

RETRACTED ARTICLE: lncRNA differentiation antagonizing nonprotein coding RNA overexpression accelerates progression and indicates poor prognosis in pancreatic ductal adenocarcinoma

Lei Chen*

Jie Liu*

Tong Tang

Yong-Chuan Zhang

Ming-Zhong Liu

Li-Ya Xu

Jun Zhang

General Surgery Department, Dazhou
Central Hospital, Dazhou 635000,
Sichuan, China

*These authors contributed equally
to this work

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

Methods: The expression of DANCER was detected in PDAC cells and tissues. The correlation of DANCER 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 DANCER 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 DANCER on proliferation. The mechanisms of DANCER exerting its function were also explored.

Results: DANCER was revealed to promote PDAC progression, with relatively higher expression levels in PDAC cell lines and tissues. Correlation analysis of the clinicopathological features and DANCER expression found that high DANCER 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 DANCER expression predicted lower OS rate and shorter PFS period. In addition, high DANCER 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 DANCER deficiency were significantly suppressed in the Transwell assay and the Matrigel assay. However, the motility of BxPC3 cells with DANCER overexpression was obviously increased. In addition, the loss of DANCER suppressed the proliferation of Panc1 cells in the CCK-8 assay and the colony formation assay, while ectopic expression of DANCER in BxPC3 cells promoted the proliferation. Besides, microRNA-33a-5p/AXL signaling pathway may be involved in mediating the function of DANCER.

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

Keywords: lncRNA DANCER, pancreatic cancer, prognosis, proliferation, metastasis

Correspondence: Jun Zhang
General Surgery Department, Dazhou
Central Hospital, No 56, South YueMiao
Street, Dazhou 635000, Sichuan, China
Tel +86 1 589 299 6013
Email zjdazhou@sohu.com

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.^{8,9} Specific patterns of lncRNA expression coordinate cell state, differentiation, development and disease.^{10–12} Interestingly, the aberrant expression of lncRNAs has been 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 down-regulated during differentiation by Kretz et al.¹⁷ Subsequently, Yuan et al¹⁸ 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 cancer²¹ and lung adenocarcinoma²² 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 in proliferation and metastasis of PDAC was defined by *in vitro* assays.

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% CO₂ at 37°C.

All the siRNAs and DANCR ectopic overexpression plasmid used in the current study were synthesized by General (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

Table 1 Relationship between lncRNA DANCR expression and clinicopathological characteristics of PDAC

| Parameters | No of patients (n=206) | DANCR (high/low) | P-value |
|-------------------------|------------------------|------------------|---------|
| Age | | | 0.067 |
| <60 years | 90 | 44/46 | |
| ≥60 years | 116 | 42/74 | |
| Gender | | | 0.133 |
| Male | 132 | 50/82 | |
| Female | 74 | 36/38 | |
| CEA | | | 0.675 |
| <4.5 µg/mL | 154 | 63/91 | |
| ≥4.5 µg/mL | 52 | 23/29 | |
| CA19-9 | | | 0.965 |
| <37 U/mL | 89 | 37/52 | |
| ≥37 U/mL | 117 | 49/68 | |
| Tumor location | | | 0.686 |
| Head and neck | 135 | 55/80 | |
| Body and tail | 71 | 31/40 | |
| Grade | | | 0.571 |
| Well + moderate | 141 | 57/84 | |
| Poor + undifferentiated | 65 | 29/36 | |
| Size | | | 0.195 |
| <4 cm | 121 | 46/75 | |
| ≥4 cm | 85 | 40/45 | |
| Neural invasion | | | 0.586 |
| No | 86 | 34/52 | |
| Yes | 120 | 52/68 | |
| Vascular invasion | | | 0.113 |
| No | 112 | 38/74 | |
| Yes | 94 | 48/46 | |
| Lymphatic invasion | | | 0.511 |
| No | 69 | 23/46 | |
| Yes | 137 | 55/82 | |
| T stage | | | 0.005 |
| T1 + T2 | 138 | 49/89 | |
| T3 + T4 | 68 | 37/29 | |
| Lymph node metastasis | | | <0.001 |
| No | 121 | 36/85 | |
| Yes | 85 | 50/35 | |
| Distant metastasis | | | 0.435 |
| No | 200 | 85/115 | |
| Yes | 6 | 3/2 | |
| TNM stage | | | <0.001 |
| Early stage (≤Ia) | 113 | 35/78 | |
| Advanced stage (>IIA) | 92 | 51/41 | |

Abbreviations: DANCR, differentiation antagonizing nonprotein coding RNA; PDAC, pancreatic ductal adenocarcinoma; CEA, carcinoembryonic antigen.

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'-GCCACTATGTAGCGGGTTTC-3', reverse 5'-ACCTGCGCTAAGAACTGAGG-3'; GAPDH: forward 5'-TGCACCACCAACTGCTTAGC-3', reverse 5'-GGCATGCACTGTGGTCATGAG-3'. All assays were performed in triplicate. Statistical analyses of the results were performed using the $2^{-\Delta\Delta C_t}$ relative quantification method.

