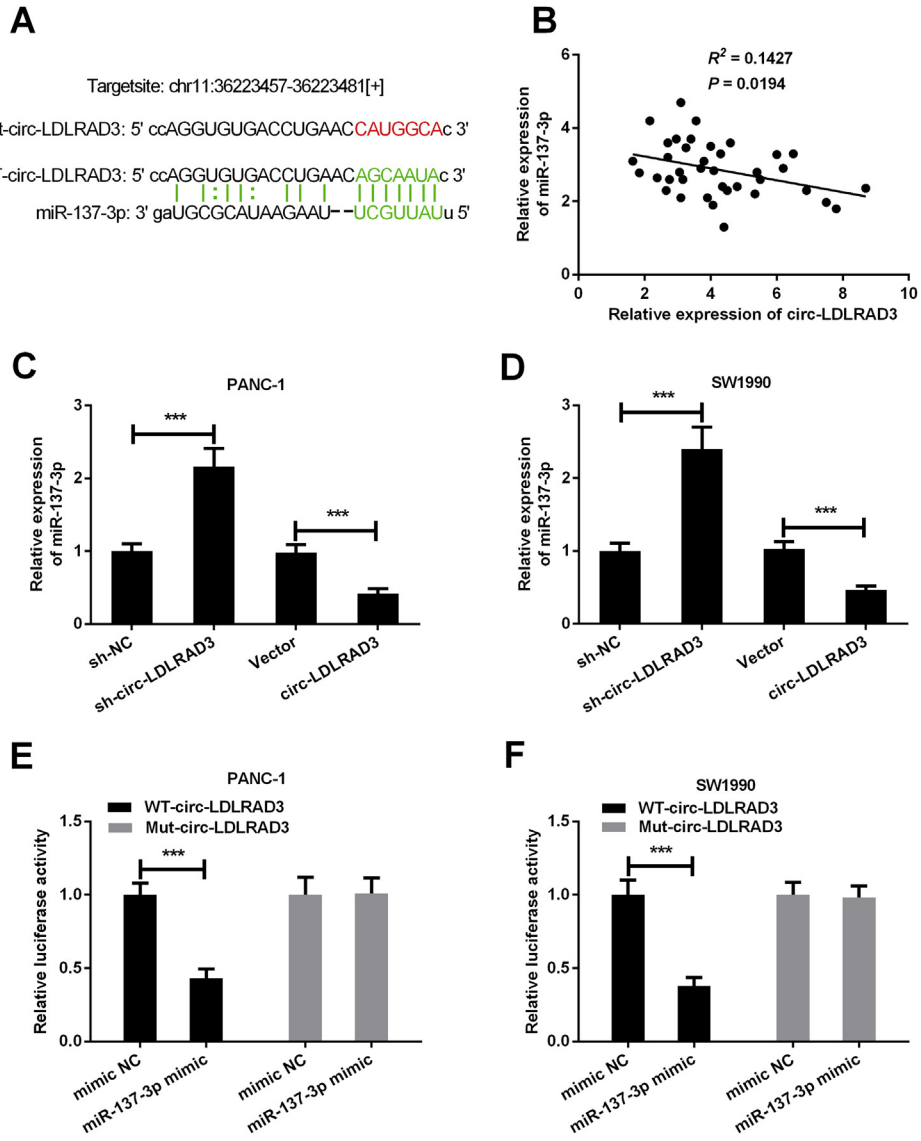

Fig. 3. circ-LDLRAD3 functions as a sink for miR-137-3p. (A) The alignment of the seed regions of miR-137-3p within circ-LDLRAD3 sequence was shown. (B) The correlation between circ-LDLRAD3 and miR-137-3p expression in pancreatic cancer tissues. (C and D) RT-qPCR analysis of miR-137-3p expression in PANC-1 and SW1990 cells after transfection of sh-circ-LDLRAD3, circ-LDLRAD3 or their matched controls. (E and F) Luciferase reporter vectors containing WT-circ-LDLRAD3 or MUT-circ-LDLRAD3 were transfected into PANC-1 and SW1990 cells together with miR-137-3p mimic or mimic NC. The relative luciferase activity was determined with the Dual-luciferase assay system. $^{***}P < 0.001$.

incubation at 37 °C, the cells were washed with PBS, fixed with formaldehyde and permeated with methanol. Following this, the cells were stained with 0.5% crystal violet at room temperature for 15 min. Non-invasive cells were scraped off using cotton swabs and invasive cells were counted under a light microscope.

