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IRF4-induced upregulation of lncRNA SOX2-OT promotes cell proliferation and metastasis in cholangiocarcinoma by regulating SOX2 and PI3K/AKT signaling

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Abstract. – OBJECTIVE: The aim of this study was to investigate the role of lncRNA SOX2-OT in the proliferation and metastasis of cholangiocarcinoma (CCA) and its underlying mechanisms.

PATIENTS AND METHODS: A total of 82 patients with CCA underwent surgery in our hospital were enrolled in this study. Five CCA cell lines (HuH-28, QBC939, HuCCT1, CCLP1, RBE) were used. The ability of proliferation and metastasis of CCA cells were detected by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay, colony formation assay, and transwell assay, respectively. Additionally, in vivo tumor metastasis assay was done. Furthermore, the Kaplan Meier method was used to validate the prognostic importance of SOX2-OT for patients with cholangiocarcinoma. Besides, the protein and mRNA expression of CCA cells were detected by quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) and Western blot, respectively.

RESULTS: The expression level of lncRNA SOX2-OT was significantly upregulated in cholangiocarcinoma tissues. Functional assays were further conducted to prove the oncogenic role of SOX2-OT on the proliferation and metastasis of cholangiocarcinoma cells. Furthermore, mechanism investigations manifested that transcription factor IRF4 upregulates SOX2-OT by promoting the transcriptional activity of SOX2-OT. SOX2-OT could positively regulate the nearby gene SOX2. SOX2-OT suppressed the nuclear transcription of PTEN, thereby activating PI3K/AKT signaling.

CONCLUSIONS: lncRNA SOX2-OT upregulated by IRF4 promotes cell proliferation and metastasis in cholangiocarcinoma via upregulating SOX2 and activating PI3K/AKT signaling pathway.

Key Words:
SOX2-OT, SOX2, Cholangiocarcinoma, Proliferation, PI3K/AKT signaling.

Introduction

Cholangiocarcinoma (CCA) is a malignant neoplasm which is highly aggressive. CCA is known for the increasing incidence[1]. So far, the efficient chemotherapy and radiotherapy for CCA has not been developed[2]. Radical resection is only effective for patients who are in the early stage[3]. Despite advances have been made in surgical techniques and chemotherapy or radiotherapy, the 5-year survival rates of patients with CCA who have received radical therapy is still less than 35%[4]. What's worse, the average overall survival of patients with unresectable tumors is usually less than 12 months[5]. The initiation and progression of CCA are very complex biological processes that are caused by the dysregulation of cancer-related genes. Therefore, it is significant to find or explore novel molecular mechanism by which the malignant phenotype of CCA was formed.

Long non-coding RNAs (lncRNAs) is a class of transcribed RNA molecules with length over 200 nucleotides[6-8]. According to previous reports[9-11], lncRNAs can modulate various biological processes in human malignant tumors. In this work, we tried to explore the specific function of a certain lncRNA in cholangiocarcinoma. Bioinformatics analysis was applied to find lncRNAs which closely associated with cholangiocarcinoma. We chose four lncRNAs which were not reported in CCA to perform Real Time-quantitative Polymerase Chain Reaction (RT-qPCR). Since lncRNA SOX2-OT was found to be significantly upregulated in CCA tissues, it was chosen to do further studies. The prognostic importance and biological function of SOX2-OT were identified in CCA. Subsequently, investigation was

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conducted to explore the molecular mechanism by which SOX2-OT exerts oncogenic function in CCA. Transcription factors can enhance the transcriptional activity of lncRNAs, thereby upregulating the expression of lncRNAs[12-14]. Here, we explored a transcription factor which can upregulate SOX2-OT in CCA. Moreover, the downstream molecular mechanism of SOX2-OT was investigated. Collectively, this study focused on the molecular mechanism by which SOX2-OT promotes CCA progression.

Patients and Methods

Tissue Specimens

A total of 82 patients with CCA underwent surgery at Qilu Hospital of Shandong University were enrolled in this study. 82 pairs of CCA tissues and adjacent non-malignant tissues were all collected from these patients and stored at -80°C. Our study had acquired the approval of the Ethics Committee of Qilu Hospital of Shandong University. The written informed consent was signed by all participants.

Cell Culture

All cell lines used in this study, including five CCA cell lines (HuH-28, QBC939, HuCCT1, CCLP1, RBE) and the intrahepatic biliary epithelial cell (HIBEC) were all commercially obtained from the Institute of Biochemistry and Cell Biology of the Chinese Academy of Sciences (Shanghai, China). Cells were maintained or preserved in Dulbecco's Modified Eagle's Medium (DMEM; Gibco, Waltham, MA, USA) which was mixed with 10% of fetal bovine serum (FBS; Gibco, Waltham, MA, USA), 100 U/mL penicillin and 100 μg/mL streptomycin. Cells were cultured in a humidified atmosphere at 37°C with 5% CO_2.

