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Microtubule-associated protein tau (MAPT) is a promising independent prognostic marker and tumor suppressive protein in clear cell renal cell carcinoma

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Abstract

Introduction: Microtubule-associated protein tau (MAPT) overexpression has been linked to poor prognosis in several cancers. MAPT-AS1 is a long noncoding RNA existing at the antisense strand of the MAPT promoter region. The clinical significance of MAPT and MAPT-AS-1 in clear cell renal cell carcinoma (ccRCC) is unknown. This study aimed to assess the expression and function of MAPT and MAPT-AS1 in ccRCC.

Methods: The expression of MAPT was determined using immunohistochemistry in ccRCC. The effects of MAPT knockdown on cell growth and invasion were evaluated and the interaction between MAPT and microtubule-associated protein tau antisense (MAPT-AS1) were analyzed. The expression of MAPT-AS1 was determined using quantitative reverse transcription polymerase chain reaction in ccRCC tissues. We investigated the effect of MAPT-AS1 knockdown on cell growth and invasion. We analyzed the regulation of MAPT and MAPT-AS1.

Results: Immunohistochemistry in 135 ccRCC cases showed that 61% of the cases were positive for MAPT. Kaplan-Meier analysis showed that the low expression of MAPT was associated with poor overall survival after nephrectomy. Knockdown of MAPT enhanced cell growth and invasion. quantitative reverse transcription polymerase chain reaction revealed a positive correlation between MAPT and MAPT-AS1. The expression of MAPT-AS1 was higher in ccRCC tissue than in nonneoplastic kidney tissue. Kaplan-Meier analysis showed that the low expression of MAPT-AS1 was associated with poor overall survival after nephrectomy by in silico analysis. MAPT-AS1 knockdown promoted cell growth and invasion activity. P53 knockout suppressed the expression of MAPT and MAPT-AS1.

Conclusion: These results suggest that MAPT and MAPT-AS1 may be promising predictive biomarkers for survival and play a tumor-suppressive role in ccRCC.

Keywords: MAPT; MAPT-AS1; Prognostic biomarker; P53; Renal cell carcinoma

Abbreviations: ccRCC, clear cell renal cell carcinoma; lncRNAs, long noncoding RNAs; MAPT, microtubule-associated protein tau; MAPT-AS1, microtubule-associated protein tau antisense RNA 1; MTT, 4,5-dimethylthiazol-2-yl-2,5-diphenyltetrazolium bromide; qRT-PCR, quantitative reverse transcription polymerase chain reaction; siRNA, short interfering RNA. TCGA-KIRC, The Cancer Genome Atlas Kidney Renal Clear Cell Carcinoma

1. Introduction

Renal cell carcinoma (RCC) accounts for approximately 90% of all renal tumors and its incidence has been steadily increasing by 2% to 4% each year [1]. Clear cell RCC (ccRCC) is the most common subtype of RCC, accounting

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for approximately 70% of all RCCs. Surgical tumor resection is the standard therapy for ccRCC because ccRCC exhibits resistance to radiotherapy and chemotherapy [2]. Around 30% of patients suffer a recurrence or metastasis after surgery [3]. Although several targeted treatments and immune therapy have been applied to patients with metastatic ccRCC, the overall survival of patients in the terminal stage of the disease is unsatisfactory [4]. Therefore, identifying biomarkers for early diagnosis and clarifying the molecular mechanisms underlying ccRCC progression will greatly improve outcomes for patients in ccRCC.

MAPT, which encodes microtubule-associated protein tau, facilitates tubulin assembly and microtubule stabilization [5]. MAPT is mainly expressed in neuronal cells and also nonneuronal cells including lymphocytes and epithelial cells [6,7]. Aggregations of MAPT are involved in several neurodegenerative disorders including Alzheimer's disease [8]. Recently, several studies have shown that aberrant expression of MAPT may have prognostic or predictive value in some cancers [9,10]. A recent study reported that MAPT was identified as a hypoxia-related gene in ccRCC. The expression of MAPT was downregulated in accordance with tumor stage. The low expression of MAPT was also associated with poor survival outcome by in silico analysis in ccRCC [11]. However, the clinical significance and biological role of MAPT in ccRCC remain unclear.

