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TPX2 promotes papillary renal cell carcinoma progression by forming a ceRNA with LINC00894

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Abstract

Purpose Papillary renal cell carcinoma (pRCC), particularly type 2, is associated with a poor prognosis. This study aimed to identify molecular mechanisms underlying pRCC progression and explore potential therapeutic targets to improve patient outcomes.

Methods TPX2 expression was analyzed in tumor samples from patients with type 2 pRCC. In vitro experiments were conducted to assess the effects of TPX2 and LINC00894 knockdown and overexpression on the proliferation and migration of Caki-2 and ACHN cells. Immunohistochemical analysis of tissue microarrays was performed to evaluate the associations between TPX2 expression and clinicopathological characteristics in type 2 pRCC patients.

Results Elevated TPX2 expression was significantly associated with a worse prognosis in type 2 pRCC patients and served as an independent risk factor for overall survival. Knockdown of TPX2 in Caki-2 and ACHN cells significantly reduced cell proliferation and migration. Additionally, LINC00894 was highly expressed in type 2 pRCC and correlated with poor prognosis. Mechanistically, miR-660-5p targeted the TPX2 3′ UTR, promoting TPX2 degradation, while LINC00894 competitively bound to miR-660-5p, protecting TPX2 from miRNA-mediated degradation and exerting a pro-oncogenic effect. Immunohistochemical analysis revealed significant correlations between TPX2 expression and clinicopathological features, including tumor thrombus volume, tumor diameter, pathological TNM stage, and Fuhrman grade.

Conclusion This study underscores the critical role of TPX2 in type 2 pRCC progression and highlights its potential as a prognostic biomarker and therapeutic target. The TPX2/LINC00894/miR-660-5p regulatory axis provides novel insights into the molecular mechanisms driving pRCC and offers a promising avenue for improving patient prognosis.

Keywords Papillary renal cell carcinoma, TPX2, miR-660-5p, LINC00894, ceRNA

Introduction

Papillary renal cell carcinoma (pRCC) stands as the second most prevalent form of renal cell carcinoma globally [1]. Emerging evidence suggests that patients with type II pRCC often face a more unfavorable prognosis compared to those with clear—cell renal cell carcinoma (ccRCC). Unfortunately, accurately predicting the prognosis of pRCC patients remains a considerable challenge [2]. Delving deeper into the pathogenesis of type 2 pRCC and exploring targeted therapeutic strategies based on its distinct pathophysiological features hold the potential

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to enhance our understanding of the disease, foster the development of novel treatment modalities, and ultimately improve patient outcomes.

Dysregulation of cell—cycle control is a well—established hallmark of tumorigenesis [3]. Xenopus kinesin—like protein 2 (XKLP2, TPX2), a key mitotic regulator, plays an indispensable role in microtubule formation during chromosome division within the cell cycle [4]. Recent research has broadened our understanding of TPX2, revealing its involvement not only in cell—cycle regulation but also in critical tumor—related processes such as metastasis and apoptosis, underscoring the multifaceted nature of this gene [5]. Nevertheless, the intricate molecular mechanisms through which TPX2 participates in these processes remain largely unexplored.

In this study, we made a notable discovery: TPX2 expression was significantly upregulated in patients with type 2 pRCC when compared to normal kidney tissue. Moreover, a strong correlation was established between higher TPX2 expression levels and poor prognosis in pRCC patients. Through our in—depth investigations, we identified that TPX2 modulates tumor progression by forming a competing endogenous RNA (ceRNA) complex with LINC00894/miR—660—5p. By integrating immunohistochemical findings with clinical prognostic data, our results indicate that TPX2 holds great promise as a potential prognostic marker for type 2 pRCC.

Materials and methods

Patient tissue samples

This study was approved by the Medical Scientific Research Ethics Committee of Peking University Third Hospital (ethics number M2017147). Written informed consent was obtained from all participants, and measures were implemented to ensure the confidentiality of patient information. Tumor and adjacent non-tumor tissues were collected from 80 patients diagnosed with type 2 papillary renal cell carcinoma (pRCC). Tissues were preserved in liquid nitrogen, formalin solution, or RNAlater solution (Qiagen). Ten samples were selected for next-generation sequencing. Additionally, 80 formalin-fixed paraffin-embedded (FFPE) type 2 pRCC tissues and their matched adjacent tissues were used to construct tissue microarrays. Pathological assessments were independently conducted by two experienced pathologists, and clinical data were collected, managed, and analyzed following standardized protocols to ensure accuracy and reliability. Further details are provided in Table 1.

Cell cultures

The human embryonic kidney cell line (HEK-293 T) and renal cancer cell lines (Caki-2, ACHN) were obtained from the American Type Culture Collection (ATCC, Manassas, Virginia, USA). Based on literature and cell line characteristics, Caki-2 cells were cultured in McCoy's 5A medium supplemented with 10% fetal bovine serum (FBS), while ACHN cells were maintained in MEM supplemented with 10% FBS. Other cell lines were cultured as previously described.

Plasmids and transfection

Short hairpin RNA (shRNA) sequences targeting TPX2 and LINC00894 were designed, cloned, and inserted into the pLKO.1-Puro vector (Addgene). Correct insertion was confirmed by bidirectional sequencing. The shRNA sequences are listed in Table S3. The target gene was synthesized and cloned into the PLVX-BSD vector (Addgene), following a similar protocol as for shRNA vector construction.

For lentivirus production, HEK-293T cells were transfected with psPAX2 (Addgene) and pMD2.G (Addgene) using Lipo3000. After 48 h of incubation, lentiviral particles were harvested, filtered, and concentrated. Target cells were infected with lentivirus in the presence of polybrene (Beyotime) and selected using puromycin or blasticidin.

Data mining and analysis

Clinical profiles of patients in The Cancer Genome Atlas (TCGA) Kidney Renal Papillary Cell Carcinoma (KIRP) cohort were accessed via https://cancergenome.nih.gov/. Kaplan–Meier (K-M) survival analysis and correlation studies were performed using GEPIA and GEPIA2 (http://gepia.cancer-pku.cn/index.html) [19, 20]. Data analysis was conducted using R Studio, incorporating packages such as limma, Cox, and NetworkD3.

