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Overexpression of COPS3 promotes clear cell renal cell carcinoma progression via regulation of Phospho-AKT(Thr308), Cyclin D1 and Caspase-3

Yang Hong, Xiaobo Huang, Lizhe An, Haiyun Ye, Kai Ma, Fengshi Zhang, Qingquan Xu*

The Department of Urology, Peking University People's Hospital, Beijing, China

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ABSTRACT

The third subunit of the COP9 signalosome (COPS3) is associated with cell proliferation and tumorigenesis process in cancer. The present study showed that the expression level of COPS3 was upregulated in malignant cell lines and COPS3 overexpression was related with clinical stage, T stage, historical grade. Kaplan-Meier survival curves showed that COPS3 may function as a prognostic factor for overall survival. CCK-8 and colony formation assays revealed that knockdown of COPS3 in ACHN and 786-O significantly impacted proliferation in vitro. In addition, flow cytometry showed that inhibition of COPS3 induced G0/G1 arrest and promoted apoptosis. COPS3 may promote kidney cancer progression by altering Phospho-AKT(Thr308), Cyclin D1 and Caspase-3 expression. Collectively, Our findings suggest that COPS3 may be a new potential target of ccRCC.

1. Introduction

ccRCC is the most common type of kidney cancer in adult tumors [1,2]. Surgery is the main treatment for this kind of cancer, while ccRCC is resistant to conventional chemotherapy, possibly through high expression of multidrug resistance genes or inactivation of apoptotic pathways [3]. Targeted therapy has shown a promising future in treating ccRCC patients. More recently, some new drugs targeting vascular endothelial growth factor receptor, such as sunitinib and sorafenib, have proved to be beneficial for ccRCC [4]. Unfortunately, large portion of kidney cancer patients treated with these inhibitors will experience disease progression. Drug treatment of kidney cancer is still unsatisfactory [5]. Therefore, it is urgent to identify novel targets for ccRCC patients.

The COPS3 encodes the third subunit of eight-subunit COP9 signalsome (CSN) complex and located on chromosome 17p11.2–12 in humans. CSN was first identified in Arabidopsis thaliana in 1996 as a negative regulator of constitutive photomorphogenesis (COP) [6,7]. Two activities associated with the CSN: a protein kinase and a deneddylase. The CSN-associated kinase phosphorylates transcription factors, which determines their stability toward the ubiquitin system. Its associated deneddylase regulates the activity of SCF (Skp1, Cullins, F-box protein) E3 ubiquitin ligases. The CSN thus appears to be a platform connecting signaling with proteolysis, which regulates the stability of many signaling proteins, including I kappa-B alpha, p105, ITPK1, c-Jun, p-53, IRF8/ICSBP, and p27 [8].

COPS3 plays an important role in both the tumorigenesis and progression of osteosarcoma, hepatocellular carcinoma and lung cancer. The overexpression of COPS3 has been found to be correlated with proliferation, metastasis and apoptosis [9–12]. Additionally, clinical data revealed that high expression of COPS3 was significantly correlated with poor outcome in osteosarcoma patients [9]. But the expression and biological function of COPS3 in ccRCC remains unclear, and the potential mechanisms underlying still need to be revealed.

Here, we observed siRNA-mediated knockdown of COPS3 expression in ACHN and 786-O ccRCC cell lines significantly impaired the proliferative ability. Statistical analysis revealed that a high expression of COPS3 was correlated with the poor prognosis. Down-regulation of COPS3 in ccRCC cell lines induced decreased expression of Phospho-AKT(Thr308), Cyclin D1 and upregulated expression of Caspase-3.