Plasmid Construction and Transfection

The whole sequence of SOX2-OT was synthesized and subcloned into a pcDNA3.1 vector (Thermo Fisher Scientific, Inc., Waltham, MA, USA). Next, pcDNA-SOX2-OT vector and the empty vector (pcDNA-NC) were transfected into the cells. To silence SOX2-OT in RBE cell, shRNAs specially target to SOX2-OT (sh-SOX2-OT) and negative control shRNA (sh-NC) were synthesized and purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). According to the instruction for users, Lipofectamine® 2000 (Invitrogen, Carlsbad, MA, USA) was used for transfection.

RT-qPCR

The isolation of total RNA from cells or tissues was conducted by using TRIzol reagent (Thermo Fisher Scientific, Inc., Waltham, MA, USA). Next, RNA was reversely transcribed into complementary Deoxyribose Nucleic Acid (cDNA) with PrimeScript™ RTMaster Mix (TaKaRa Biotechnology Co., Ltd., Dalian, China). RT-qPCR was conducted using random primers which were purchased from Augct DNA-Syn Biotechnology Co., Ltd. (Beijing, China). The relative expression level of genes was determined with $2^{-\Delta\Delta Cq}$ method. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as the internal reference.

MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyl Tetrazolium Bromide] Assay

CCLP1 and RBE cells (3×10^3) were incubated in a 96-well plate in DMEM (200 μL/well) in a humidified atmosphere (37°C + 5% CO_2). After incubation, MTT solution (Merck KGaA, Darmstadt, Germany) was added into each well. After the culture medium was discarded, 150 μL dimethyl sulfoxide (DMSO; Sigma-Aldrich, St. Louis, MO, USA) was added into each well and mixed for 10 min, thereby dissolving the crystallization. The absorbance was recorded by using a microplate reader to measure the wavelength of 570 nm at different time points (12, 24, 48, 72, and 96 h).

Colony Formation Assay

CCLP1 and RBE cells were plated in 6-well plates at a density of 500 cells per well and then incubated in Roswell Park Memorial Institute-1640 (RPMI-1640) medium (HyClone, South Logan, UT, USA) which was added into 10% of FBS at 37°C. 14 days later, cells were fixed with 4% methanol for 15 min and stained with 0.1% of crystal violet for 30 min. The visible colonies were calculated manually.

Flow Cytometry Analysis

After transfection was terminated, CCLP1 and RBE cells were stained with an Annexin V-fluorescein isothiocyanate (FITC)/propidium iodide (PI) kit (BD Biosciences, San Jose, CA, USA) in accordance with the instructions for users. The apoptosis condition of cells was analyzed by using a flow cytometer and CellQuest software version 0.9.3.1 (BD Biosciences, San Jose, CA, USA).

Transwell Assay

After necessary transfection, migration and invasion of CCLP1 and RBE cells were examined

with transwell chambers (8 μm pore size, Corning). To examine the cell invasion, the top of the insert was coated with Matrigel (BD Biosciences, Franklin Lakes, NJ, USA). Cells were cultured in the upper well. 24 hours later, cells invaded into the Matrigel layer were calculated. To measure the number of cell migration, cells were cultured in the upper well. 24 hours later, the cells migrated through the transwell plate were assessed. Cells were then fixed with 3% paraformaldehyde and stained with 1% crystal violet. A light microscope was utilized to calculate the number of migratory and invasive cells.

In vivo Tumor Metastasis Assay

All animal studies had acquired the approval of the Animal Experimental Committee of Qilu Hospital of Shandong University. The BALB/C nude mice (male, 4-5 weeks old) were obtained from the Experimental Animal Center of Qilu Hospital of Shandong University. To conduct *in vivo* metastasis assays, 1×10^7 BCPAP/sh-SOX2-OT and BCPAP/sh-NC cells were separately injected into nude mice through the spleen. 60 days later, all mice were sacrificed so as to observe the lung metastasis. Finally, the metastatic tissues were photographed and detected by hematoxylin and eosin (H&E) staining (Boster, Wuhan, China).

Luciferase Reporter Assay

The different fragment binding sequences of IRF4 and SOX2-OT promoter were synthesized and cloned into the pGL4 reporter vector. The luciferase activity was assessed by utilizing the dual-luciferase reporter assay system (Promega, Madison, WI, USA) and normalized to the Renilla.

Western Blot Analysis

Total protein was isolated with radioimmunoprecipitation assay (RIPA) buffer (Beyotime, Shanghai, China) and stored at -20°C. The protein concentration was assessed by using the bicinchoninic acid (BCA) protein assay kit (Thermo Fisher Scientific, Inc., Waltham, MA, USA). The protein samples were averagely isolated by 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred to polyvinylidene difluoride membranes (Sangon Biotech Co., Ltd., Shanghai, China). Subsequently, the membranes were blocked with 5% defatted milk at 37°C for about 1 h. Next, the membranes were incubated with the primary antibodies at 4°C overnight and with secondary antibody at 37°C for 1 h. All antibodies used in this experiment were obtained from Abcam (Cambridge, MA, USA). The candidate proteins referred to glyceraldehyde 3-phosphate dehydrogenase (GAPDH). The protein bands were visualized with the enhanced chemiluminescence detection reagent (Thermo Fisher Scientific, Inc., Waltham, MA, USA).

