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Original Article

Oncogenic and prognostic role of CKAP2L in hepatocellular carcinoma

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Abstract: Cytoskeleton-associated protein 2-like (CKAP2L) exerts crucial function in the cell-cycle progression and mitotic spindle formation of neural stem/progenitor cells. However, in hepatocellular carcinoma (HCC), the expression pattern, clinical significance and biologic role of CKAP2L remain unexplored. We analysed The Cancer Genome Atlas (TCGA) database and found that CKAP2L was dramatically upregulated in HCC tissues at the mRNA level compared to adjacent tissues, which was validated in 48 paired HCC and para-tumor tissues using quantitative real-time PCR (qRT-PCR). Immunohistochemical analysis of tissue microarray revealed that CKAP2L was also significantly overexpressed at the protein level. Further clinical and survival analysis of the TCGA cohort revealed that increased CKAP2L expression was strongly associated with reduced overall survival. We further validated that higher CKAP2L protein expression was associated with worse prognosis in the Peking Union Medical College Hospital (PUMCH) cohort. Univariate and multivariate Cox regression analyses in the TCGA and PUMCH cohort suggested that CKAP2L overexpression was an independent risk factor for poor prognosis in HCC patients. Then, we validated that CKAP2L silencing inhibited HCC cell proliferation, migration, and invasion abilities. Knockdown of CKAP2L in Huh7 cells suppressed the growth of xenograft tumors in vivo. Furthermore, qRT-PCR and western blotting results demonstrated that the expression of Class I Phosphoinositide 3-Kinase PIK3CA/p110α and PIK3CB/p110β isoforms reduced obviously in Huh7 cells after depleting CKAP2L. This study demonstrated for the first time that high CKAP2L expression in HCC tissues is significantly correlated with poor prognosis in HCC patients and greatly facilitate the malignancy of HCC, thus providing a new prognostic biomarker and potential therapeutic target.

Keywords: CKAP2L, hepatocellular carcinoma, prognosis, therapeutic target

Introduction

Globally, liver cancers are currently the fourth most frequent cause of cancer-correlated death and rank sixth in terms of incident cases [1]. HCC is the predominant histologic subtype of primary liver cancer in many countries, constituting approximately 90% of cases [2, 3]. Despite considerable and clinical meaningful progression in HCC therapeutic strategies, including surgical resection, chemotherapy, radiotherapy and target therapy, the 5-year survival rates of in advanced stage HCC patients remain grim, primarily owing to tumor relapse and distant metastasis [4-8]. The initiation and development of HCC is considered a complex and multi-step process [9]. Understanding the molecular profiling of HCC will facilitate biological insights, detection of novel therapeutic targets, and the characterization of accurate subsets with prognostic implications influencing HCC clinical surveillance [10].

CKAL2L, also known as radial fiber and mitotic spindle (Radmis), is a mitotic spindle protein-coding gene located on 2q14.1. Yumoto et al. have demonstrated the crucial role of CKAL2L in cell-cycle progression of neural progenitors and mitotic spindle formation [11]. Furthermore, CKAL2L was validated as a critical constituent of human centrosome, as it is localized to the spindle, the spindle pole and the midbody [12]. CKAL2L is involved in aetiology of Filippi Syndrome. CKAP2L mutations result in upregulation of altered spindle microtubules due to multipolar configurations and abnormalities in

chromosome segregation. These findings indicate that CKAP2L deficiency hinders apoptosis at the interdigital spaces by exerting its adverse influence on mitosis and triggering mitotic checkpoints [13]. Overexpression of CKAP2L facilitates lung adenocarcinoma invasion and is associated with poor prognosis [14]. However, in HCC, the expression and biologic function of CKAP2L in the development of HCC remain to be elucidated.

In the present study, we first evaluated the clinical significance of CKAP2L in HCC patients. In vitro results indicated that CKAP2L promoted HCC cell proliferation, migration, and invasion. We also determined the oncogenic effect of CKAP2L in tumor growth using xenograft mouse model. Finally, we explored the potential mechanism of how CKAP2L impacts HCC cell proliferation and motility. Our evidence demonstrated that CKAP2L might be involved in the development of HCC and could serve as a novel prognostic biomarker.

Materials and methods

Gene expression data

Gene expression profiling and clinical datasets derived from the TCGA network were utilized to analyze the relationship between the expression signature of CKAP2L mRNA and clinical prognosis of HCC patients [10, 15, 16].

Patients and tissues

A total of 361 HCC patients and 49 adjacent para-carcinoma controls from the TCGA cohort were enrolled in this study. Age, gender, tumor grade, tumor-node-metastasis (TNM) stage, AFP value, vascular invasion, overall survival, and corresponding vital status and other clinicopathologic characteristics were included.

