DANCR Acts as a Diagnostic Biomarker and Promotes Tumor Growth and Metastasis in Hepatocellular Carcinoma

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Abstract. *Background: Long non-coding RNA differentiation antagonizing non-protein coding RNA (DANCR) is considered to be an oncogene in many cancer types. However, the role of DANCR in diagnosis and progression of hepatocellular carcinoma (HCC) is still unknown. Patients and Methods: In this study, 146 participants including healthy volunteers (HVs) and patients with chronic hepatitis B (CHB), cirrhosis and HCC were recruited. Results: Firstly, quantitative reverse transcription-PCR analysis showed that DANCR was up-regulated in tumor tissues and plasma of patients with HCC, and its expression was highly correlated with microvascular and liver capsule invasion of HCC. Receiver operating characteristic analysis showed that plasma DANCR exhibited significantly increased discriminatory power for differentiating patients with HCC from HVs and non-HCC patients compared to alpha fetoprotein, which has been used as a biomarker for HCC diagnosis. Furthermore, knockdown of DANCR repressed the β -catenin pathway and inhibited HCC cell proliferation and metastasis both in vitro and in vivo. Conclusion: This study revealed, for the first time, the importance of DANCR in clinical diagnosis and disease progression of HCC.*

Hepatocellular carcinoma (HCC) is one of the most common and malignant diseases worldwide (1). Most cases of HCC are associated with viral hepatitis infection and cirrhosis (2). In recent years, abdominal ultrasound, computed tomography, magnetic resonance imaging, liver biopsy and serum biomarkers have become common diagnostic tools for HCC.

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Accordingly, the most common treatments for HCC are liver resection, liver transplantation and radiofrequency ablation. However, the overall survival rate for patients with HCC remains unsatisfactory partly because of delayed diagnosis and recurrence after treatment (3). Therefore, new diagnostic biomarkers and treatment targets are still needed for HCC.

In the past few years, long non-coding RNAs (lncRNAs), with a size of longer than 200 nucleotides, were demonstrated as new diagnostic biomarkers and treatment targets for many diseases, including cancer (4, 5). Many lncRNAs have been reported to participate in the pathogenesis of tumors by affecting different biological processes, including cell proliferation, apoptosis, differentiation and migration (6, 7). The relatively stable structure of lncRNA in human plasma indicates that lncRNAs might be potential circulating biomarkers for tumor occurrence and progression (8, 9). Although more and more lncRNAs have been suggested as important regulators in HCC tumorigenesis (10, 11), the roles of the majority of lncRNAs in HCC diagnosis and treatments remain to be explored.

Many studies reported that the lncRNA differentiation antagonizing non-protein coding RNA (DANCR), which was found to suppress the differentiation of epidermal cells (12), plays an important role in tumor progression. For instance, DANCR promoted invasion of prostate cancer cells by silencing expression of TIMP metalloproteinase inhibitor 2/3 (TIMP2/3) (13). Another study showed that overexpression of DANCR was associated with advanced tumor progression and poor prognosis in patients with colorectal cancer (14). For HCC, recently, Yuan *et al.* indicated that DANCR increased stemness features of HCC *via* de-repression of catenin beta 1 (CTNNB1) (15). However, the diagnostic efficacy of DANCR for HCC and the effects of DANCR on HCC cell proliferation and invasion are still unknown.

In this study, we recruited a cohort of 146 participants including healthy volunteers (HVs) and patients with chronic hepatitis B (CHB), cirrhosis and HCC, and investigated the potential use of circulating DANCR in plasma as a diagnostic biomarker for HCC. We also explored the effects of DANCR on HCC cell proliferation and invasion. Our findings

Table I. Clinicopathological characteristics of healthy volunteers (HVs) and patients with chronic hepatitis B (CHB), cirrhosis and hepatocellular carcinoma (HCC).