Statistical Analysis

Statistical Product and Service Solutions (SPSS) software (version 19.0; IBM Corp., Armonk, NY, USA) was utilized to analyze all data. Data obtained from more than two independent experiments displayed as the mean ± standard deviation (SD). Differences between two groups or more than two groups were analyzed with the Student's *t*-test or One-way ANOVA (LSD post-hoc test). Survival curve was generated and analyzed by the Kaplan-Meier method and the log-rank test. Expression correlation was analyzed with Spearman's correlation analysis. $p<0.05$ was considered to be statistically significant.

Results

SOX2-OT is Upregulated in CCA Tissues and Closely Associated with the Prognosis of CCA Patients

We found some lncRNAs associated with CCA from a public bioinformatics website (https://www.genecards.org/). Four of these lncRNAs (SOX2-OT, SNHG1, ZFAS1, SNHG12) were chosen for further analysis due to they are not reported in CCA. We examined the level of these four lncRNAs in CCA tissues and adjacent normal tissues by applying qRT-PCR. The results revealed that only SOX2-OT was significantly upregulated in CCA tissues (Figure 1A). Furthermore, the level of SOX2-OT was tested in CCA cells (HuH-28, QBC939, HuCCT1, CCLP1, RBE) and the intrahepatic biliary epithelial cell (HIBEC). Unsurprisingly, the level of SOX2-OT was much higher in CCA cells (Figure 1B). Using the mean value of SOX2-OT expression as the threshold, CCA samples used in this study were divided into two groups: SOX2-OT above mean (n=44) and SOX2-OT below mean (n=38). The clinical significance of SOX2-OT was analyzed and shown in Table I. The Kaplan Meier analysis revealed that the high expression of SOX2-OT indicated the lower overall survival rate of CCA patients (Figure 1C).

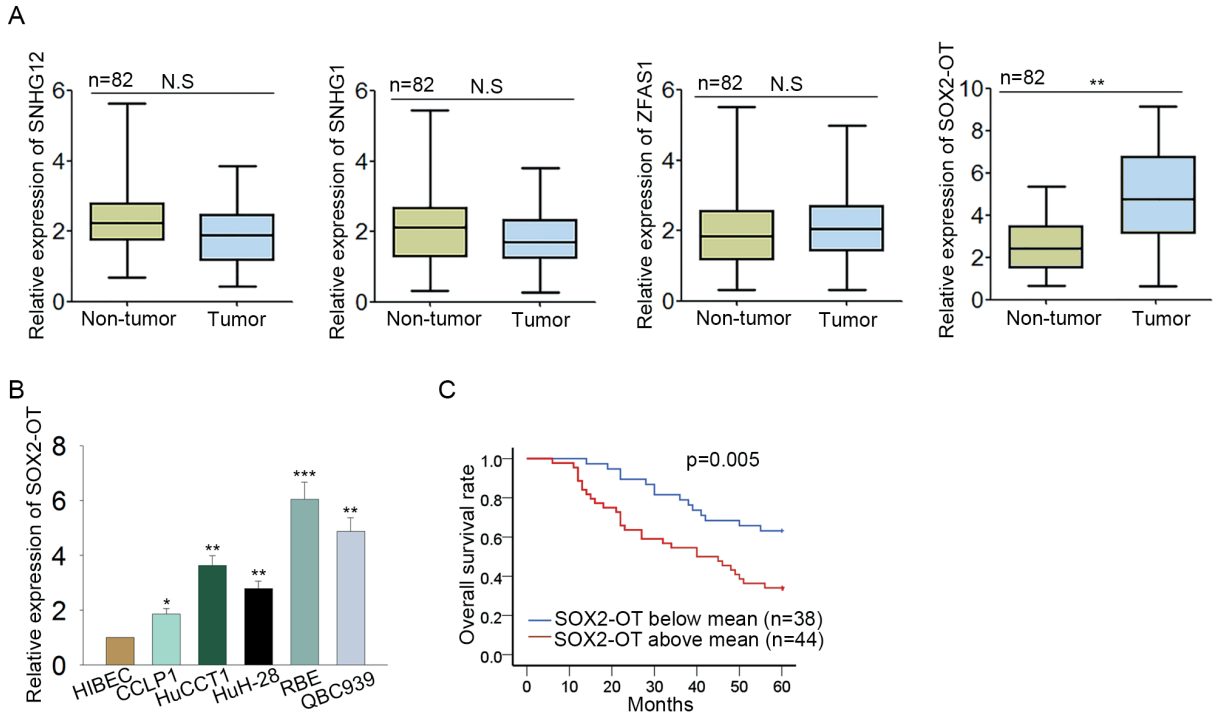

Figure 1. SOX2-OT is upregulated in CCA tissues and closely associated with the prognosis of CCA patients. ***A,*** The expression pattern of four lncRNAs associated with CCA was examined in CCA tissues and corresponding non-malignant tissues with qRT-PCR. ***B,*** The level of lncRNA SOX2-OT was tested in CCA cells (HuH-28, QBC939, HuCCT1, CCLP1, RBE) and the intrahepatic biliary epithelial cell (HIBEC). ***C,*** Kaplan Meier method was applied to analyze the relevance between SOX2-OT expression and the overall survival of CCA patients. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ *vs.* control group. N.S: no significance.