Statistical analysis

Statistical analyses were performed using GraphPad Prism version 9.0 (La Jolla, CA, USA). A two-tailed t-test was used to determine statistical significance, with thresholds set at *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

Results

Identification and characteristics of TPX2 in pRCC

To identify potential genes implicated in type 2 papillary renal cell carcinoma (pRCC), we analyzed tumor and adjacent normal tissues from 10 consented patients with type 2 pRCC. Transcriptome sequencing of paired fresh samples revealed differentially expressed mRNAs between tumor and normal tissues. Genes with $|\log2FC| > 1$ and $p < 0.05$ were selected as significant, identifying 676 differentially expressed genes (DEGs) (Fig. 1A). To validate these findings, we analyzed data from 321 Kidney Renal Papillary Cell Carcinoma (KIRP) patients in the TCGA database, including 289 tumor and

Table 1 Correlations between TPX2 expression in tissues and clinicopathologic characteristics of type 2 pRCC patients

Characteristics	TPX2 expression		*P* value
	Low	High	
n	40	39	
Gender, *n* (%)			0.486
Male	28 (35.4%)	30 (38%)	
Female	12 (15.2%)	9 (11.4%)	
Age, mean ± sd	58.7 ± 12.035	59.436 ± 11.534	0.782
BMI, median (IQR)	25.15 (22.999, 27.298)	24.251 (22.626, 26.598)	0.468
Concomitant symptoms, *n* (%)			< 0.001
Yes	12 (15.2%)	27 (34.2%)	
No	28 (35.4%)	12 (15.2%)	
Tumor thrombus, *n* (%)			< 0.001
Yes	6 (7.6%)	29 (36.7%)	
No	34 (43%)	10 (12.7%)	
Maximum diameter of tumor, median (IQR)	4.35 (2.925, 5.725)	8.5 (5.5, 10.7)	< 0.001
T.stage, *n* (%)			< 0.001
≥T3a	11 (13.9%)	29 (36.7%)	
<T3a	29 (36.7%)	10 (12.7%)	
N.stage, *n* (%)			< 0.001
N1	6 (7.6%)	22 (27.8%)	
N0	34 (43%)	17 (21.5%)	
M.stage, *n* (%)			0.030
M1	3 (3.8%)	10 (12.7%)	
M0	37 (46.8%)	29 (36.7%)	
Fuhrman grading, *n* (%)			0.001
1	3 (3.8%)	0 (0%)	
2	19 (24.1%)	7 (8.9%)	
3	18 (22.8%)	26 (32.9%)	
4	0 (0%)	6 (7.6%)	

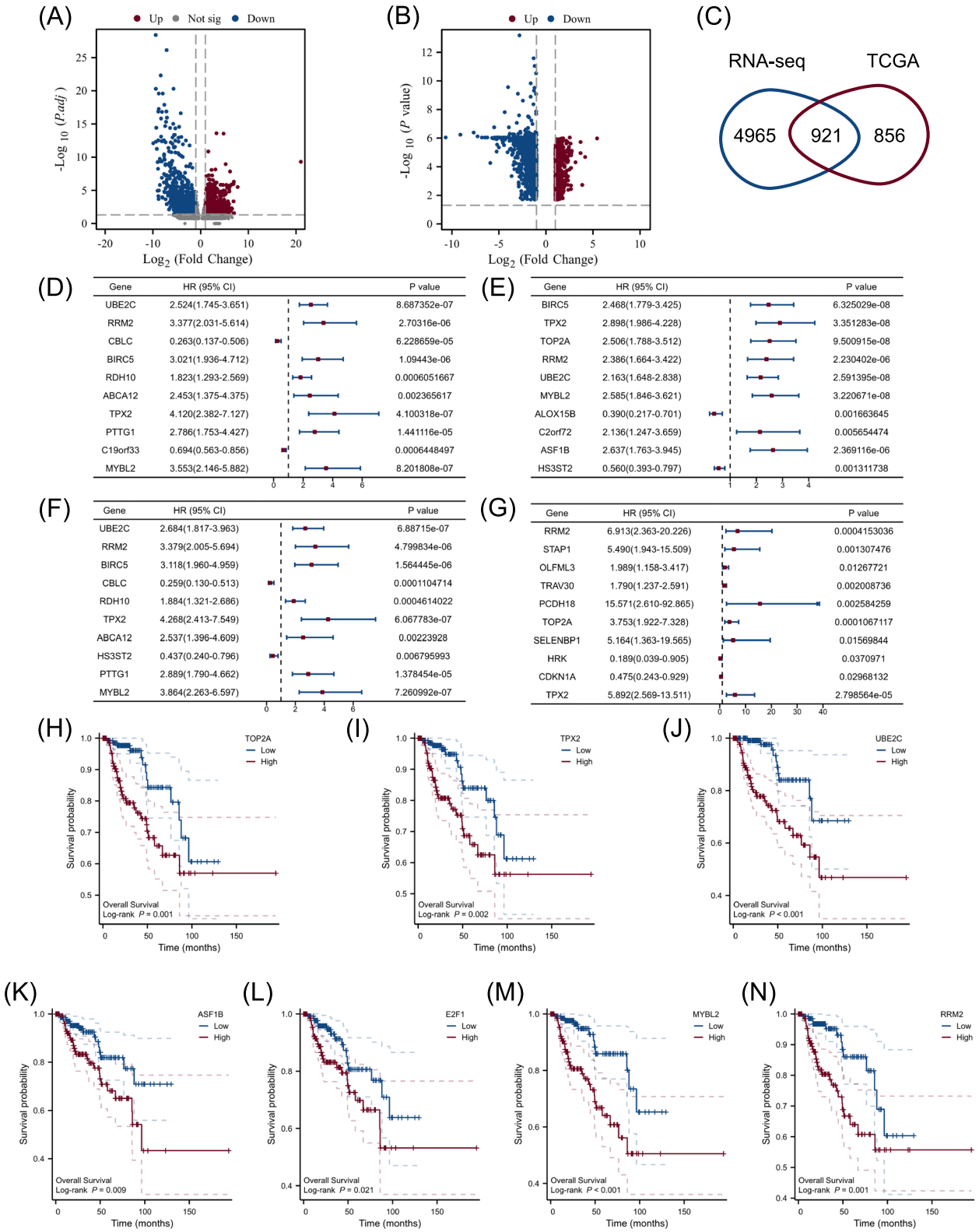
32 normal samples. Additionally, 85 type 2 pRCC tumor samples and 9 adjacent normal samples were examined, revealing 921 overlapping DEGs across datasets (Fig. 1B-C).

Survival analysis was performed using the day of surgery as the starting point and death or loss to follow-up as the endpoint. Patients were stratified into high- and low-expression groups based on median gene expression levels. Genes significantly associated with overall survival (OS) (p-adj < 0.05) were ranked, and the top 10 were visualized in a forest plot (Fig. 1D). We also evaluated progression-free survival (PFS), disease-specific survival (DSS), and disease-free interval (DFI) (Fig. 1E-G).

The DEGs identified in both public databases and clinical samples, including E2F1, UBE2C, MYBL2, ASF1B, TPX2, RRM2, and TOP2A, were significantly correlated with clinical prognostic indicators. Among these, TPX2 exhibited the most distinct expression pattern and was associated with poor prognosis (Fig. 1H-N).

(See figure on next page.)