To evaluate the potential application of CKAP2L as a biomarker for HCC, we investigated relative CKAP2L mRNA levels among 48 pairs of fresh HCC and their corresponding non-cancerous tissues by qRT-PCR. In this present study, immunohistochemistry was also performed to further investigate CKAP2L expression in 36 normal liver tissues, 31 para-tumor tissues and 158 HCC samples. Additionally, CKAP2L expression in HCC and its association with patients' clinicopathologic characteristics and prognosis were analyzed. HCC tissue samples were consecutively chosen from 158 HCC patients who underwent hepatectomy at PUMCH between January 2010 and December 2011. Normal liver tissues were collected from patients with hepatolithiasis who were treated in the same hospital. Complete clinicopathologic and follow-up data are available for the 158 HCC samples. The study protocol was approved by the Ethics Committee of PUMCH. Informed consent was obtained from each patient.

RNA isolation, qRT-PCR

Total RNA was extracted from HCC specimens or cell lines using Trizol reagent (Invitrogen, Carlsbad, CA, USA). The reverse transcription for mRNAs was performed utilizing Oligo dT primer. qRT-PCR was performed using a standard protocol from the SYBR Green PCR kit (Tsingke, Beijing, China). qRT-PCR was carried out using LightCycler® 480 real-time PCR (Roche, Basel, Switzerland) and the data were normalized to GAPDH mRNA expression. Relative expression of CKAP2L, p110α, p110β was computed utilizing the $2^{-\Delta\Delta CT}$ method [17]. The primers for real-time PCR are listed below:

Primer	sequence
CKAP2L-F	GAGCCAAAACACCAAGCCTTA
CKAP2L-R	GGAGTTTAATGCTGATGGACCTT
GAPDH-F	GGAGCGAGATCCCTCCAAAAT
GAPDH-R	GGCTGTTGTCATACTTCTCATGG
p110α-F	AAGAGCCCCGAGCGTTTCT
p110α-R	CATCAAGTGGATGCCCCACA
p110β-F	AACGCCAGGCAGTGTATGTT
p110β-R	GGGGCCCTAAGAATGGTCG

Tissue microarray construction and immunohistochemistry

Tissue microarrays (TMA) were constructed in the previous study [18]. Briefly, hematoxylin and eosin (H&E) sections were assessed and an appropriate area of tumor was marked on the corresponding paraffin block. While avoiding necrotic tissue, the representative 1.5 mm diameter tissue cores were removed by a hollow needle and re-embedded into a recipient paraffin block at a defined position. Two TMA blocks were then built in PUMCH.

TMA slides were pretreated at 65°C for 2 hours, followed by deparaffinization. Antigen retrieval

was performed using citrate buffer (pH 6) at a temperature of 97°C for 20 min. Endogenous peroxidase activity was blocked by incubating the sections with 3% hydrogen peroxide for 10 minutes at room temperature. Non-specific binding of the antibody was blocked by incubating with 5% normal goat serum in phosphate-buffered saline containing 0.1% Tween 20 (PBST) for 1 hour at room temperature. The slides were then incubated with primary antibodies against CKAP2L (1:500, Abcam, UK) overnight at 4°C. After washing, slides were incubated for 2 hours at room temperature with the secondary antibody conjugated to horseradish peroxidase (HRP; 1:100; DAKO). HRP activity was detected using the Liquid DAB+ Substrate Chromogen System (DAKO). Finally, sections were counterstained with hematoxylin and photographed.

CKAP2L expression was evaluated using the "hybrid scoring system" (H-score) criteria based both on the percentage of positively stained cells and on the intensity of staining. In brief, the H-score was calculated as the sum of the product of the staining intensity in tumor cells (0, no staining; 1, weak staining; 2, moderate staining; three, intense staining) and the extent of cells showing that staining intensity (0-100%). Therefore, the possible H-score ranged from 0 to 300 and the scores for each core reported by the two independent readers were averaged. The observation of staining results by microscope and H scores were evaluated as described in a previous study [18-20].

Cell culture

The HCC cell lines SMMC-7721, Huh7 and were obtained from Shanghai Cell Bank, Chinese Academy of Sciences, and cultured as recommended by the supplier. The cells were cultured in DMEM medium containing 10% fetal bovine serum in a humidified 37°C incubator supplemented with 5% CO_2.

Western blotting

Cells were collected in RIPA buffer with protease inhibitor cocktails (AMRESCO) and phosphatase inhibitor cocktail II and III (MCE) and lysed on ice for 30 min with a short vortex every 10 min. Lysates were centrifuged for 15 min at 13000×g and 4°C, supernatants were collected, and protein concentrations were determined by the BCA Protein Assay Reagent (Pierce). Lysates were size fractionated by SDS-PAGE and transferred onto nitrocellulose membranes. For western blotting analysis, the membranes were incubated with primary antibodies against CKAP2L (Proteintech, Wuhan, China), p110α, p110β (CST, #9655T) and β-tubulin at 4°C overnight. After three washes with TBST, the membrane was incubated with a secondary antibody at room temperature for 2 h. Then, the signals were detected by enhanced chemiluminescence or fluorescence according to the manufacturer's recommendations.