SOX2-OT Positively Regulates the Proliferation and Metastasis of CCA Cells

According to the data of Figure 1B, CCLP1 cell presented the lowest expression level of SOX2-OT, whereas RBE cell presented the highest expression level of SOX2-OT. Therefore, SOX2-OT was overexpressed in CCLP1 cell by transfecting with pcDNA-SOX2-OT (Figure 2A, left), while SOX2-OT was silenced in RBE cell by transfecting with sh-SOX2-OT (Figure 2A, right). Cells transfected with empty vector (pcDNA-SOX2-OT) or control shRNA (sh-NC) were used as control group. MTT and colony formation assay revealed that the proliferative ability of CCLP1 cell was improved by pcDNA-SOX2-OT (Figure 2B-2C, left), whereas the proliferative ability of RBE cell was markedly weakened by sh-SOX2-OT (Figure 2B-2C, right). Additionally, the apoptosis rate of CCLP1 cell was decreased by pcDNA-SOX2-OT, whereas that of RBE cell was increased by sh-SOX2-OT (Figure 2D). Furthermore, the migratory ability of CCLP1 cell was significantly enhanced by pcDNA-SOX2-OT (Figure 2E, left), whereas the migratory ability of RBE cell was markedly suppressed by sh-SOX2-OT (Figure 2E, right). Similarly, the invasive ability of RBE and CCLP1 cells was also positively regulated by SOX2-OT (Figure 2F). *In vivo* experiment was further performed to demonstrate the effect of SOX2-OT on the metastasis of CCA cells. As illustrated in Figure 2G, H&E-stained lung sections revealed that control tumors showed stronger metastatic ability than SOX2-OT-knockdown tumors.

Transcription Factor IRF4 Enhances the Transcription Activity of SOX2-OT

All findings above suggested that upregulation of SOX2-OT is an oncogenic factor for the tumorigenesis of CCA. Here, we further investigated the reason why SOX2-OT was upregulated in CCA. According to the data of UCSC (http://genome.ucsc.edu/), IRF4 is an upstream transcription factor of SOX2-OT. As presented in Figure 3A, an IRF4 binding site in the SOX2-OT promoter region was predicted using JASPAR (http://jaspar.genereg.net). Top five putative binding sequences between IRF4 and SOX2-OT promoter were

chosen for further analysis. The diagram shown in Figure 3B revealed the affinity of IRF4 to the promoter region of SOX2-OT. ChIP assay further verified the affinity of IRF4 to the part 2 (P2) of SOX2-OT promoter (Figure 3C). To demonstrate the combination between IRF4 and SOX2-OT promoter, three putative binding sites in the P2 segment were inserted into the pGL4 vector. Luciferase reporter assay certified that the luciferase activity of three binding sites was positively regulated by IRF4 (Figure 3D). Furthermore, it was uncovered that IRF4 was expressed much higher in CCA tissues and cell lines (Figure 3E-3F). Naturally, the positive expression correlation between SOX2-OT and IRF4 was analyzed by the Spearman's correlation analysis (Figure 3G). To further certify the regulatory relationship between IRF4 and SOX2-OT, IRF4 was separately overexpressed or silenced in two different CCA cell lines (CCLP1 and RBE) (Figure 3H). Afterwards, the expression level of SOX2-OT was uncovered to be positively modulated by IFR4 in CCA cell lines (Figure 3I).

LncRNA SOX2-OT Exerts Function Through Upregulating SOX2 and Activating PI3K/AKT Signaling

The data of UCSC revealed that SOX2 is a nearby gene of SOX2-OT (Figure 4A). Furthermore, we analyzed the regulatory relationship between SOX2-OT and SOX2 in CCA. At first, the higher level of SOX2 was observed in CCA tissues (Figure 4B). The positive correlation between SOX2-OT expression and SOX2 expression in CCA tissues was then analyzed by the Spearman's correlation analysis (Figure 4C). The results of qRT-PCR and Western blotting validated the positive regulatory effects of SOX2-OT on SOX2 (Figure 4D-4E). The PI3K/AKT signaling pathway is closely associated with the cell proliferation and metastasis of human malignant tumors[15,16]. Whereas, PTEN is a well known inhibitor of PI3K/AKT signaling pathway. Here, we examined the protein levels of the core factors of the PTEN/PI3K/AKT pathway in CCLP1 and RBE cells. The results revealed that SOX2-OT inhibited the expression

Table I. Correlation between the expression of lncRNA SOX2-OT and clinicopathological features of patients with CCA (n=82).