Fig. 1 Identification and Characterization of TPX2 in pRCC. **A**-**B** Volcano plot depicting significantly upregulated and downregulated genes. **C** Venn diagrams illustrating overlapping differentially expressed genes identified from the TCGA database and next-generation sequencing data. **D**-**G** Forest plots highlighting 10 genes significantly associated with overall survival (OS), progression-free interval (PFI), disease-specific survival (DSS), and disease-free interval (DFI). **H**-**N** Kaplan–Meier survival curves demonstrating the prognostic significance of E2F1, UBE2C, MYBL2, ASF1B, TPX2, PRRM2, and TOP2A in pRCC patients



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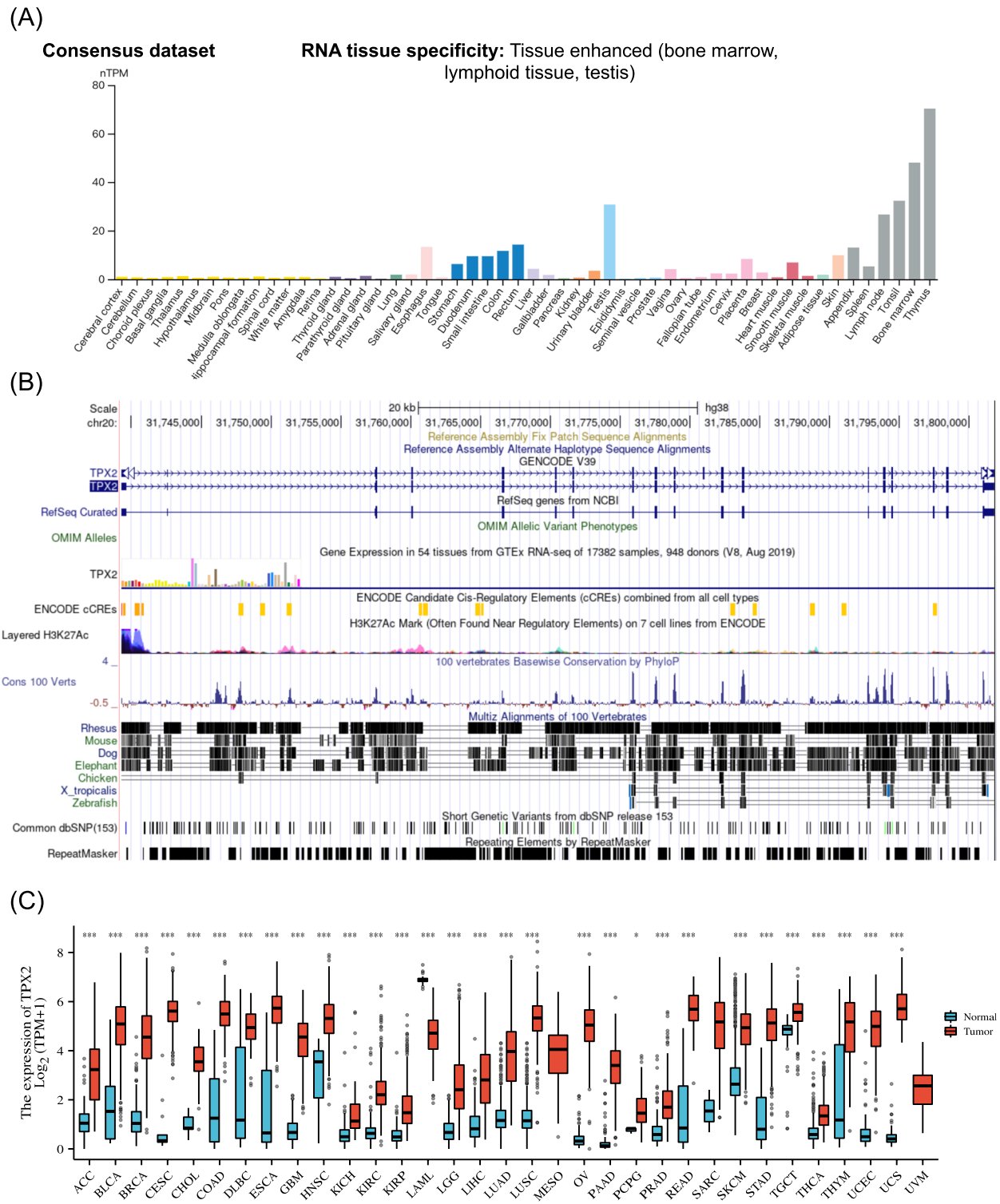


Fig. 2 Expression and Conservation of TPX2 in Human Tissues and Tumors. **A** Tissue-specific expression of TPX2 based on the Human Protein Atlas (HPA) and Genotype-Tissue Expression (GTEx) datasets.**B** Evolutionary conservation of the TPX2 gene in Homo sapiens visualized using the UCSC Genome Browser. **C** Pooled analysis of TPX2 expression across various tumor types using Oncomine data. Statistical significance is denoted as $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$

Analysis of TPX2 gene expression and its prognostic relevance across cancers

Analysis of TPX2 expression using the HPA, GTEx, UCSC, and Oncomine databases revealed high expression in the thymus, bone marrow, tonsils, and lymph nodes, but low expression in the kidney, indicating limited tissue specificity (Fig. 2A). TPX2 also demonstrated evolutionary conservation in *H. sapiens* (Fig. 2B).

TPX2 mRNA was upregulated in various tumor tissues compared to normal tissues, suggesting a potential oncogenic role across multiple cancer types (Fig. 2C). Prognostic analysis using TCGA data revealed that high TPX2 expression was associated with poor OS in cancers such as adrenocortical carcinoma (ACC) (Fig. S1A-B). Additionally, TPX2 expression correlated with pathological stages of kidney papillary cell carcinoma (KIPC) (Fig. S1C).

Genetic alterations in TPX2 were analyzed using cBioPortal, revealing the highest alteration frequency in cervical cancer (>25%), primarily involving copy number amplifications (Fig. S2A). A total of 150 TPX2 mutations were identified, including 104 missense, 5 deletion, 20 fusion, and 1 in-frame mutation (Fig. S2B).

Biological role of TPX2 in renal cell carcinoma

To investigate TPX2's role in pRCC, we selected Caki-2 and ACHN cell lines for functional studies. TPX2 knockdown using shRNA reduced its protein levels, while overexpression increased them (Fig. 3A).

TPX2 knockdown significantly impaired cell proliferation, as demonstrated by CCK-8 and colony formation assays, whereas overexpression enhanced proliferative capacity (Fig. 3B-C). Migration and invasion assays revealed that TPX2 knockdown reduced cell motility and invasiveness, while overexpression increased these properties (Fig. 3D-E).

In vivo experiments using BALB/c nude mice showed that TPX2 knockdown resulted in smaller tumors, whereas overexpression led to larger tumor volumes (Fig. 3F). These findings highlight TPX2's critical role in promoting tumorigenesis and metastasis in pRCC.

TPX2 and the immune microenvironment in type 2 pRCC

Previous studies have demonstrated the involvement of cancer-associated fibroblasts (CAFs) in the regulation of various tumor-infiltrating immune cells [6]. To explore TPX2's role in the tumor immune microenvironment, we analyzed its correlation with CAFs and immune cell markers using the TIMER tool. TPX2 expression positively correlated with B-cell markers (CD19, CD79A) and exhibited associations with markers of CD8+T cells (CD8A, CD8B), CD4+T cells (CD4), macrophages (NOS2, IRF5, PTGS2), and neutrophils (CEACAM8, ITGAM, CCR7) (Fig. S3B-F). These findings suggest that TPX2 may influence immune cell infiltration and activity in type 2 pRCC.

The LINC00894/hsa-miR-660-5p axis regulates TPX2 expression

The elevated expression of TPX2 in type 2 papillary renal cell carcinoma (pRCC) and its oncogenic potential prompted an investigation into the mechanisms underlying its overexpression. Noncoding RNAs (ncRNAs) are known to play pivotal roles in regulating gene expression at the transcriptome level, influencing both physiological and pathological processes [7]. To explore whether TPX2 is modulated by the competing endogenous RNA (ceRNA) network in type 2 pRCC, we employed a systematic approach.