Transwell assay and Matrigel assay

The transfected cells and corresponding control cells (1×10^5) 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), precoated (in Transwell assay) or coated (in Matrigel assay) with 2 µL Matrigel (BD Biosciences). The bottom chamber was filled with medium containing 20% FBS, working as chemo-attractant. After 24-hour incubation, nonmigrating cells in the upper chamber were removed and then the migrating 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

Triplicate 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 (2×10^4 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

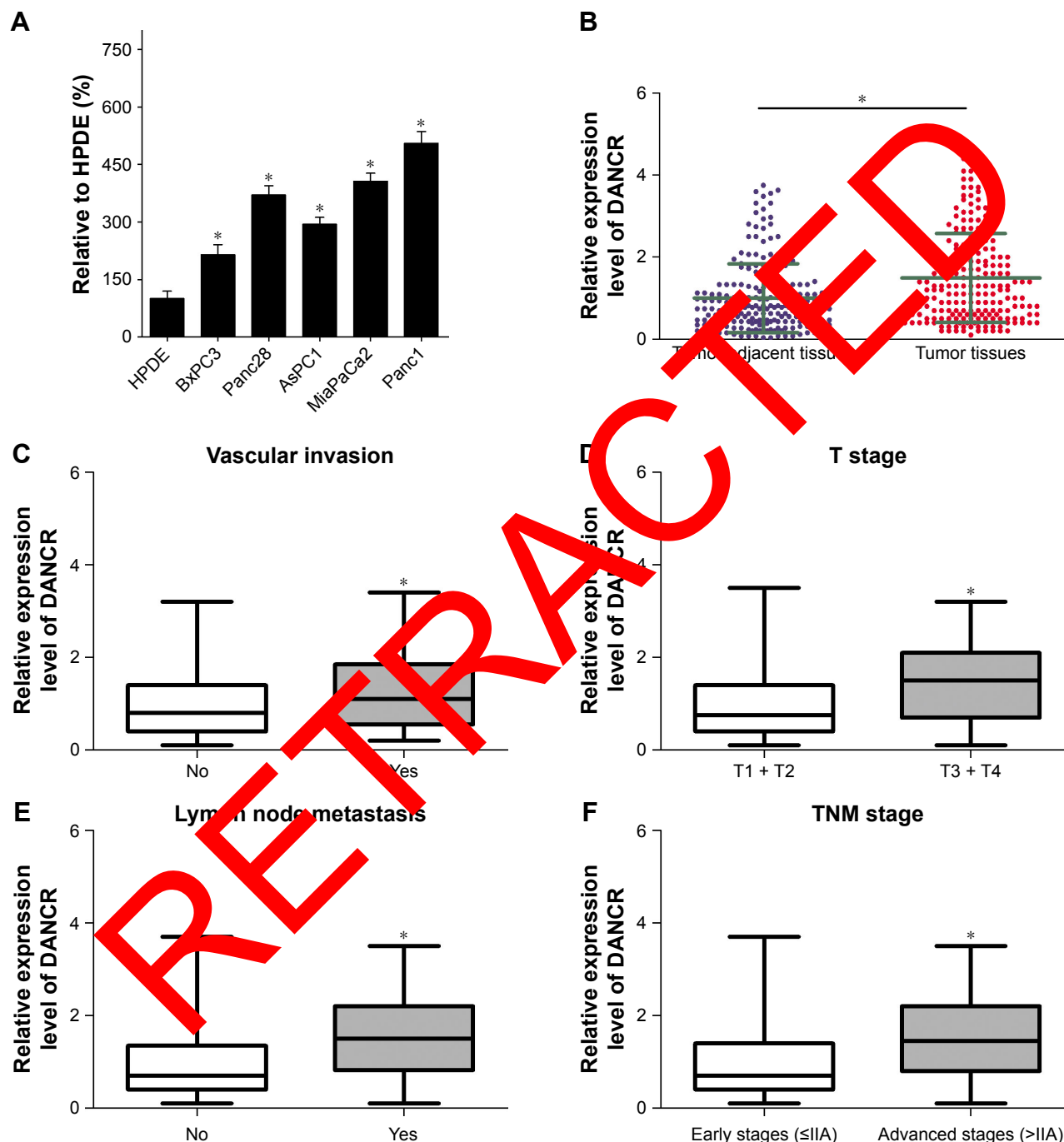


Figure 1 DANCR overexpression implicates cancer progression in PDAC.

Notes: (A) Expression of DANCR in five PDAC cell lines (BxPC3, Panc28, AsPC1, MiaPaCa2 and Panc1) 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. $*P < 0.05$.

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

out by the qRT-PCR assay, which revealed that DANCER was overexpressed in five PDAC cell lines compared with HPDE cells ($P<0.05$; Figure 1A). For further determination of the expression pattern of DANCER in PDAC, DANCER expression was evaluated in 206 PDAC tissues and paired tumor-adjacent tissues. Interestingly, PDAC tissues displayed a notably higher DANCER expression level than paired tumor-adjacent tissues ($P<0.05$; Figure 1B). The PDAC tissues were dichotomized with the mean expression level of DANCER serving as the cutoff value, including the high DANCER expression group ($n=86$) and the low DANCER expression group ($n=120$). The relationship between DANCER expression and clinicopathological features of PDAC was statistically analyzed, which found that high DANCER 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 DANCER expression level. Overall, high DANCER expression indicates advanced tumor stage, and DANCER may promote the clinical progression of PDAC.

