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NAA10 promotes proliferation of renal cell carcinoma by upregulating UPK1B

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Abstract. – OBJECTIVE: The purpose of this study was to illustrate the role of NAA10 in aggravating the malignant progression of renal cell carcinoma (RCC) by upregulating UPK1B.

PATIENTS AND METHODS: NAA10 levels in RCC tissues and paracancerous tissues were detected. Thereafter, the potential relationship between NAA10 level and clinical parameters of RCC patients was analyzed. After knockdown of NAA10, changes in proliferative potential of 786-O and Caki-1 cells were examined by cell counting kit-8 (CCK-8), colony formation and 5-Ethynyl-2'-deoxyuridine (EdU) assay. Finally, the regulatory role of NAA10 in the downstream gene UPK1B and the involvement of UPK1B in the development of RCC were determined via rescue experiments.

RESULTS: NAA10 was upregulated in RCC tissues than paracancerous tissues. Tumor staging was much worse in RCC patients expressing a higher level of NAA10. Knockdown of NAA10 inhibited proliferative potential and downregulated UPK1B in RCC cells. Besides, NAA10 level was identified to be positively linked to UPK1B level in RCC tissues. At last, overexpression of UPK1B was able to abolish the inhibitory effect of silenced NAA10 on RCC proliferation.

CONCLUSIONS: NAA10 level is closely linked to tumor staging and poor prognosis in RCC patients. NAA10 aggravates the malignant progression of RCC by upregulating UPK1B and may be a specific biomarker in RCC.

Key Words:
NAA10, UPK1B, Renal cell carcinoma (RCC), Proliferation.

Introduction

Renal cell carcinoma (RCC) is prevalent in renal malignancies, ranking third in genitourinary malignancies[1,2]. With the wide application of abdominal imaging examination, the detective rate of localized, early stage renal cancers has increased[3,4]. At present, radical nephrectomy is the gold standard for the treatment of localized renal cancer[5,6]. However, some RCC patients develop postoperative tumor recurrence or distant metastasis[7,8]. Although great process had been made in the development of molecular biology and targeted VEGFR therapy in recent years[8,9], about 50% RCC patients are insensitive to targeted therapy[10]. In addition, the majority of RCC patients develop drug resistance within 1 year. It is necessary to seek for new biomarkers, thus improving clinical outcomes of RCC[11,12].

N-alpha-acetyltransferase 10 (NAA10) is derived from the acetylation of the N-terminal alpha position of the protein by a group of highly conserved N-acetyltransferase protein complexes (NatA, NatB, NatC, NatD, and NatE). In particular, NatA complex consists of the catalytic subunit NAA10, the helper subunit NAA15P and the unknown functional subunit NAA50p[13-16]. Biological functions of NAA10 in human diseases have been identified[17]. The aim of this paper was to explore the regulatory effects of NAA10 on the malignant development of RCC and the underlying mechanism.

Patients and Methods

RCC Patients and Tumor Tissues

RCC tissues (n=66) and paracancerous tissues (n=66) were surgically resected from RCC

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patients treated in The First Hospital of Shanxi Medical University from June 2016 to May 2019. In this study, the diagnosis and selection (inclusion/exclusion) of RCC patients was based on the guideline proposed by the Union for International Cancer Control (UICC). This investigation was approved by the Ethics Committee of The First Hospital of Shanxi Medical University and conducted after informed consent of each subject was obtained.

Cell Culture

RCC cell lines (ACHN, Caki-1, 769P, Caki-2 and 786-O) and renal tubular epithelial cell line (HK-2) were obtained from American Type Culture Collection (ATCC; Manassas, VA, USA). Cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM; Gibco, Rockville, MD, USA) with 10% fetal bovine serum (FBS; Gibco, Rockville, MD, USA), 100 U/mL penicillin and 100 μg/mL streptomycin in a 5% CO_2 incubator at 37°C.

Transfection

Sh-NAA10, sh-NC, pcDNA3.1-UPK1B and pcDNA3.1-NC were constructed by GenePharma (Shanghai, China). Cells were cultured to 30-40% confluence, transfected using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) for 48 h, and collected for the following use.