Long noncoding RNAs (lncRNAs) are defined as nonprotein coding RNAs of >200 nucleotides [12]. LncRNAs are involved in cancer biology and may serve as biomarkers for tumor diagnosis and prognosis [13,14]. A large number of studies have identified several lncRNAs that are involved in tumorigenesis and cancer progression in ccRCC [15]. Moreover, recent studies have shown that promoter-associated lncRNAs may play an essential role in gene expression [16,17]. MAPT-AS1 is a lncRNA located at the antisense strand of the MAPT promoter region. However, the expression and function of MAPT and the interaction between MAPT and MAPT-AS1 have not been fully elucidated in ccRCC.

In this study, we analyzed both the expression of MAPT and MAPT-AS1 in ccRCC and the effect of their knockdown by using short interfering RNA (siRNA) in RCC cell lines. We investigated the interaction between MAPT and MAPT-AS1 and examined the regulation of MAPT and MAPT-AS1.

2. Materials and methods

2.1. Tissue samples

Samples of ccRCC were collected for immunohistochemistry from 135 patients diagnosed as having ccRCC who underwent nephrectomy at Hiroshima University Hospital (Supplementary Table 1). Tumor staging was performed according to the tumor, regional lymph nodes, metastasis (TNM) stage grouping system [18]. ccRCC tissue samples (Supplementary Table 2) for quantitative reverse transcription polymerase chain reaction (qRT-PCR) were also collected from 41 of these same patients.

2.2. Stability and α-amanitin treatment

We plated 786-O cells transfected with MAPT-AS1 siRNA or negative control into 6-well plates. We treated the cells 24 hours later with α-amanitin (100 nM) and harvested them for RT-PCR at 12- and 24-hour post-treatment.

2.3. Immunohistochemistry, in silico analysis, western blotting, RNA interference, qRT-PCR analysis, cell growth and invasion assays, generation of p53 knockout cells, and statistical analysis

These methods are described in detail in the Supplementary Materials.

3. Results

3.1. Expression of MAPT in ccRCC

We performed immunohistochemistry to analyze the expression of MAPT in 135 ccRCC tissue samples (Hiroshima cohort, Supplementary Table 1). Weak or no staining of MAPT was observed in the non-neoplastic kidney, whereas stronger and more extensive staining was observed in ccRCC tissue (Fig. 1A). Staining of MAPT was mainly detected in the cell membrane and cytoplasm of ccRCC tissue (Fig. 1B). When >20% of tumor cells were stained, the specimen was considered positive for MAPT. In total, 83 (61%) of the 135 ccRCC cases were positive for MAPT. The expression of MAPT was associated with low nuclear grade and low T and M stages (Table 1). A Kaplan-Meier analysis revealed that overall survival in MAPT-positive ccRCC cases compared with MAPT-negative ccRCC cases from the Hiroshima cohort ($P < 0.001$, Fig. 1C). Furthermore, we performed univariate and multivariate Cox proportional hazard analyses to evaluate the potential use of MAPT expression as a prognostic factor. In the multivariate model, low MAPT expression was independently associated with poor overall survival (hazard ratio 0.51; 95% confidence interval 0.26−0.98; $P = 0.043$) as well as pT, pN, and pM stages (Table 2). To verify these findings, we analyzed The Cancer Genome Atlas (TCGA) Kidney Renal Clear Cell Carcinoma (KIRC) database via the online GEPIA database [19]. The expression of MAPT was higher in ccRCC samples than in non-neoplastic kidney samples (Fig. 1D). Stage plot analysis showed that the expression of MAPT was decreased in accordance with the tumor stage (Fig. 1E). Additionally, a Kaplan-Meier analysis showed that low expression of MAPT was associated with poor prognosis in ccRCC from the TCGA-KIRC database, which was consistent with the finding in our patients (Fig. 1F). Collectively, these results suggest that MAPT may be a