Transfection of small interfering RNA

For small interfering RNA (siRNA) silencing of CKAP2L, RNA interference assay was performed by using synthetic siRNA duplexes. According to sequences of human CKAP2L gene sequence (NM_001304361.1), siRNA targeting CKAP2L were designed and synthesized from Ribobio (Guangzhou, China). The targeting sequences were as follows: siRNA, 5'-CAAAGTTGTTGGCAAGTAA-3'. SiRNAs was transfected into cells using Lipofectamine RNAiMAX transfection reagent (Thermo Fisher Scientific, Waltham, MA) according to the manufacturer's instructions. After 24 hours of transfection, cells were harvested for cell proliferation, migration, invasion. SiRNA could effectively reduce endogenous CKAP2L expression in SMMC-7721 and Huh7 cells by qRT-PCR and western blotting.

Plasmid construct and transfection

Lentivirus was produced through the co-transfection of HEK293FT cells with short hairpin RNA constructs targeting CKAP2L (Control, shCKAP2L: 5'-CAAAGTTGTTGGCAAGTAA-3'; Tsingke, Beijing, China) and a lentiviral packaging mix (Invitrogen, Carlsbad, USA) following with the manufacturer's instructions. Lentivirus-containing supernatant was harvested at 48 h post-transfection, centrifuged, and stored at -80°C. For viral transductions, the scrambled Control or shCKAP2L lentiviruses was incubated with Huh7 cells for 48 h at 37°C in a humidified cell culture incubator. Stable CKAP2L knockdown Huh7 cells were selected using puromycin (1 μg/ml) for 14 d, and the CKAP2L protein expression level was identified through western blotting.

Cell proliferation assay

Cell viability was measured with the Cell Counting Kit-8 (CCK-8) (Dojindo, Shanghai, China) according to the manufacturer's instructions. Cells were plated at a density of 1×10^3 cells per well in 96-well plates and incubated at 37°C. Proliferation rates were determined at 0, 24, 48, 72 and 96 h post-transfection, and quantification was performed on a microtiter plate reader (Spectra Rainbow, Tecan) using the Clone Select Imager System (Genetix) according to the manufacturer's protocol. Values represent the mean ± standard deviation (SD) of four data points from a representative experiment, and experiments were repeated more than three times with similar results.

Transwell migration and invasion assay

Double-chamber migration assays were performed using transwell chambers (24-well plate, 8 μm pores; BD Biosciences). The upper well of the transwell (Corning Inc, Corning, NY) was coated with Matrigel (BD Biosciences, San Jose, CA) at 37°C in 5% CO_2 incubator for 1 hour for invasion experiment. In brief, the lower chambers were filled with 600 μl DMEM containing 10% FBS. HCC cells with different treatments were suspended in serum-free medium, seeded in the upper chambers and incubated at 37°C for 24 h. Then, the cells on the upper surface of the filters were removed using cotton wool swabs. The migrated cells on the lower side of the membrane were fixed in 95% methanol and stained with 0.1% crystal violet dye, and the number of cells migrating to the lower surface was counted in three randomly selected high-magnification fields (100×) for each sample.

Subcutaneous tumor models

All animal experiments were carried out in accordance with the National Animal Experimentation Guidelines upon approval of the experimental protocol by the Institutional Animal Experimentation Committee of PUMCH. For subcutaneous xenograft experiments, 6-weeks-old female BALB/c mice were selected to assess tumorigenicity. The Huh7 cell line (6×10^6 cells/mouse) with stable knockdown and the corresponding controls were subcutaneously injected into the nude mice. Tumor size was evaluated three times a week by measuring the width and length of the tumors utilizing a calliper.

Statistical analysis

Student's t test was adopted for the comparison between two groups. Chi-square test was applied to evaluate the association of CKAP2L expression with the clinicopathologic characteristics of HCC patients. The log-rank test was carried out to distinguish differences in survival time. Kaplan Meier and Cox regression analyses were utilized to evaluate the association between candidate gene and overall survival as well as the prognosis of HCC. $P<0.05$ was considered significant. All statistical analyses were performed utilizing the SPSS statistical software package (version 22.0; IBM, Armonk, NY, USA) and GraphPad Prism 7 (GraphPad Software, La Jolla, CA, USA).

Results

Upregulation of CKAP2L mRNA and protein in HCC patients

First, we investigated the TCGA database and revealed that CKAP2L mRNA expression was significantly increased in 361 HCC samples compared with 49 adjacent normal tissues (**Figure 1A**, $P<0.001$). To validate the expression pattern of CKAP2L mRNA in HCC, we selected 48 HCC tissues and paired non-tumour tissues to evaluate the CKAP2L mRNA levels by conducting qRT-PCR. Consistent with TCGA network results, CKAP2L mRNA was dramatically upregulated in HCC specimens compared with their corresponding normal tissues (**Figure 1B**). To further verify the protein expression profiling of CKAP2L in HCC, TMA construction and immunohistochemistry was selected to evaluate CKAP2L protein levels in 36 normal liver tissues, 31 para-tumour tissues and 158 HCC samples. CKAP2L protein expression in HCC was dramatically increased compared with normal liver tissue or para-tumour tissue (**Figure 1C**, **1D**). Collectively, these results suggest that CKAP2L expression patterns are upregulated in HCC.