Cell Proliferation Assay

Cells were inoculated in a 96-well plate with 2×10^3 cells per well. At the appointed time points, absorbance value at 490 nm of each sample was recorded using the cell counting kit-8 (CCK-8) kit (Dojindo Laboratories, Kumamoto, Japan) for plotting the viability curves.

Colony Formation Assay

Cells were inoculated in a 6-well plate with 200 cells per well and cultured for 2 weeks. Culture medium was replaced once in the first week and twice in the second week. Next, visible colonies were washed in phosphate-buffered saline (PBS), fixed in methanol for 20 min and dyed in 0.1% crystal violet for 20 min and were captured and calculated.

5-Ethynyl-2′-Deoxyuridine (EdU) Assay

Cells in a 96-well plate (4×10^3 cells/well) were incubated with EdU solution (Beyotime, Shanghai, China) in the dark for 30 min and dyed with 4',6-diamidino-2-phenylindole (DAPI) for another 30 min. At last, EdU-positive ratio was calculated by the number of EdU-positive cells to that of DAPI-labeled nuclei.

Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

RNAs extracted by TRIzol reagent (Invitrogen, Carlsbad, CA, USA) were purified by DNase I treatment, and reversely transcribed into complementary deoxyribose nucleic acids (cDNAs) using Primescript RT Reagent (TaKaRa, Otsu, Shiga Japan). The obtained cDNAs underwent qRT-PCR using SYBR®Premix Ex Taq™ (TaKaRa, Otsu, Shiga, Japan). β-actin was the internal reference. Each sample was performed in triplicate, and relative level was calculated by $2^{-\Delta\Delta Ct}$. NAA10: forward: 5'-GGAAGAGGATCAGTGAAGTGGA-3' and reverse: 5'-CGGAGGTGAATTGCCTTTGC-3', UPK1B: forward: 5'-TGCTCCAGGACAATTGCTGT-3' and reverse: 5'-GCCTCCAGGTTGAGAGGTTC-3', and β-actin: forward: 5›-CCTGGCACCCAGCACAAT-3› and reverse: 5'-GCTGATCCACATCTGCTGGAA-3'.

Western Blot

Cells were washed in PBS and collected. The mixture was lysed in radio immunoprecipitation assay (RIPA(solution and centrifuged for isolating cellular proteins. Later, the concentration of extracted protein samples was measured by bicinchoninic acid assay (BCA) method, and each sample was adjusted to the same volume. Protein samples were separated on 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and loaded on polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). Subsequently, membranes were immersed in Tris-Buffered Saline and Tween-20 (TBST) containing 5% skim milk for 2 h to block non-specific antigens. Membranes were then reacted with primary antibodies at 4°C overnight, and secondary antibodies on the next day for 1 h at room temperature. Finally, band exposure was achieved by enhanced chemiluminescence (ECL) method and grey value analyses were finally conducted.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 22.0 (IBM, Armonk, NY, USA) was used for data analyses. Data were expressed as mean ± standard deviation. Differences between groups were analyzed by the *t*-test. Chi-square test was

used for analyzing the relationship between NAA10 level and clinical parameters of RCC patients. Pearson correlation test was applied for evaluating the relationship between expression levels of NAA10 and UPK1B in RCC tissues. $p<0.05$ was considered as statistically significant.

Results

NAA10 Was Upregulated In RCC

Compared with paracancerous tissues, NAA10 was upregulated in RCC tissues (Figure 1A). Similarly, NAA10 was highly expressed in RCC cell lines than in HK-2 cell line (Figure 1B). Among the five tested RCC cell lines, 786-O and Caki-1 cells expressed the highest level of NAA10, so they were selected for the following experiments.

NAA10 Level Was Linked to Tumor Staging In RCC Patients

Included 66 RCC patients were assigned into two groups according to the median level of NAA10. Through analyzing clinical parameters of RCC patients, it was found that NAA10 level was positively correlated with tumor staging in RCC patients but not with age, gender and metastasis (Table I). It is suggested that NAA10 can be a novel biomarker in predicting the malignant progression of RCC.

Knockdown of NAA10 Suppressed Proliferative Potential In RCC

Transfection efficacy of sh-NAA10 was tested in 786-O and Caki-1 cells (Figure 1C). Viability was markedly reduced in RCC cells transfected with sh-NAA10 (Figure 2A). Compared with those transfected with sh-NC, RCC cells transfected with sh-NAA10 showed fewer visible colonies (Figure 2B). In addition, EdU-positive rate was lower in RCC cells with NAA10 knockdown than those of controls (Figure 2C). The above data demonstrated that NAA10 was able to promote proliferative potential in RCC.