Abbreviations: COPS3, The third subunit of the COP9 signalosome; ccRCC, clear cell renal cell carcinoma; qRT-PCR, quantitative real-time PCR; CCK-8, Cell Counting Kit-8; CSN, COP9 signalosome; COP, constitutive photomorphogenesis; siRNA, small interfering RNA; FBS, Fetal bovine serum; SDS, Sodium Dodecyl Sulphate; PAGE, polyacrylamide gel electrophoresis; PBS, phosphate bufffered solution; FITC, Fluorescein isothiocyanate; PI, Propidium Iodide; IgG, Immunoglobulin class G; HRP, Horse radish peroxidase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; Co-IP, Co-Immunoprecipitation; PARP, poly (ADP-ribose) polymerase; IHC, Immunohistochemical; OGS, osteogenic sarcoma; HCC, hepatocellular carcinoma; COP1, constitutively photomorphogenic 1

* Corresponding author.
E-mail addresses: 1446347933@qq.com (Y. Hong), huang6299@sina.com (X. Huang), qdalz@126.com (L. An), yehypku@sina.com (H. Ye), suacaca@hotmail.com (K. Ma), 762322069@qq.com (F. Zhang), xuqingquan@pkuph.edu.cn (Q. Xu).

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Table 1
Clinicopathological variables and evaluation of COPS3 immunostaining in clear cell renal cell carcinoma tissues.

Characteristics	All cases	Scores for COPS3 staining		P-value
		High	Low	
Type				0.032
Non-tumor	102	50	52	
ccRCC	105	67	38	
Gender				0.980
male	72	46	26	
female	33	21	12	
Age(years)				0.400
< 60	64	42	21	
> =60	41	24	17	
Clinical stage				0.001
I-II	64	33	31	
III-IV	41	34	7	
Histological grade				0.010
1–2	86	50	36	
3–4	19	17	2	
T stage				0.010
T1-T2	90	53	37	
T3-T4	15	14	1	

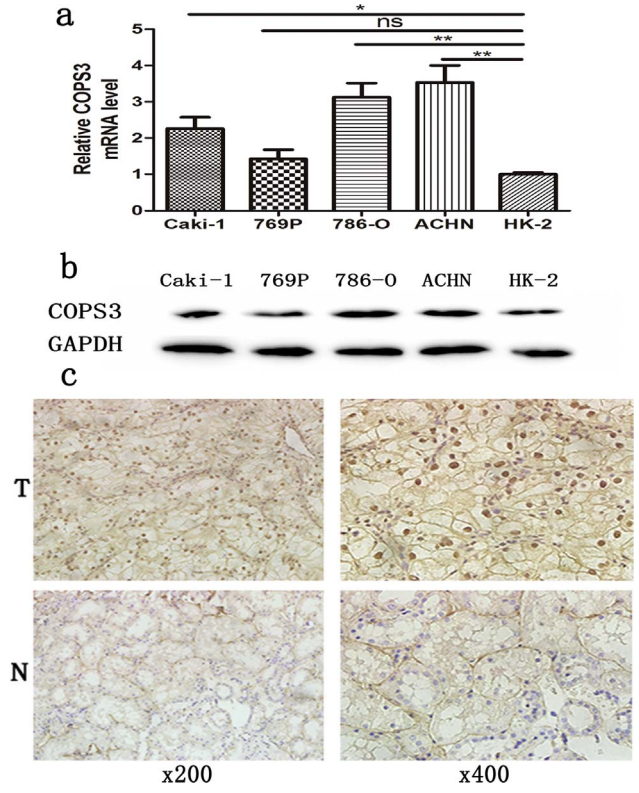

Fig. 1. COPS3 was regulated in ccRCC tissues and cell lines. a and b COPS3 was detected in ccRCC cell lines at both mRNA and protein level by western blotting and qRT-PCR respectively.($^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$, $^{ns}P > 0.05$) **c** COPS3 protein in tumor tissues(T) and adjacent normal tissues(N) detected by immunohistochemistry. Magnification: *left*(x200), *right*(x400).