Increased CKAP2L expression is associated with age, AFP value, histologic grade, and TNM stage in HCC patients

We analysed the correlation between CKAP2L expression patterns and the clinicopathologic

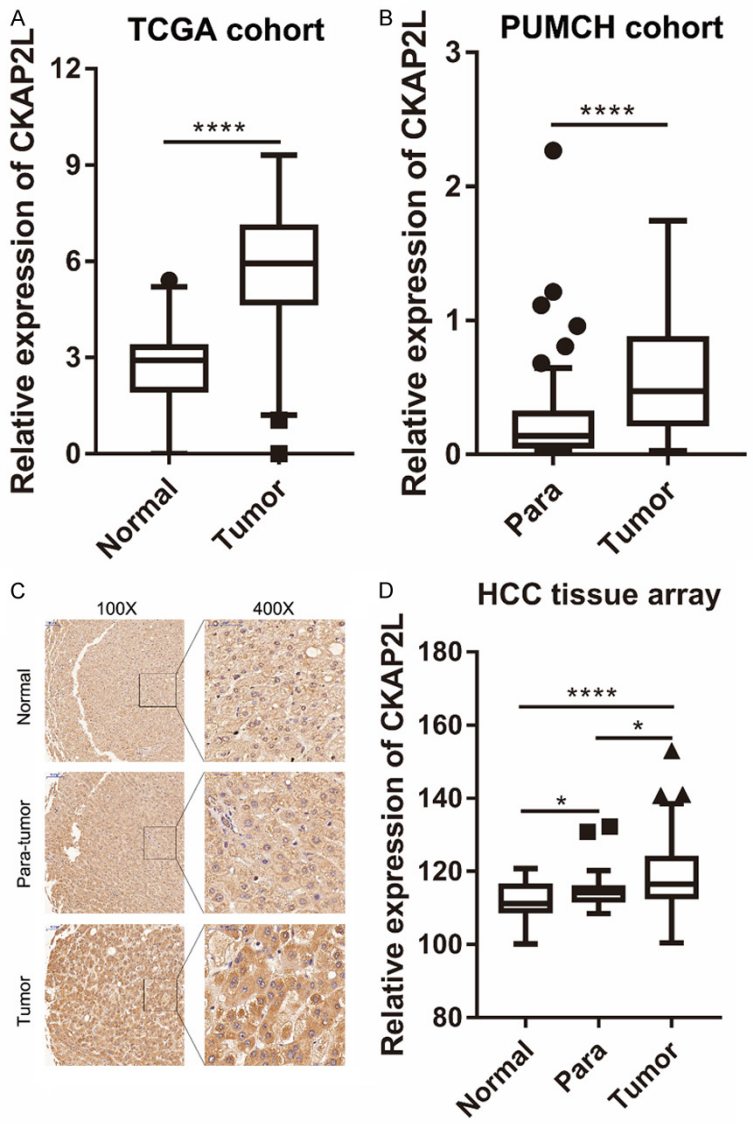


Figure 1. CKAP2L expression in HCC. CKAP2L expression was detected by qRT-PCR and immunohistochemical staining in HCC and paired non-tumor tissues. A. Expression level of 361 HCC samples compared with 49 adjacent normal tissue from TCGA database (****P<0.0001). B. qRT-PCR analysis of the CKAP2L expression in 48 paired HCC and para-tumor tissues from PUMCH (****P<0.0001; **P<0.01; ns, P>0.05). C. Representative photographs of CKAP2L expression in TMA sections of normal liver, para-tumor and HCC tissues (Original magnifications of ×40; bar = 50 μm). D. Comparison of H-scores of CKAP2L between normal liver (n = 36), para-tumor (n = 31) and HCC tissues (n = 158) (****P<0.0001; *P<0.05).

findings of 361 HCC patients by utilizing the RNA-seq and clinical information from TCGA cohort. Increased CKAP2L expression was significantly associated with age (P=0.0153), AFP value (P<0.001), histological grade (P<0.0001), and TNM stage (P=0.0085). However, no significant relationship was identified between CKAP2L expression and gender and vascular invasion (**Table 1**).

CKAP2L overexpression is related with poor prognosis in HCC patients

We conducted a Kaplan-Meier analysis in the TCGA HCC database and demonstrated that patients with increased CKAP2L expression exhibit prominently reduced overall survival time (P=0.0024) (**Figure 2A**). Immunohistochemical staining of 158 HCC samples from PUMCH was performed to evaluate CKAP2L protein expression in HCC tissues. We then performed prognosis analysis. The results confirmed that high CKAP2L protein expression is associated with reduced overall survival time (P=0.0207) (**Figure 2B**).