NAA10 Positively Regulated UPK1B

Transfection of sh-NAA10 downregulated UPK1B in both 786-O and Caki-1 cells (Figure 3A). Subsequently, pcDNA3.1-UPK1B and corresponding pcDNA3.1-NC were constructed. Transfection of pcDNA3.1-UPK1B markedly upregulated UPK1B and NAA10 in RCC cells (Figure 3B, 3C). Similar to NAA10, UPK1B was upregulated in RCC tissues (Figure 3D).

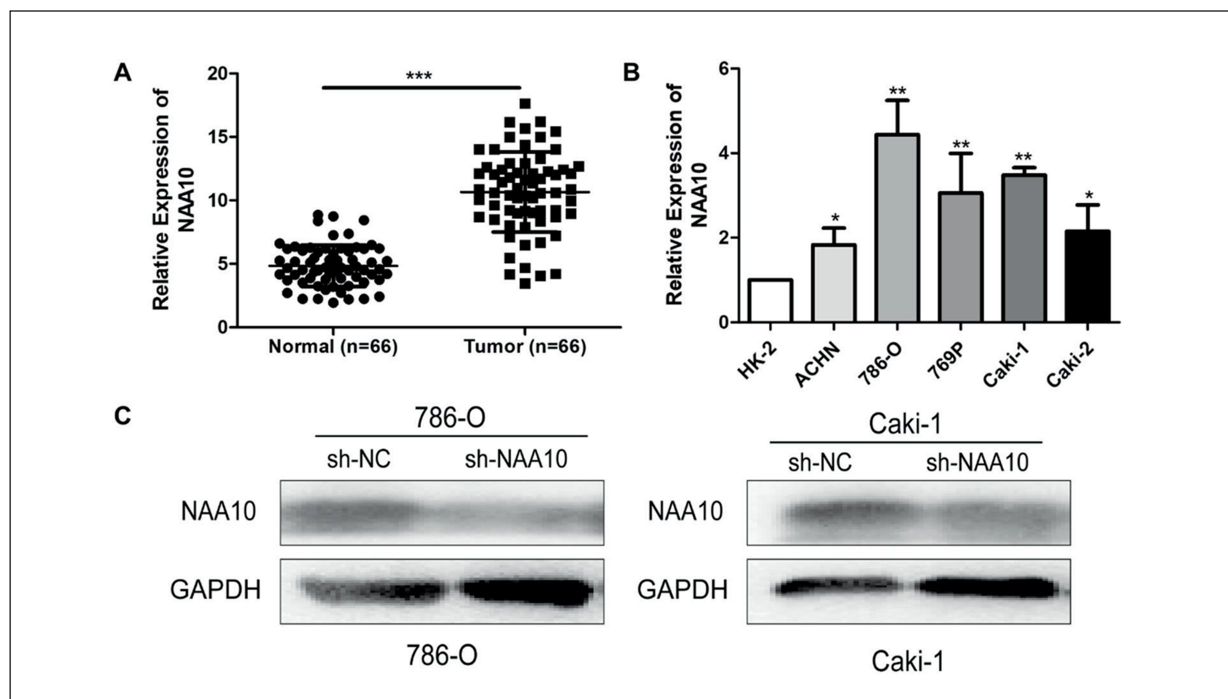

Figure 1. NAA10 was upregulated in RCC. **A,** NAA10 levels in RCC tissues (n=66) and paracancerous tissues (n=66). **B,** NAA10 level in HK-2 and RCC cell lines. **C,** Protein and mRNA levels of NAA10 in 786-O and Caki-1 cells transfected with sh-NC or sh-NAA10. Data were expressed as mean±SD. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

Table I. Association of NAA10 expression with clinicopathologic characteristics of renal cell carcinoma.

Parameters	No. of cases	NAA10 expression		*p*-value
		Low (%)	High (%)	
Age (years)				0.782
< 60	28	16	12	
≥ 60	38	23	15	
Gender				0.585
Male	32	20	12	
Female	34	19	15	
T stage				0.021
T1-T2	38	27	11	
T3-T4	28	12	16	
Lymph node metastasis				0.282
No	40	26	14	
Yes	26	14	13	
Distance metastasis				0.195
No	45	29	16	
es	21	10	11	

Moreover, a positive correlation was identified between expression levels of NAA10 and UPK1B in RCC tissues (Figure 3E).