2.8. Luciferase reporter assay

circ-LDLRAD3 sequence containing the miR-137-3p binding sites were fused into luciferase reporter vector (WT-circ-LDLRAD3), meanwhile, site-directed mutagenesis of the miR-137-3p binding sites in circ-LDLRAD3 sequence was also introduced into luciferase reporter vector (MUT-circ-LDLRAD3). PANC-1 and SW1990 cells were co-transfected with miR-137-3p mimic or mimic NC together with WT-circ-LDLRAD3 or MUT-circ-LDLRAD3 using Lipofectamine™ 2000. The luciferase activity was determined 24 h post transfection using Dual-Luciferase Reporter System (Promega, Madison, WI, USA) as directed by the manufacturer.

2.9. Western blot

PANC-1 and SW1990 cells were lysed with RIPA lysis buffer. Total proteins were loaded onto a 14% sodium dodecyl sulfate-polyacrylamide gel and then transferred onto polyvinylidene fluoride membranes. The membranes were probed with anti-PTN or anti-GAPDH antibody (Boster, Wuhan, China) overnight at 4 °C after blocking with 5% skim milk. Afterwards, membranes were immunoblotted with secondary antibodies (Boster) at room temperature for 1 h. Immunochemical detection was performed using chemiluminescence (Boster) as directed by the manufacturer.

2.10. RT-qPCR

Total RNA from tissues and cells was prepared by using TRIzol reagent (Invitrogen) in keeping with the manufacturer's instructions. For RNase R treatment, the extracted RNA was treated with/without RNase R (3 U/ μg) for 20 min at 37 °C, followed by purification with an RNeasy MinElute cleaning Kit (Qiagen, Valencia, CA). For miRNA analysis, total RNA was converted into cDNA using a First-Strand cDNA Synthesis Kit (Takara, Dalian, China), followed by RT-qPCR analysis using SYBR Premix Ex Taq™ kit (Takara). For the quantification of circ-LDLRAD3 and PTN, total RNA was used to determine the expression of circ-LDLRAD3 and PTN using One Step TB Green™ PrimeScript™ RT-PCR Kit (Takara). RT-qPCR was performed on iQ SYBR Green Supermix (Bio-Rad, Hercules, CA, USA) with the following primers: circ-LDLRAD3 5′ primer (5′-CTT GCT GGA CCA GAG AAC-3′) and 3′ primer (5′-CAT GAG

