European Review for Medical and Pharmacological Sciences 2020; 24: 2368-2379

Long noncoding RNA SOX2OT maintains the stemness of pancreatic cancer cells by regulating DEK *via* interacting with miR-200a/141

C.-S. LIU[1], Q. ZHOU[2], Y.-D. ZHANG[3], Y. FU[4]

[1]Department of Hematology, the First Hospital of Jilin University, Changchun, China
[2]Department of Hepatobiliary, the First Hospital of Jilin University, Changchun, China
[3]Department of Rheumatology, the First Hospital of Jilin University, Changchun, China
[4]Department of Hepatobiliary and Pancreas Surgery, the First Hospital of Jilin University, Changchun, China

Abstract. – OBJECTIVE: Long noncoding RNA sex determination region of Y chromosome (SRY)-related HMG-box (SOX) is involved in the development of various cancers. However, the molecular mechanism of SOXOT, an overlapping transcript of SOX, in pancreatic cancer (PC) is still undefined. We aimed to explore the epigenetic function of SOX2OT and its downstream factors in advanced PC.

PATIENTS AND METHODS: The levels of 2OT, miRNA, and DEK proto-oncogene (D pancreatic cancer tissues and cell lines e evaluated by quantitative polymerase chain action (qPCR). The log-rank test was applied evaluate the role of high SOX2O in sho ening the overall survival of ic cance patients. The Chi-squared was e to assess the relation betwe OX2OT ession and clinicopathologic fea Colony assay teste cell eration of PC cells with SOX2 nockdow w cytometry and Wester were use determine the stemness tum lls *in vitro*. he underlying reg ory mech between SOX2OT and miR a/141 was pr d by bioinformatics an rified by RNA tra ction, qPCR, and We blotti Mice xenograft models were app to mine the promoting effects of SOX2 C *in vi*

RESU The ession of SOX2OT in PC t s an s is strongly elevated. High lev of SO T expression are more likely esent in patients with advanced TNM ve CD44, and poor overall surviv. SOX2OT overexpression promotes proliferion and stemness maintaining of PC cells *in* and boosts tumor growth *in vivo*. Furthermore, SOX2OT upregulates DEK expression by binding to miR-200a/141 as a competing endogenous RNA.

CONCLUSIONS: DEK induced by SOXOT-miR-200a/141 axis may markedly promote stem cell property of PC, resulting in an advanced stage and rio vival. Thes ndings suggest the SOX2OT-D is as a novel therapeutic ta PC.

K ords:
ncreatic can Long noncoding RNA SOX2 o pping trans t, DEK proto-oncogene, Stemne IR-200a/1

Introduction

Although the incidence of pancreatic cancer (PC) ranks over tenth among all cancers, its new cases (458 918) and deaths (432 242) are so close[1], suggesting that PC is a destructive malignancy. Early diagnosis and therapy can effectively reduce the harm of pancreatic cancer. The lack of effective early diagnosis and prognostic markers has led to a lack of treatment options for tumor discovery[2]. Approximately 50% of patients are diagnosed with metastases (liver, abdominal cavity, etc.), resulting in a 5-year survival rate of less than 8%[2,3]. Although existing treatments such as surgery and radiation/chemotherapy are known to help prolong survival and relieve symptoms, there is not much improvement in overall patient survival[4]. Therefore, genetic studies of the aberrant expression underlying PC development will help us fully understand the tumorigenesis of PC, furthering the identification of promising therapeutic targets that may ameliorate the inferior survival of patients.

Low survival rates and tumor stem cell characteristics are mediated by long non-coding RNAs (lncRNAs)[5,6]. LncRNAs are transcripts with more than 200 bases and cannot encode proteins[7] but inhibit microRNA (miRNA) function through

Corresponding Author: Yu Fu, MD, Ph.D; e-mail: fuyuchangchun@sina.com

interactions[8]. Generally, carcinogenic lncRNAs are abnormally expressed in malignant tumors, enhancing cell proliferation, metastasis, and stem cell characteristics[9]. Inhibition of these carcinogenic lncRNAs can impair cellular malignant biological behavior, thereby controlling cancer growth and improving therapeutic outcomes[10]. The dysregulation of lncRNA is linked to the proliferation, metastasis and stem cell characteristics of pancreatic cancer cells[11-14]. LncRNA actin filament-associated protein 1 antisense RNA 1 (AFAP1-AS1) is found to be related to the stemness features[12]. LncRNA growth arrest-specific 5 (GAS5) not only represses epithelial-mesenchymal transition but also inhibits tumor stem cell-mediated chemotherapy resistance *via* increasing suppressor of cytokine signaling 3 (SOCS3) expression[13]. Linc-dynein cytoplasmic 2 heavy chain 1(DYNC2H1)-4 plays an important role in the maintenance of pancreatic self-renewal[14].

Here, we address that the anomalous levels of the LncRNA SOX2 overlapping transcript (SOX-2OT) in PC cancerous tissues and explore the underlying biological mechanism. We show that increased lnc SOX2OT is linked with CD[illegible] a marker for stemness, and also a strong pr[illegible] for poor survival. All these suggest its pat[illegible]-netic significance for maintaining the dest[illegible] tiveness of PC. Investigating the downstre[illegible] epigenetic regulation of lnc S[illegible] may he[illegible] us in developing a novel ther[illegible]atic ta[illegible] for PC.

Patient[illegible]d M[illegible]ds

Patients, Co[illegible]ding Tissu[illegible] and Follow-up

The Et[illegible] Committee[illegible]e First Hospital of Jilin U[illegible]rsity approved t[illegible]udy. All patients gav[illegible]eir wri[illegible]informed consent before this inve[illegible]tio[illegible]fter a biopsy or surgery, a total of 96 PC [illegible]s were [illegible]ected from April 2014 to [illegible]ber 2[illegible] All [illegible]ents diagnosed by the biopsy [illegible]tology[illegible]nstant clinical metastasis (M1 st[illegible] the 7th A[illegible]CC TNM staging system) is con-[illegible]gnetic resonance imaging (MRI) or [illegible]one scanning. Tissue specimens were frozen in [illegible]0°C for further experiments. 5-year-follow-up [illegible]mplemented to attain overall survival.

RNA Extraction and Quantitative PCR

Total RNA of tissue and cell line was extracted using RNAiso Plus (TaKaRa, Beijing, China) according to the instruction. The extracted RNA was synthesized to cDNA by the PrimeScript™ RT reagent Kit (TaKaRa, Beijing, China). Quantitative PCR was done using SYBR® Green Realtime PCR Master Mix (TOYOBO, Shanghai, China) on the Applied Biosystems Veriti Thermal Cycler (Thermo Fisher Scientific, W[illegible] MA, USA) following the manufactur[illegible] col. The quantitation of the target [illegible] expression was assessed using the endog[illegible] control by the $2^{-\Delta\Delta}$Ct method (glyceral[illegible]ehyde[illegible]phate dehydrogenase, GAPDH). [illegible]t Flex [illegible]m-eter (Thermo Fisher Sci[illegible]ic, W[illegible]ltham, USA) was used to e[illegible]te the [illegible]lity of prepared RNA, an[illegible] cD[illegible] w[illegible]easur[illegible]. All sequences for pr[illegible]rs we[illegible]own i[illegible]pple-mentary Tabl[illegible]

Cell Cultu[illegible]

The [illegible] lines Capa[illegible] BxPC-3, Hs 766T, and nor[illegible]pa[illegible]creatic ducta[illegible]helial cell line HP-D[illegible] were purchased from the Chinese Academy o[illegible]iences (Sh[illegible]hai Institute of Cell Biology, S[illegible]hai, China[illegible]ll the cell lines were cultured in [illegible] fetal b[illegible]e serum-supplementing Roswell P[illegible]rial Institute-1640 (RPMI-1640) medium (Thermo Fisher Scientific, Waltham, [illegible]USA). All of them were in a 37°C atmo-ph[illegible]ith 5% CO^2.