Clinical factor	HVs (n=43)	CHB (n=29)	Cirrhosis (n=22)	HCC (n=52)	p-Value (versus HCC)		
					HVs	CHB	Cirrhosis
Gender (Male/female)	26/17	15/14	13/9	34/18	0.673	0.246	0.609
Age (years)	58.79±9.67	55.24±11.89	54.86±7.62	60.27±9.27	0.820	0.073	0.081
ALT (U/l)	17.52±8.49	78.41±67.35	29.35±18.59	67.78±123.04	0.009	0.910	0.174
AST (U/l)	18.91±6.24	61.83±56.43	34.95±16.56	69.98±152.3	0.032	0.973	0.376
Alkaline phosphatase (U/l)	87.81±52.77	73.26±25.63	90.96±44.16	120.93±163.13	0.313	0.139	0.570
Prothrombin time (s)	10.45±1.35	11.71±1.43	14.74±4.24	11.11±1.43	0.338	0.506	0.000
Albumin (g/dl)	37.19±4.47	37.93±5.79	33.22±7.60	37.02±5.43	0.847	1.000	0.002
Total bilirubin (mg/dl)	15.24±4.60	34.69±68.36	28.24±19.08	21.00±11.25	0.744	0.183	0.783

ALT: Alanine aminotransferase; AST: aspartate aminotransferase.

provided new insights into the roles of *DANCR* in HCC tumorigenesis and suggest the potential value of *DANCR* in the clinical diagnosis of HCC.

Materials and Methods

Patients and samples. Tissue samples were collected from patients with HCC who underwent partial liver resection at Department of Hepatobiliary and Pancreatic Surgery, the Affiliated Hospital of Qingdao University, from June 2014 to August 2016. Plasma samples were collected from HVs and patients with CHB, cirrhosis and HCC before therapy. Patients with CHB or cirrhosis were diagnosed and selected randomly at Department of Infectious Diseases ranged from June 2015 to August 2016. HCC, CHB and cirrhosis were confirmed by laboratory tests, imaging and histological techniques. The clinicopathological characteristics of patients and HVs are listed in Table I. This work was carried out in accordance with the Declaration of Helsinki for experiments involving humans. This study was also approved by the Ethics Committee of Affiliated Hospital of Qingdao University (application number: 20140105) and all participants provided their informed consent to participate in this study.

Cell culture. HCC cell lines SMMC-7721 and HCCLM3 were obtained from the Cell Resource Center of Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences (Shanghai, China) and cultured in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum (Gibco, Grand Island, NY, USA) in a humidified atmosphere of 5% CO₂ at 37°C.

Quantitative reverse transcription-PCR (qRT-PCR). Total RNAs of tissues or plasma were extracted using Trizol (Invitrogen, Carlsbad, CA, USA). For the extraction of RNA from plasma, 1 pmol SV40 miRNA mimic (Qiagen, Valencia, CA, USA) per 200 µl plasma was added as spike-in RNA for normalization. After reverse transcription, cDNA was amplified by using SYBR-Green Premix (Takara, Otsu, Japan). The expression of *DANCR*, vimentin, cyclin D1 (*CCND1*) and v-myc avian myelocytomatosis viral oncogene homolog (*MYC*) in tissues and cell lines were normalized to the expression of β-actin. The data were analyzed by delta Ct method. The primers used in this study are listed in Table II.

Transfection and luciferase reporter assay. siRNAs targeting *DANCR* and negative control (NC) were purchased from GenePharma Company (Shanghai, China). Oligonucleotides were transfected into cells by Hiperfect transfection reagent (Qiagen). TOP/FLASH reporter gene including β-catenin-binding sites was obtained from Millipore Corporation (Billerica, MA, USA). The reporter constructs were transfected into cells using Lipofectamine 3000 (Invitrogen). Luciferase activity assay was conducted using Dual Luciferase Assay System (Promega, Madison, WI, USA). pRL-TK plasmid (Promega) was used to normalize the transfection efficiency.