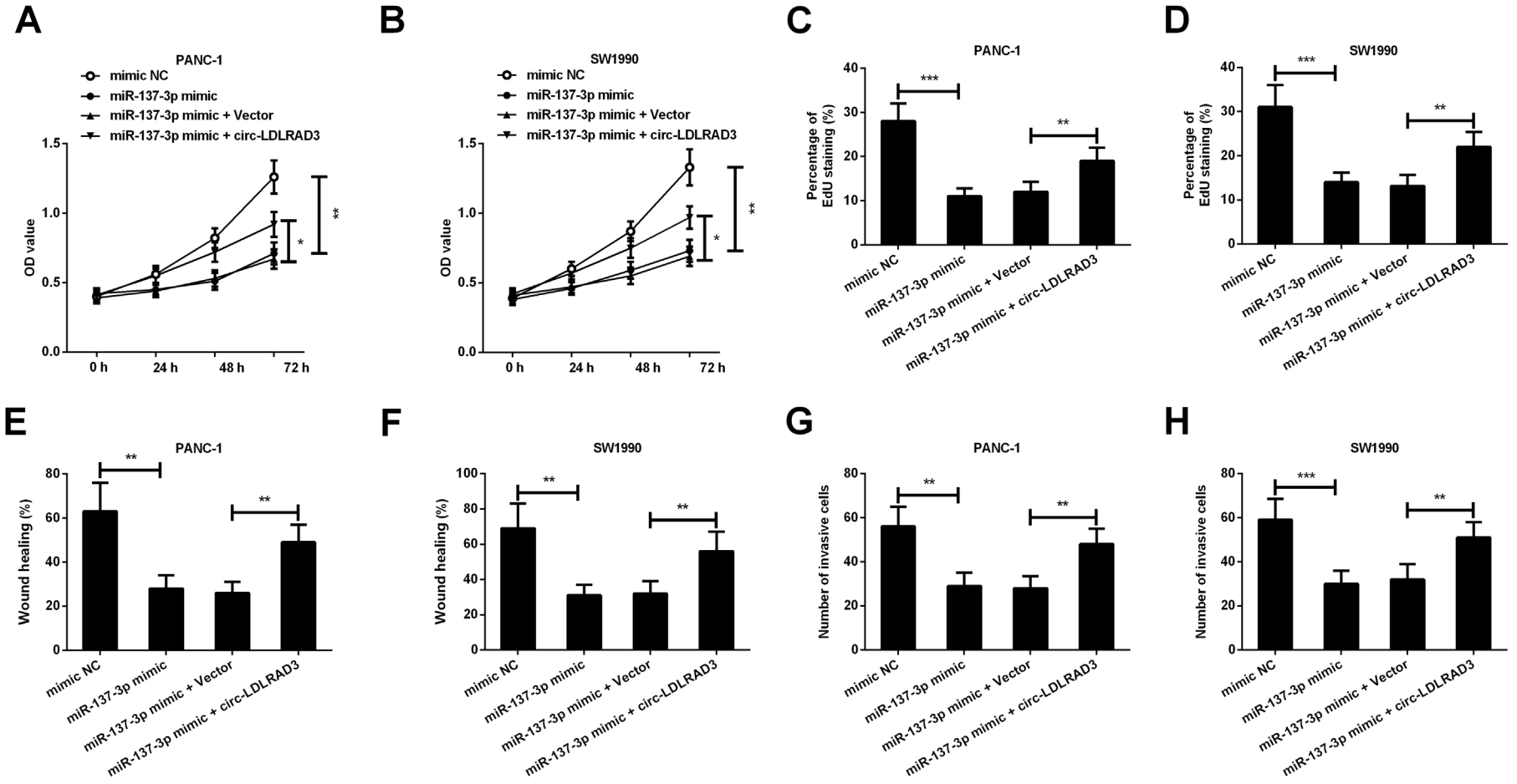

Fig. 4. Overexpression of circ-LDLRAD3 mitigates the inhibition effect of miR-137-3p on the biological behavior of pancreatic cancer cells. PANC-1 and SW1990 cells were co-transfected with miR-137-3p mimic or mimic NC and circ-LDLRAD3 or Vector. (A and B) The viability of PANC-1 and SW1990 cells was measured using CCK-8 assay. (C and D) The proliferation of PANC-1 and SW1990 cells was assessed using EdU assay. (E and F) The migration of PANC-1 and SW1990 cells was determined using wound-healing assay. (G and H) The invasion of PANC-1 and SW1990 cells was measured using transwell invasion assay. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