Gene Overexpression and Cells Transfection

For gene overexpression, lentiviral vectors (pcD-ciR vector, Geenseed Biotech, Guangzhou, China) were used to construct SOX2OT expressing particles (oe-SOX2OT, SOX2OT sequence was shown in **Supplementary Table I**). SOX-2OT-cDNA or NC-cDNA (MOI = 20) with polybrene was transfected into BxPC-3 and Hs 766T. 24 h after transfection, a new medium replacement was done to the culture medium. Stably transfected cells were selected by puromycin (1 μg/ml). The Puromycin (1 μg/ml, 2-3 times) selection was made until green fluorescence was shown in all cancer cells *via* the fluorescence microscope (Olympus IX71, Japan).

Proliferation Ability of Tumor Cells

PC cells were cultured in 96-well plates (5×10^4 cells/well) for 2 weeks. Then, tumor cells were implanted with 500 cells/well for colony formation assay. Finally, 4% paraformaldehyde (5 min) and 1% crystal violet (10 min) were used to fix and stain the colonies. All colonies were observed by a microscope.

Table I. Relation between SOX2OT expression and clinicopathological features in PC (n = 96).

		SOX2OT expression		
	Total no.	Low n = 48	High n = 48	*p*-value
Gender				0.68
Male	46	24	22	
Female	50	24	26	
Age				0.413
≤ 60	44	20	24	
> 60	52	28	24	
Tumor size				
≤ 2 cm	44	26	18	
> 2 cm	52	22	3	
TNM stage				0.037
I+II	38	24	1	
III+IV	58	24	34	
Lymphatic invasion				0.200
No	62	34	28	
Yes	34	14	20	
Vascular invasion				0.676
No	58	30		
Yes	38	18	2	
Liver metastasis				0.186
No	66	36	30	
Yes	30	12	18	
CD44				0.003*
Negative	62	38	24	
Positive	34	10	24	

Note: *$p < 0.05$ represents statistical difference.

Flow Cytometry

The surface markers anal[y]s[is] of the [tu]mor cells was performed by flow cy[to]metry. Fif[ty] thousand cells stained with anti[body] [mi]c[e] PE-CD24, BD Bios[cie]nces, F[ran]klin Lakes, NJ, USA) were mea[sured] by BD [Ac]curi C6 Flow Cytometry (BD [Bio]sciences, Frank[lin] Lakes, NJ, USA).

Animal Experiment and Tumor Growth In Vivo

4-week-old BALB/c-nude mice were purchased from the Laboratory Animal Center of Jilin University, fed in an atmosphere with a 12 h light/dark cycle under specific pathogen-free conditions. Cells (2×10^5 cells in 100 ul) transfected with an expression vector (oe-SOX-2OT) or control (oe-NC) were respectively subcutaneously into mice (each group n = 5). The tumor sizes of every mouse were detected every week. In the fifth week, tumor weight was measured after the mice were sacrificed. All the procedures of these experiments attained approval from the Institutional Animal Care and Use Committee of the First Hospital of Jilin University.

Dual-Luciferase Reporter Analysis

The wildtype lncRNA SOX2OT sequences containing miRRNA-binding sites or mutant sites were cloned to psiCHECK-2 plasmid (Promega, Madison, WI, USA). Then, following the manufacturer's instruction, the *Renilla* Luciferase reporter vector was transfected into PC cells along with the synthetic vectors, miRNA, anti-miRNA, or relative controls. The above transfected PC cells were seeded for incubation 48 h. The Luciferase activities of the target gene were tested by a Dual Luciferase-Reporter Assay System (Promega, Madison, WI, USA).

Western Blotting Assay

Radioimmunoprecipitation assay (RIPA) buffer (Solarbio, Beijing, China) was applied to extract total proteins, supplementing with 1% phosphorylation and protease inhibitors (Solarbio, Beijing, China). According to the manufacturer's protocol, the concentration of the protein samples was tested by the bicinchoninic acid assay (BCA) protein assay kit (Tiangen, Beijing, China). After denatured at 96°C for 10 min, 9% sodium do-

decyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE; Solarbio, Beijing, China) used to divide the target proteins. The polyvinylidene difluoride (PVDF) membrane (Solarbio, Beijing, China) was used for transfer. After incubation with 5% non-fat milk for a blockade of non-specific signals, PVDF membranes were incubated with primary antibodies against Nanog (1:4000), OCT4 (1:3000), DEK (1:2000), and GAPDH (1:4000) (Cell Signaling Technology, Danvers, MA, USA) overnight at 4°C. Then, the PVDF membrane was dealt with horseradish peroxidase (HRP) conjugated secondary antibody (1:8000, Cell Signaling Technology, Danvers, MA, USA). The protein blots were photographed using a Western imaging system (General Electric Company, Boston, MA, USA). The density of bands was quantified by Image J software (Bio-Rad, Hercules, CA, USA).

Statistical Analysis

All data were analyzed using GraphPad Prism 6 software (San Diego, CA, USA), and presented as the mean ± standard deviation. Comparison between two groups was carried out by Student's *t*-test. Each experiment was done indepe[n]dently three times. $p < 0.05$ was defined as a[t] difference.

Result[s]

Elevated SOX2OT E[x]pressio[n] Highly Related to CD44 a[nd] Poor Survival in PC

By analyzing [t]pairs [o]f tissues [i]n patients, we fou[nd] SOX2OT [e]xpressi[on] inclined in PC tissu[es] to adjace[nt] normal tissues (Figure 1A, $p < 0$[.]). Then, we observed that the ex[p]ression of SO[X2]OT was higher in PC