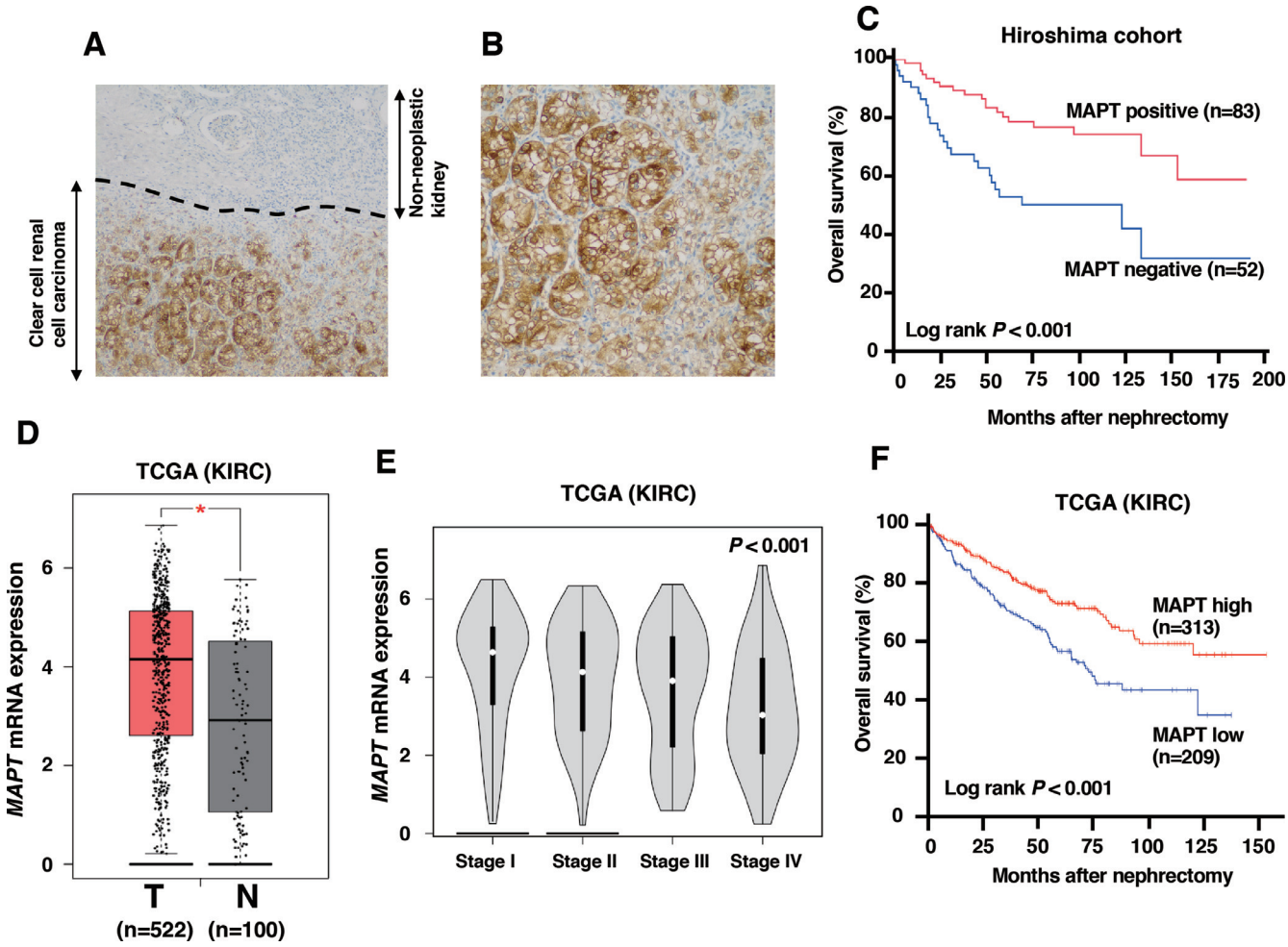

Fig. 1. The expression of MAPT in clear cell renal cell carcinoma (ccRCC). (A) Immunohistochemical staining of MAPT in the non-neoplastic kidney and ccRCC. Original magnification: 100× . (B) Immunohistochemical staining of MAPT in ccRCC. Original magnification: 400× . (C) A Kaplan-Meier plot of overall survival of ccRCC patients after nephrectomy from the Hiroshima cohort. (D) The mRNA expression levels of MAPT were obtained from the TCGA-KIRC dataset. N: normal, T: tumor. *$P < 0.01$. (E) The mRNA expression levels of MAPT were compared between the various TNM stages. One-way ANOVA was used to analyze the differences between mRNA expression levels of MAPT and the various TNM stages in the ccRCC patients. (F) A Kaplan-Meier plot of overall survival of ccRCC patients after nephrectomy from the TCGA-KIRC dataset.

clinically relevant marker for predicting outcome after nephrectomy.

3.2. Knockdown of MAPT promoted cell proliferation and invasion in ccRCC cell lines

To determine the functional significance of MAPT in ccRCC, we investigated the effect of the modulation of MAPT on cell proliferation and invasion. We used 786-0 and Caki-1 cells, which are well-known ccRCC cell lines. [20]. Western blotting showed that the expression of MAPT was higher in 786-O than in Caki-1 cells (Fig. 2A). We used RNA interference targeting MAPT in 786-O cells and confirmed the efficiency of MAPT knockdowns by western blotting and qRT-PCR (Fig. 2B and C). We performed a 4,5-dimethylthiazol-2-yl-2,5-diphenyltetrazolium bromide (MTT) assay and invasion assay, which revealed that knockdown of MAPT-enhanced cell growth and invasion activity in 786-O cells (Fig. 2D and E).