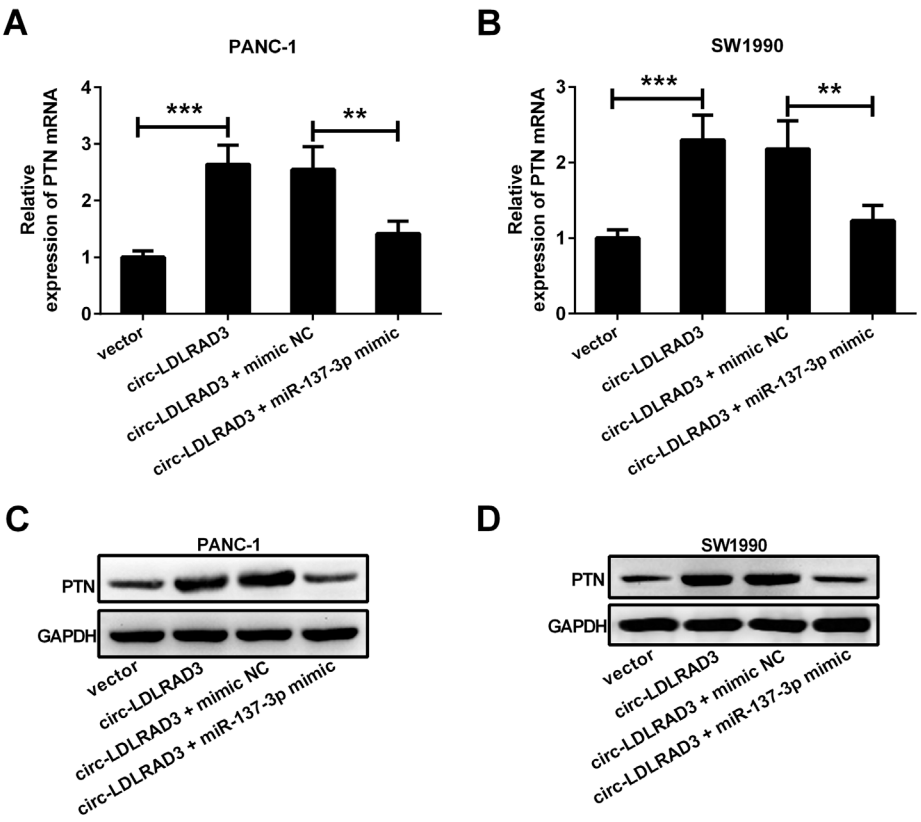

Fig. 5. circ-LDLRAD3 regulates the expression of PTN through sponging miR-137-3p. PANC-1 and SW1990 cells were co-transfected with circ-LDLRAD3 or Vector with miR-137-3p mimic or mimic NC. (A and B) RT-qPCR analysis of PTN expression in PANC-1 and SW1990 cells. (C and D) Western blot analysis of PTN expression in PANC-1 and SW1990 cells. $^{**}P < 0.01$; $^{***}P < 0.001$.

GTT GTT CCG CTT C-3′); PTN 5′ primer (5′-CCA TTT CCC TTC CGT TCC-3′) and 3′ primer (5′-AGG TTG CTA CCG CTG AGT CC-3′); GAPDH 5′ primer (5′-GGG AAA CTG TGG CGT GAT-3′) and 3′ primer (5′-GAG TGG GTG TCG CTG TTG A-3′); miR-137-3p 5′ primer (5′-ACA CTC ATT ATT GCT TA-3′) and 3′ primer (5′-CTA CGC GTA TTG AGA GTA C-3′); U6 5′ primer (5′-CTC GCT TCG GCA GCA CA-3′) and 3′ primer (5′-AAC GCT TCA CGA ATT TGC GT-3′). GAPDH was regarded as a housekeeping gene for circ-LDLRAD3 and PTN, whereas, U6 was employed as an internal control for miR-137-3p. The $2^{-\Delta\Delta Ct}$ method was applied to evaluate the expression of circ-LDLRAD3, miR-137-3p and PTN.

2.11. In vivo study

Animal studies were performed in accordance with the guiding principles of institutional animal ethics committee and with approval by the animal care and use committees at The First Affiliated Hospital, and College of Clinical Medicine of Henan University of Science and Technology. SW1990 cells were stably transfected with sh-NC or sh-circ-LDLRAD3. Afterwards, these cells were subcutaneously inoculated into Balb/c-nude mice (Slac, Shanghai, China). Tumor size was monitored weekly using calipers following inoculation of SW1990 cells expressing sh-NC or sh-circ-LDLRAD3. The tumor volumes were calculated according to the formula: volume = 0.5 × length × width^2. 5 weeks after inoculation, mice were euthanized, and the tumor weight was measured.