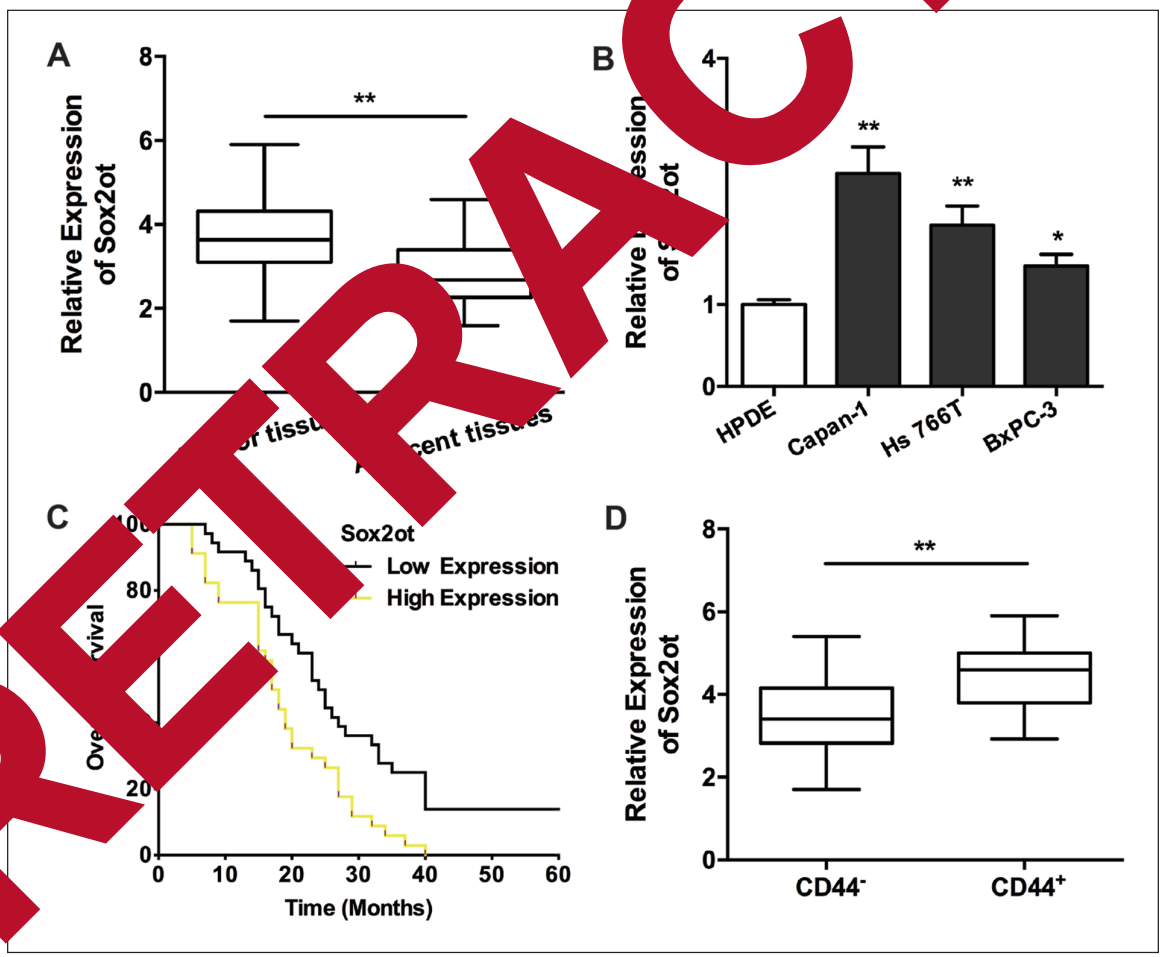

Figure 1. Elevated SOX2OT expression is highly related to CD44 and poor survival in PC. **A**, Expression of lncRNA SOX2OT in PC tissues and adjacent normal tissues shown by qPCR. **B**, lncRNA SOX2OT expression in PC cells (Capan-1, Hs766T, BxPC-3) and HPDE by qPCR. **C**, Overall survival of PC patients with high and low lncRNA SOX2OT expression. **D**, Expression of lncRNA SOX2OT in PC tissues with different state of CD44 (Negative, n = 62; Positive n = 34). $^{**}p < 0.01$, $^{*}p < 0.05$.

cells (Capan-1, Hs766T, BxPC-3) than normal pancreatic ductal epithelial cell line (HPDE) (Figure 1B, $p < 0.01$). Based on Kaplan-Meier analysis, a higher expression of SOX2OT was associated with inferior overall survival (Figure 1C, $p < 0.05$). Moreover, PC patients with late TNM stage and positive CD44 expression (stemness) exhibited notably increased SOX2OT expression than PC tissues with less advanced TNM stages (Table I, $p = 0.037$) or negative CD44 expression (Table I, $p = 0.003$; Figure 1D, $p < 0.01$), respectively. Altogether, our results indicated that SOX2OT is up-regulated in PC tissues, and it demonstrates an oncogenic role in PC by maintaining stemness.

SOX2OT Promotes PC Cell Proliferation and Stem Cell Feature In Vitro

A synthesized lentiviral SOX2OT-ov pres ing (oe-SOX2OT) vector was tra ted into two cell lines (Hs766T and BxPC increase SOX2OT expression (Figure A, B, 0.01). Then, we applied molecular eriments itro to verify the regulatory f ion of SOX2

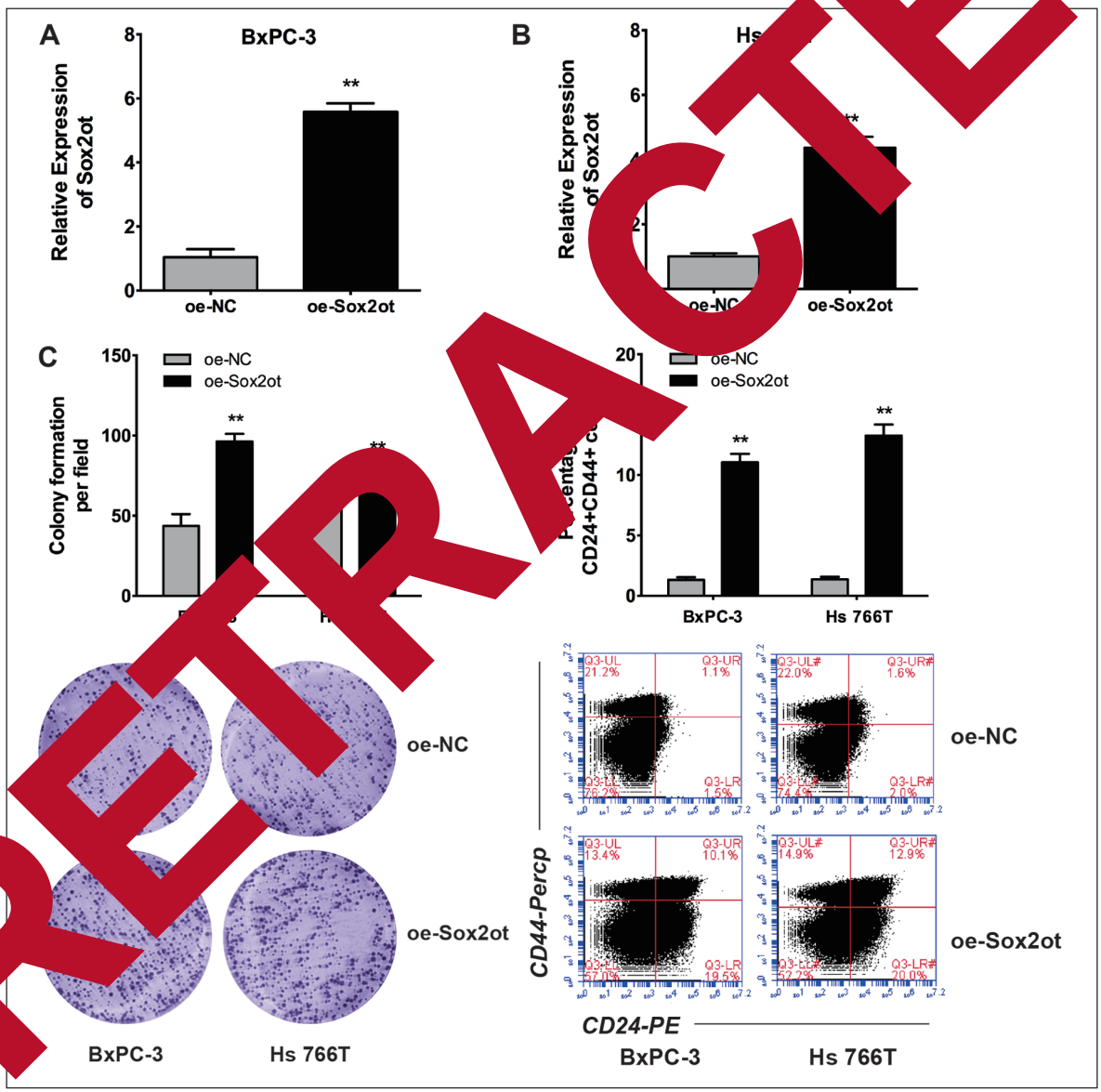

Figure 2. SOX2OT promotes PC cell proliferation and stem cell feature *in vitro*. **A, B**, SOX2OT expression was increased by lentiviral vectors in PC cells. **C**, Proliferative ability of PC cells transfected with overexpressing vector or vector control measured by colony assay (400×). **D**, The $CD24^+CD44^+$ rate of PC cells transfected with overexpressing vector or control showed by flow cytometry. $^{**}p < 0.01$, $^{*}p < 0.05$.