CKAP2L upregulation is an independent risk factor for predicting HCC patient prognosis

To further explore whether high CKAP2L expression is an independent risk factor for predicting the prognosis of HCC patients, we established a univariate and multivariate Cox proportional risk regression model based on the TCGA dataset and the PUMCH cohort. The patients' age, sex, AFP level, CKAP2L expression level, Edmondson-Steiner pathologic grading, TNM staging, and vascular invasion were included in the Cox model. In univariate analysis of Cox proportional risk regression model, high CKAP2L expression and TNM staging are risk factors of poor prognosis of HCC patients (HR= 1.73 and 2.42, respectively). To adjust other clinicopathologic parameters, we performed multivariate Cox proportional risk regression model analysis, and the results suggested that high CKAP2L expression and TNM staging were independent risk factors for the dismal prognosis of HCC patients. The HRs were 1.68 (95% confidence interval (1.15-2.46)) and 2.27 (95%

Table 1. Patients' clinicopathologic data and the expression patterns of CKAP2L from the TCGA cohort

Variable	Total number (n=361)	CKAP2L expression		*P* value
		Low	High	
Age				
≤60	173	75	98	0.0153
>60	187	105	82	
Gender				
Male	244	133	111	0.165
Female	117	48	69	
AFP value (μg/L)				
<200	196	115	81	<0.0001
>200	75	20	55	
TNM stage				
Stage I-II	249	134	115	0.0085
Stage III-IV	88	33	55	
Histologic grade				
Grade 1-2	224	134	90	<0.0001
Grade 3-4	132	44	88	
Vascular invasion				
Yes	105	48	57	0.2969
None	200	104	96	

confidence interval (1.56-3.31)) (**Table 2**). The results were validated by the clinical follow-up data of PUMCH cohort HCC patients, and the results of the univariate factor Cox proportional risk regression model reveals that high CKAP2L expression, TNM staging, AFP (>200 μg/L), CA-199 (>37 u/mL), pathologic grading, and tumour number represent risk factors for the poor prognosis of HCC patients. Multivariate Cox proportional risk regression model analysis results further validated that high CKAP2L expression and TNM staging are independent risk factors for the adverse prognosis of HCC patients, the risks are 1.60 (95% confidence interval (1.01-2.55)) and 2.23 (95% confidence interval (1.147-4.348)), respectively (**Table 3**). The above results indicate that high CKAP2L expression can be used as a potential molecular marker for predicting the prognosis and stratification of HCC patients.

Down-regulation of CKAP2L expression inhibited cell proliferation, migration, and invasion abilities

Overexpression of CKAP2L correlated with poor prognosis of HCC patients, it was hypothesized that downregulation of CKAP2L expression could inhibit the viability, proliferation, migration, and invasion of HCC cells. After transfection with CKAP2L siRNA, qRT-PCR and western blot analysis were performed to confirm that CKAP2L were successfully knocked down at both transcriptional and protein levels in SMMC-7721 and Huh7 cells (**Figure 3A**). CCK-8 analysis indicated that downregulating CKAP2L expression significantly decreased cell proliferation in SMMC-7721 and Huh7 cells (**Figure 3B**). Transwell migration and invasion assays demonstrated that knockdown of CKAP2L expression dramatically repressed cell migration and invasion abilities (**Figure 3C**, **3D**).

CKAP2L facilitates cell growth in vivo

To evaluate the tumorigenic ability of CKAP2L, Huh7 cells stably transfected with Control and sh-CKAP2L were subcutaneously injected into the BALB/c nude mice respectively. Knockdown of CKAP2L in Huh7 cells markedly reduced the tumor size (**Figure 4A**, **4B**) and weight of xenografts (**Figure 4C**). These in vitro and in vivo results demonstrate the oncogenic role of CKAP2L in HCC.

CKAP2L knockdown suppresses PI3K signaling pathway

Afterwards, we investigated the effect of knockdown of CKAP2L on PI3K signaling pathway in Huh7 cells by qRT-PCR and western blot. Overwhelming evidence has demonstrated that Class I Phosphoinositide 3-Kinase PIK3CA/p110α and PIK3CB/p110β isoforms maintain distinct functions in PI3K signaling and cellular transformation [21, 22]. Our results showed that mRNA and protein levels of p110α, and p110β in Huh7 cells were significantly decreased in the si-CKAP2L group compared with the control group (**Figure 5A**, **5B**). This observation indicated that CKAP2L might serve as a positive regulator in HCC progression by regulating the PI3K signaling pathway.