	SOX2-OT Expression			
Variable	**Low**	**High**	**χ^2-value**	***p*-value**
Age				
<60	7	18	0.122	0.727
≥60	21	26		
Gender				
Male	27	27	0.851	0.356
Female	11	17		
Tumor site				
Intrahepatic	10	14	0.298	0.585
Extrahepatic	28	30		
Differentiation grade				
Well/moderately	26	32	0.183	0.669
Poor/undifferentiatited	12	12		
Tumor stage				
T1-T2	19	8	11.421	0.002
T3-T4	19	36		
TNM stage				
I-II	28	16	9.347	0.001
III-IV	10	28		
Postoperative recurrence				
Absent	23	12	9.216	0.002
Present	15	32		
Lymph node invasion				
Absent	19	12	4.479	0.034
Present	19	32		

Low/high by the sample mean. Pearson χ^2-test. $p<0.05$ was considered statistically significant.

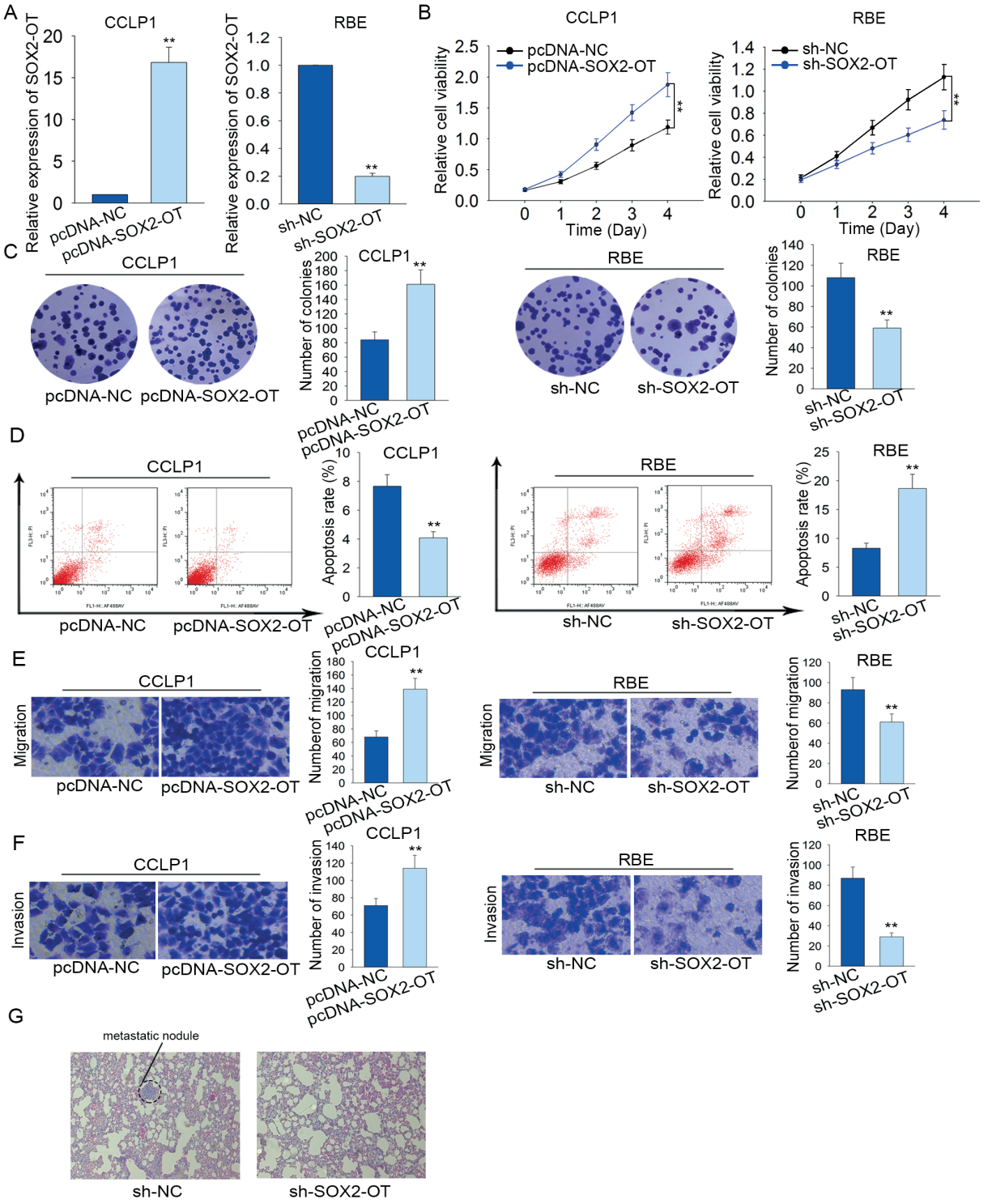

Figure 2. SOX2-OT positively regulates the proliferation and metastasis of CCA cells. ***A,*** SOX2-OT was overexpressed in CCLP1 cell by transfecting with pcDNA-SOX2-OT. A cell transfected with empty vector (NC) was used as control group for next assays. SOX2-OT was silenced in RBE cell by transfecting with sh-SOX2-OT. RBE cell transfected with control shRNA (sh-NC) was used as control group for subsequent experiments. ***B-C,*** MTT and colony formation assay were conducted to examine the proliferative ability of CCLP1 and RBE cells at 48 hour's post-transfection. ***D,*** Apoptosis rate was examined in CCLP1 and RBE cells which underwent different transfections. ***E-F,*** Transwell assay detected the effects of SOX2-OT overexpression and knockdown on cell migration and invasion. ***G,*** H&E staining of metastatic tumor tissues injected with BCPAP/sh-NC and BCPAP/sh-SOX2-OT were shown. $^{**}p<0.01$ *vs.* control group.