the biological behavior of tumor cells. Colony formation assays revealed that the proliferation of Hs766T and BxPC-3 (Figure 2C, $p < 0.05$) increased notably after SOX2OT overexpression. Also, the percentage of $CD24^{+}CD44^{+}$ cells (Figure 2D, $p < 0.05$) and the expression of Nanog, OCT4 (distinctive transcription factors of cancer stem cell) (Figure 3A, B, $p < 0.05$) were inclined after the SOX2OT level was increased. Therefore, these data suggest that lncRNA SOX2OT can mediate the stem-cell-abilities of PC cells *in vitro,* including proliferation and distinctive surface markers.

SOX2OT Accelerates PC Tumor Growth In Vivo

Hs766T and BxPC-3 cells transfected with oe-SOX2OT or oe-NC were incubated in immune-deficient mice to induce xenograft tumor models. The experiment revealed that increased SOX2OT accelerated the growth (Tumor size, figure 4 A, B, $p < 0.05$; Tumor weight, figure 4 C, D, $p < 0.05$) of PC tumor nodes, compared to that in vector control mice. The macroscopic observation of tumor nodes is shown in Figure 4 E, F. Thus, these results suggest that SOX2OT dysregulation is involved in PC tumor formation *in vivo*.

SOX2OT Modulates PC Stemness Targeting MiR-200 Family

Bioinformatic analysis by miRTa predicted the complementary binding potent tween SOX2OT and miR-200a/2 41 (Fig A). Assays validated that th ciferas acti miR-200a/200b/141 fa was ficantly pressed by the wil ty OX T rath than the mutant form C ce gure 5D, D, $p < 0.01$). Compa o the oe- eat ells, the expression 0a/200b/1 ined in PC cells trans ted w e-SOX2O (Figure 5E, $p < 0.01$). Further ver that SOX2OT could tar 200 family, expression of miR-2 200b/141 in PC cells (BxPC-3 and Hs 766T, F re 5F, $p < 0.$ and cancerous tissues (Figure

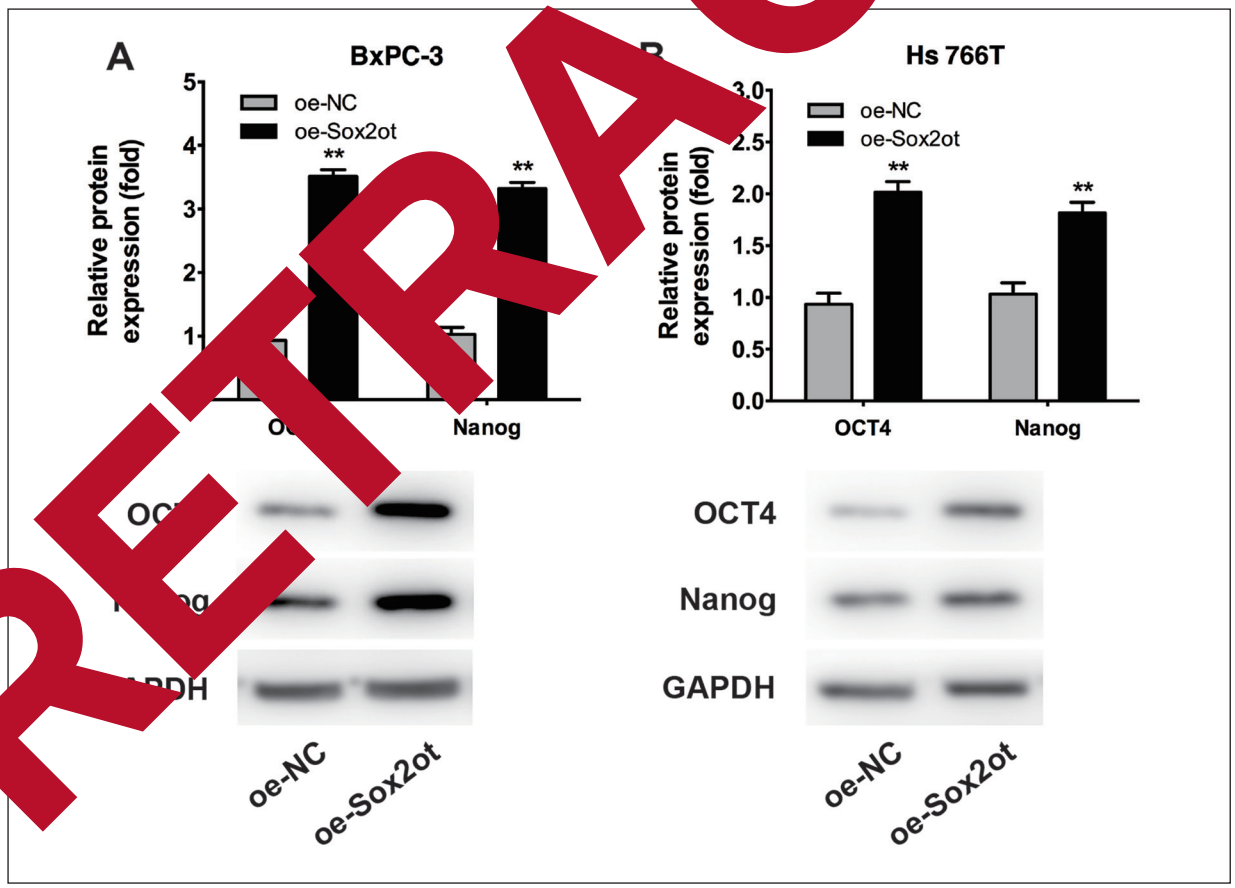

Figure 3. SOX2OT promotes stem cell feature of PC *in vitro.* **A, B**, Western blot assay showed the OCT4 and Nanog protein expression in PC cells transfected with overexpressing vector or control in BxPC-3(A) and Hs 766T(B). $^{**}p < 0.01$, $^{*}p < 0.05$.

