IncRNA differentiation antagonizing nonprotein coding RNA overexpression accelerates progression and indicates poor prognosis in pancreatic ductal adenocarcinoma

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Background: IncRNA differentiation antagonizing nonprotein coding RNA (IncRNA DANCR) has been suggested to play an oncogenic role in multiple cancers. However, to the best of our knowledge, the clinical significance and role of DANCR in pancreatic ductal adenocarcinoma (PDAC) has not been illuminated till now. The present study aims to identify the functional role of DANCR in PDAC.

Methods: The expression of DANCR was detected in PDAC cells and tissues. The correlation of DANCR expression and PDAC clinicopathological features was analysed. Kaplan-Meier method was used to depict the overall survival (OS) rate and shorter progression-free survival (PFS) of PDAC patients, and Log-rank test was performed to analyse the difference. Univariate and multivariate COX regression model were utilized to analyse the risk factors for prognosis. Transwell assay and Matrigel assay were conducted to detect the effect of DANCR on the migration and invasion of PDAC cells, respectively. Colony formation assay and Cell Counting Kit-8 (CCK-8) assay were performed to evaluate the function of DANCR on proliferation. The mechanisms of DANCR exerting its function were also explored.

Results: DANCR was revealed to promote PDAC progression, with relatively higher expression levels in PDAC cell lines and tissues. Correlation analysis of the clinicopathological features and DANCR expression found that high DANCR expression was statistically correlated with vascular invasion ($P=0.013$), advanced T stage ($P=0.005$), lymph node metastasis ($P<0.001$) and advanced TNM stage ($P<0.001$). Notably, survival analysis discovered that high DANCR expression predicted lower OS rate and shorter PFS period. In addition, high DANCR expression was identified as an independent risk factor for poor OS (HR = 1.199, 95% CI = 1.113–1.290, $P<0.001$) and PFS (HR = 1.199, 95% CI = 1.114–1.290, $P<0.001$) of PDAC. Moreover, in vitro assays detected that the migration and invasion of Panc1 cells with DANCR deficiency were significantly suppressed in the Transwell assay and the Matrigel assay. However, the motility of BxPC3 cells with DANCR overexpression was obviously increased. In addition, the loss of DANCR suppressed the proliferation of Panc1 cells in the CCK-8 assay and the colony formation assay, while ectopic expression of DANCR in BxPC3 cells promoted the proliferation. Besides, microRNA-33a-5p/AXL signaling pathway may be involved in mediating the function of DANCR.

Conclusion: Overexpression of IncRNA DANCR in PDAC is associated with cancer progression and predicts poor OS and PFS. DANCR could promote the proliferation and metastasis of PDAC cells. DANCR may serve as a potential prognostic marker and therapeutic target in PDAC.

Keywords: IncRNA DANCR, pancreatic cancer, prognosis, proliferation, metastasis
Introduction

Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal malignancies. The 5-year survival rate increased only from 3% to 8% over the past 40 years.\(^1\) The reasons for such dismal survival include lack of early detection, presentation at late stages and inadequate current therapies.\(^2\) Most PDAC patients lack specific symptoms at early stage, and only about 20% of PDAC patients have the opportunity to receive curative resection. Carbohydrate antigen (CA) 19-9 is the most commonly used tumor marker for the diagnosis of PDAC in clinical practice; the median sensitivity and specificity of CA19-9 for the diagnosis are only 75.5% and 77.6%, respectively, with a low positive predictive value of 0.5%-0.9%, which does not qualify it as a useful screening parameter.\(^3\)-\(^5\) No conventional biomarkers have proven to be a specific and reliable tool for the early detection of PDAC.\(^5\)

Considering the complexity of cancer biology, combination of different markers as diagnostic or prognostic indices appears promising. Therefore, more explorations are needed to validate novel diagnostic and prognostic markers.