Cell proliferation assays. Cell Counting Kit-8 (CCK-8, Dojindo, Kumamoto, Japan) was used to evaluate cell proliferation. Briefly, cells were seeded into a 96-well plate and transfected with corresponding oligonucleotides. At different time points, 10 µl CCK-8 solution was added into each well of the plate. The plates were incubated at 37°C for 1 h and the absorbance at 450 nm was measured. The OD450 value reflected the cell viability.

Transwell invasion assays. Cell invasion assays were conducted using 24-well transwell chambers with 8.0-µm pore size polycarbonate membrane (Corning Incorporated, Corning, NY, USA). Cells were seeded on the top side of the membrane pre-coated with Matrigel (BD, Franklin Lakes, NJ, USA). After incubation in a humidified atmosphere of 5% CO₂ at 37°C, cells in the upper chamber were removed with cotton swabs. Invaded cells on the lower membrane surface were fixed with methanol and stained with 5% crystal violet. The cells were counted under a Leica DMI4000B microscope (Leica, Heidelberg, Germany).

HCC xenograft mouse model. *DANCR*-knockdown lentivirus and the NC lentivirus were purchased from Genechem corporation (Shanghai, China). HCCLM3 cells (5×10⁶) infected with the corresponding lentivirus were injected subcutaneously to the posterior flank of the BALB/c nude mice purchased from the Shanghai Institute of Materia Medica (Shanghai, China). One to two weeks later, the subcutaneous tumors were implanted into the livers of new nude mice. About 4 weeks after implantation, the mice were euthanized and the tumors were removed. The tumor volumes were calculated as follows: tumor volume (mm³) = (length × width²)/2. The lungs of mice were fixed with formaldehyde and the fixed tissues were serial sectioned and stained with hematoxylin-eosin.

Table II. Primers used in this study.

Primer	Full name	Sequence
<i>DANCR</i>	Differentiation antagonizing non-protein coding RNA	Forward: GCCACTATGTAGCGGGTTTC Reverse: ACCTGCGCTAAGAACTGAGG
<i>VIM</i>	Vimentin	Forward: AAAGTGTGGCTGCCAAGAAC Reverse: AGCCTCAGAGAGGTCAGCAA
<i>MYC</i>	v-Myc avian myelocytomatosis viral oncogene homolog	Forward: CAAGAGGCGAACACACAACGT Reverse: GGGCCTTTTCATTGTTTCCA
<i>CCND1</i>	Cyclin D1	Forward: CTGGAGGTCTGCCGAGGAACA Reverse: CTTAGAGGCCACGAACATGCA
<i>ACTB</i>	β -Actin	Forward: CATCCTCACCTGAAGTACCCC Reverse: AGCCTGGATGCAACGTACATG

Then lung metastatic nodules were observed under a Leica DMI4000B microscope (Leica). All animal studies were conducted in the Animal Institute of Qingdao University according to the protocols approved by the Medical Experimental Animal Care Commission of Qingdao University (application number: QDFYMAL-2015122503).

Western blot. Total proteins were extracted from cells transfected with indicated siRNAs using RIPA lysis buffer, separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred onto a 0.45 μ m polyvinylidene fluoride membrane (Millipore). The following primary antibodies were used in the immunoblotting assays: β -actin and β -catenin (Cell Signaling Technology, Danvers, MA, USA).

Statistical analysis. Statistical analysis was performed using the SPSS program (version 18.0; Chicago, IL, USA). Data are presented as the mean $\pm$ standard deviation (SD). Statistical significance was calculated by Student's *t*-test, χ^2 test or one-way ANOVA followed by Newman-Keul's test. Pearson analysis was used in correlation analysis. Receiver operating characteristic (ROC) and the area under the ROC curve (AUC) was applied to evaluate the diagnostic value and the Youden index was applied to determine the optimal cutoff points of *DANCR* and alpha fetoprotein (AFP) values in the diagnosis of HCC. The statistical significance of differences between two AUCs was calculated and compared by using Delong's algorithm. A value of $p < 0.05$ was considered statistically significant.