2.12. Statistical analysis

The results were given as mean ± standard deviation. Statistical analyses were carried out using one-way analysis of variance or student's *t* test with SPSS software (version 20.0; IBM, SPSS, Chicago, IL, USA). Difference was defined as $P < 0.05$.

3. Results

3.1. Characterization of circ-LDLRAD3 in pancreatic cancer

There is no denying that circRNAs are characterized by the presence of covalently linked ends. To identify the circular characteristics of circ-LDLRAD3, we treated total RNA from pancreatic cancer cells (PANC-1 and SW1990) with RNase R for 30 min, and found that the expression of LDLRAD3 mRNA but not circ-LDLRAD3 was markedly reduced following treatment with RNase R, providing strong evidence that circ-LDLRAD3 is resistant to RNase R treatment (Fig. 1A). Also, we found that the expression of LDLRAD3 in SW1990 cells was time-dependently decreased following treatment with actinomycin D, while circ-LDLRAD3 was not (Fig. 1B). Additionally, a marked elevation of circ-LDLRAD3 expression was discovered in pancreatic cancer cell lines (BXPC-3, Hs 766T, CFPAC-1, PANC-1 and SW1990), especially in PANC-1 and SW1990 cells, relative to HPDE cells (Fig. 1C). In line with this, we found that circ-LDLRAD3 expression was strikingly increased in pancreatic cancer tissues compared with that in their corresponding adjacent non-cancerous tissues (Fig. 1D). Interestingly, pancreatic cancer patients with high circ-LDLRAD3 expression had a poor overall survival than patients with low circ-LDLRAD3 expression (Fig. 1E).

3.2. Knockdown of circ-LDLRAD3 inhibits proliferation, migration and invasion of pancreatic cancer in vitro

In order to characterize the role of circ-LDLRAD3 in pancreatic cancer, we knockdown circ-LDLRAD3 by sh-circ-LDLRAD3A in PANC-1 and SW1990 cells. The transfection efficiency was confirmed using RT-qPCR. Compared with cells transfected with sh-NC, PANC-1 and SW1990 cells transfected with sh-circ-LDLRAD3A exerted reduced expression of circ-LDLRAD3 (Fig. 2A and B). As determined by CCK-8 assay, knockdown of circ-LDLRAD3 inhibited the viability of PANC-1 and SW1990 cells (Fig. 2C and D). Meanwhile, the results of EdU assay revealed that the percentage of EdU staining cells was markedly