Overexpression of UPK1B Reversed Inhibitory Effects of Silenced NAA10 on Proliferative Potential In RCC

Downregulated NAA10 in RCC cells with NAA10 knockdown was found to be upregulated by overexpression of UPK1B (Figure 4A). Interestingly, viability (Figure 4B) and EdU-positive rate (Figure 4C) decreased by knockdown of NAA10 were partially reversed by overexpression of UPK1B. It was demonstrated that UPK1B was involved in NAA10-regulated development of RCC.

Discussion

Clear cell renal cell carcinoma (ccRCC) is the major pathological subtype of RCC, accounting for higher than 80% of all RCC cases[3-5]. Effective treatment for advanced metastatic RCC is lacked[5,6]. Although drugs that target VEGFR can effectively alleviate the development of RCC, only 50% RCC patients can benefit from these drugs[8,9]. Finding a novel target and biomarker for RCC contributes to clinical treatment[10-12].

Gene transcription and mRNA translation are two essential processes for eukaryotic gene expressions[18,19]. When the translation of mRNA in eukaryotes is out of control, dysregulated genes lead to uncontrolled cell growth or even tumorigenesis[18,19]. Therefore, targeting abnormally translated factors in human tumors may be a potential targeted therapeutic strategy[20,21]. NAA10 is involved in almost all processes of translation initiation and tumor development[17]. However, the mechanism of NAA10 in ccRCC is not clear. Therefore, this objective of this study was firstly to elucidate the oncogenic role of NAA10 in the progression of ccRCC. Our previous research showed that NAA10 was upregulated in RCC tissues than in normal renal tissues, and its level was positively linked to histopathological staging of ccRCC patients. Here, higher level of NAA10 was identified in RCC tissues compared with paracancerous tissues. Tumor metastasis and malignant proliferation are two vital events in tumor deterioration[22,23]. The findings of this study showed that knockdown of NAA10 suppressed viability, clonality and EdU-positive rate in RCC, suggesting that NAA10 stimulated proliferative potential in RCC.

Through online prediction, UPK1B was considered as the downstream gene of NAA10. In this paper, UPK1B was positively regulated by NAA10 in RCC cells. Compared with that in paracancerous tissues, NAA10 was upregulated in RCC tissues. Notably, overexpression of UPK1B was able to abolish the inhibitory effect of silenced NAA10 on RCC proliferation. Our results demonstrated a positive feedback loop that NAA10 promotes proliferative potential in RCC by positively regulating NAA10 level.