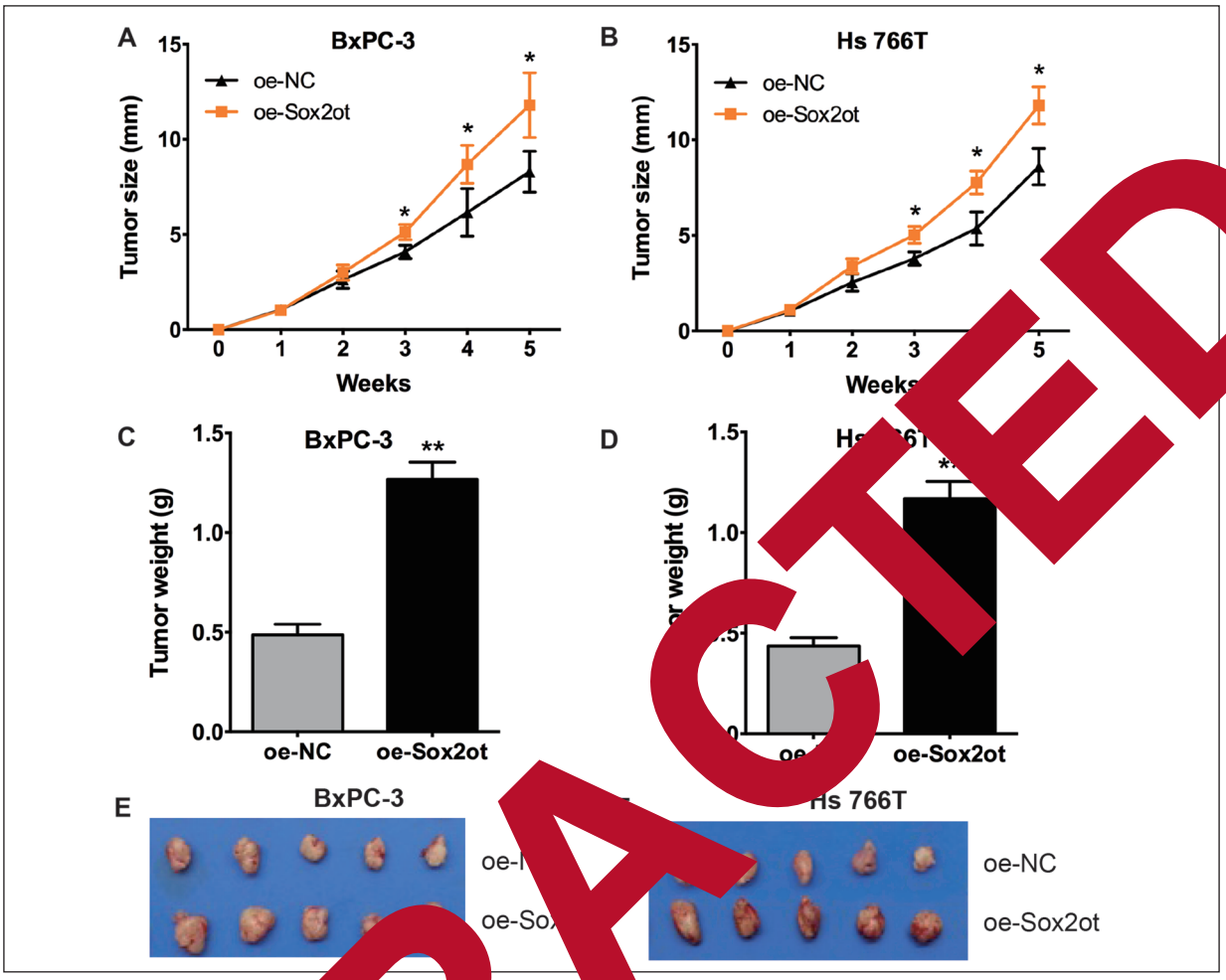

Figure 4. SOX2OT accele... tumo... **A, B**, Tumor size between SOX2OT overexpressing mice and the control mice. **C, D**, Tum... ight... nudes ... overexpression of SOX2OT. **E, F**, The macroscopic observation of tumor nudes. $^{**}p < 0.01$ ... 0.05.

5G, $p < 0.01$) was ma... ly lower than normal ones, sh... by qPCR. ...lectively, our data sugge... that SOX2OT ... cted with miR-200... 0b/141... competing endogenous RNA to i... ate... mness in CRC.

...20... 41 ...ctly Targets DEK in Pa... evelo... t

...ed on b... nformatics analysis using Tar... predicted that DEK proto-oncogene (DEK, protein coded by oncogene at 6p22.3), which exhibits increased expression in PC, may ... downstream target for miR-200a and miR-14... but not miR-200b. Their complementary binding relation (Figure 6A) was verified by a Luciferase reporter assay (Figure 5C, D, $p < 0.01$). DEK expression was inclined in cancer cells transfected with oe-SOX2OT, compared to that in vector controls (Figure 5E, $p < 0.01$). Altogether, these results indicate that DEK is an effector protein targeted by SOX2OT /miR-200 axis in PC.

Discussion

Despite extensive research on the molecular mechanisms of tumor cell stem cell characteristics, the underlying causes of malignant tumor invasion, metastasis and drug resistance, there is still a lack of an effective intervention strategy. Exploring the significant molecular characteristics of the stem-cell-phenotype in cancerous cells may lead to the discovery of more effective therapeutic targets. We investigated the molecular mechanism of SOX2OT/DEK regulatory axis