lncRNAs are defined as endogenous cellular RNAs of more than 200 nucleotides in length and lack an open reading frame of significant length (<100 amino acids).\(^6\)-\(^8\) lncRNAs are found in almost every branch of life and involved in numerous important biological phenomena, such as imprinting genomic loci, shaping chromosome conformation and allosterically regulating enzymatic activity.\(^9\)-\(^9\) Specific patterns of lncRNA expression coordinate cell state, differentiation, development and disease.\(^10\)-\(^12\) More interestingly, the aberrant expression of lncRNAs is discovered to be involved in cancer initiation and progression through transcriptional and posttranscriptional regulations.\(^13\),\(^14\) In addition, mounting evidence showed that lncRNAs are expressed in a tissue-specific manner, which makes them an ideal biomarker for cancer diagnosis and therapeutic target.\(^15\) lncRNAs have shown potential as biomarkers in the diagnosis and prognosis of bladder cancer, prostate cancer, gastric cancer, pancreatic cancer, breast cancer and many other cancer types.\(^16\)

lncRNA differentiation antagonizing nonprotein coding RNA (DANCR) was first identified as an 855 bp lncRNA downregulated during differentiation by Kretz et al.\(^17\) Subsequently, Yuan et al\(^18\) reported that DANCR could increase the stemness features and predict prognosis in hepatocellular carcinoma. After that, the oncogenic role of DANCR in gastric cancer,\(^19\) colorectal cancer,\(^20\) prostate cancer,\(^20\) and lung adenocarcinoma\(^22\) has been reported. However, the clinical significance and role of DANCR in PDAC has not been illuminated yet.

Our study aimed to measure the expression level of DANCR in PDAC cell lines and tissues, and the significance of DANCR in the clinical progression of PDAC was verified. Moreover, the prognostic value of DANCR was analyzed. In addition, the functional role of DANCR was analyzed.

Materials and methods

Cell lines and cell culture

The human pancreatic cancer cell lines Panc1, Panc28, AsPC1, MiaPaCa2 and BxPC3 and the human pancreatic ductal epithelial cell line HPDE were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). Cells were cultured in Roswell Park Memorial Institute-1640 medium (AsPC1, BxPC3 and HPDE) or DMEM (HyClone; Thermo Fisher Scientific, Waltham, MA, USA; Panc1, Panc28 and MiaPaCa2) supplemented with 10% FBS (Hyclone), 100 U/mL penicillin and 100 mg/mL streptomycin. All cells were cultured in a humidified incubator with 5% CO\(_2\) at 37°C.

All the siRNAs and DANCR ectopic overexpression plasmid used in the current study were synthesized by GenePharma (Shanghai, China). Transfection was conducted using the Lipofectamine™ 2000 transfection reagent (Thermo Fisher Scientific) according to the protocol recommended by the manufacturer. The transfected cells were utilized for further investigations 48 hours later.

Clinical specimens

The 206 PDAC tissues and paired tumor adjacent tissues were collected from surgical resections at the general surgery department of Dazhou Central Hospital. The tumor-adjacent tissues, defined as normal tissues in routine pathological results, were obtained 2 cm away from the PDAC tissues. These tissues were divided into two groups, the low DANCR expression group (n=120) and the high DANCR expression group (n=86), with the mean DANCR expression level serving as the cutoff value. The clinicopathological characteristics of the PDAC patients are summarized in Table 1. All the patients involved in the current study received radical surgical resection without preoperative chemotherapy or radiotherapy. The collected specimens were snap frozen in liquid nitrogen and stored at −80°C until being used. Tissue specimen collections were made with full informed consent of all patients following institutional ethical guidelines that were reviewed and approved by the ethics committee of Dazhou Central Hospital.