Results

DANCR was up-regulated in tumor tissues and plasma of patients with HCC. qRT-PCR analysis showed that the expression levels of *DANCR* were significantly higher in tumor tissue than that in paired adjacent non-tumor liver tissue (ANLT) of patients with HCC (Figure 1A). We also found that the plasma *DANCR* levels in patients with HCC was higher than that in HVs and patients with CHB and cirrhosis (Figure 1B). These results indicated that *DANCR* was up-regulated in tumor tissues and plasma in patients with HCC. The correlation of *DANCR* expression between tumor tissues and plasma from patients with HCC was then

analyzed. The results showed that there was a significant positive relationship between plasma *DANCR* and the corresponding tumor tissue *DANCR* levels (Figure 1C).

Surprisingly, in the 10 patients with HCC who underwent liver resection, plasma *DANCR* was significantly lower after surgery than that before surgery (Figure 1D), which suggested that plasma *DANCR* might derive from tumor tissues in patients with HCC.

Diagnostic evaluation of plasma *DANCR* in distinguishing patients with HCC. The diagnostic evaluation of plasma *DANCR* in differentiating patients with HCC from controls was conducted by calculating the area under the ROC curve and compared with that of AFP, which has been a useful biomarker for HCC diagnosis. The results showed that AUC for plasma *DANCR* was 0.868 which was higher than that for AFP (AUC=0.744, Figure 2A and B, Table III) when differentiating patients with HCC from HVs and non-HCC patients. In view of the fact that AFP was usually up-regulated in patients with chronic hepatitis or cirrhosis and exhibited poor sensitivity for diagnosing HCC (16), we evaluated the power of plasma *DANCR* in differentiating patients with HCC from those with CHB and cirrhosis. The results showed that the AUC for plasma *DANCR* was 0.864 which was significantly superior to that for AFP (AUC=0.650, Figure 2C and D, Table IV) when differentiating HCC patients from patients with CHB and cirrhosis. At the optimal cutoff values, the sensitivities for plasma *DANCR* and AFP were 80.8% and 55.8%, while the specificities were 84.3% and 76.5%, respectively (Table IV). All these results indicate that plasma *DANCR* appears to be a better biomarker than AFP in diagnosing patients with HCC.

Correlation analysis of *DANCR* levels with clinicopathological characteristics of HCC. In order to explore the correlation between clinicopathological variables and *DANCR* levels in tumor tissues and plasma, firstly, patients with HCC were divided into two groups, a high-expression group and a low-

Figure 1. Expression levels of differentiation antagonizing non-protein coding RNA (DANCR) in tissues and plasma from patients with hepatocellular carcinoma (HCC). A: Expression levels of DANCR in 52 pairs of tumor tissues and adjacent non-tumor liver tissues (ANLTs) from patients with HCC were measured by quantitative reverse transcription-PCR and normalized to β -actin expression. The data were analyzed by delta Ct method. Left panel: DANCR expression levels in tumor tissues and ANLTs were compared with paired Student's t-test. Right panel: The bars represent the relative DANCR expressions with the ratio of its levels in tumor tissue versus ANLT (logarithmic scale). B: Plasma DANCR expressions in healthy volunteers (HVs) (n=43) and patients with chronic hepatitis B (CHB) (n=29), cirrhosis (n=22) and HCC (n=52). DANCR expressions were compared by ANOVA followed by Newman-Keul's test. C: The correlation between tumor tissue and plasma DANCR levels in patients with HCC (n=52). D: Plasma DANCR levels before and after curative resection in patients with HCC (n=10). ** $p<0.01$, *** $p<0.001$.

Figure 2. The diagnostic power of plasma differentiation antagonizing non-protein coding RNA (DANCR) and alpha fetoprotein (AFP) for patients with hepatocellular carcinoma (HCC). Receiver operating characteristic (ROC) analysis of plasma DANCR (A) and AFP (B) in differentiating patients with HCC (n=52) from healthy volunteers (HVs) and patients with chronic hepatitis B (CHB) and cirrhosis (n=94). ROC analysis of plasma DANCR (C) and AFP (D) in differentiating patients with HCC (n=52) from patients with CHB and cirrhosis (n=51).