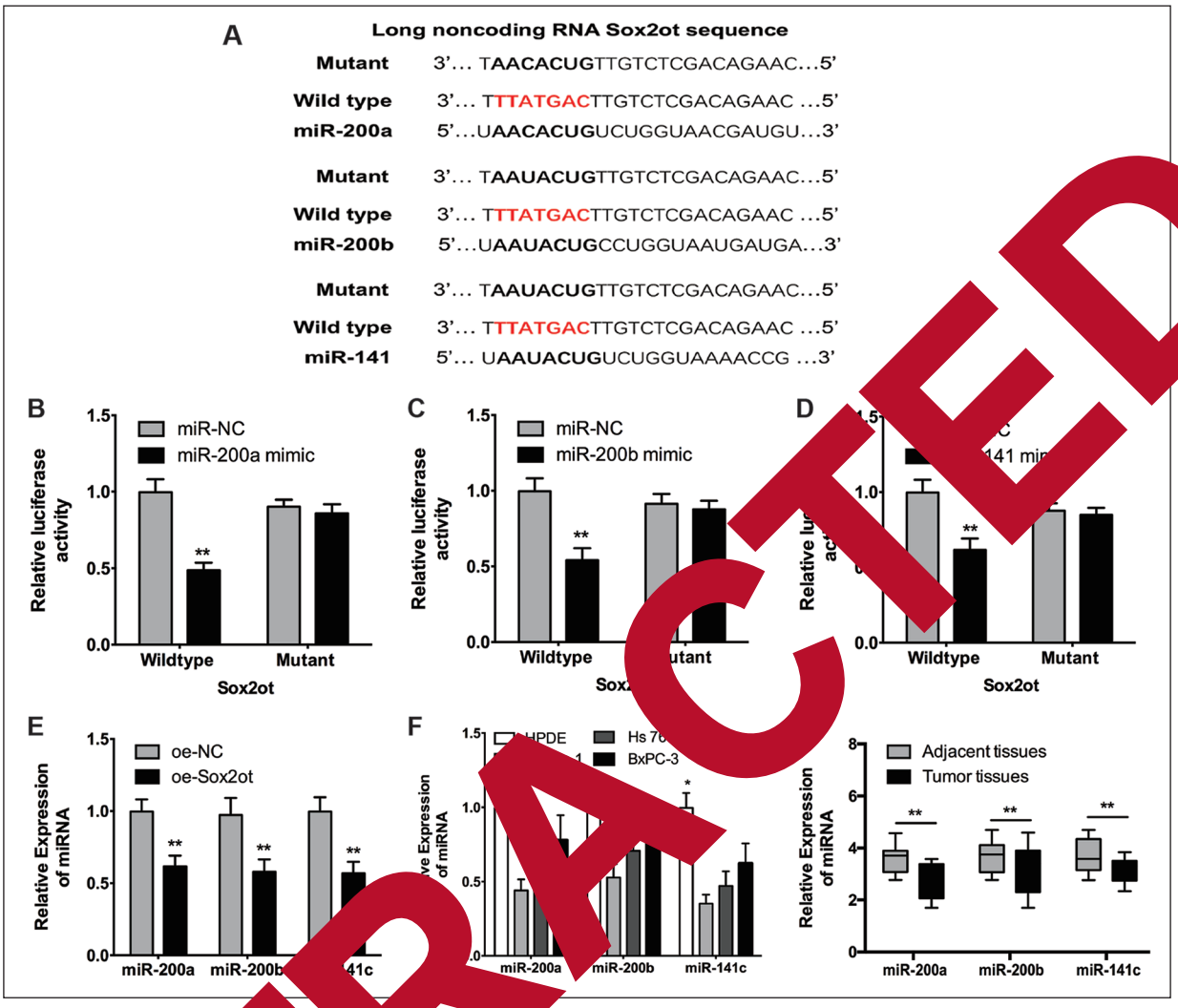

Figure 5. SOX2OT ulates Pc ness by targeting miR-200 family. **A**, The schematic diagram presents the complementary bin es within S T and miR-200a/200b/141. **B, C, D**, Luciferase reporter assay confirmed the molecular bindi w OX2OT and 00 family. **E**, qPCR showed the miR-200a/200b/141 expression in PC cells transfected with erexpr vector or co rol. **F**, MiR-200a/200b/141 expression in PC cells (Capan-1, Hs766T, BxPC-3) and HPDE **G**, MiR-20 b/141 expression in PC tissues and adjacent normal tissues shown by qPCR. $^{**}p < 0.01$, $^{*}p < 0.05$

in the enhance tem cell characteristics ncre an ells and explored potential ne nterve in tumor progression. Previ- ou research s have confirmed that SOX2OT as an oncogene for various can- ers[15]. SOX2OT was found to be significantly rrelated with overall survival in esophageal mous cell carcinoma[16]. Besides, SOX2OT levels in serum were strongly associated with larger tumor size, advanced stage, and positive lymph node of lung squamous cell carcinoma[17]. Moreover, SOX2OT accelerates the expression of Sox2, thus promoting epithelial-mesenchymal transition and stem cell like properties of PC in a competitively binding way[18]. Since Sox2 shares part of the gene sequence with the adjacent Sox-2ot gene, most of the current studies believe that the final regulatory target of SOX2OT is SOX2. Little is known about the non-SOX2 regulatory axis mediated by SOX2OT, especially in PC. In this study, we found that the aberrant expression of SOX2OT is strongly related to tumor stem cell characteristics and may be an independent predictor of inferior overall survival of pancreatic cancer (Figure 1). The silencing of SOX2OT in pancreatic cancer cells inhibits the proliferation

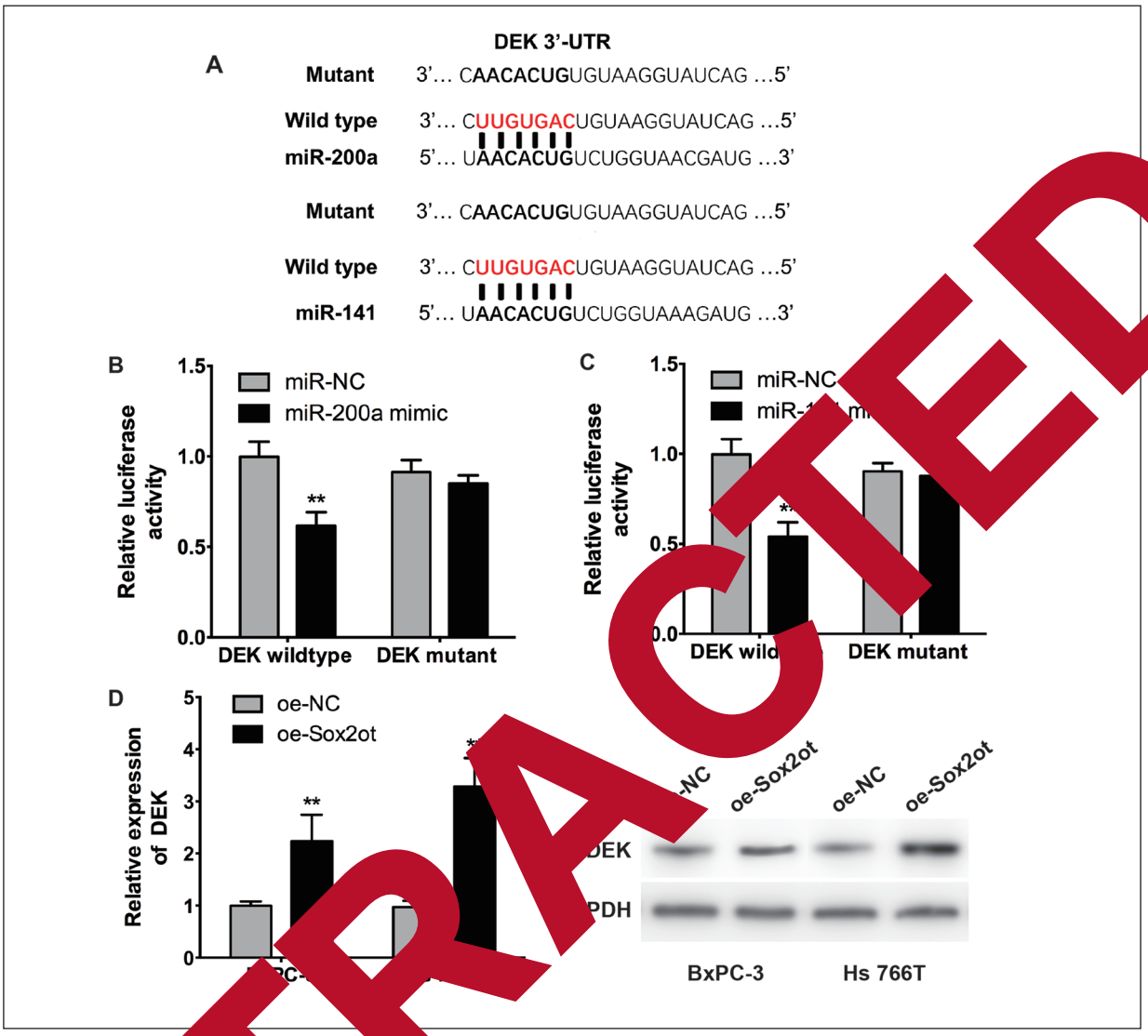

Figure 6. miR-[illegible]/141 [illegible]ly targets DE[illegible]n PC development. **A**, Bioinformatics tools reveal the complementary binding sites within t[illegible]-200a/141 a[illegible]K 3'-UTR. **B, C**, Luciferase reporter assay validated the molecular binding between miR-200a (B)/1[illegible] and DEK 3'-U[illegible]. **D**, Western blot assay showed the DEK protein expression in PC cells transfected with SOXOT[illegible] expressing vector or [illegible]l. $^{**}p < 0.01$, $^{*}p < 0.05$.

vitr[illegible]ll li[illegible] (Figure 2) and the growth of[illegible]mors *in*[illegible] (Figure 4). SOX2OT can alter th[illegible]ological [illegible]ehavior of tumor cells through [illegible]olecules *via* miRNA regulation. [illegible]ltogether, these results indicate that SOX2OT [illegible]es participate in stemness maintaining and [illegible] prognosis in PC patients.