RNA isolation, RNA extraction and quantitative real time (qRT)-PCR

Total RNA was extracted from tissues or cultured cells using TRIzol reagent (Thermo Fisher Scientific). One microgram of
total RNA was reversely transcribed in a final volume of 20 μL under standard conditions using PrimeScript RT Reagent Kit (TaKaRa, Dalian, China). After the reverse transcription, 1 μL of the complementary DNA was used for subsequent reactions. The qRT-PCR reactions were performed using an ABI7500 System (Thermo Fisher Scientific) and SYBR Green PCR Master Mix (TaKaRa). GAPDH was used as endogenous control. The primers used in this study are as follows: DANCR: forward 5'-GCCACTATGAGCGGTTTCT-3', reverse 5'-ACCTGCGCTAAAGAATTGAG-3'; GAPDH: forward 5'-TGCACCCACAACTGGTTAC-3', reverse 5'-GGCATGACCTGGTTGATGAG-3'. All assays were performed in triplicate. Statistical analyses of the results were performed using the 2-ΔΔCt relative quantification method.

Transwell assay and Matrigel assay
The transfected cells and corresponding control cells (1×10⁶) were suspended with fresh medium (200 μL) and added into the upper side of Transwell chambers (8 μm pore size; BD Biosciences, San Jose, CA, USA), uncoated (in Transwell assay) or coated (in Matrigel assay) with 50 μL Matrigel (BD Biosciences). The bottom chamber was filled with medium containing 20% FBS, working as chemo-attractant. After 24-hour incubation, nonmigrated cells in the upper chamber were removed and then the migrated cells in the downside of the chamber were fixed with 4% paraformaldehyde for 30 minutes and stained with Giemsa (1:10 dilution) for 30 minutes at room temperature. The cell numbers were counted in five random fields of each chamber under the microscope.

Colony formation assay
Duplicate cultures of transfected PDAC cells and corresponding control cells (500 cells/well) were seeded in six-well plates and maintained at 37°C in a 5% CO₂ atmosphere, and the fresh medium was added every 2 days. Two weeks later, formed colonies were fixed with methanol and stained with 0.1% crystal violet (Sigma-Aldrich Co., St Louis, MO, USA). Colonies with more than 50 cells were counted. Each experiment was repeated in triplicate.

Cell Counting Kit-8 (CCK-8) assay
The CCK-8 (Dojindo, Kumamoto, Japan) assay was conducted according to the manufacturer’s protocol. Briefly, transfected PDAC cells and corresponding control cells (2×10⁴ cells per well) were plated in 24-well plates in triplicate. CCK-8 reagent was added at the indicated time points (0, 24, 48, 72 and 96 hours), and the cells were cultured for a further 4 hours at 37°C. Absorbance at 450 nm was measured using a microplate reader.

Statistical analyses
All statistical analyses were performed using SPSS 16.0 (SPSS Inc., Chicago, IL, USA). Continuous data were analyzed using an independent t-test between two groups. Categorical data were analyzed using the chi-squared test or Fisher’s exact test as appropriate. Overall survival (OS)
rate and progression-free survival (PFS) rate were calculated using the Kaplan–Meier method and the log-rank test for comparisons. Multivariate survival analyses were performed on all factors that were significant in univariate analyses using the Cox regression model. A \( P \)-value of <0.05 was considered to represent statistical significance.

Results

DANCR overexpression implicates cancer progression in PDAC

Comparison of DANCR expression in PDAC cell lines with human pancreatic ductal epithelial cell line, HPDE, was carried out by quantitative real-time polymerase chain reaction (qRT-PCR) assay. The expression level of DANCR was measured to be significantly higher in PDAC cell lines (BxPC3, Panc28, AsPC1, MiaPaCa2 and Panc1) compared to HPDE. (Figure 1A)

The expression level of DANCR in PDAC tissues relative to paired tumor-adjacent tissues was detected by qRT-PCR assay and calculated. (Figure 1B) The expression level of DANCR in PDAC tissues with vascular invasion and T-stage T1 and T2 detected by qRT-PCR assay was compared with PDAC tissues without vascular invasion and T-stage T3 and T4. (Figure 1C, 1D) The expression level of DANCR in PDAC tissues with lymph node metastasis detected by qRT-PCR assay was compared with PDAC tissues without lymph node metastasis. (Figure 1E) The expression level of DANCR in PDAC tissues with TNM early stages detected by qRT-PCR assay was compared with PDAC tissues with TNM advanced stages. *\( P \)-value <0.05.