DANCER overexpression indicates poor prognosis in PDAC

To evaluate the prognostic significance of DANCER in PDAC, the OS rate and PFS rate of PDAC patients with high DANCER expression and low DANCER 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 DANCER

expression had a significantly higher OS rate and PFS rate. Univariate analysis found that high DANCER 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 DANCER 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 DANCER 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 DANCER 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 DANCER expression predicts poor OS and PFS, and high DANCER expression is an independent risk factor of poor OS and PFS.

DANCER accelerates metastasis and proliferation of PDAC cells

The aforementioned observations suggested that DANCER expression level may be associated with with clinical progression and poor prognosis of PDAC. The following assays would try to detect the functional role of DANCER in metastasis and proliferation of PDAC. The expression of DANCER 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 DANCER obviously inhibited the migration and invasion of Panc1 cells, respectively (Figure 3C). Therefore, the migration and invasion abilities were evidently accelerated after DANCER

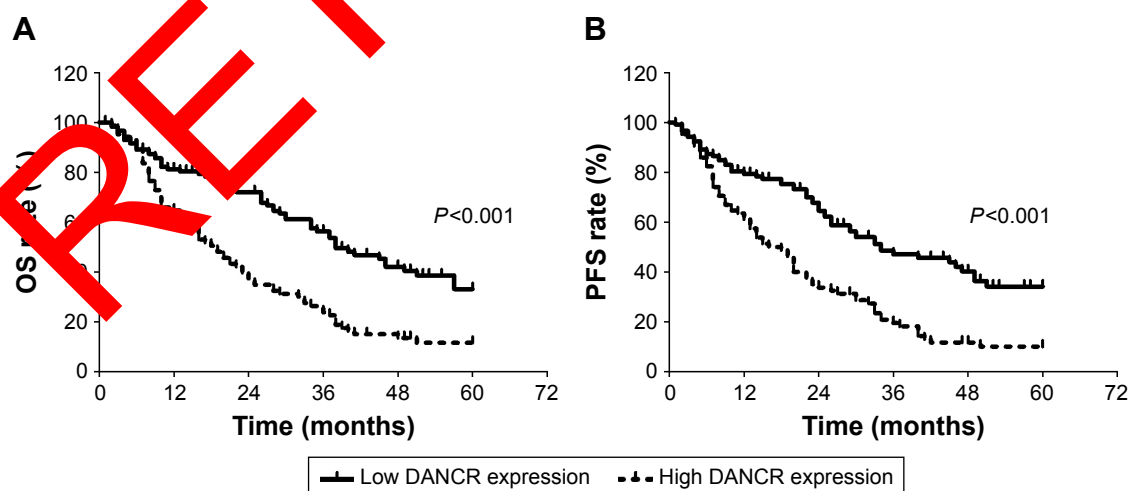


Figure 2 DANCER overexpression indicates poor prognosis in PDAC.

Notes: (A) The OS rate of PDAC patients with low DANCER expression and high DANCER expression was depicted with the Kaplan–Meier analysis and compared with the log-rank test. (B) The PFS rate of PDAC patients with low DANCER expression and high DANCER expression was depicted with the Kaplan–Meier analysis and compared with the log-rank test.

Abbreviations: DANCER, differentiation antagonizing nonprotein coding RNA; OS, overall survival; PDAC, pancreatic ductal adenocarcinoma; PFS, progression-free survival.

Table 2 Univariate and multivariate analysis of clinicopathologic features for OS of PDAC patients

| Parameters | Univariate analysis | | | Multivariate analysis | | |
|---|---------------------|--------------|-----------|-----------------------|-------------|-----------|
| | HR | 95% CI | P-value | HR | 95% CI | P-value |
| Age: ≥ 60 years vs < 60 years | 0.837 | 0.54–1.181 | 0.311 | | | |
| Gender: male vs female | 1.446 | 0.992–2.109 | 0.055 | | | |
| CEA: ≥ 4.5 $\mu\text{g/mL}$ vs < 4.5 $\mu\text{g/mL}$ | 0.940 | 0.631–1.400 | 0.760 | | | |
| CA19-9: < 37 U/mL vs ≥ 37 U/mL | 1.030 | 0.729–1.455 | 0.867 | | | |
| Tumor location: head and neck vs body and tail | 1.101 | 0.765–1.585 | 0.604 | | | |
| Grade: well + moderate vs poor | 1.027 | 0.717–1.469 | 0.886 | | | |
| Size: < 4 cm vs ≥ 4 cm | 0.952 | 0.670–1.351 | 0.783 | | | |
| Neural invasion: yes vs no | 1.179 | 0.834–1.667 | 0.350 | | | |
| Vascular invasion: no vs yes | 1.528 | 1.084–2.154 | 0.015 | 1.463 | 1.033–2.072 | 0.032 |
| Lymphatic invasion: no vs yes | 0.763 | 0.535–1.089 | 0.137 | | | |
| T stage: T1 + T2 vs T3 + T4 | 1.863 | 1.310–2.648 | 0.001 | 0.964 | 0.511–1.818 | 0.909 |
| Lymph node metastasis: no vs yes | 1.795 | 1.275–2.529 | 0.001 | 1.344 | 0.550–3.285 | 0.516 |
| Distant metastasis: no vs yes | 11.172 | 4.320–28.890 | < 0.001 | 6.112 | 1.99–16.213 | < 0.001 |
| TNM stage: early stages (\leq IIa) vs advanced stages ($>$ IIa) | 1.621 | 1.151–2.283 | 0.006 | 1.843 | 0.37–1.885 | 0.677 |
| DANCR: high vs low | 1.224 | 1.150–1.302 | < 0.001 | 1.199 | 1.113–1.290 | < 0.001 |