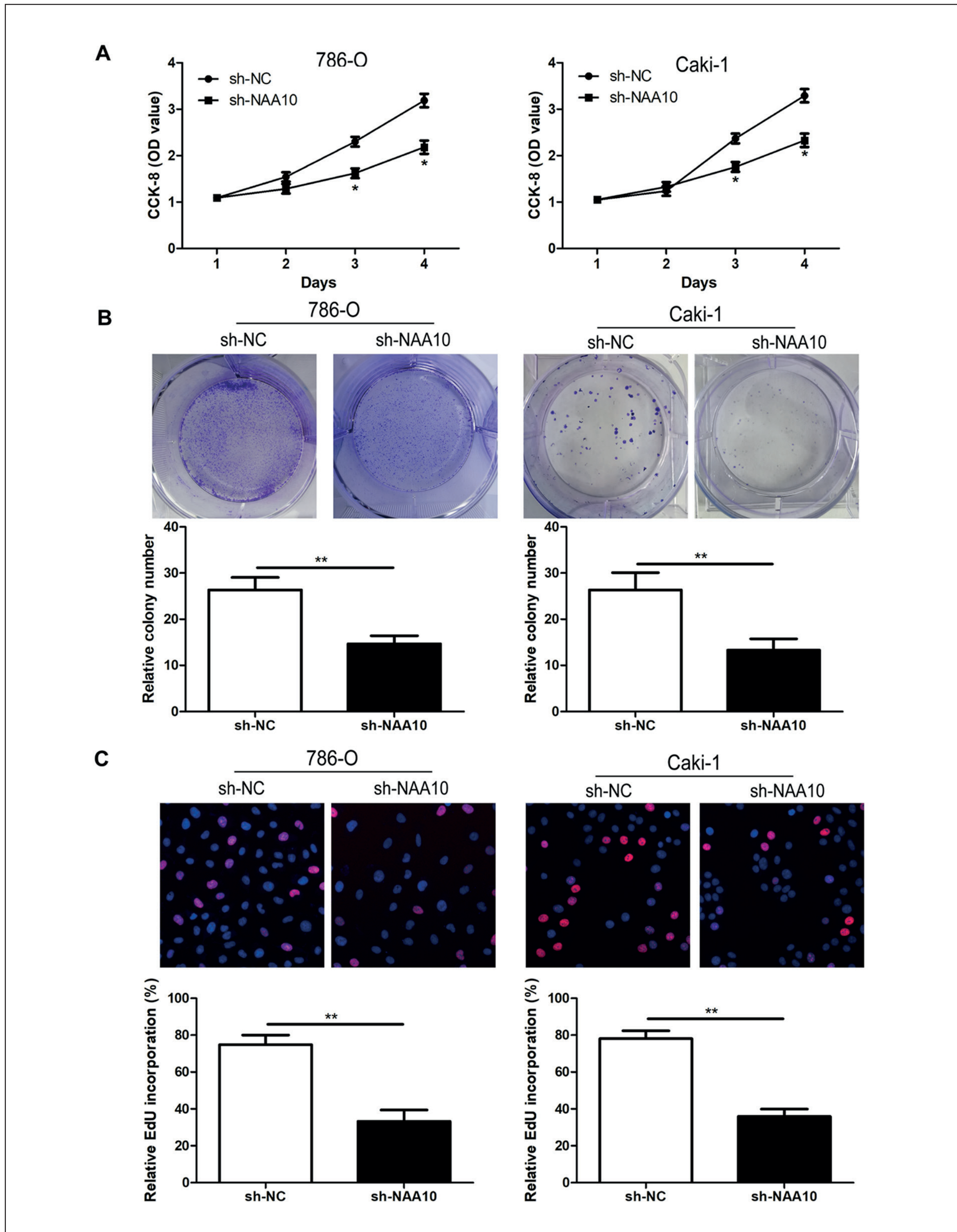

Figure 2. Knockdown of NAA10 suppressed proliferative potential in RCC. ***A*,** Viability in 786-O and Caki-1 cells transfected with sh-NC or sh-NAA10. **B,** Colony number in 786-O and Caki-1 cells transfected with sh-NC or sh-NAA10 (magnification 10×*)*. **C,** EdU-positive rate in 786-O and Caki-1 cells transfected with sh-NC or sh-NAA10 (magnification 40×). Data were expressed as mean±SD. *$p < 0.05$, **$p < 0.01$.