Abbreviations: DANCR, differentiation antagonizing nonprotein coding RNA; PDAC, pancreatic ductal adenocarcinoma; qRT-PCR, quantitative real time polymerase chain reaction.

Figure 1 DANCR overexpression implicates cancer progression in PDAC.

Notes: (A) Expression of DANCR in five PDAC cell lines (BxPC3, Panc28, AsPC1, MiaPaCa2 and Panc 1) and human pancreatic ductal epithelial cell line, HPDE, was measured by qRT-PCR assay. (B) The expression level of DANCR in PDAC tissues relative to paired tumor-adjacent tissues was detected by qRT-PCR assay and calculated. (C) The expression level of DANCR in PDAC tissues with vascular invasion evaluated by qRT-PCR assay was compared with PDAC tissues without vascular invasion. (D) The expression level of DANCR in PDAC tissues with T-stage T1 and T2 detected by qRT-PCR assay was compared with PDAC tissues with T-stage T3 and T4. (E) The expression level of DANCR in PDAC tissues with lymph node metastasis detected by qRT-PCR assay was compared with PDAC tissues without lymph node metastasis. (F) The expression level of DANCR in PDAC tissues with TNM early stages detected by qRT-PCR assay was compared with PDAC tissues with TNM advanced stages. *\( P \)-value <0.05.
out by the qRT-PCR assay, which revealed that DANCR was overexpressed in five PDAC cell lines compared with HPDE cells ($P<0.05$; Figure 1A). For further determination of the expression pattern of DANCR in PDAC, DANCR expression was evaluated in 206 PDAC tissues and paired tumor-adjacent tissues. Interestingly, PDAC tissues displayed a notably higher DANCR expression level than paired tumor-adjacent tissues ($P<0.05$; Figure 1B). The PDAC tissues were dichotomized with the mean expression level of DANCR serving as the cutoff value, including the high DANCR expression group ($n=86$) and the low DANCR expression group ($n=120$). The relationship between DANCR expression and clinicopathological features of PDAC was statistically analyzed, which found that high DANCR expression correlated with vascular invasion ($P=0.013$), advanced T stage ($P=0.005$), lymph node metastasis ($P<0.001$) and advanced TNM stage ($P<0.001$; Table 1). In addition, patients with vascular invasion (Figure 1C), advanced T stage (Figure 1D), lymph node metastasis (Figure 1E) and advanced TNM stage (Figure 1F) exhibited much higher DANCR expression level. Overall, high DANCR expression indicates advanced tumor stage, and DANCR may promote the clinical progression of PDAC.

DANCR overexpression indicates poor prognosis in PDAC

To evaluate the prognostic significance of DANCR in PDAC, the OS rate and PFS rate of PDAC patients with high DANCR expression and low DANCR expression were depicted with the Kaplan–Meier analysis and compared with the log-rank test. As shown in Figure 2A and B, patients with low DANCR expression had a significantly higher OS rate and PFS rate. Univariate analysis found that high DANCR expression (HR $=1.224$, 95% CI $=1.120–1.302$, $P<0.001$) was one of the six risk factors related to poor OS of PDAC (Table 2). Besides, multivariate analysis further identified high DANCR expression as an independent risk factor of poor OS of PDAC ($HR =1.199$, 95% CI $=1.113–1.290$, $P<0.001$; Table 2). Similarly, univariate analysis found that high DANCR expression (HR $=1.219$, 95% CI $=1.146–1.296$, $P<0.001$) was one of the six risk factors related to poor PFS of PDAC (Table 3). In addition, multivariate analysis further identified high DANCR expression as an independent risk factor of poor PFS of PDAC ($HR =1.199$, 95% CI $=1.114–1.290$, $P<0.001$; Table 3). Taken together, high DANCR expression predicts poor OS and PFS, and high DANCR expression is an independent risk factor of poor OS and PFS.