Abbreviations: DANCR, differentiation antagonizing nonprotein coding RNA; OS, overall survival; PDAC, pancreatic ductal adenocarcinoma.

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.^{23,24} 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 | | | |
| Gender: male vs female | 1.444 | 0.991–2.105 | 0.056 | | | |
| CEA: ≥ 4.5 $\mu\text{g/mL}$ vs < 4.5 $\mu\text{g/mL}$ | 0.950 | 0.638–1.415 | 0.801 | | | |
| CA19-9: < 37 U/mL vs ≥ 37 U/mL | 0.981 | 0.695–1.385 | 0.913 | | | |
| Tumor location: head and neck vs body and tail | 1.161 | 0.810–1.664 | 0.416 | | | |
| Grade: well + moderate vs poor | 1.056 | 0.739–1.508 | 0.765 | | | |
| Size: < 4 cm vs ≥ 4 cm | 0.992 | 0.699–1.406 | 0.963 | | | |
| Neural invasion: yes vs no | 1.165 | 0.824–1.646 | 0.388 | | | |
| Vascular invasion: no vs yes | 1.416 | 1.004–1.996 | 0.047 | 1.343 | 0.948–1.903 | 0.097 |
| Lymphatic invasion: no vs yes | 0.795 | 0.556–1.136 | 0.208 | | | |
| T stage: T1 + T2 vs T3 + T4 | 1.775 | 1.245–2.530 | 0.002 | 0.892 | 0.488–1.631 | 0.711 |
| N stage: N1 vs N0 | 1.769 | 1.256–2.493 | 0.001 | 1.405 | 0.595–3.315 | 0.438 |
| M stage: M1 vs M0 | 10.762 | 4.170–27.780 | < 0.001 | 6.081 | 2.292–16.131 | < 0.001 |
| TNM stage: early stages (\leq IIa) vs advanced stages ($>$ IIa) | 1.593 | 1.131–2.244 | 0.008 | 0.831 | 0.379–1.821 | 0.643 |
| DANCR: high vs low | 1.219 | 1.146–1.296 | < 0.001 | 1.199 | 1.114–1.290 | < 0.001 |

Abbreviations: DANCR, differentiation antagonizing nonprotein coding RNA; PDAC, pancreatic ductal adenocarcinoma; PFS, progression-free survival.