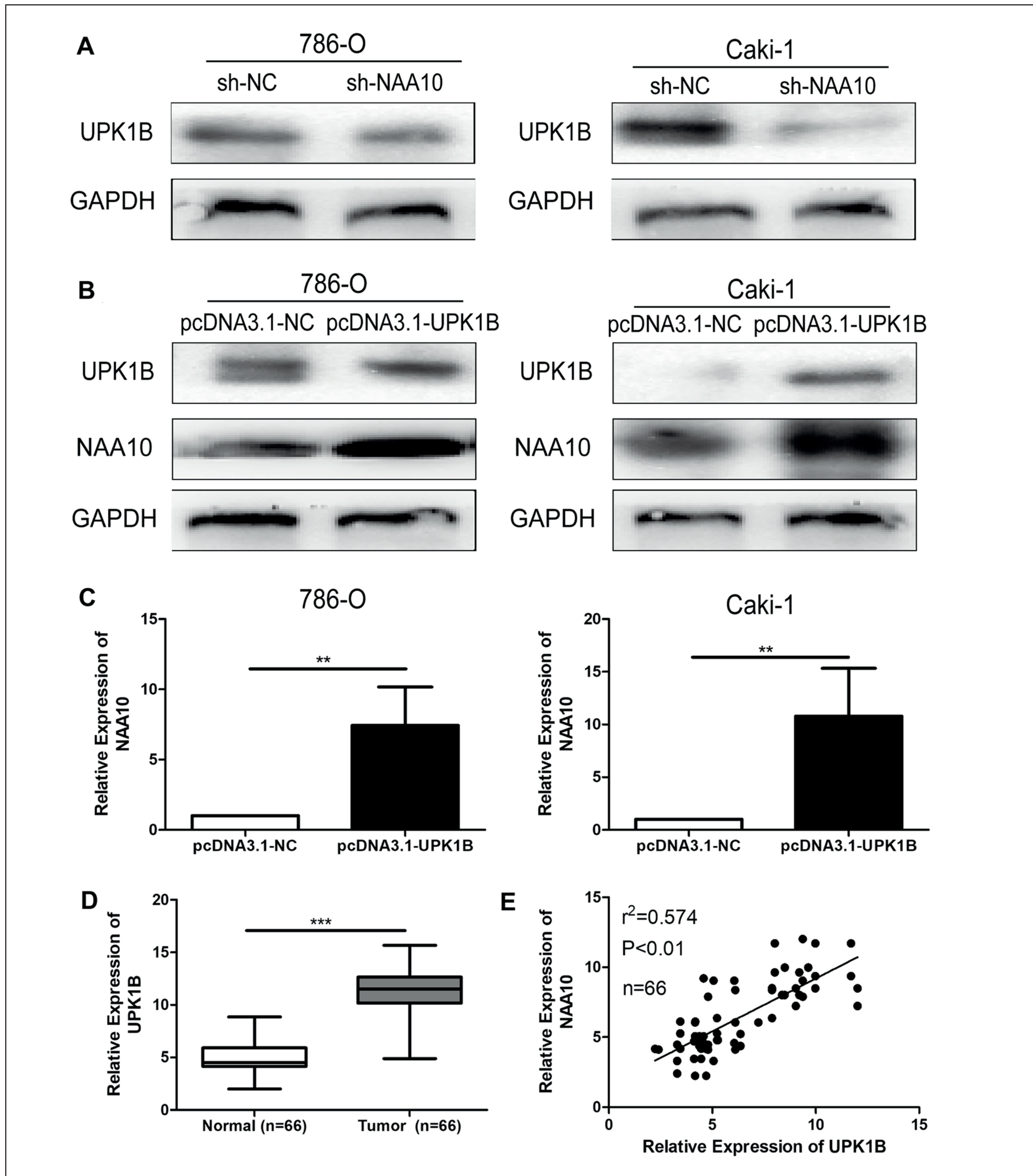

Figure 3. NAA10 positively regulated UPK1B. **A,** Protein and mRNA levels of UPK1B in 786-O and Caki-1 cells transfected with sh-NC or sh-NAA10. **B,** Protein levels of UPK1B and NAA10 in 786-O and Caki-1 cells transfected with pcDNA3.1-NC or pcDNA3.1-UPK1B. **C,** The mRNA level of NAA10 in 786-O and Caki-1 cells transfected with pcDNA3.1-NC or pcDNA3.1-UPK1B. **D,** UPK1B levels in RCC tissues (n=66) and paracancerous tissues (n=66). **E,** A positive correlation between expression levels of NAA10 and UPK1B in RCC tissues. Data were expressed as mean±SD. $^{**}p < 0.01$, $^{***}p < 0.001$.