DANCR accelerates metastasis and proliferation of PDAC cells

The abovementioned observations suggested that DANCR expression level may be associated with clinical progression and poor prognosis of PDAC. The following assays would try to detect the functional role of DANCR in metastasis and proliferation of PDAC. The expression of DANCR was silenced and upregulated in Panc1 cells and BxPC3 cells, respectively (Figure 3A and B). The Transwell assay and the Matrigel assay found that the loss of DANCR obviously inhibited the migration and invasion of Panc1 cells, respectively (Figure 3C). Therefore, the migration and invasion abilities were evidently accelerated after DANCR

![Figure 2](image_url)  
**Figure 2** DANCR overexpression indicates poor prognosis in PDAC.  
**Notes:** (A) The OS rate of PDAC patients with low DANCR expression and high DANCR expression was depicted with the Kaplan–Meier analysis and compared with the log-rank test. (B) The PFS rate of PDAC patients with low DANCR expression and high DANCR expression was depicted with the Kaplan–Meier analysis and compared with the log-rank test.  
**Abbreviations:** DANCR, differentiation antagonizing nonprotein coding RNA; OS, overall survival; PDAC, pancreatic ductal adenocarcinoma; PFS, progression-free survival.
overexpression in BxPC3 cells (Figure 3D). Furthermore, the colony formation assay and the CCK-8 assay found that DANCR deficiency notably decreased the colony numbers and OD value of Panc1 cells (Figure 4A and B). Accordingly, DANCR ectopic expression remarkably increased the colony numbers and OD value of BxPC3 cells (Figure 4C and D). These results confirmed that DANCR could accelerate the metastasis and proliferation of PDAC cells.

**DANCR may function through upregulating AXL via microRNA-33a-5p inhibition**

DANCR has been reported to competitively interact with miR-634 and miR-33a-5p, thus regulating downstream protein expression in glioma and osteosarcoma, respectively. The expression of miR-634 and miR-33a-5p was also investigated in PDAC cells by qRT-PCR. The results showed that miR-33a-5p was noticeably downregulated in BxPC3 with DANCR overexpression (Figure 5A), accompanied with increased expression of AXL (Figure 5B), the downstream protein of miR-33a-5p. Accordingly, when DANCR was silenced in Panc1 cells, the expression of miR-33a-5p was upregulated (Figure 5C), while the expression of AXL was significantly suppressed (Figure 5D). However, the expression of miR-634 and its downstream protein, RAB1A, was not changed when DANCR was knocked down or overexpressed (Figure 5A–D). These evidences indicate that DANCR may also function through upregulating AXL via microRNA-33a-5p inhibition in PDAC cells.