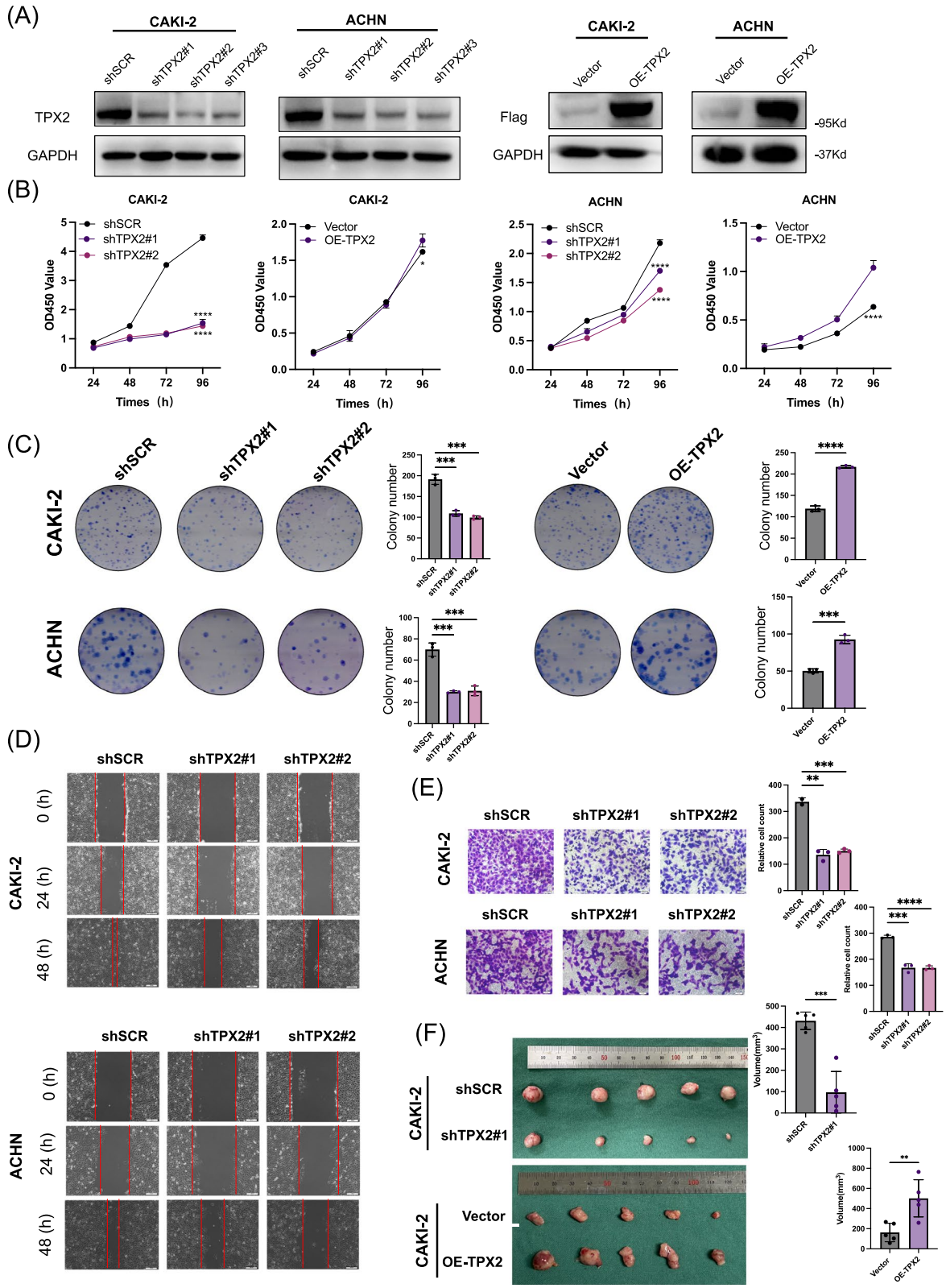
Using the starBase platform (https://starbase.sysu.edu.cn/), we predicted potential RNA interactions by selecting candidate genes based on differential expression and survival prognosis. Coexpression analysis was performed, and only interactions with an absolute correlation coefficient > 0.2 and $p < 0.001$ were included in the ceRNA network. Visualization using the networkD3 package confirmed that the TPX2: LINC00894/hsa-miR-660-5p axis met all screening criteria (Fig. S4A-H), suggesting its potential regulatory role in TPX2 expression.

LINC00894 modulates the biological behavior of renal cell carcinoma

To assess the clinical relevance of LINC00894 in type 2 pRCC, we analyzed its expression in 10 pairs of tumor tissues and adjacent normal renal tissues using quantitative real-time polymerase chain reaction (qRT–PCR). LINC00894 was significantly upregulated in 7 out of 10 type 2 pRCC tissues compared to normal tissues (Fig. 4A), indicating its potential involvement in tumorigenesis.

(See figure on next page.)

Fig. 3 Oncogenic Role of TPX2 in Proliferation, Migration, and Invasion In Vitro and In Vivo. **A** Western blot analysis of TPX2 protein levels in Caki-2 and ACHN cells transfected with scrambled shRNA (shSCR), TPX2 shRNA (shTPX2), empty vector, or TPX2 overexpression plasmid (OE-TPX2). **B** Cell proliferation assay showing relative proliferation rates after 96 h. **C** Colony formation assay and quantification of colonies formed by shSCR, shTPX2, vector, and OE-TPX2 cells. **D** Wound healing assay demonstrating reduced migration in TPX2-knockdown cells (scale bars = 100 μm). **E** Transwell invasion assay showing decreased invasion in TPX2-knockdown cells (scale bars = 20 μm). **F** Representative images and quantification of tumor xenografts in nude mice ($n = 5$) from shSCR, shTPX2#1, vector, and OE-TPX2 groups. Statistical significance is denoted as $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$



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To elucidate the functional role of LINC00894, we performed gain- and loss-of-function experiments in Caki-2 and ACHN cells. Knockdown of LINC00894 significantly suppressed cell proliferation, whereas its overexpression enhanced colony formation (Fig. 4C). In vivo studies further demonstrated that subcutaneous injection of Caki-2 cells with shLINC00894#1 resulted in smaller tumor volumes in BALB/c nude mice, while overexpression of LINC00894 led to larger tumor volumes compared to controls (Fig. 4D). These findings suggest that LINC00894 promotes tumorigenesis in vivo.

Additionally, LINC00894 knockdown impaired the migratory and invasive abilities of Caki-2 and ACHN cells, while its overexpression enhanced these properties (Fig. 5A). Transwell assays corroborated these results, showing that LINC00894 knockdown reduced cell invasiveness, whereas overexpression increased it (Fig. 5B). Collectively, these data highlight the oncogenic role of LINC00894 in regulating the malignant behavior of type 2 pRCC.