Discussion

HCC is one of the most commonly diagnosed and deadliest cancers globally [3, 23]. In the past decades, multiple therapies, including surgical resection, chemotherapy, and radiothera-

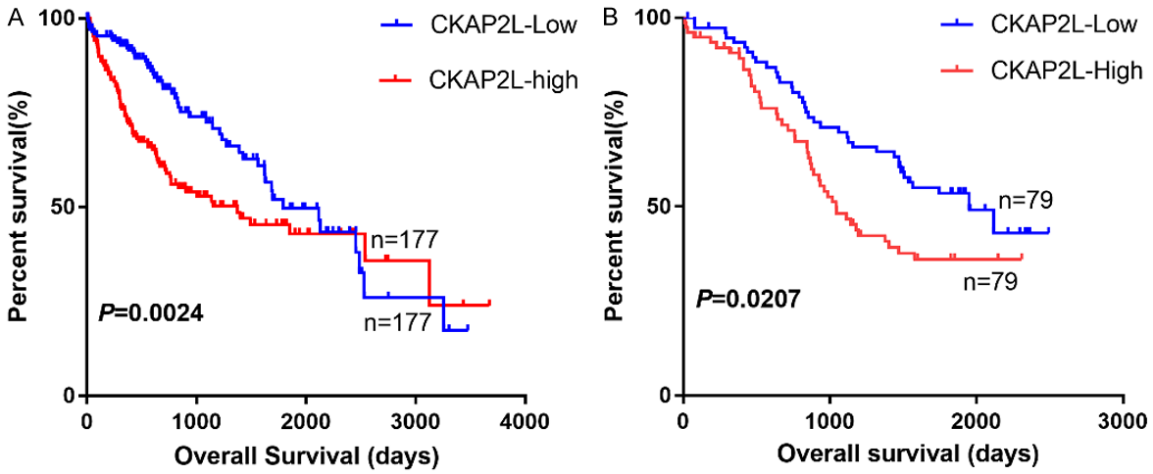


Figure 2. High CKAP2L expression is correlated with poor survival for HCC patients. Patients were then divided into CKAP2L-high and low expression group according to mRNA or protein expression. Kaplan-Meier analyses was performed to evaluate the correlation of CKAP2L expression and patients' survival. A. In the TCGA cohort, higher CKAP2L correlated with worse overall survival (P = 0.0024). B. In the PUMCH cohort, patients with higher protein expression also had significantly shorter overall survival.

Table 2. Cox regression analysis of candidate prognostic indicators for TCGA cohort HCC patients

Variable	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Age (>60 y)	1.25 (0.88-1.77)	0.216	1.38 (0.95-2.01)	0.089
Gender (male sex)	0.83 (0.58-1.18)	0.29		
AFP (>200 μg/L)	1.06 (0.66-1.69)	0.825		
Tumor grade (G3/4)	1.08 (0.75-1.55)	0.683		
TNM stage (Stage III/IV)	2.42 (1.67-3.51)	0.000003***	2.32 (1.60-3.38)	0.00002***
Vascular invasion	1.39 (0.92-2.11)	0.121		
CKAP2L overexpression	1.73 (1.21-2.47)	0.002**	1.68 (1.15-2.46)	0.007**

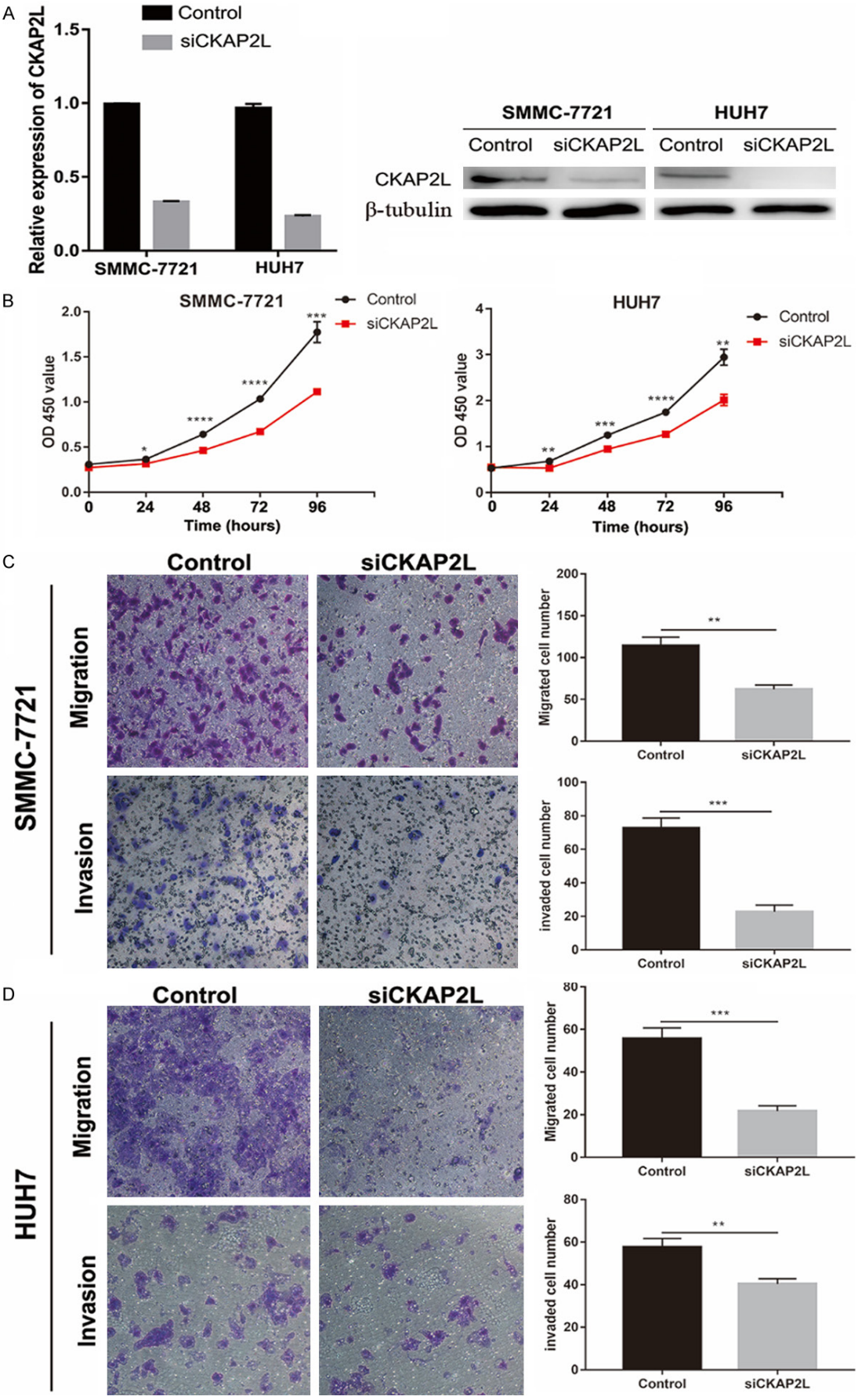
Note: **P<0.01, ***P<0.001.