**Table 3** Univariate and multivariate analysis of clinicopathologic features for PFS of PDAC patients

| Parameters | Univariate analysis | Multivariate analysis |
|------------|---------------------|----------------------|
|            | HR  | 95% CI       | P-value | HR  | 95% CI       | P-value |
| Age: ≥60 years vs <60 years | 0.863 | 0.612–1.218 | 0.402 | 1.343 | 0.948–1.903 | 0.097 |
| Gender: male vs female | 1.444 | 1.091–1.925 | 0.056 | 1.391 | 1.047–1.861 | 0.021 |
| CEA: ≥4.5 μg/mL vs <4.5 μg/mL | 0.977 | 0.725–1.327 | 0.822 | 1.334 | 0.930–1.930 | 0.122 |
| CA19-9: ≤37 U/mL vs >37 U/mL | 0.981 | 0.765–1.265 | 0.860 | 0.991 | 0.748–1.351 | 0.932 |
| Tumor location: head and neck vs body and tail | 0.892 | 0.691–1.153 | 0.511 | 1.199 | 0.925–1.555 | 0.151 |
| Grade: well vs moderate vs poor | 1.141 | 0.876–1.492 | 0.302 | 1.115 | 0.865–1.454 | 0.491 |
| Size: <4 cm vs ≥4 cm | 0.992 | 0.699–1.406 | 0.963 | 1.199 | 0.925–1.555 | 0.151 |
| Vascular invasion: yes vs no | 1.165 | 0.824–1.646 | 0.388 | 1.114 | 0.865–1.454 | 0.491 |
| Lymphatic invasion: no vs yes | 1.416 | 1.004–1.996 | 0.047 | 1.343 | 0.948–1.903 | 0.097 |
| T stage: T1 + T2 vs T3 + T4 | 1.376 | 1.125–1.680 | 0.002 | 1.000 | 0.760–1.311 | 1.000 |
| N stage: N1 vs N0 | 1.000 | 0.767–1.283 | 1.000 | 0.556–1.136 | 0.047 |
| M stage: M1 vs M0 | 0.916 | 0.709–1.182 | 0.428 | 0.916 | 0.709–1.182 | 0.428 |
| TNM stage: early stages (≤IIa) vs advanced stages (>IIa) | 1.593 | 1.131–2.244 | 0.008 | 1.199 | 0.925–1.555 | 0.151 |
| DANCR: high vs low | 1.219 | 1.146–1.296 | 0.001 | 1.199 | 0.925–1.555 | 0.151 |

Abbreviations: DANCR, differentiation antagonizing nonprotein coding RNA; PDAC, pancreatic ductal adenocarcinoma; PFS, progression-free survival.
Conclusion
Increasing data suggest that lncRNAs play pivotal roles in the progression of PDAC, which indicated that lncRNAs were involved in tumor growth, survival, epithelial-mesenchymal transition (EMT), tumor microenvironment, cancer stem cells (CSCs) and chemoresistance in PDAC.\textsuperscript{25} LncRNAs can mediate the expression of miRNA-targeted genes through functioning as miRNA sponge.\textsuperscript{26,27} EMT is

Figure 3 DANC is an antagonizing nonprotein coding RNA in PDAC cells.

Notes: (A) The relative expression level of DANC in Panc1 cells after DANC interference was determined by qRT-PCR assay. (B) The relative expression level of DANC in BxPC3 cells after DANC overexpression was confirmed by qRT-PCR assay. (C) The migration and invasion abilities of Panc1 cells with DANC silencing were analyzed with Transwell assay and Matrigel assay, respectively (right panel). Typical images are shown in the left panel. (D) The migration and invasion abilities of BxPC3 cells with DANC overexpression were revealed with Transwell assay and Matrigel assay, respectively (right panel). Typical images are shown in the left panel. *P<0.05.

Abbreviations: DANC, differentiation antagonizing nonprotein coding RNA; PDAC, pancreatic ductal adenocarcinoma; qRT-PCR, quantitative real time polymerase chain reaction.
an initial step in cancer metastasis. Accumulating studies found that IncRNAs participate in the EMT of PDAC. For example, IncRNA regulator of reprogramming was reported to promote the aggressive biological behaviors of PDAC by acting as a regulator of ZEB1, which is a primary transcriptional factor in the EMT progress, and thus increase the invasion and metastasis of PDAC. CSCs are also an important way by which IncRNAs exert their functions in modulating the development of PDAC. Chemoradioresistance is an essential reason leading to the relapse of cancer patients. Multiple studies have focused on the potential role of IncRNAs in chemoradioresistance. Combination of IncRNAs and conventional chemotherapeutic reagents is considered as a promising way for improving the sensitivity of adjuvant therapy. There are also studies that revealed that IncRNAs could modulate the epigenetic modifications and autophagy in PDAC. Obviously, IncRNAs can function in a wide range of cancer biology; however, clinical trials investigating IncRNAs in the treatment of PDAC are rare, and further studies are needed for utilizing IncRNAs in clinical practice.