Competitively binding to target microRNAs for post-transcriptional regulation is a distinctive interaction style of lncRNAs[19]. In our study, bioinformatics analysis predicted the interaction between the SOX2OT and miR-200 families (miR-200a/200b/141) (Figure 5A). The Luciferase activity of the miR-200 family was inhibited by wild-type SOX2OT but was not inhibited by the mutant, thus demonstrating the direct-binding between them (Figure 5B, C, D). Furthermore, our results show that SOX2OT overexpression promotes the increased expression of the miR-200 family in tumor cells (Figure 5E). Therefore, since the oncogenic role of SOX2OT and its competitive repression on the miR-200 family, the miR-200 family is a significant suppressor of tumor development. Diaz-Riascos et al[20] found that

miR-200a, miR-429, and miR-205 are frequently overexpressed in pancreatic tumors in the mi-200 family, while cadherin-1(CDH1) is downregulated. Functional validation indicated that miR-141 and miR-429 inhibit the tumorigenic potential of pancreatic cancer cells[20]. The restraining function of miR-200 family members on migration and invasion of pancreatic cancer cells can be mediated by the suppression of the sonic hedgehog (Shh) pathway *via* modulating the expression of cyclin D1 and Bcl-2[21]. An intensive investigation found that ribonuclease monocyte chemoattractant protein-1 (MCPiP1) can counteract Dicer1 activity during miRNA-200 family maturation, leading to a dysregulation of this family in pancreatic cancer[22]. Our results represent the novel investigation on the important role of the miR-200 family in stemness maintenance in PC breast, and our observations are in accordance with other researches of the miR-200 family in breast cancer and glioma[23,24].

DEK is a structural protein within chromatin, possessing the chaperone activity of histone H3.3[25]. Several molecular functions in nuclei are regulated by DEK activity, inclu g transcription, DNA repair, and homo recombination[26-28]. Besides, DEK was fou o be of importance for long-term self-renev stem cells, including hematopoietic ones a cancerous ones[29]. In mamm rs, ove expressing miR-489 can ease genitor cell populations (CD49 D61hi) medi-ating oncogenes incl di EI suppressing tumo owth metastasis[30]. Increased DEK vates the mmosphere formation (ste aracteristi d motility of breast ancer partly *via* β-catenin activatio Shibata et a und that the aberrant c hosomal-gain o DEK oncogene loc p22.3) significantly associated with poo gn in high-grade neuroendocrine carcin ilencin DEK by small hairpin lea of lung cancer stem cell pr rty[32]. gh DEK is addressed to be in ed in th metastasis and inferior overall patients with PC[33-35], few studies ave demonstrated the role of DEK in stemness maintaining in PC cancer cells. Our research has shown that the SOX2OT-miR200 axis increases the endonuclease level of DEK in PC cells with a stem-cell-feature (Figure 6). Altogether, our results suggest that DEK acts as a modulator of stem cell property in PC cancer cells *via* a lncRNA-miRNA-interaction way.

Conclusions

We have demonstrated that high levels of SOX-2OT expression are associated with stem cell characteristics in PC. SOX2OT can strongly promote the proliferation and stemness maint of PC *in vitro*. Furthermore, DEK is u by increased SOX2OT *via* comple ary binding to miR-200a/141, resulting in growth of PC. These data suggest that he lnc SOX-2OT- miR-200a/141-DEK a ay be a is-ing therapeutic target for

Conflict of In t

The Authors d they have no of interests.

Data bility Stat t

Th sets analyzed during current study are avail-a om the corresponding author on reasonable request.

Di ure of F ncial Arrangements

The anuscript preparation are funded by Yu Fu.

ut s' Contribution

Guarantor of integrity of the entire study: Yu Fu; Study concepts: Yu Fu; Study design: Yu Fu; Literature research: Qiang Zhou; Clinical studies: Yandong Zhang; Animal experiments: Chuishui Liu, Yandong Zhang; Molecular assay: Chuishui Liu; Data acquisition: Chuishui Liu; Statistical analysis: Chuishui Liu, Qiang Zhou; Manuscript preparation: Chuishui Liu; Manuscript editing: Chuishui Liu; Manuscript review: Yu Fu, Yandong Zhang, Qiang Zhou.

References

1) Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018; 68: 394-424.

2) Zhao L, Kong H, Sun H, Chen Z, Chen B, Zhou M. LncRNA-PVT1 promotes pancreatic cancer cells proliferation and migration through acting as a molecular sponge to regulate miR-448. J Cell Physiol 2018; 233: 4044-4055.

3) Zhang SH, Liu GF, Li XF, Liu L, Yu SN. Efficacy of different chemotherapy regimens in treatment of advanced or metastatic pancreatic cancer: a network meta-analysis. J Cell Physiol 2018; 233: 3352-3374.

4) Zhou Y, Shan T, Ding W, Hua Z, Shen Y, Lu Z, Chen B, Dai T. Study on mechanism about long non-

coding RNA MALAT1 affecting pancreatic cancer by regulating Hippo-YAP signaling. J Cell Physiol 2018; 233: 5805-5814.

5) Xie SY, Li G, Han C, Yu YY, Li N. RKIP reduction enhances radioresistance by activating the Shh signaling pathway in non-small-cell lung cancer. Onco Targets Ther 2017; 10: 5605-5619.

6) Chen M, Liu P, Chen Y, Chen Z, Shen M, Liu X, Li X, Li A, Lin Y, Yang R, Ni W, Zhou X, Zhang L, Tian Y, Li J, Chen J. Long noncoding RNA FAM201A mediates the radiosensitivity of esophageal squamous cell cancer by regulating ATM and mTOR expression via miR-101. Front Genet 2018; 9: 611.

7) Mattick JS, Rinn JL. Discovery and annotation of long noncoding RNAs. Nat Struct Mol Biol 2015; 22: 5-7.

8) Akhade VS, Pal D, Kanduri C. Long noncoding RNA: genome organization and mechanism of action. Adv Exp Med Biol 2017; 1008: 47-74.

9) Chen HH, Zong J, Wang SJ. LncRNA GAPLINC promotes the growth and metastasis of glioblastoma by sponging miR-331-3p. Eur Rev Med Pharmacol Sci 2019; 23: 262-270.

10) Gu J, Wang Y, Wang X, Zhou D, Shao C, Zhou M, He Z. Downregulation of lncRNA GAS5 confers tamoxifen resistance by activating miR-222 in breast cancer. Cancer Lett 2018; 434: 1-10.

11) Fu Z, Chen C, Zhou Q, Wang Y, Zhao Y, Zha[illegible] W, Zheng S, Ye H, Wang L, He Z, Lin Q, Li [illegible] R. LncRNA HOTTIP modulates cancer ste[illegible]ell properties in human pancreatic cancer by [illegible]lating HOXA9. Cancer Lett 2017; 410: 68-81.

12) Wu XB, Feng X, Chang QM, Zh[illegible]Y, Wang [illegible] Liu J, Hu ZQ, Liu JZ, Wu W[illegible]P, Liu XC. Cross-talk among AFAP[illegible], AC[illegible] and microRNA-384 regulates [illegible]emness [illegible]pancreatic cancer cells and t[illegible]mo[illegible]city [illegible] Exp Clin Cancer R[illegible]2019;

13) Liu B, Wu S, M[illegible]an S, Xiao [illegible]n L, Zhang F, Shang M, M[illegible]cRNA GAS[illegible]erses EMT and tumo[illegible]m [illegible]ediated ge[illegible]tabine resistanc[illegible] and metas[illegible] by targeting miR-221/SOC[illegible] pancreatic [illegible]r. Mol Ther Nucleic Ac[illegible]018; 13: 472-482.