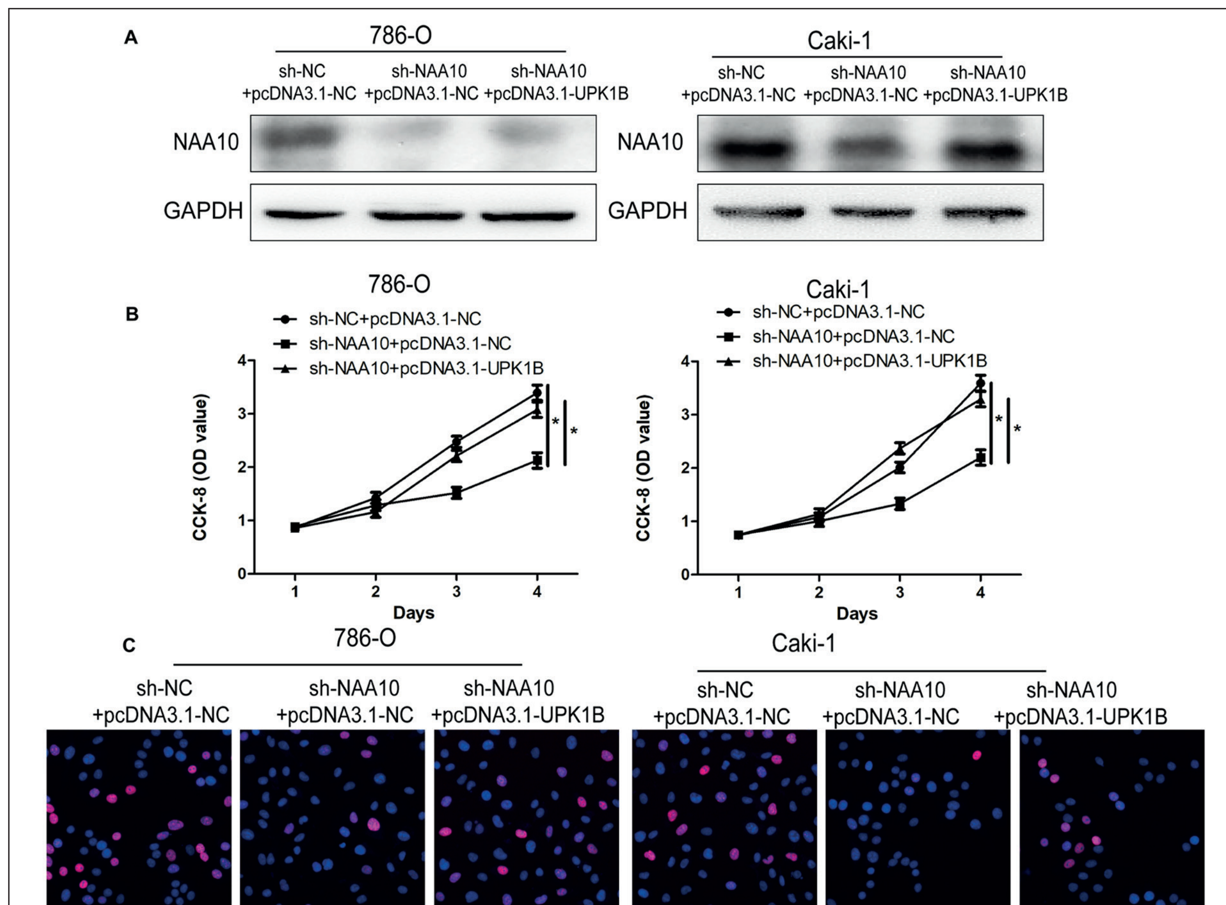

Figure 4. Overexpression of UPK1B reversed inhibitory effects of silenced NAA10 on proliferative potential in RCC. **A,** Protein and mRNA levels of NAA10 in 786-O and Caki-1 cells transfected with sh-NC+pcDNA3.1-NC, sh-NAA10+pcDNA3.1-NC or sh-NAA10+pcDNA3.1-UPK1B. **B,** Viability in 786-O and Caki-1 cells transfected with sh-NC+pcDNA3.1-NC, sh-NAA10+pcDNA3.1-NC or sh-NAA10+pcDNA3.1-UPK1B. **C,** EdU-positive rate in 786-O and Caki-1 cells transfected with sh-NC+pcDNA3.1-NC, sh-NAA10+pcDNA3.1-NC or sh-NAA10+pcDNA3.1-UPK1B (magnification 40×). Data were expressed as mean±SD. $*p < 0.05$, $**p < 0.01$.