Table III. Power of alpha fetoprotein (AFP) and plasma differentiation antagonizing non-protein coding RNA (DANCR) in differentiating patients with hepatocellular carcinoma (HCC) from healthy volunteers (HVs) and patients with chronic hepatitis B (CHB) and cirrhosis.

HCC versus HVs, CHB and cirrhosis	AUC	Cut-off value	Sensitivity (%)	Specificity (%)	Youden index	p-Value (versus AFP)
AFP	0.744	8.84 (ng/ml)	65.4	77.7	0.431	0.026
Plasma DANCR	0.868	1.65 (Δ Ct)	83.8	72.7	0.565	

AUC: Area under the receiver operating characteristic curve.

Table IV. Power of alpha fetoprotein (AFP) and plasma differentiation antagonizing non-protein coding RNA (DANCR) in differentiating patients with hepatocellular carcinoma (HCC) from those with chronic hepatitis B (CHB) and cirrhosis.

HCC versus CHB+cirrhosis	AUC	Cut-off value	Sensitivity (%)	Specificity (%)	Youden index	p-Value (versus AFP)
AFP	0.650	18.97 (ng/ml)	55.8	76.5	0.323	
Plasma DANCR	0.864	1.64 (Δ Ct)	80.8	84.3	0.651	0.001

expression group, according to the DANCR level in tumor tissues (Table V). Correlation analysis indicated that there were more patients with poor tumor differentiation, microvascular invasion and liver capsule invasion in the high expression group.

Patients with HCC were then once more divided into two groups according to the cutoff value of plasma DANCR level for differentiating patients with HCC from HVs and non-HCC patients. Correlation analysis showed that there was a higher proportion of patients with microvascular invasion and liver capsule invasion in the high expression group (Table VI). These results suggest that the DANCR level might be correlated with metastasis of HCC.

Silencing DANCR inhibited HCC cell proliferation and metastasis in vitro and in vivo. In order to explore the biological function of DANCR in HCC cells, HCC cell lines SMMC-7721 and HCCLM3 were transfected with siRNAs for targeting DANCR (siDANCR) and negative control (siNC), respectively. CCK-8 assays were performed to evaluate the effects of DANCR on HCC cell proliferation. Compared with the control group transfected with siNC, transfection of both SMMC-7721 cells and HCCLM3 cells with siDANCR led to a significant decrease in cell viability (Figure 3A and B). Transwell assays with matrigel were conducted to explore the effects of DANCR on HCC cell invasion. The results indicated that knockdown of DANCR inhibited the invasive activity of both SMMC-7721 cells and HCCLM3 cells (Figure 3C and D). These results together suggest that silencing DANCR inhibits the proliferation and invasion of HCC cells *in vitro*.

We also explored the function of DANCR *in vivo* using a HCC xenograft mouse model. The results showed that knockdown of DANCR in the xenografted tumor cells led to smaller tumor volumes than that in xenografted control cells (Figure 3E). Moreover, the lung metastasis rate decreased from 100% in the control group to 33.3% in the group with DANCR knockdown (Figure 3F). These results suggest an important role for DANCR knockdown in inhibition of HCC growth and metastasis *in vivo*.

Silencing DANCR repressed β -catenin pathway in HCC cells. Previous reports indicated that the activation of β -catenin pathway promoted tumor cell proliferation and

Table V. Clinicopathological characteristics of patients with hepatocellular carcinoma (HCC) categorized according to the differentiation antagonizing non-protein coding RNA (DANCR) level of tumor tissues.