Since being discovered in 2012, DANCR has attracted much attention for its critical role in cancer biology. DANCR is now regarded as an oncogene for promoting cancer growth and metastasis in hepatocellular carcinoma, glioma, gastric cancer, osteosarcoma, lung adenocarcinoma, prostate cancer and colorectal cancer. DANCR was also implicated to be a diagnostic and prognostic marker. Mechanistically, DANCR could directly interact with miR-634 and this interaction resulted in the inhibition of RAB1A expression, thus accelerating the progression of glioma. By competitively binding to miR-33a-5p, DANCR could upregulate the expression of the receptor tyrosine kinase AXL and increase the function of CSCs in osteosarcoma. The mechanisms of DANCR mediating cancer progression are relatively rare, which deserves further investigations.
This study discovered that DANCIR was overexpressed in PDAC tissues compared with tumor-adjacent tissues. Further detection found that high DANCIR expression was correlated with vascular invasion ($P=0.013$), advanced T stage ($P=0.005$), lymph node metastasis ($P<0.001$) and advanced TNM stage ($P<0.001$), all of which are critical factors evaluating cancer progression and prognosis. Moreover, high DANCIR expression correlated with poor OS and PFS. Multivariate analysis identified high DANCIR expression as an independent survival risk factor for OS and PFS. In addition, DANCIR was confirmed to facilitate growth and metastasis of PDAC cells. These results indicated that DANCIR is a promising prognostic marker and therapeutic target in PDAC. In addition, DANCIR may function through upregulating AXL via microRNA-33a-5p inhibition.

In conclusion, our study confirmed the overexpression of DANCIR in PDAC cells and tissues. The clinical significance and prognostic value of DANCIR were also detected. In vitro assays demonstrated the oncogenic role of DANCIR. These observations demonstrated that DANCIR plays a crucial role in the progression of PDAC, and DANCIR might potentially serve as a prognostic marker and therapeutic target for PDAC patients.

**Disclosure**

The author reports no conflicts of interest in this work.

**References**

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. *CA Cancer J Clin*. 2016;66(1):7–30.
2. Gupta R, Amanam I, Chung V. Current and future therapies for advanced pancreatic cancer. *J Surg Oncol*. 2017;116(1):25–34.
3. Malesci A, Tommasini MA, Bonato C, et al. Determination of CA 19-9 antigen in serum and pancreatic juice for differential diagnosis of pancreatic adenocarcinoma from chronic pancreatitis. *Gastroenterology*. 1987;92(1):60–67.
4. Paganuzzi M, Onetto M, Marroni P, et al. CA 19-9 and CA 50 in benign and malignant pancreatic and biliary diseases. *Cancer*. 1988;61(10):2100–2108.
5. Loosen SH, Neumann UP, Trautwein C, Roderburg C, Luedde T. Current and future biomarkers for pancreatic adenocarcinoma. *Tumour Biol*. 2017;39(6):1010428317692231.

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**Figure 5** DANCIR functions through upregulating AXL via microRNA-33a-5p inhibition. 

**Notes:**

(A) Expression of miR-33a-5p and miR-634 was measured by qRT-PCR in BxPC3 cells with DANCIR overexpression. (B) Expression of AXL and RAB1A was detected by qRT-PCR in BxPC3 cells with DANCIR overexpression. (C) Expression of miR-33a-5p and miR-634 was measured by qRT-PCR in Panc1 cells with DANCIR deficiency. (D) Expression of AXL and RAB1A was detected by qRT-PCR in Panc1 cells with DANCIR deficiency. *P* $< 0.05$. 

**Abbreviations:** DANCIR, differentiation antagonizing nonprotein coding RNA; qRT-PCR, quantitative real time polymerase chain reaction.
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