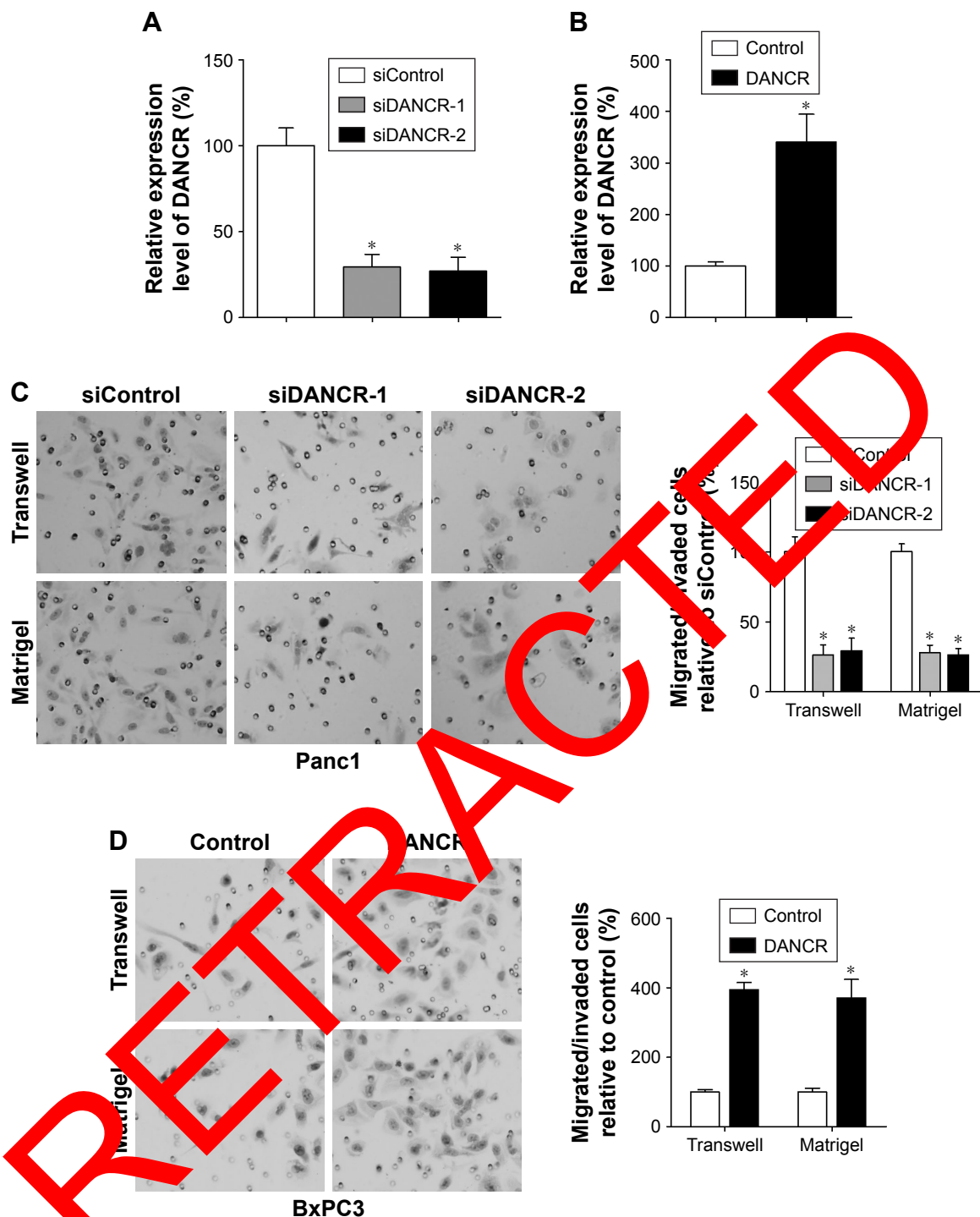


Figure 3 DANCER accelerates metastasis of PDAC cells.

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

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

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.²⁵ lncRNAs can mediate the expression of miRNA-targeted genes through functioning as miRNA sponge.^{26,27} EMT is

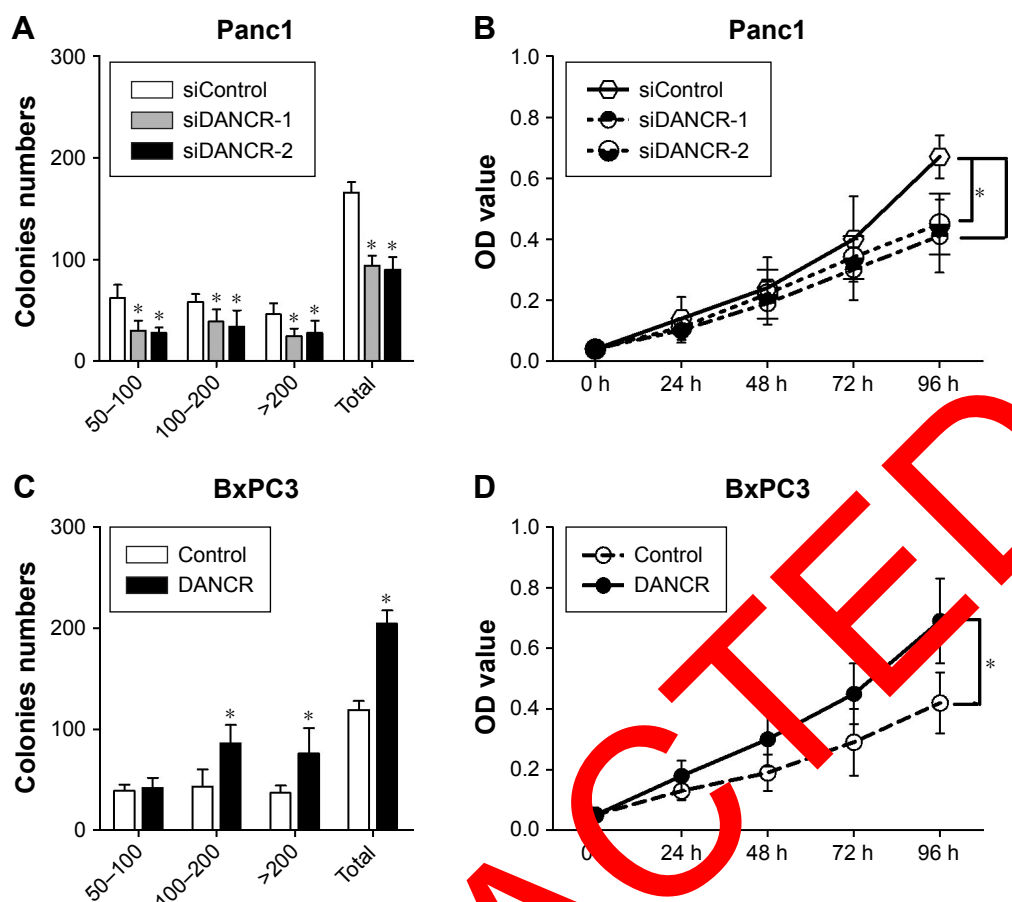


Figure 4 DANCR increases proliferation of PDAC cells.