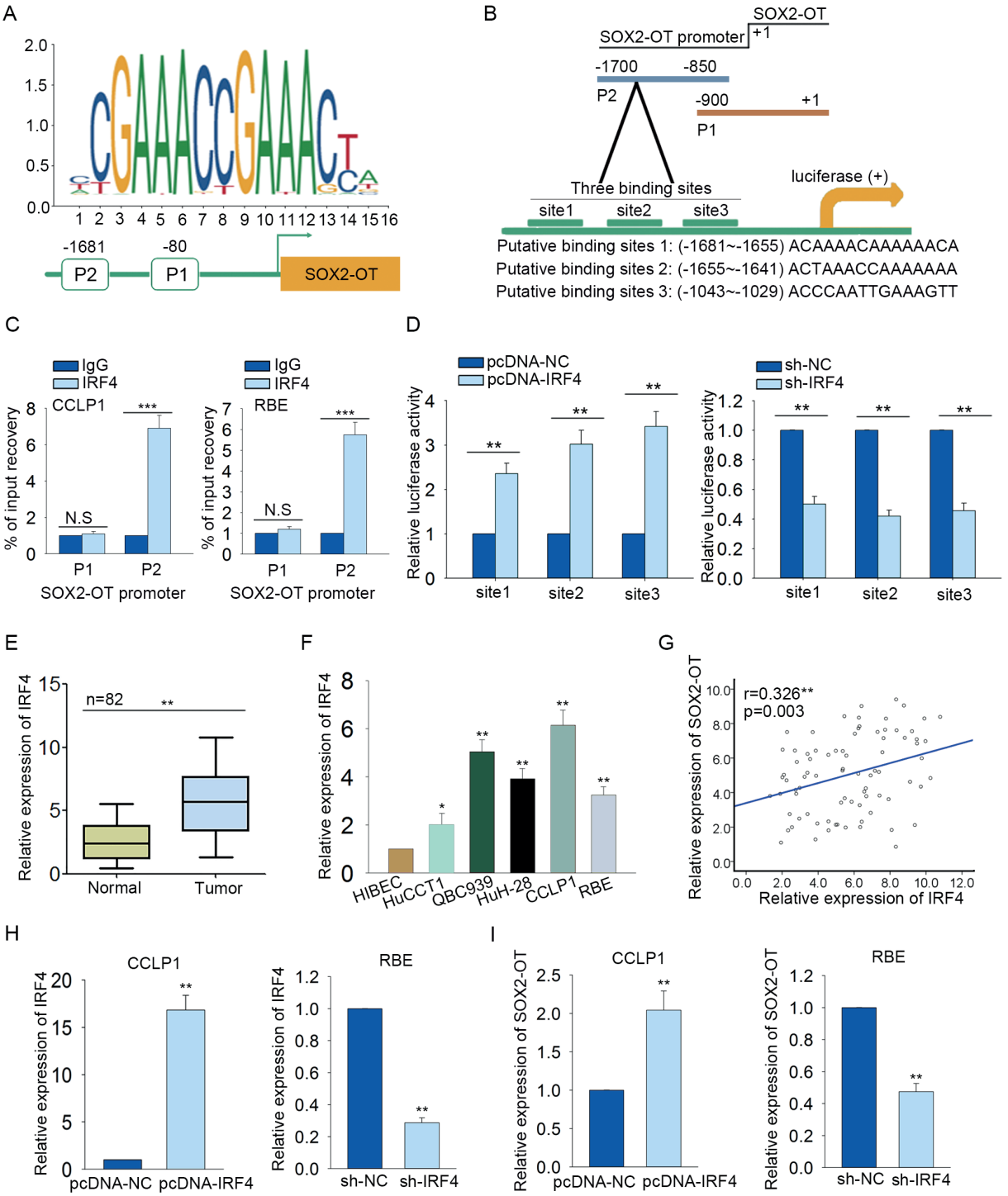

Figure 3. Transcription factor IRF4 enhances the transcription activity of SOX2-OT. ***A,*** IRF4 binding site in the SOX2-OT promoter region was predicted using JASPAR. ***B,*** Diagram revealed the affinity of IRF4 to the promoter region of SOX2-OT was generated. ***C,*** ChIP assay detected the affinity of IRF4 to the two parts (P1 and P2) of SOX2-OT promoter. ***D,*** The combination between IRF4 and the SOX2-OT promoter was identified with luciferase reporter assay. ***E-F,*** The expression level of IRF4 in CCA tissues and cell lines was examined. Results were obtained with qRT-PCR. ***G,*** The expression correlation between SOX2-OT and IRF4 was analyzed with Spearman's correlation analysis. ***H,*** IRF4 was overexpressed or silenced in CCLP1 cell and RBE cell by transfecting with pcDNA-IRF4 or sh-IRF4, respectively. ***I,*** The expression of SOX2-OT was measured in response to IRF4 overexpression or knockdown. $^{*}p<0.05$, $^{**}p<0.01$ *vs.* control group.