LINC00894 and miR-660-5p synergistically regulate TPX2 expression

To validate the regulatory relationship between miR-660-5p and TPX2, we performed dual luciferase reporter assays in HEK293 cells. Cotransfection with miR-660-5p mimics and Luc-wt plasmids containing the predicted binding sites for TPX2 or LINC00894 significantly inhibited luciferase activity, while no effect was observed with Luc-mut plasmids (Fig. 6A). Furthermore, miR-660-5p transfection reduced TPX2 protein levels (Fig. 6B), confirming that miR-660-5p targets the 3' UTR of TPX2 and suppresses its expression.

Given the critical role of TPX2 in spindle assembly and mitosis, we investigated whether miR-660-5p affects these processes. Transfection of miR-660-5p mimics in Caki-2 cells disrupted spindle formation and arrested mitosis (Fig. 6C), suggesting that miR-660-5p influences chromosomal stability by targeting TPX2.

In vivo, subcutaneous injection of Caki-2 cells followed by miR-660-5p agomir treatment significantly inhibited tumor growth compared to controls (Fig. 5C), underscoring the therapeutic potential of miR-660-5p in type 2 pRCC.

TPX2 as a prognostic marker in type 2 pRCC

Our study revealed a strong association between high TPX2 expression and the aggressive behavior of type 2 pRCC, suggesting its utility as a prognostic marker. Immunohistochemical (IHC) staining of tissue microarrays from 80 type 2 pRCC patients showed that TPX2 was predominantly localized in the cytosol, with positive cells exhibiting light to dark brown staining (Fig. 7A). Using a semiquantitative scoring system, patients were stratified into high and low TPX2 expression groups based on a cutoff value of 150.

Baseline characteristics analysis (Table 1) indicated that TPX2 expression was independent of sex, age, and BMI but correlated with concomitant symptoms, tumor thrombus, tumor diameter, TNM stage, and Fuhrman grade. Kaplan–Meier survival analysis demonstrated that high TPX2 expression was associated with worse overall survival ($p < 0.001$) (Fig. 7B). Cox univariate analysis further identified high TPX2 expression as an independent risk factor for overall survival (Table 2).

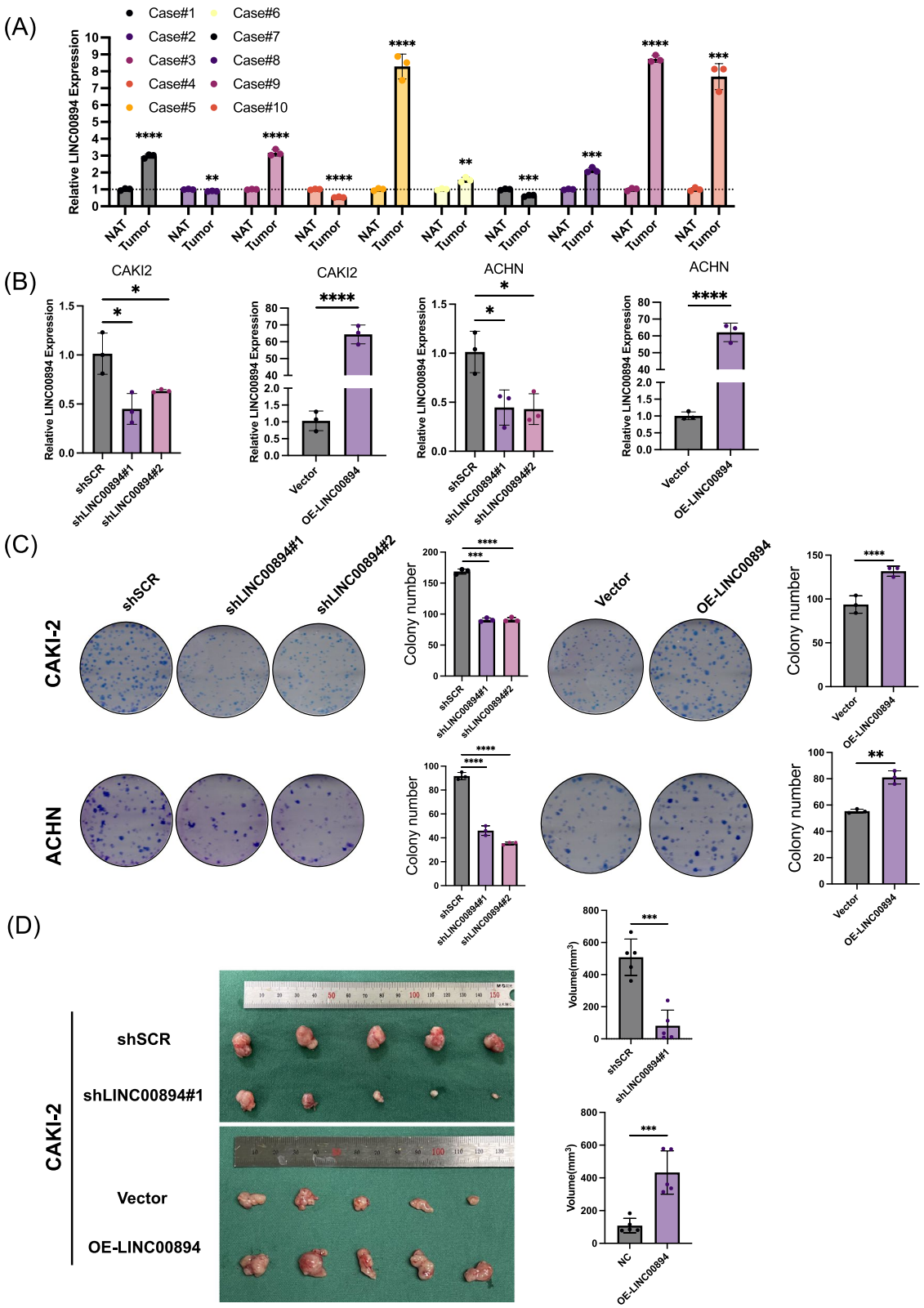
Moreover, we uncovered a novel ceRNA regulatory network involving TPX2, LINC00894, and miR-660-5p (Fig. 7C), highlighting the intricate interplay between these molecules in type 2 pRCC progression.

Discussion

Papillary renal cell carcinoma (pRCC) represents the second most prevalent histologic subtype of renal tumors, constituting approximately 7–14% of all renal cell carcinoma cases [8]. Historically, pRCC has been categorized into two distinct subtypes: type 1 and type 2. Type 1 pRCC is typically characterized as a low-grade tumor with a relatively indolent clinical course, whereas type 2 pRCC is associated with high-grade histopathological features, including abundant eosinophilic cytoplasm and pseudostratified tumor nuclei, often indicative of a more aggressive phenotype [8]. However, emerging molecular studies have challenged this binary classification, revealing that type 2 pRCC is a heterogeneous entity encompassing multiple molecular subtypes with distinct biological behaviors and clinical outcomes [9]. This complexity has led to inconsistencies in the classification and prognostic stratification of pRCC, underscoring the

(See figure on next page.)