Notes: (A) Colony formation assay was performed to verify the proliferation ability of Panc1 cells after DANCR silencing. The 50–100/100–200/>200 brackets represent cell numbers in each colony. (B) The proliferation ability of Panc1 cells with DANCR deficiency was evaluated by CCK-8 assay. (C) Colony formation assay was performed to verify the proliferation ability of BxPC3 cells after DANCR overexpression. The 50–100/100–200/>200 brackets represent cell numbers in each colony. (D) The proliferation ability of BxPC3 cells with DANCR overexpression was evaluated by CCK-8 assay. * $P < 0.05$.

Abbreviations: CCK-8, Cell Counting Kit-8; DANCR, differentially expressed nonprotein coding RNA; h, hour(s); PDAC, pancreatic ductal adenocarcinoma.

an initial step in cancer metastasis.²⁸ Accumulating studies found that lncRNAs participate in the EMT of PDAC.^{29–32} For example, lncRNA 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 process, and thus increase the invasion and metastasis of PDAC.²⁹ CSCs are also an important way by which lncRNAs exert their functions in modulating the development of PDAC.^{33,34} Chemoradioresistance is an essential reason leading to the relapse of cancer patients. Multiple studies have focused on the potential role of lncRNAs in chemoradioresistance.^{35–39} Combination of lncRNAs and conventional chemotherapeutic reagents is considered as a promising way for improving the sensitivity of adjuvant therapy.³⁹ There are also studies that revealed that lncRNAs could modulate the epigenetic modifications and autophagy in PDAC.^{40–42} Obviously, lncRNAs can function in a wide range of cancer biology; however, clinical

trials investigating lncRNAs in the treatment of PDAC are rare, and further studies are needed for utilizing lncRNAs in clinical practice.

Since being discovered in 2012,¹⁷ 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,⁴³ glioma,²⁴ gastric cancer,⁴⁴ osteosarcoma,²³ lung adenocarcinoma,²² prostate cancer²¹ and colorectal cancer.⁴⁵ DANCR was also implicated to be a diagnostic and prognostic marker.^{19,20,43} Mechanistically, DANCR could directly interact with miR-634 and this interaction resulted in the inhibition of RAB1A expression, thus accelerating the progression of glioma.²⁴ 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.²³ The mechanisms of DANCR mediating cancer progression are relatively rare, which deserves further investigations.

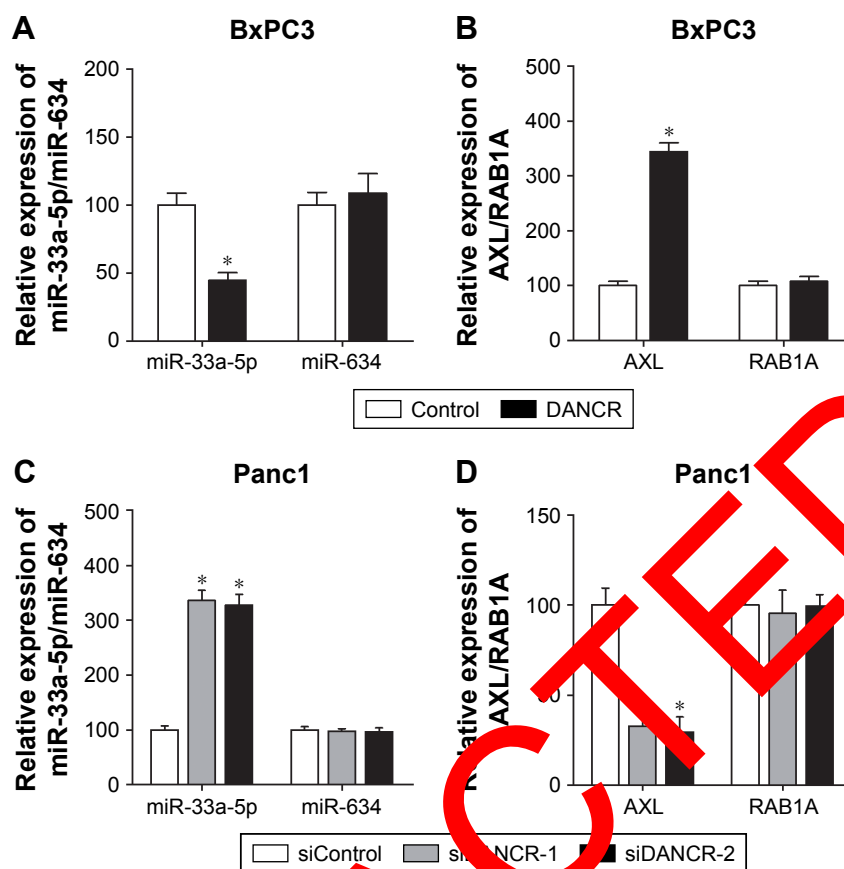


Figure 5 DANCR functions through upregulating AXL via microRNA-33a-5p inhibition. **Notes:** (A) Expression of miR-33a-5p and miR-634 was measured by qRT-PCR in BxPC3 cells with DANCR overexpression. (B) Expression of AXL and RAB1A was detected by qRT-PCR in BxPC3 cells with DANCR overexpression. (C) Expression of miR-33a-5p and miR-634 was measured by qRT-PCR in Panc1 cells with DANCR deficiency. (D) Expression of AXL and RAB1A was detected by qRT-PCR in Panc1 cells with DANCR deficiency. * $P < 0.05$. **Abbreviations:** DANCR, differentiation antagonizing non-protein coding RNA; qRT-PCR, quantitative real time polymerase chain reaction.