of PTEN and activated the PI3K/AKT signaling pathway (Figure 4F). To investigate how did SOX2-OT downregulate PTEN in CCA cells, further mechanism experiments were conducted. To find the transcription factors which could modulate the expression of PTEN by interacting with SOX2-OT, a pull-down assay was conducted by using the biotin-labeled SOX2-OT promoter, and the results were analyzed using the mass spectrometry (Figure 4G). Six of 23 candidate proteins were chosen for subsequent experiments due to they have been reported as nuclear receptor coactivators or transcription factors. The expression of PTEN was found to be significantly regulated by FOXA1 in CCLP1 and RBE cells (Figure 4H). Furthermore, ChIP assay and luciferase reporter assay demonstrated that FOXA1 suppressed the transcription activity of PTEN by binding to the promoter region of PTEN (Figure 4I-4J). According to the results of subcellular fractionation assay, the expression of FOXA1 was enriched in the nucleus of CCA cells (Figure 4K). Furthermore, the levels of PTEN was decreased in the nucleus by FOXA1 (Figure 4L), suggesting that FOXA1 could weaken the translocation to the nucleus of PTEN.

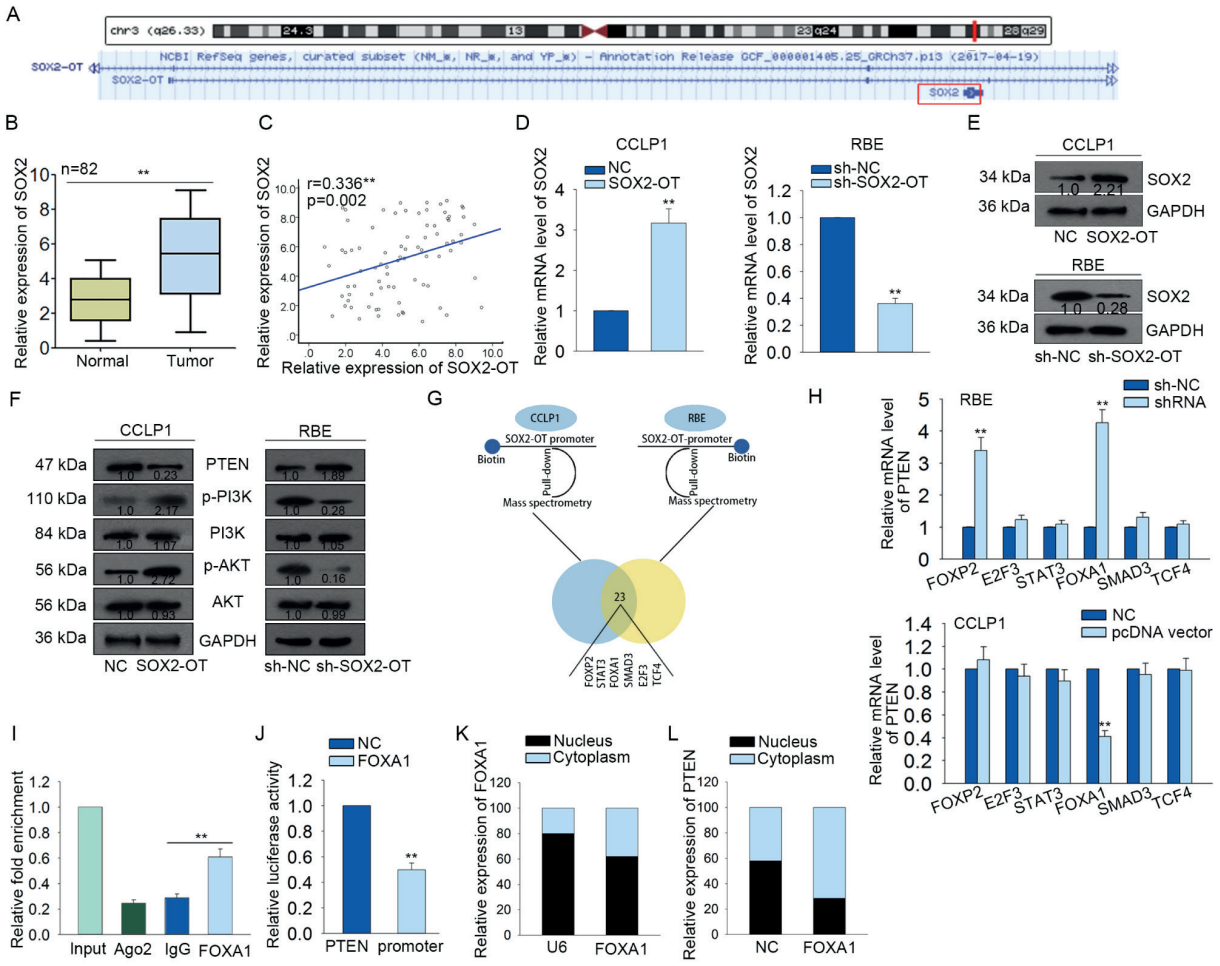

Figure 4. LncRNA SOX2-OT exerts function through upregulating SOX2 and activating PI3K/AKT signaling pathway. ***A,*** Data of UCSC illustrated the potential interaction between SOX2 and SOX2-OT. ***B,*** qRT-PCR tested the level of SOX2 in CCA tissues and corresponding non-tumor tissues. ***C,*** The expression relevance between SOX2-OT and SOX2 in CCA tissues was analyzed by Spearman's correlation analysis. ***D-E,*** The effects of SOX2-OT on the mRNA level and protein level of SOX2 were tested. ***F,*** The levels of PTEN and phosphorylated PI3K and AKT (p-PI3K and p-AKT) were examined in indicated CCA cells. ***G,*** The combination between SOX2-OT and the potential upstream transcription factors of PTEN was analyzed by pull-down assay and one-shot mass spectrometry. ***H,*** The expression of PTEN was detected in response to the expression changes of six transcription factors. ***I-J,*** ChIP assay and luciferase reporter assay were performed to identify the affinity of FOXA1 to the PTEN promoter. ***K,*** The localization of FOXA1 in the nucleus or cytoplasm of CCA cell was determined. ***L,*** The effects of FOXA1 on the nucleus translocation of PTEN was examined by subcellular fractionation assay. $^{**}p<0.01$, *vs.* control group.

Discussion

Based on the bioinformatics analysis, four lncRNAs were chosen for further detection because they are potentially correlated with CCA. All these lncRNAs were examined in CCA tissues and corresponding non-tumor tissues. As a result, lncRNA SOX2-OT was the only one which was upregulated in CCA tissues. Therefore, SOX2-OT was further studied in CCA. To identify the research value of SOX2-OT for CCA, the prognostic value was identified. The research results suggested that high expression of SOX2-OT is a prognostic factor for patients with CCA. Therefore, it is valuable to investigate the biological function of SOX2-OT in CCA progression. Data of functional assays demonstrated that SOX2-OT is a tumor promoter in CCA.