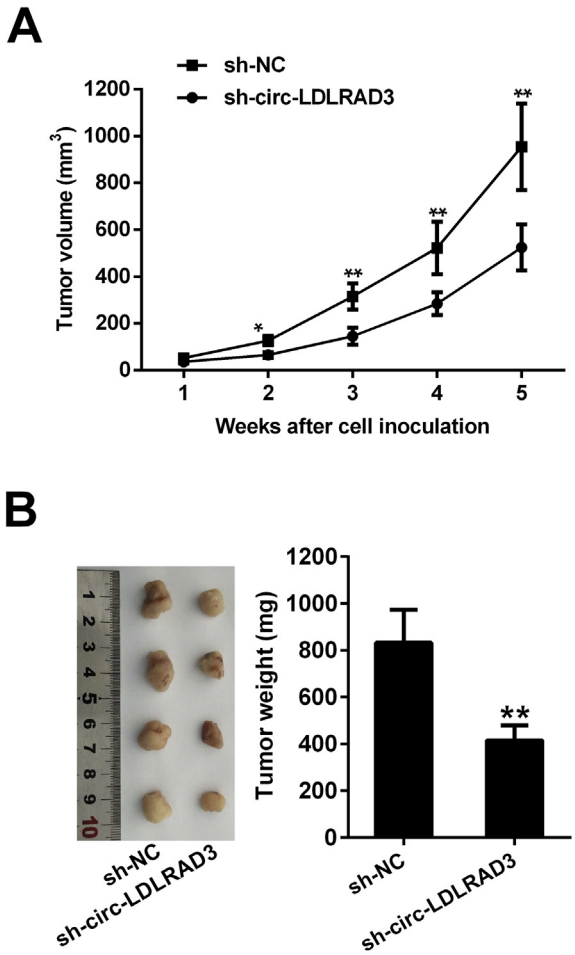

Fig. 6. Knockdown of circ-LDLRAD3 inhibits the growth of pancreatic cancer xenograft tumor *in vivo*. A mouse model of pancreatic cancer was established by subcutaneously inoculating SW1990 cells stably expressing sh-NC or sh-circ-LDLRAD3. (A) Tumor volume was monitored and recorded every week. (B) At the 5th week after inoculation, tumors were excised and weighed. $^{*}P < 0.05$; $^{**}P < 0.01$.

reduced following transfection of sh-circ-LDLRAD3A (Fig. 2E and F). Moreover, silencing of circ-LDLRAD3 obviously inhibited the migration and invasion of PANC-1 cells, and similar results were observed in SW1990 cells (Fig. 2H–J).

3.3. circ-LDLRAD3 functions as a sink for miR-137-3p

To identify the circ-LDLRAD3 target, we used a bioinformatics search (starBase) to predict the putative miRNA target of circ-LDLRAD3, and found that the sequence of circ-LDLRAD3 contains the putative binding sites of miR-137-3p (Fig. 3A). Moreover, a negative correlation between circ-LDLRAD3 and miR-137-3p expression was seen in pancreatic cancer tissues (Fig. 3B). Also, knockdown of circ-LDLRAD3 caused a marked increase in miR-137-3p expression. Conversely, overexpression of circ-LDLRAD3 led to reduced expression of miR-137-3p in PANC-1 cells (Fig. 3C). While, the similar results were found in SW1990 cells (Fig. 3D). To further confirm whether miR-137-3p is a direct target of circ-LDLRAD3, luciferase reporter assay was conducted in PANC-1 and SW1990 cells. Transfection of miR-137-3p mimic but not mimic NC remarkably decreased the relative luciferase activity of reporter containing WT-circ-LDLRAD3 in PANC-1 and SW1990 cells. However, the relative luciferase activity of reporter containing Mut-circ-LDLRAD3 in PANC-1 and SW1990 cells was unaffected upon miR-137-3p mimic or mimic NC transfection (Fig. 3E and F).

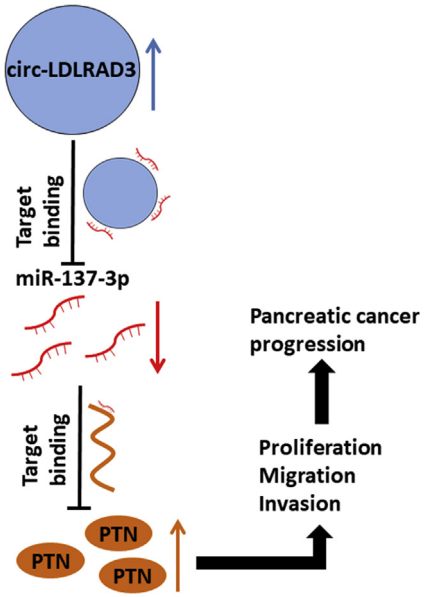

Fig. 7. The schematic diagram of circ-LDLRAD3 in pancreatic cancer. Increased circ-LDLRAD3 causes the upregulation of PTN by acting as a miR-137-3p sponge, thus accelerating the progression of pancreatic cancer.

3.4. Overexpression of circ-LDLRAD3 mitigates the inhibition effect of miR-137-3p on the biological behavior of pancreatic cancer cells