This study discovered that DANCR was overexpressed in PDAC tissues compared with tumor-adjacent tissues. Further detection found that high DANCR 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 DANCR expression correlated with poor OS and PFS. Multivariate analysis identified high DANCR expression as an independent survival risk factor for OS and PFS. In addition, DANCR was confirmed to facilitate growth and metastasis of PDAC cells. These results indicated that DANCR is a promising prognostic marker and therapeutic target in PDAC. In addition, DANCR may function through upregulating AXL via microRNA-33a-5p inhibition.

In conclusion, our study confirmed the overexpression of DANCR in PDAC cells and tissues. The clinical significance and prognostic value of DANCR were also detected. In vitro assays demonstrated the oncogenic role of DANCR.

These observations demonstrated that DANCR plays a crucial role in the progression of PDAC, and DANCR 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.

6. Chen LL, Carmichael GG. Long noncoding RNAs in mammalian cells: what, where, and why? *Wiley Interdiscip Rev RNA*. 2010;1(1):2–21.
7. Lipovich L, Johnson R, Lin CY. MacroRNA underdogs in a microRNA world: evolutionary, regulatory, and biomedical significance of mammalian long non-protein-coding RNA. *Biochim Biophys Acta*. 2010;1799(9):597–615.
8. Ponting CP, Oliver PL, Reik W. Evolution and functions of long non-coding RNAs. *Cell*. 2009;136(4):629–641.
9. Rinn JL, Chang HY. Genome regulation by long noncoding RNAs. *Annu Rev Biochem*. 2012;81:145–166.
10. Flynn RA, Chang HY. Long noncoding RNAs in cell-fate programming and reprogramming. *Cell Stem Cell*. 2014;14(6):752–761.
11. Batista PJ, Chang HY. Long noncoding RNAs: cellular address codes in development and disease. *Cell*. 2013;152(6):1298–1307.
12. Adams BD, Parsons C, Walker L, Zhang WC, Slack FJ. Targeting noncoding RNAs in disease. *J Clin Invest*. 2017;127(3):761–771.
13. Evans JR, Feng FY, Chinnaiyan AM. The bright side of dark matter: lncRNAs in cancer. *J Clin Invest*. 2016;126(8):2775–2782.
14. Bartonicsek N, Maag JL, Dinger ME. Long noncoding RNAs in cancer: mechanisms of action and technological advancements. *Mol Cancer*. 2016;15(1):43.
15. Cabili MN, Trapnell C, Goff L, et al. Integrative annotation of human large intergenic noncoding RNAs reveals global properties and specific subclasses. *Genes Dev*. 2011;25(18):1915–1927.
16. Chandra Gupta S, Nandan Tripathi Y. Potential of long non-coding RNAs in cancer patients: From biomarkers to therapeutic targets. *Int J Cancer*. 2017;140(9):1955–1967.
17. Kretz M, Webster DE, Flockhart RJ, et al. Suppression of progenitor differentiation requires the long noncoding RNA ANCR. *Genes Dev*. 2012;26(4):338–343.
18. Yuan SX, Wang J, Yang F, et al. Long noncoding RNA DANCER increases stemness features of hepatocellular carcinoma by derepression of CTNNB1. *Hepatology*. 2016;63(2):499–511.
19. Hao YP, Qiu JH, Zhang DB, Yu CG. Long non-coding RNA DANCER as a prognostic indicator, promotes cell growth and tumorigenicity in gastric cancer. *Tumour Biol*. 2017;39(6):1–9.
20. Liu Y, Zhang M, Liang L, Li J, Chen YX. Over-expression of lncRNA DANCER is associated with advanced tumor progression and poor prognosis in patients with colorectal cancer. *Int J Clin Exp Pathol*. 2015;8(9):11480–11484.
21. Jia J, Li F, Tang XS, et al. Long noncoding RNA DANCER promotes invasion of prostate cancer through epigenetically silencing expression of TIMP2/3. *Oncotarget*. 2016;7(25):37878–37881.
22. Lu QC, Rui ZH, Guo ZL, et al. lncRNA-DANCER contributes to lung adenocarcinoma progression by sponging miR-496 to modulate mTOR expression. *J Cell Mol Med*. 2018;22(9):1527–1537.
23. Jiang N, Wang X, Xie X, et al. lncRNA DANCER promotes tumor progression and cancer stemness features in osteosarcoma by upregulating AXL via miR-322 inhibition. *Cancer Lett*. 2017;405:46–55.
24. Xu D, Yu J, Guo G, et al. LncRNA DANCER functions as a competing endogenous RNA to regulate RAB17 expression by sponging miR-634 in glioma. *Biosci Res*. 2017;16(1):BSR20171664.
25. Duguang L, Jiang Xiaowei Q, et al. The involvement of lncRNAs in the development and progression of pancreatic cancer. *Cancer Biol Ther*. 2017;18(12):927–931.
26. Zhang X, Gao F, Zhou L, et al. UCA1 Regulates the Growth and Metastasis of Pancreatic Cancer by Sponging miR-135a. *Oncol Res*. 2017;25(9):1529–1541.