2. Material and methods

2.1. Tissue microarray and samples

A tissue microarray with 150 spots of human ccRCC (75) and paired non-tumor tissues(75) was obtained from Shanghai Xinchao Biotech Co. Ltd. (Shanghai, China) Thirty patients with ccRCC undergoing resection at Department of Urology, Peking University People's Hospital. The research was approved by Ethics Committee of Peking University People's Hospital and informed consent was obtained from all participants. The characteristics of patients are presented in Table 1.

Table 2
Univariate and multivariate analysis using Cox regression model for overall survival of patients with clear cell renal cell carcinoma (ccRCC).

Characteristics	Univariate analysis			Multivariate analysis		
	HR	95% CI	P value	HR	95% CI	P value
COPS3 (High/Low)	3.131	1.070–9.166	0.037	Not in model		
Gender (Male/Female)	0.756	0.319–1.792	0.525	Not in model		
Age (≥60/ < 60)	0.865	0.379–1.975	0.731	Not in model		
T stage (T1-T2/T3-T4)	0.332	0.137–0.804	0.015	Not in model		
Histological grade (1–2/3–4)	0.227	0.094–0.548	0.001	0.309	0.123–0.775	0.012
Clinical stage (I-II/III-IV)	0.202	0.090–0.453	0.000	0.290	0.125–0.672	0.004

2.2. Cell lines and si-RNAs

Four RCC cell lines ACHN, 769 P, 786-O, Caki-1 were purchased from the American Type Culture Collection (ATCC, USA). The Cell lines were cultured in RPMI-1640 (Hyclone, Logan, UT, USA) with 10% FBS. Si-RNAs targeting COPS3 were obtained from GenePharma (Shanghai, China). The following sequences were noted below: si-RNA1, 5′-GCCU GCCCUUCCAUAUCUUTT-3′ (sense) and 5′-AAGAUAUGGAAGGGCAG GCTT-3′ (antisense); si-RNA2, 5′-GCGAGGAAUUGGCAUCCUUTT-3′ (sense) and 5′-AAGGAUGCCAAUUCCUCGCTT-3′(antisense); and negative control (si-NC) 5′-UUCUCCGAACGUGUCACGUTT-3′ (sense) and 5′-ACGUGACACGUUCGGAGAATT-3′ (antisense). The si-RNAs were transfected into ACHN and 786-O cells using Lipofectamine 3000 Transfection kit (Invitrogen, USA) according to the manufacturer's instructions. Subsequently, the efficiency of COPS3 silencing was determined using qRT-PCR and western blotting.

2.3. qRT-PCR

Total RNA was isolated from cells using simple Total RNA Kit (Tiangen, Beijing, China). Besides, 1.6 μg total RNA using FastQuant RT Kit (Tiangen, Beijing, China). Subsequently, qRT-PCR was performed using an iQ5 real-time detection system (Bio-Rad Laboratories, Hercules, CA, USA). Besides, the mRNA levels of target genes were normalized to GAPDH and the change of gene expression levels were calculated using $2^{-\Delta\Delta Ct}$ method. The primers used were as follows: COPS3 (forward, 5′-CAACCAACAACCCCTCAGAAC-3′ and reverse, 5′-TTATCGCGAGTGAAGGTTTCAC-3′); and GAPDH (forward,5′-CACC CACTCCTCCACCTTTG-3′ and reverse, 5′-CCACCACCCTGTTGCTG TAG-3′).

2.4. Immunohistochemical analysis

The samples and microarray incubated with anti-COPS3 monoclonal antibody (1:200 dilutions, Bioworld, USA). Immunodetection was performed using the Envision ABC kit (Gene Tech company limited, Shanghai, China). Results of COPS3 staining were assessed by two experienced pathologists using double blind method. Each slice for calculating the score of COPS3 staining was as follows: the extent of staining in a x200 field was scored as 0, 0%; 1, 1–25%; 2, 26–50%; 3, 51–100%. Besides, the intensity of staining was scored as 0, blue; 1, light brown; 2, brown; 3, dark brown. The results of each field were