Fig. 4 LINC00894 Promotes pRCC Progression In Vitro and In Vivo. **A** qRT-PCR analysis of LINC00894 expression in 10 pairs of type 2 pRCC tissues and adjacent normal renal tissues. **B** Validation of LINC00894 overexpression and knockdown in Caki-2 and ACHN cell lines. **C** Colony formation assay and quantification of colonies formed by shSCR, shLINC00894, vector, and OE-LINC00894 cells. **D** Representative images and quantification of tumor xenografts in nude mice ($n = 5$) from shSCR, shLINC00894#1, vector, and OE-LINC00894 groups. Statistical significance is denoted as $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$



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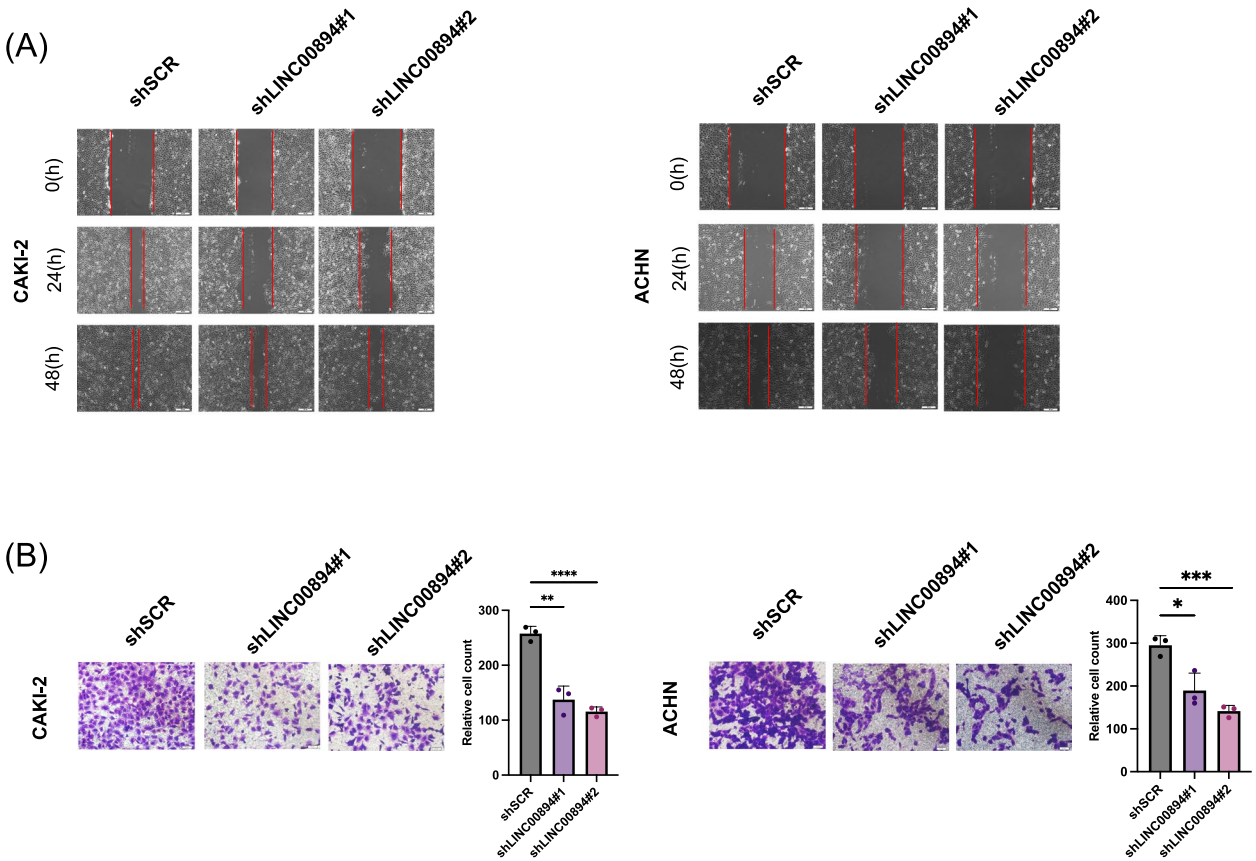

Fig. 5 Role of LINC00894 in Renal Carcinoma Cell Migration and Invasion. **A** Wound healing assay showing migration of Caki-2 and ACHN cells (scale bars = 100 μm). **B** Transwell invasion assay showing invasion of Caki-2 and ACHN cells (scale bars = 20 μm)

need for a more nuanced understanding of its molecular underpinnings.

While pRCC generally exhibits a more favorable prognosis compared to clear cell renal cell carcinoma (ccRCC), recent evidence suggests that this advantage is primarily confined to type 1 pRCC, with type 2 pRCC often associated with poorer outcomes [2]. These findings emphasize the critical need for further research to elucidate the molecular heterogeneity of type 2 pRCC and its implications for prognosis and therapeutic strategies. A deeper understanding of the molecular drivers of type 2 pRCC is essential to develop personalized treatment approaches and improve patient outcomes.

In this study, we conducted a comprehensive analysis integrating whole-transcriptome sequencing and database mining in patients with type 2 pRCC. Our results revealed a significant upregulation of TPX2, a key regulator of mitotic spindle formation, in tumor tissues. TPX2, also known as Xenopus kinesin-like protein 2, plays a pivotal role in mitosis by localizing and activating Aurora A kinase, thereby ensuring proper chromosome segregation and genomic stability [10]. Beyond its mitotic functions, TPX2 has been implicated in oncogenesis through its interaction with the tumor suppressor protein 53BP1, which it counteracts to promote DNA replication and prevent cell cycle arrest at sites of DNA damage [11]. The oncogenic potential of TPX2 has been further