Table 3. Cox regression analysis of potential prognostic predictors for PUMCH cohort HCC patients

Variable	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Age (>60 y)	1.16 (0.70-1.79)	0.65	1.33 (0.81-2.20)	0.258
Gender (male sex)	0.93 (0.50-1.72)	0.817		
AFP (>200 μg/L)	1.95 (1.25-3.06)	0.003**	1.33(0.81-2.20)	0.04*
CA199 (>37 U/mL)	1.90 (1.19-3.06)	0.008**		
Tumor grade (G3/4)	1.93 (1.24-3.02)	0.004**		
TNM stage (Stage III/IV)	3.14 (1.88-5.24)	0.000012****	2.23 (1.15-4.35)	0.018*
HBV antibody (+)	0.70 (0.41-1.18)	0.18		
Tumor number (≥2)	1.87 (1.14-3.05)	0.013*	1.29 (0.70-2.20)	0.41
CKAP2L overexpression	2.00 (1.28-3.12)	0.002**	1.60 (1.01-2.55)	0.046*

HR: hazard ratio; CI: confidence interval; *P<0.05; **P<0.01; ****P<0.0001.

py and liver transplantation, were developed for the treatment of HCC [7]. Therapeutic strategies are inhibited by a complicated phenotypic and molecular heterogeneity, and predictive biomarkers of response in advanced stage patients are lacking [8]. Despite extraordinary progress in our understanding of molecular subclasses of HCC, identification of critical


A
Control
siCKAP2L
Relative expression of CKAP2L
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Time (hours)
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HUH7
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Figure 3. Silencing CKAP2L expression decreased proliferation, migration and invasion of HCC cells. A. qRT-PCR and western blot analysis were performed to verify that CKAP2L was successfully silenced at both transcriptional and protein levels in SMMC-7721 and Huh7 cells. B. CCK-8 analysis indicated that silencing CKAP2L expression significantly decreased cell proliferation in SMMC-7721 and Huh7 cells. C, D. Transwell study demonstrated that knockdown of CKAP2L expression significantly impaired cell migration and invasion abilities. *P<0.05; **P<0.01; ****P<0.0001.

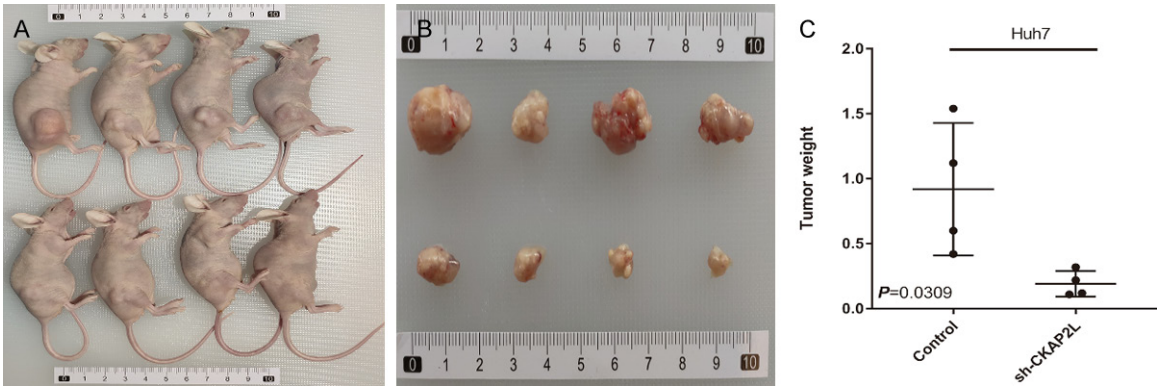

Figure 4. CKAP2L promoted HCC cell growth in vivo. (A, B) Stable CKAP2L knockdown inhibited subcutaneous tumorigenicity, as indicated by tumor size and weight (C). *P<0.05.