Numerous studies have revealed that lncRNAs can act as tumor suppressors[17-19] or oncogenes[20-24] in human malignant tumors. As we all know, lncRNAs usually exert function through being regulated by some biological molecules. The present work has certified that the upregulation of lncRNAs is induced by transcription factors. Here, we also investigated the upstream molecular mechanism of SOX2-OT. Mechanism experiments revealed that SOX2-OT was upregulated by transcription factor IRF4. Moreover, the findings of UCSC indicated that SOX2 is a nearby gene of SOX2-OT. The expression pattern of SOX2 was consistent with that of SOX2-OT. Naturally, the positive expression correlation between SOX2-OT and SOX2 was analyzed. qRT-PCR and Western blot analysis determined the positive regulatory effects of SOX2-OT on SOX2. Several studies[25,26] have revealed that activation of PI3K/AKT signaling pathway is closely associated with lncRNA-mediated progression of malignant tumors. In this study, we examined the correlation between SOX2-OT and PI3K/AKT signaling pathway. The phosphorylation of PI3K and AKT was found to be positively regulated by SOX2-OT. PTEN is a famous inhibitor of PI3K/AKT signaling. Here, PTEN was uncovered to be downregulated by SOX2-OT. To detect how did SOX2-OT downregulate PTEN in CCA cells, further mechanism investigation was carried out. The upstream transcription factors of PTEN were found from UCSC. By applying pull-down assay and mass spectrometry, six transcription factors were screened out. The regulatory relationship between these six transcription factors and PTEN was further analyzed in two CCA cells. The result manifested that only FOXA1 could significantly regulate PTEN in CCA cells. Mechanism experiments including ChIP assay and luciferase reporter assay revealed the combination between FOXA1 and the promoter region of PTEN. Both results suggested that FOXA1 could inhibit the transcription activity of PTEN in CCA cells. Furthermore, FOXA1 was found to be located in the nucleus of the CCA cell, indicating that FOXA1 can regulate gene expression at transcriptional level. Simultaneously, the levels of PTEM was decreased in the nucleus when FOXA1 was overexpressed. It suggested that FOXA1 could weaken the translocation to the nucleus of PTEN. Taken together, SOX2-OT suppressed PTEN transcription by interacting with FOXA1. Combining with the findings above, we confirmed that SOX2-OT activated PI3K/AKT signaling pathway by inhibiting PTEN transcription.

Conclusions

We showed that IRF4-induced upregulation of SOX2-OT promoted the progression of CCA by upregulating SOX2 and activating PI3K/AKT signaling pathway. This study revealed a novel molecular mechanism which centered with SOX2-OT. Our research findings may help to find novel therapeutic targets for CCA.

Conflict of Interest

The Authors declare that they have no conflict of interest.

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