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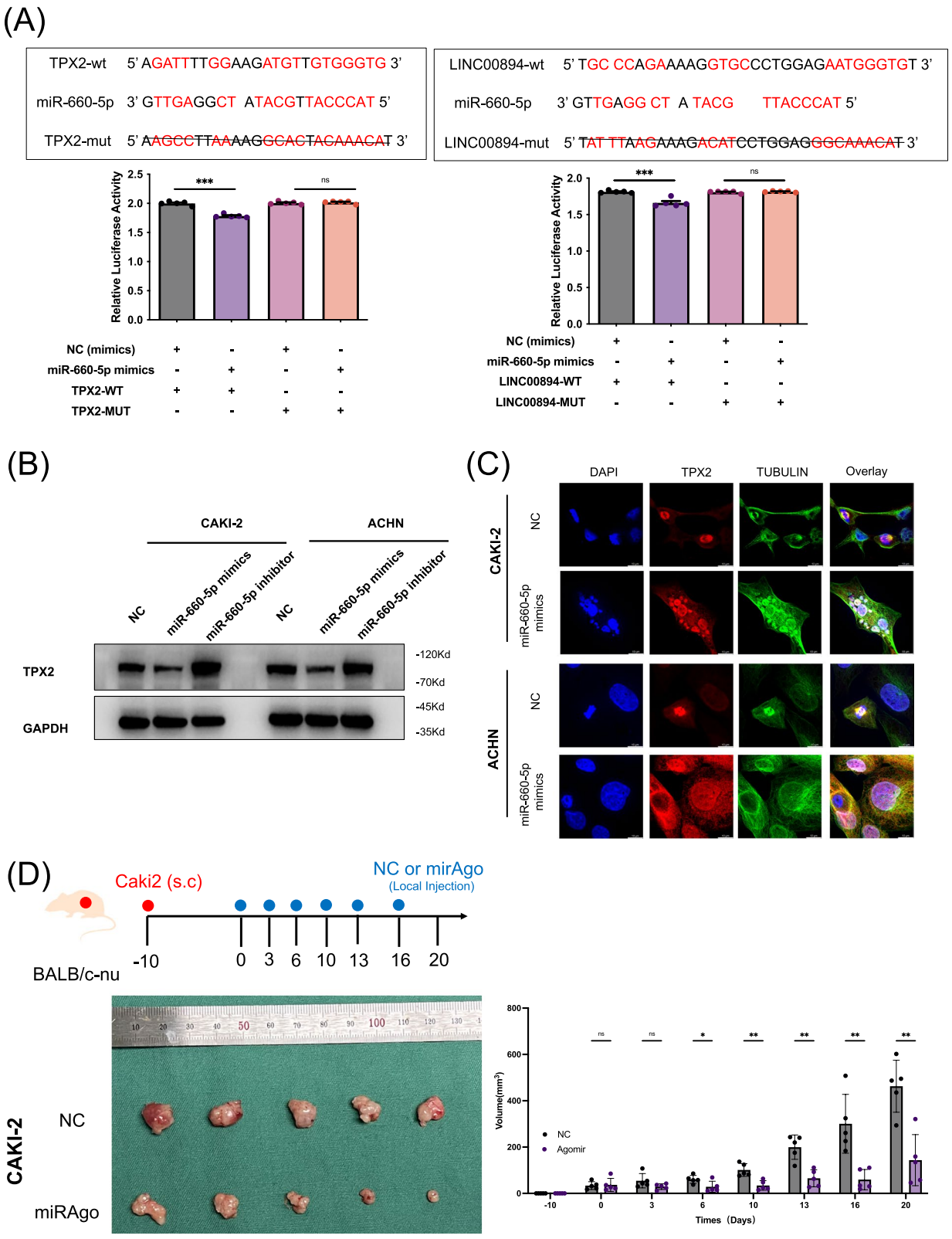
Fig. 6 LINC00894 and miR-660-5p Regulate TPX2 Expression. **A** Predicted binding sites of TPX2 and LINC00894 with miR-660-5p. Dual luciferase reporter assays in HEK-293 cells cotransfected with reporter vectors and miR-660-5p mimics. **B** Western blot analysis of TPX2 protein levels in Caki-2 and ACHN cells transfected with miR-660-5p mimics or inhibitor. **C** Laser scanning confocal microscopy (LSCM) images showing miR-660-5p's effect on mitotic spindle formation. **D** Representative images of subcutaneous xenograft tumors in nude mice injected with Caki-2 cells and treated with miR-660-5p agomir (2.5 nmol) every 3 days for 20 days. Statistical significance is denoted as $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$



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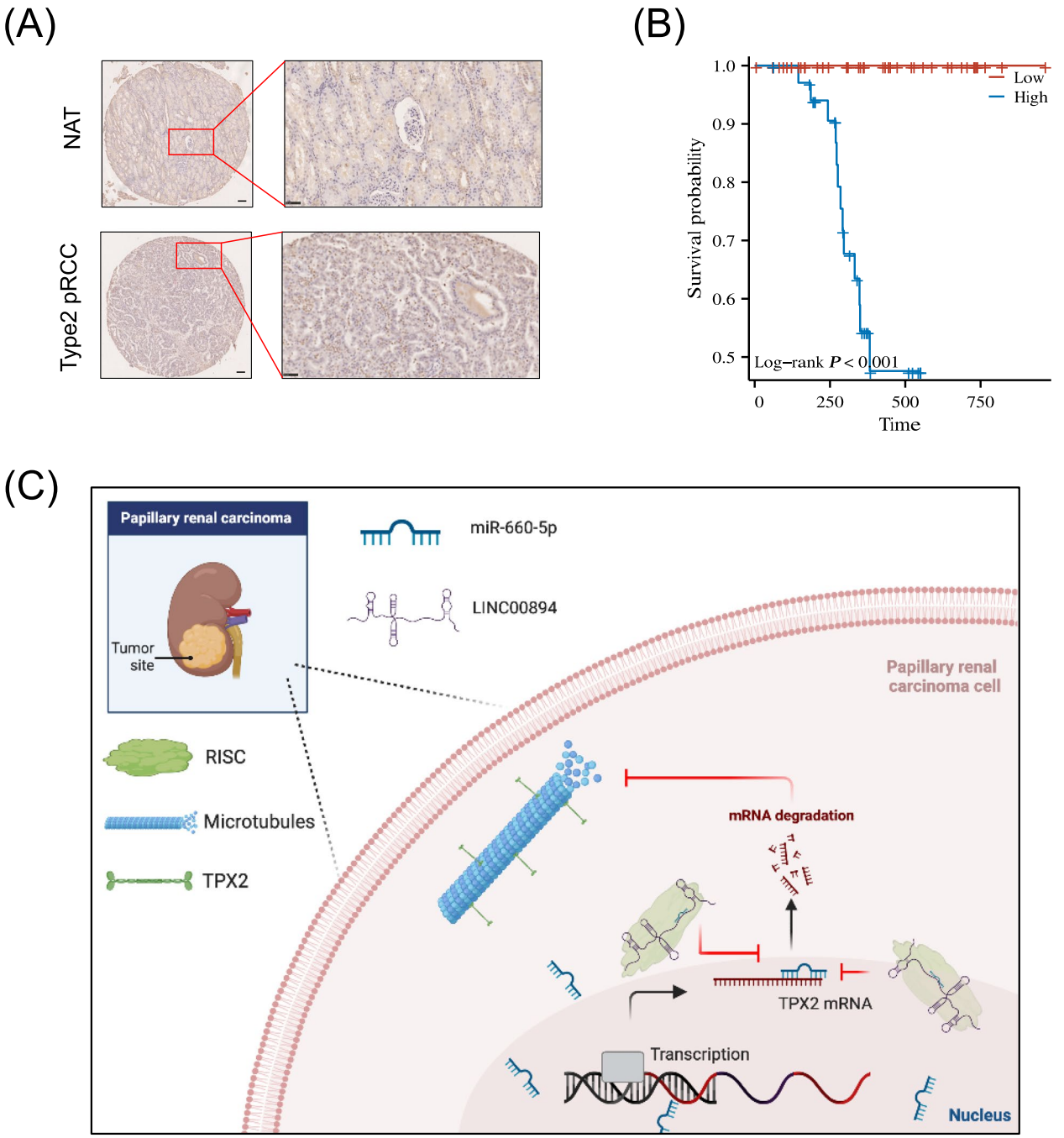

Fig. 7 TPX2 as a Prognostic Marker in Type 2 pRCC. **A** Immunohistochemical staining of TPX2 in adjacent normal and tumor tissues. **B** Kaplan–Meier survival analysis showing the correlation between TPX2 expression and overall survival (OS) in type 2 pRCC patients. **C** Schematic diagram of the LINC00894/miR-660-5p/TPX2 regulatory axis, created using BioRender. Statistical significance is denoted as $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$

corroborated in various malignancies, including non-small cell lung cancer (NSCLC), where its overexpression has been linked to enhanced cell migration, invasion, and chemoresistance to docetaxel [12]. Similarly, in ovarian cancer, TPX2 has been shown to interact with Lamin A/C, contributing to tumorigenesis and genomic instability [13]. Moreover, TPX2-mediated activation of the transcription factor PXR has been associated with drug resistance in hepatocellular carcinoma [14], highlighting its multifaceted role in cancer progression.