14) [illegible]Y, Zha[illegible] Li K, Gong L, Yang Q, Huang X, [illegible]C[illegible] M, Yang H. Linc-DYNC2H1-4 pro[illegible] and C[illegible]phenotypes by acting as a s[illegible] of miR[illegible] in pancreatic cancer cells. Cell D[illegible]Di[illegible]7; 8: e2924.

15) [illegible]hou W, [illegible], Wang R, Han Y, Wang C, Yang F, [illegible]n Y, Liang H, Qi L, Wang C, Guo Z, Gu Y. Identif[illegible] driver copy number alterations in diverse cancer types and application in drug repositioning. Mol Oncol 2017; 11: 1459-1474.

Tian W, Jiang C, Huang Z, Xu D, Zheng S. Comprehensive analysis of dysregulated lncRNAs, miRNAs and mRNAs with associated ceRNA network in esophageal squamous cell carcinoma. Gene 2019; 696: 206-218.

17) Teng Y, Kang H, Chu Y. Identification of an exosomal long noncoding RNA SOX2-OT in plasma as a promising biomarker for lung squamous cell carcinoma. Genet Test Mol Biomarkers 2019; 23: 235-240.

18) Li Z, Jiang P, Li J, Peng M, Zhao X, Zhang X, Chen K, Zhang Y, Liu H, Gan L, Bi H, Zhen P, Zhu J, Li X. Tumor-derived exosomal lnc-Sox2ot promotes EMT and stemness by acting as a ceRNA in p[illegible]ic ductal adenocarcinoma. Oncogen[illegible]; 3822-3838.

19) Wang J, Liu ZH, Yu LJ. Long n[illegible]ding RNA LINC00641 promotes cell growth a[illegible]igration through modulating miR-3[illegible]ZBTB[illegible]is in acute myeloid leukemia. [illegible]ev Med Ph[illegible]ol Sci 2019; 23: 7498-750[illegible]

20) Diaz-Riascos ZV, Gi[illegible]MM, F[illegible]at J, Serra[illegible] T, Busquets J, Bu[illegible], Capell[illegible]G. Expression and [illegible]of m[illegible]As from [illegible]miR-200 family i[illegible] tumor fo[illegible]n a[illegible]etastatic propensit[illegible]creatic can[illegible]ner Nucleic Acid[illegible]9; [illegible]91-503.

21) Fu J, Shrivastava [illegible]rivastava SK, Srivastava RK, S[illegible]. Triacetyl[illegible]ratrol upregulates miR-[illegible]and suppresse[illegible]Shh pathway in pancreatic cancer: a potentia[illegible] therapeutic agent. Int J Oncol 2019; [illegible]1306-1316.

2[illegible]oudouresque[illegible]ret C, Dobric A, Silvy F, Soubeyran [illegible]vanna J, [illegible]ardo D, Berthois Y. Ribonuclease [illegible]utes to the loss of micro-RNA-200 fam[illegible]ers in pancreatic cancer cells. Oncotarget 2018; 9: 35941-35961.

[illegible]B, Du R, Zhou L, Xu J, Chen S, Chen J, Yang X, [illegible]X, Shao ZM, Zhang L, Yu Z, Xie N, Guan JL, Liu S. miR-200c/141 regulates breast cancer stem cell heterogeneity via targeting HIPK1/beta-catenin axis. Theranostics 2018; 8: 5801-5813.

24) Zhang L, Zhang W, Li Y, Alvarez A, Li Z, Wang Y, Song L, Lv D, Nakano I, Hu B, Cheng SY, Feng H. SHP-2-upregulated ZEB1 is important for PDGFRalpha-driven glioma epithelial-mesenchymal transition and invasion in mice and humans. Oncogene 2016; 35: 5641-5652.

25) Ivanauskiene K, Delbarre E, McGhie JD, Kuntziger T, Wong LH, Collas P. The PML-associated protein DEK regulates the balance of H3.3 loading on chromatin and is important for telomere integrity. Genome Res 2014; 24: 1584-1594.

26) Privette Vinnedge LM, Kappes F, Nassar N, Wells SI. Stacking the DEK: from chromatin topology to cancer stem cells. Cell Cycle 2013; 12: 51-66.

27) Smith EA, Gole B, Willis NA, Soria R, Starnes LM, Krumpelbeck EF, Jegga AG, Ali AM, Guo H, Meetei AR, Andreassen PR, Kappes F, Vinnedge LM, Daniel JA, Scully R, Wiesmuller L, Wells SI. DEK is required for homologous recombination repair of DNA breaks. Sci Rep 2017; 7: 44662.

28) Kavanaugh GM, Wise-Draper TM, Morreale RJ, Morrison MA, Gole B, Schwemberger S, Tichy ED, Lu L, Babcock GF, Wells JM, Drissi R, Bissler JJ, Stambrook PJ, Andreassen PR, Wiesmuller L, Wells SI. The human DEK oncogene regulates DNA damage response signaling and repair. Nucleic Acids Res 2011; 39: 7465-7476.

29) Broxmeyer HE, Kappes F, Mor-Vaknin N, Legendre M, Kinzfogl J, Cooper S, Hangoc G, Markovitz DM. DEK regulates hematopoietic stem engraftment and progenitor cell proliferation. Stem Cells Dev 2012; 21: 1449-1454.

30) Patel Y, Soni M, Awgulewitsch A, Kern MJ, Liu S, Shah N, Singh UP, Chen H. Overexpression of miR-489 derails mammary hierarchy structure and inhibits HER2/neu-induced tumorigenesis. Oncogene 2019; 38: 445-453.

31) Privette Vinnedge LM, McClaine R, Wagh PK, Wikenheiser-Brokamp KA, Waltz SE, Wells SI. The human DEK oncogene stimulates beta-catenin signaling, invasion and mammosphere formation in breast cancer. Oncogene 2011; 30: 2741-2752.

32) Shibata T, Kokubu A, Miyamoto M, Hosoda F, Gotoh M, Tsuta K, Asamura H, Matsuno Y, Kondo T, Imoto I, Inazawa J, Hirohashi S. DEK oncoprotein regulates transcriptional modifiers and sustains tumor initiation activity in high-grade neuroendocrine carcinoma of the lung. Oncogene 2010; 29: 4671-4681.

33) Zhao T, Qiu B, Zhou S, Ding G, Cao L, Wu Z. Expression of DEK in pancreatic cancer and its correlation with clinicopathological feat[illegible] prognosis. J Cancer 2019; 10: 911-91[illegible]

34) Sun J, Bi F, Yang Y, Zhang Y, Jin A, [illegible]in Z. DEK protein overexpression predicts [illegible]prognosis in pancreatic ductal adenocarcinom[illegible]ol Rep 2017; 37: 857-864.

35) Wu X, Wu G, Wu Z, Ya[illegible]i G. MiR-20[illegible] presses the proliferat[illegible]nd me[illegible]tasis in [illegible] creatic ductal ade[illegible]cinom[illegible]rough dow[illegible]- regulation of DE[illegible] G[illegible] Tr[illegible] Oncol [illegible]16; 9: 25-31.