Conclusions

NAA10 level is closely linked to histopathological staging in RCC patients, which aggravates the malignant progression of RCC by upregulating UPK1B. Thus, NAA10 may be a specific biomarker in RCC.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Jonasch E, Gao J, Rathmell WK. Renal cell carcinoma. BMJ 2014; 349: g4797.
2) Inamura K. Renal cell tumors: understanding their molecular pathological epidemiology and the 2016 WHO Classification. Int J Mol Sci 2017; 18: 2195.
3) Krajewski KM, Shinagare AB. Novel imaging in renal cell carcinoma. Curr Opin Urol 2016; 26: 388-395.
4) Park JJ, Jeong BC, Kim CK, Seo SI, Carriere KC, Kim M, Park BK, Jeon SS, Lee HM, Choi HY. Postoperative outcome of cystic renal cell carcinoma defined on preoperative imaging: a retrospective study. J Urol 2017; 197: 991-997.
5) Dell'Atti L, Borghi C, Galosi AB. Laparoscopic approach in management of renal cell carcinoma during pregnancy: state of the art. Clin Genitourin Cancer 2019; 17: e822-e830.
6) Yang TY, Lin WR, Chiu AW. Spontaneous regression of adrenal metastasis from renal cell carcinoma after sunitinib withdrawal: case report and literature review. BMC Urol 2018; 18: 105.
7) Jaen-Torrejimeno I, Rojas-Holguin A, Lopez-Guerra D, Ramia JM, Blanco-Fernandez G. Pancreatic

resection for metastatic renal cell carcinoma. A systematic review. HPB (Oxford) 2020; 22: 479-486.

8) Barata PC, Rini BI. Treatment of renal cell carcinoma: current status and future directions. CA Cancer J Clin 2017; 67: 507-524.

9) Nadal R, Amin A, Geynisman DM, Voss MH, Weinstock M, Doyle J, Zhang Z, Viudez A, Plimack ER, McDermott DF, Motzer R, Rini B, Hammers HJ. Safety and clinical activity of vascular endothelial growth factor receptor (VEGFR)-tyrosine kinase inhibitors after programmed cell death 1 inhibitor treatment in patients with metastatic clear cell renal cell carcinoma. Ann Oncol 2016; 27: 1304-1311.

10) Cetin B, Kosar A. Game of thrones: immunotherapy versus molecular targeted therapy in renal cell cancer scenarios. Int Urol Nephrol 2019; 51: 2107-2117.

11) Mantia CM, McDermott DF. Vascular endothelial growth factor and programmed death-1 pathway inhibitors in renal cell carcinoma. Cancer 2019; 125: 4148-4157.

12) Barth DA, Slaby O, Klec C, Juracek J, Drula R, Calin GA, Pichler M. Current concepts of non-coding RNAs in the pathogenesis of non-clear cell renal cell carcinoma. Cancers (Basel) 2019; 11: 1580.

13) Lee D, Jang MK, Seo JH, Ryu SH, Kim JA, Chung YH. ARD1/NAA10 in hepatocellular carcinoma: pathways and clinical implications. Exp Mol Med 2018; 50: 88.

14) Vo T, Jeong CH, Lee S, Kim KW, Ha E, Seo JH. Versatility of ARD1/NAA10-mediated protein lysine acetylation. Exp Mol Med 2018; 50: 86.

15) Lee MN, Kweon HY, Oh GT. N-alpha-acetyltransferase 10 (NAA10) in development: the role of NAA10. Exp Mol Med 2018; 50: 87.

16) Wu Y, Lyon GJ. NAA10-related syndrome. Exp Mol Med 2018; 50: 85.

17) Dorfel MJ, Lyon GJ. The biological functions of Naa10 - from amino-terminal acetylation to human disease. Gene 2015; 567: 103-131.

18) Yan X, Hoek TA, Vale RD, Tanenbaum ME. Dynamics of translation of single mRNA molecules in vivo. Cell 2016; 165: 976-989.

19) Kapur M, Monaghan CE, Ackerman SL. Regulation of mRNA translation in neurons-A matter of life and death. Neuron 2017; 96: 616-637.

20) Leibovitch M, Topisirovic I. Dysregulation of mRNA translation and energy metabolism in cancer. Adv Biol Regul 2018; 67: 30-39.

21) Kim HJ. Cell fate control by translation: mRNA translation initiation as a therapeutic target for cancer development and stem cell fate control. Biomolecules 2019; 9: 665.

22) Fecci PE, Champion CD, Hoj J, McKernan CM, Goodwin CR, Kirkpatrick JP, Anders CK, Pendergast AM, Sampson JH. The evolving modern management of brain metastasis. Clin Cancer Res 2019; 25: 6570-6580.

23) Zhu C, Wei Y, Wei X. AXL receptor tyrosine kinase as a promising anti-cancer approach: functions, molecular mechanisms and clinical applications. Mol Cancer 2019; 18: 153.