Table 2 Risk factors affecting OS in patients with type 2 pRCC

Characteristics	Total (*N*)	Univariate analysis		Multivariate analysis	
		Hazard ratio (95% CI)	*P* value	Hazard ratio (95% CI)	*P* value
Age	75	0.987 (0.945—1.032)	0.576		
BMI	75	1.009 (0.830—1.227)	0.928		
Sex	75		0.348		
Male	55	Reference			
Female	20	0.512 (0.113—2.312)	0.384		
Fuhrman grading	75		0.518		
1	3	0.000 (0.000 - Inf)	0.999		
2	25	0.518 (0.137—1.955)	0.332		
3	42	Reference			
4	5	1.560 (0.331—7.360)	0.574		
T.stage	75		**0.001**		
≥T3a	37	Reference		Reference	
<T3a	38	0.130 (0.029—0.590)	**0.008**	1.162 (0.000—2783.886)	0.970
N.stage	75		**0.005**		
N0	48	Reference		Reference	
N1	27	5.106 (1.558—16.735)	**0.007**	0.504 (0.069—3.663)	0.498
M.stage	75		**0.003**		
M0	63	Reference		Reference	
M1	12	7.135 (2.168—23.480)	**0.001**	2.515 (0.297—21.273)	0.397
Concomitant symptoms	75		0.157		
Yes	37	Reference			
No	38	0.440 (0.135—1.435)	0.174		
Tumor thrombus	75		**0.001**		
Yes	32	Reference		Reference	
No	43	0.129 (0.028—0.582)	**0.008**	0.144 (0.000—426.529)	0.634
Maximum diameter of tumor	75	1.179 (1.072—1.297)	**<0.001**	0.892 (0.720—1.105)	0.295
Score	75	1.061 (1.027—1.097)	**<0.001**	1.072 (1.023—1.123)	**0.003**

To explore the functional significance of TPX2 in pRCC, we employed two well-characterized renal carcinoma cell lines, Caki-2 and ACHN [15, 16]. Our experimental data demonstrated that TPX2 significantly promotes cell proliferation, migration, and metastasis in both cell lines, aligning with previous findings that link high TPX2 expression to poor prognosis in pRCC patients. These results suggest that TPX2 may serve as a critical driver of the aggressive phenotype observed in type 2 pRCC.

In addition to protein-coding genes, noncoding RNAs (ncRNAs) have emerged as key regulators of gene expression and cellular homeostasis, with their dysregulation implicated in various human diseases, including cancer [17]. The competitive endogenous RNA (ceRNA) hypothesis, proposed by Salmena et al. in 2011, posits that ncRNAs, including long noncoding RNAs (lncRNAs) and microRNAs (miRNAs), can modulate gene expression by competing for shared miRNA binding sites, thereby forming intricate regulatory networks [18]. In our study, we constructed a ceRNA network based on lncRNA–miRNA-mRNA interactions and identified LINC00894 as a promising prognostic marker in type 2 pRCC. Functional assays revealed that LINC00894 plays a significant role in promoting the migration and invasion of Caki-2 and ACHN cells. Furthermore, we elucidated a regulatory mechanism involving LINC00894, miR-660-5p, and TPX2, demonstrating that miR-660-5p directly targets both TPX2 and LINC00894. This interaction was validated using luciferase reporter assays, confirming the ceRNA-mediated regulation of TPX2 by LINC00894.

Our findings suggest a novel regulatory axis in type 2 pRCC progression, wherein elevated LINC00894 expression competitively binds miR-660-5p, thereby alleviating miR-660-5p-mediated suppression of TPX2. This mechanism facilitates the unhindered translation of TPX2, leading to its overexpression and subsequent promotion of cancer progression. This study not only highlights the oncogenic role of TPX2 in type 2 pRCC but also provides

insights into the complex regulatory networks involving ncRNAs that govern tumor biology.

In conclusion, our work underscores the importance of TPX2 and its regulatory network in the pathogenesis of type 2 pRCC. The identification of LINC00894 as a key ceRNA modulating TPX2 expression offers new avenues for therapeutic intervention and prognostic stratification in this challenging subtype of renal cancer. Future studies should focus on validating these findings in larger patient cohorts and exploring the therapeutic potential of targeting the LINC00894/miR-660-5p/TPX2 axis in type 2 pRCC.

Conclusions

Our findings underscore the oncogenic role of TPX2 in type 2 pRCC and its potential as a prognostic marker. We identified a novel ceRNA axis involving LINC00894/miR-660-5p that regulates TPX2 expression and demonstrated the functional significance of LINC00894 in renal cell carcinoma progression. These insights provide a foundation for further exploration of therapeutic strategies targeting this regulatory network in type 2 pRCC.

Abbreviations

pRCC	Papillary renal cell carcinoma
ccRCC	Clear cell renal cell carcinoma
XKLP2	TPX2, Xenopus kinesin-like protein 2

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12920-025-02120-9.

Supplementary Material 1: Figure S1. TPX2 Expression and Overall Survival in Various Tumor Types. (A) Survival map showing TPX2 expression across different tumor types. (B-C) Kaplan-Meier survival analysis of TPX2 expression in patients with various tumors, generated using GEPIA2. Figure S2. TPX2 Gene Alterations in TCGA Tumor Types. (A) Types of TPX2 gene alterations across TCGA tumor types. (B) Mutation sites in the TPX2 gene, analyzed using cBioPortal. Figure S3. TPX2 and the Immune Microenvironment in Type 2 pRCC. (A) Correlation between TPX2 expression and immune cell infiltration. (B) Analysis of immune cell infiltration using the EPIC and TIDE algorithms. Figure S4. Regulation of TPX2 by the LINC00894/miR-660-5p Axis. (A-C) Correlation analysis of hsa-miR-660-5p, TPX2, and LINC00894 expression levels in the TCGA dataset. (D-E) Differential expression of LINC00894 and hsa-miR-660-5p in type 2 pRCC tumor tissues. (F-G) Kaplan-Meier survival analysis of LINC00894 and hsa-miR-660-5p in type 2 pRCC patients. (H) ceRNA regulatory network of TPX2 in the TCGA dataset.

Supplementary Material 2.

Supplementary Material 3.

Acknowledgements

N/A.

Authors' contributions

DZS and YWW developed the study concept and design; DZS, YWW and LC acquired, analyzed and interpreted the data, and performed the statistical analysis; DZS, YWW and MLL wrote and edited the manuscript; and ZY and YY provided technical and material support. All the authors have read and approved the final paper.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

The project was approved by the Medical Scientific Research Ethics Committee of the Peking University Third Hospital (ethics number M2017147). The animal ethical and welfare has been reviewed and approved by the China-Japan Friendship Hospital Animal Ethical and Welfare Committee (ethics number zryhyy21-22–01-10). All patients provided written informed consent, and appropriate measures were taken to protect the confidentiality of their personal information.

Consent for publication

Written informed consent for publication of their details was obtained from the patient.

Competing interests

The authors declare no competing interests.

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