Characteristic	DANCR level of tumor tissues		p-Value
	Low (n=23)	High (n=29)	
	(HCC/ ANLT $\leq$ 1.5)	(HCC/ ANLT $>$ 1.5)	
Age ($\leq$ 60/ $>$ 60 years)	9/14	13/16	0.680
Gender (male/female)	17/6	17/12	0.250
Differentiation (well, moderate/poor)	12/11	6/23	0.018
Tumor size ($>$ 5/ $\leq$ 5 cm)	18/5	19/10	0.341
Number of tumors (1/ $\geq$ 2)	21/2	24/5	0.370
TNM stage (I, II/III, IV)	18/5	21/8	0.629
Vascular invasion (-/+)	19/4	12/17	0.003
Invasion of liver capsule (-/+)	16/7	11/18	0.023
Hepatitis B (-/+)	10/13	13/16	0.922
Cirrhosis (-/+)	12/11	12/17	0.438

ANLT: Adjacent non-tumor liver tissues. Bold numbers indicate significant differences ($p<0.05$).

Table VI. Clinicopathological characteristics of patients with hepatocellular carcinoma (HCC) categorized according to the plasma differentiation antagonizing non-protein coding RNA (DANCR) level.

Characteristic	Plasma DANCR level		p-Value
	Low (n=11)	High (n=41)	
	(Δ Ct $\leq$ 1.65)	(Δ Ct $>$ 1.65)	
Age ($\leq$ 60/ $>$ 60 years)	4/7	18/23	0.653
Gender (male/female)	7/4	27/14	0.891
Differentiation (well-moderate/poor)	6/5	12/29	0.118
Tumor size ($>$ 5/ $\leq$ 5 cm)	8/3	29/12	0.897
Number of tumors (1/ $\geq$ 2)	10/1	35/6	0.632
TNM stage (I-II/III-IV)	7/4	32/9	0.327
Vascular invasion (-/+)	10/1	21/20	0.017
Invasion of liver capsule (-/+)	10/1	17/24	0.004
Hepatitis B (-/+)	4/7	19/22	0.554
Cirrhosis (-/+)	6/5	18/23	0.530

ANLT: Adjacent non-tumor liver tissues. Bold numbers indicate significant differences ($p<0.05$).

Figure 3. Effects of differentiation antagonizing non-protein coding RNA (DANCR) knockdown on hepatocellular carcinoma (HCC) cell proliferation (A, B) and metastasis (C, D) in vitro and in vivo. CCK-8 analysis of SMMC-7721 (A) and HCCLM3 (B) cells transfected with negative control siRNA (siNC) or siDANCR, respectively. Transwell invasion assays of SMMC-7721 cells (C) and HCCLM3 cells (D) transfected with siNC or siDANCR, respectively. The number of cells passing through the membrane was counted and compared. E: The sizes of local liver tumors of HCC mouse model induced by using HCCLM3 cells infected with siNC or siDANCR lentivirus were calculated and compared with unpaired Student's t-test. Results are presented as the mean $\pm$ S.D. (n=6). F: The percentage of mice with and without lung metastatic nodules was calculated. * p <0.05, ** p <0.01, *** p <0.001.

Figure 4. Effects of differentiation antagonizing non-protein coding RNA (DANCR) knockdown on β -catenin signaling pathway in hepatocellular carcinoma (HCC) cells. A: Representative western blot analysis of β -catenin in SMMC-7721 cells transfected with negative control siRNA (siNC) or siDANCR. B: Reporter gene analysis of β -catenin activity in SMMC-7721 cells with siNC or siDANCR transfection. Data were normalized to the activity of β -catenin in cells with siNC transfection. Results are presented as the mean $\pm$ S.D. (n=3). Quantitative reverse transcription-PCR analysis of vimentin (C), cyclin D1 (CCND1) (D) and v-myc avian myelocytomatosis viral oncogene homolog (MYC) (E) expression in SMMC-7721 cells transfected with siNC or siDANCR. Data were normalized to the corresponding gene expression levels in cells transfected with siNC. Results are presented as the mean $\pm$ S.D. (n=3).

metastasis (17). In investigating the molecular basis for the effects of *DANCR* in HCC cells, western blot analysis showed that β -catenin expression levels were lower in SMMC-7721 cells transfected with siDANCR than that in control cells (Figure 4A). When β -catenin reporter gene and siDANCR were transfected together into SMMC-7721 cells, we found that the activity of β -catenin was significantly reduced by siDANCR (Figure 4B). Further analysis showed that genes downstream of β -catenin, namely vimentin, *CCND1* and *MYC*, were all down-regulated by siDANCR in

SMMC-7721 cells (Figure 4C-E). These results strongly suggest that silencing of *DANCR* inhibits β -catenin signaling in HCC cells.