To investigate if circ-LDLRAD3-mediated growth promotion of pancreatic cancer cells requires miR-137-3p, we co-transfected PANC-1 and SW1990 cells with miR-137-3p mimic or mimic NC and circ-LDLRAD3 or Vector. Upregulation of miR-137-3p decreased the viability of PANC-1 and SW1990 cells, and forced expression of circ-LDLRAD3 mitigated the effect of miR-137-3p (Fig. 4A and B). Similarly, overexpression of miR-137-3p in PANC-1 and SW1990 cells resulted in inhibition of cell proliferation as evidenced by a reduced percentage of EdU staining cells, which was blocked by circ-LDLRAD3 overexpression (Fig. 4C and D). Simultaneously, upregulation of miR-137-3p repressed the migration of PANC-1 and SW1990 cells, however, this repression was partially reversed by circ-LDLRAD3 overexpression (Fig. 4E and F). Furthermore, ectopic expression of miR-137-3p inhibited the invasion of PANC-1 and SW1990 cells, while this action was abrogated by circ-LDLRAD3 overexpression (Fig. 4G and H).

3.5. circ-LDLRAD3 regulates the expression of PTN via miR-137-3p

To determine whether circ-LDLRAD3 and miR-137-3p regulate the expression of PTN, we transfected PANC-1 and SW1990 cells with circ-LDLRAD3 alone or with miR-137-3p mimic. As detected by RT-qPCR, overexpression of circ-LDLRAD3 markedly increased the expression of PTN mRNA in PANC-1 and SW1990 cells, which was attenuated by miR-137-3p overexpression (Fig. 5A and B). In line with this, the protein level of PTN was reduced in PANC-1 and SW1990 cells following circ-LDLRAD3 overexpression, however, this change induced by circ-LDLRAD3 overexpression was blocked by miR-137-3p overexpression (Fig. 5C and D).

3.6. Knockdown of circ-LDLRAD3 inhibits the growth of pancreatic cancer xenograft tumor in vivo

Given the tumor-promoting role of circ-LDLRAD3 *in vitro*, we further explore whether circ-LDLRAD3 knockdown inhibits the growth of pancreatic cancer *in vivo*. SW1990 cells stably expressing sh-NC or sh-

circ-LDLRAD3 were subcutaneously inoculated into Balb/c-nude mice. As expected, the volume of xenograft tumors from sh-circ-LDLRAD3-expressing SW1990 cells was much lower than tumors from sh-NC-expressing SW1990 cells (Fig. 6A). Meanwhile, an obvious reduction in tumor weight was noted following inoculation of SW1990 cells stably expressing sh-circ-LDLRAD3 (Fig. 6B). Our data demonstrate that increased circ-LDLRAD3 causes the upregulation of PTN by acting as a miR-137-3p sponge, ensuing promoting the progression of pancreatic cancer (Fig. 7).

4. Discussion

Discoveries of circRNAs open up new avenues for cancer biology research, which is of important directive significance for targeting therapy of cancer. The clinical importance of circRNAs in cancers has been widely discussed. For instance, Li et al. using microarray to explored the expression profile of circRNAs in pancreatic ductal adenocarcinoma and found 351 differential expressed circRNAs (209 were upregulated and 142 were downregulated) between pancreatic ductal adenocarcinoma tissues and their paired non-cancerous tissues, providing novel insights into pancreatic ductal adenocarcinoma biology [16]. Tumor stratification, on the basis of special circRNA expression levels, could be utilized as diagnostic and prognostic biomarkers for pancreatic cancer [17]. circRNA_100782 was found to be upregulated in pancreatic ductal adenocarcinoma and silencing of circRNA_100782 repressed the proliferation of BxPC3 cells through miR-124/interleukin-6 receptor/signal transducer and activator of transcription 3 pathway [18]. Moreover, circ_0007534 as an oncogene facilitated cell proliferation, migration and invasion, and repressed cell apoptosis by sponging miR-625 and miR-892b in pancreatic ductal adenocarcinoma [19]. Additionally, functional studies in PANC1 cells revealed that knockdown of circ_0030235 repressed cells proliferation, migration and invasion, promoted cell apoptosis through sponging miR-1253 and miR-1294 [17]. Besides, circZMYM2 sponged with miR-335-5p and promoted the expression of jumonji domain containing protein 2C, ultimately promoting the proliferation and invasion and inhibiting the apoptosis of pancreatic cancer cells [20]. As described by Yang et al., circ-LDLRAD3, located at chr11:36248634–36248980, was upregulated in pancreatic cancer, and tightly related with venous invasion and lymphatic invasion [11]. Nevertheless, no functional studies have been performed addressing the role of circ-LDLRAD3 in pancreatic cancer. In this work, the upregulation of circ-LDLRAD3 was discovered in pancreatic cancer tissues and cell lines. Downregulation of circ-LDLRAD3 led to inhibition of cell proliferation, migration and invasion in PANC-1 and SW1990 cells *in vitro*, and repression of tumor growth *in vivo*, which adds to the growing body of evidence implicating circRNA-mediated regulation of cancer.