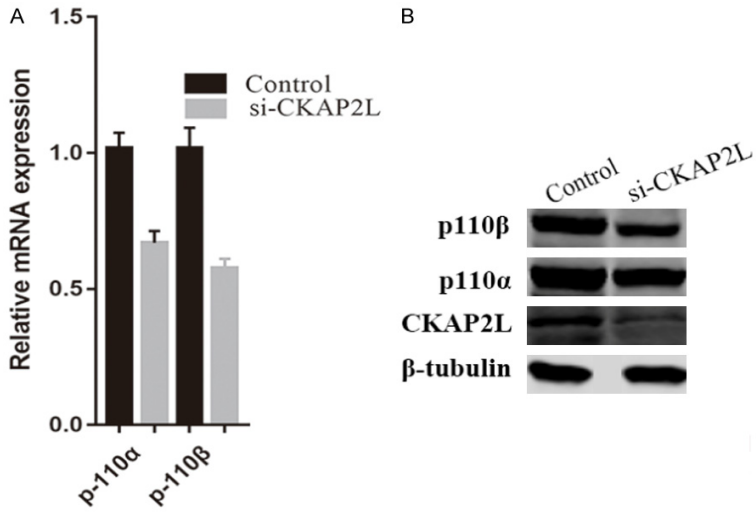

Figure 5. CKAP2L activates the PI3K signaling pathway. A. qRT-PCR results of PIK3CA/p110α and PIK3CB/p110β expression in CKAP2L knockdown Huh7 cells. B. Western blotting results of PIK3CA/p110α and PIK3CB/p110β expression in CKAP2L knockdown Huh7 cells. β-tubulin was used as an internal control.

molecular drivers of tumor progression and predictive prognostic biomarker for patient stratification is urgently needed.

CKAP2L is composed of 9 exons and encodes a 745-amino acid polypeptide called cytoskeletal associated protein 2 like protein. The CKAP2L protein contains the cytoskeleton-associated protein 2 (CKAP2) C-terminal domain (CKAP2_C superfamily), which contains 319 amino acids at the C-terminus and is an important paralogue of CKAP2. The CKAP2 gene encodes a cytoskeleton-related protein, exhibits microtubule-stabilizing properties, plays a role in regulating cell division, and is involved in the regulation of aneuploidy, cell cycle, and apoptosis in a p53/TP53-dependent manner [13, 24, 25]. The CKAP2L protein is localized to the centrosome, cytoplasm, mitotic spindle, and spindle pole. Mitotic spindle proteins are essential for mitotic spindle formation and cell cycle progression in neural stem/progenitor cells. Mutations in this gene are associated with spindle tissue defects, such as mitotic spindle defects, chromosome segregation, and abnormalities [11]. The c.571dupA CKAP2L mutation was associated with the congenital disease Filippi syndrome [13, 26].

The clinical significance and the possible mechanism of CKAP2L in the development of HCC remain to be elucidated.

In the current study, we investigated the expression and clinical significance of the CKAP2L gene in HCC. We first analyzed the TCGA cohort and discovered that CKAP2L was highly upregulated in HCC. In addition, we used the Kaplan-Meier curve and Log-rank tests to identify a distinctive association between high CKAP2L expression in HCC patients and reduced overall survival. Multivariate Cox regression analysis further demonstrated that high CKAP2L expression is an independent risk factor for predicting prognosis in HCC patients. Analyzing clinical data, we found that CKAP2L overexpression was significantly associated with age, AFP levels, pathologic grade, and TNM stage. We have demonstrated that knockdown of CKAP2L expression inhibited the proliferation, migration, and invasion of SMMC-7721 and Huh7 cells. The growth promotion effect of CKAP2L was also evaluated by xenograft tumor growth. We observed that silencing of CKAP2L dramatically reduced the expression of p110α, and p110β at mRNA and protein level. This data indicated that CKAP2L might promote cell proliferation partially by regulating the PI3K signaling pathway. However, further investigation is needed to elucidate how CKAP2L activates the PI3K signaling pathway.

In conclusion, we discovered and verified for the first time that CKAP2L was prominently upregulated in HCC, and silencing CKAP2L expression significantly attenuated proliferation, migration, and invasion ability of HCC cells. Our findings indicated that CKAP2L could act as an original prognostic biomarker and therapeutic target of HCC.

Acknowledgements

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Disclosure of conflict of interest

None.

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