27. Wang G, Pan J, Zhang L, Wei Y, Wang C. Long non-coding RNA CRNDE sponges miR-384 to promote proliferation and metastasis of pancreatic cancer cells through upregulating IRS1. *Cell Prolif*. 2017;50(6):e12389.
28. Geiger TR, Peeper DS. Metastasis mechanisms. *Biochim Biophys Acta*. 2009;1796(2):293–308.
29. Zhan HX, Wang Y, Li C, et al. lincRNA-ROR promotes invasion, metastasis and tumor growth in pancreatic cancer through activating ZEB1 pathway. *Cancer Lett*. 2016;374(2):261–271.
30. Wu BQ, Jiang Y, Zhu F, Sun DL, He XZ. Long Noncoding RNA PVT1 Promotes EMT and Cell Proliferation and Migration Through Downregulating p21 in Pancreatic Cancer Cells. *Technol Cancer Res Treat*. 2017;16(6):819–827.
31. Chen S, Zhang JQ, Chen JZ, et al. The over expression of long non-coding RNA ANRIL promotes epithelial-mesenchymal transition by activating the ATM-E2F1 signaling pathway in pancreatic cancer: An in vivo and in vitro study. *Int J Biol Macromol*. 2017;102:718–728.
32. Li H, Wang X, Wen C, et al. Long noncoding RNA NORAD, a novel competing endogenous RNA, enhances the hypoxia-induced epithelial-mesenchymal transition to promote metastasis in pancreatic cancer. *Mol Cancer*. 2017;16(1):169.
33. Jiao F, Hu H, Han T, et al. Long noncoding RNA MALAT1 enhances stem cell-like phenotypes in pancreatic cancer cells. *Int J Mol Sci*. 2015;16(4):6677–6693.
34. Fu Z, Chen C, Zhou Q, et al. LncRNA HOTAIR modulates cancer stem cell properties in human pancreatic cancer by regulating HOXA9. *Cancer Lett*. 2017;393:68–74.
35. Jiang Y, Li Z, Teng S, et al. The long non-coding RNA HOTAIR affects the radiosensitivity of pancreatic ductal adenocarcinoma by regulating the expression of Wnt inhibitory factor 1. *Tumour Biol*. 2016;37(3):3957–3967.
36. Wang L, Song P, Wang J, Huang M, Tian B. Gemcitabine treatment causes resistance and malignancy of pancreatic cancer stem-like cells via induction of lncRNA HOTAIR. *Exp Ther Med*. 2017;14(5):1473–1480.
37. Gao JQ, Wang J, Chen DH, et al. Long non-coding RNA GAS5 antagonizes chemoresistance of pancreatic cancer cells through down-regulation of miR-181c-5p. *Biomed Pharmacother*. 2018;97:809–817.
38. Li Z, Gao X, Zhou Y, et al. The long non-coding RNA HOTTIP promotes progression and gemcitabine resistance by regulating HOXA13 in pancreatic cancer. *J Transl Med*. 2015;13:84.
39. Xiong G, Feng M, Yang G, et al. The underlying mechanisms of non-coding RNAs in the chemoresistance of pancreatic cancer. *Cancer Lett*. 2017;397:94–102.
40. Li C, Zhao Z, Zhou Z, Liu R. linc-ROR confers gemcitabine resistance to pancreatic cancer cells via inducing autophagy and modulating the miR-124/PTBP1/PKM2 axis. *Cancer Chemother Pharmacol*. 2016;78(6):1199–1207.
41. Qian K, Liu G, Tang Z, et al. The long non-coding RNA NEAT1 interacted with miR-101 modulates breast cancer growth by targeting EZH2. *Arch Biochem Biophys*. 2017;615:1–9.
42. Lian Y, Wang J, Feng J, et al. Long non-coding RNA IRAIN suppresses apoptosis and promotes proliferation by binding to LSD1 and EZH2 in pancreatic cancer. *Tumour Biol*. 2016;37(11):14929–14937.
43. Ma X, Wang X, Yang C, et al. DANCER Acts as a Diagnostic Biomarker and Promotes Tumor Growth and Metastasis in Hepatocellular Carcinoma. *Anticancer Res*. 2016;36(12):6389–6398.
44. Mao Z, Li H, du B, et al. LncRNA DANCER promotes migration and invasion through suppression of lncRNA-LET in gastric cancer cells. *Biosci Res*. 2017;37(6):BSR20171070.
45. Tong X, Gu PC, Xu SZ, Lin XJ. Long non-coding RNA-DANCER in human circulating monocytes: a potential biomarker associated with postmenopausal osteoporosis. *Biosci Biotechnol Biochem*. 2015;79(5):732–737.

RETRACTED

OncoTargets and Therapy

Dovepress

Publish your work in this journal

OncoTargets and Therapy is an international, peer-reviewed, open access journal focusing on the pathological basis of all cancers, potential targets for therapy and treatment protocols employed to improve the management of cancer patients. The journal also focuses on the impact of management programs and new therapeutic agents and protocols on

patient perspectives such as quality of life, adherence and satisfaction. The manuscript management system is completely online and includes a very quick and fair peer-review system, which is all easy to use. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <http://www.dovepress.com/oncotargets-and-therapy-journal>