The classic role of circRNAs is to function as a sink for miRNA, as in the case of hsa_circ_0006215 and ciRS-7 [21,22]. They reportedly interact with miRNAs and serve as candidates for competing endogenous RNAs, thereby upregulating the expression of their target genes. In our experiments, the interaction between circ-LDLRAD3 and miR-137-3p was predicted by StarBase software and identified by luciferase reporter assay. Interestingly, aberrant function of miR-137 has been reported in a host of cancers. miR-137 has been shown to be underexpressed in pancreatic cancer and significantly associated with tumor size, tumor node metastasis stage and worst prognosis, suggesting the prognostic role of miR-137. Moreover, miR-137 acted as a tumor suppressor in pancreatic cancer by targeting MORF4-related gene-binding protein [23]. Functional experiments in AsPC-1 and PANC-1 cells revealed that miR-137, through targeting KLF12-associated Wnt/β-catenin pathways, suppressed pancreatic cancer cell stemness [24]. miR-137 was also shown to sensitize PANC-1 cells to doxorubicin via inhibiting autophagy resulting through inhibition of autophagy-related gene 5 [25]. In addition, increased expression of miR-137 has been causally linked to the proliferation and senescence of pancreatic cancer cells [26]. However, the potential involvement of miR-137-3p in circ-LDLRAD3-mediated growth regulation of pancreatic cancer cells has not been characterized. Here, we provided evidence that miR-137-3p exerted an inhibitory effect on pancreatic cancer cell proliferation, migration and invasion, however, this effect could be mitigated by circ-LDLRAD3 overexpression, showing that circ-LDLRAD3 could play a crucial role in pancreatic oncogenesis by targeting miR-137-3p.

As our previous study suggested that higher expression of PTN was closely related with perineural invasion, large bloody ascites, liver metastasis, and reduced survival time, demonstrating the prognostic role of PTN in pancreatic cancer [27]. Upregulation of PTN was considered as a principal contributor to the perineural invasion of pancreatic cancer [28]. More importantly, PTN has been identified as a target of miR-137. A recent study in hypertrophic scar fibroblasts also found that there is a negative correlation between miR-137 and PTN in hypertrophic scars. Overexpression of miR-137 repressed the proliferation and metastasis of hypertrophic scar fibroblasts and this effect was mediated by inhibiting PTN expression [29]. Additionally, miR-137 repressed the growth and metastasis of pancreatic cancer, and reduced 5-fluorouracil-induced cytotoxicity in PANC-1 and MIA PaCa-2 cells. However, these actions could be rescued by knockdown of PTN, indicating that miR-137 regulates pancreatic cancer progression by targeting PTN [30]. As mentioned above, we speculated that circ-LDLRAD3 might regulate pancreatic cancer progression through miR-137-3p/PTN axis. Herein, we found that circ-LDLRAD3 negatively controls the expression of PTN through miR-137-3p. This finding indicated that circ-LDLRAD3 repressed pancreatic cancer progression as ceRNA to target PTN expression by sponging miR-137-3p, representing a new mechanism for pancreatic cancer.

In conclusion, our findings suggest that circ-LDLRAD3/miR-137-3p/PTN axis is another regulator of pancreatic cancer cell proliferation and metastasis. Elucidating the pathogenic role of circ-LDLRAD3 and its underlying mechanism may provide a new potential therapeutic target for the treatment of pancreatic cancer.

Declaration of competing interest

The authors declare that there are no conflicts of interest.

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