Discussion

Although great advances have been achieved in HCC diagnosis in recent years, many patients are still diagnosed with HCC at an advanced stage and the overall 5-year survival rate of HCC remains very low (3). Currently, AFP is one of

the most common serum biomarkers for HCC diagnosis (18). However, AFP was reported to exhibit poor diagnostic effectiveness for HCC (16). Considering these reasons, it is necessary to search for a more reliable strategy for HCC diagnosis. In this study, we found that the level of plasma *DANCR* was much higher in patients with HCC than that in HVs and non-HCC patients, and further ROC analysis showed that the diagnostic performance of plasma *DANCR* was better than that of AFP in discriminating patients with HCC from HVs and non-HCC patients. These results suggest that plasma *DANCR* would be a more useful biomarker than AFP.

Recently, several studies have shown the positive correlation of lncRNAs in plasma and tumor tissues in patients with cancer (9, 19). In this study, we also found that plasma *DANCR* levels exhibited a positive relationship with tumor tissue *DANCR* levels. Combined with the finding that plasma *DANCR* levels of patients with HCC were much lower after surgery than before surgery, plasma *DANCR* might derive from tumor tissues. However, the in-depth mechanism underlining the positive correlation of *DANCR* between plasma and tumor tissues remains unknown. The transport of lncRNAs between tumor tissues and plasma might be conducted by means of some kind of microparticles such as exosomes. We would try to explore this hypothesis in future study.

Many reports have indicated that the deregulation of lncRNAs plays important roles in occurrence and progression of many cancer types by regulating tumor cell proliferation and invasion. Our previous study indicated that lncRNA X inactive specific transcript (*XIST*) was down-regulated in female patients with HCC and inhibited HCC cell proliferation and metastasis (17). Another lncRNA lung adenocarcinoma associated transcript 1 (*LUADT1*) was reported to suppress p27 expression and promote lung adenocarcinoma cell proliferation (7). In this study, our results indicated that down-regulation of *DANCR* inhibited HCC cell proliferation and invasion *in vitro* and *in vivo*. In agreement with previous reports for prostate cancer and colorectal cancer (13, 14), *DANCR* may also act as an oncogene in HCC progression. Numerous oncogenes or tumor-suppressor genes have been confirmed as targets for lncRNAs in many cancer types, including HCC (20, 21). lncRNAs can affect cellular behavior by diverse mechanisms, including direct interaction with DNA, microRNA and protein, and through genetic diversity. For example, the interaction between *miR-141* and lncRNA *H19* was found to regulate gastric cancer cell proliferation and migration (22). Oncofetal lncRNA plasmacytoma variant translocation 1 (*PVT1*) promoted proliferation and stem cell-like property of HCC cells by interacting with nucleolar protein 2 (*NOP2*) (23). In this study, β -catenin protein levels and the β -catenin downstream genes vimentin, *CCND1* and *MYC* were all

down-regulated by siDANCR in HCC cells. However, the specific mechanism for the regulatory function of *DANCR* remains to be further explored.

In summary, our results showed for the first time that lncRNA *DANCR* was up-regulated in tumor tissues and plasma of patients with HCC, and plasma *DANCR* might be a useful biomarker for HCC diagnosis. Functionally, knockdown of *DANCR* inhibited HCC cell proliferation and invasion by suppressing β -catenin signaling. Our study revealed important roles of *DANCR* in HCC diagnosis and progression.

Conflicts of Interest

The Authors declare no conflicts of interest.

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