3.3. Interaction between MAPT and MAPT-AS1 expression

MAPT-AS1 is located at the antisense strand of the MAPT promoter region (Fig. 3A). Some recent studies have shown a positive correlation between MAPT and MAPT-AS1 in breast cancer [21,22], and another study showed an inverse correlation between MAPT and MAPT-AS1 in Parkinson's disease [23]. To examine the interaction between MAPT and MAPT-AS1 in ccRCC, we analyzed the expression of MAPT and MAPT-AS1 in 41 ccRCC tissues by qRT-PCR. There was a significantly positive correlation between MAPT and MAPT-AS1 in ccRCC ($P < 0.001$, $R = 0.69$) (Fig. 3B), which was consistent with the findings from the TCGA-KIRC database (Fig. 3C). To verify this interaction, we analyzed the effect of MAPT-AS1 knockdown on the expression of MAPT. qRT-PCR revealed that the expression of MAPT-AS1 was higher in 786-O cells than in Caki-1 cells (Fig. 3D). We used RNA interference targeting MAPT-AS1 in 786-O cells and

Table 1
Relationship between microtubule-associated protein tau (MAPT) expression and clinicopathologic characteristics in the 135 clear cell renal cell carcinomas

	MAPT expression		*P* value[a]
	Positive (%)	Negative	
Age			
≤65 (*n* = 71)	41 (57%)	30 (42%)	N.S.
≥66 (*n* = 64)	42 (65%)	22 (34%)	
Sex			
Female (*n* = 34)	24 (70%)	10 (29%)	N.S.
Male (*n* = 101)	59 (58%)	42 (42%)	
Nuclear grade (Fuhrman)			
G1/2 (*n* = 94)	67 (71%)	27 (29 %)	<0.001
G3/4 (*n* = 41)	16 (39%)	25 (61%)	
T stage			
T1/2 (*n* = 88)	65 (73%)	23 (27%)	<0.001
T3/4 (*n* = 47)	18 (38%)	29 (62%)	
N stage			
N0 (*n* = 116)	74 (64%)	42 (36%)	N.S.
N1/2/3 (*n* =19)	9 (47%)	10 (53%)	
M stage			
M0 (*n* = 114)	76 (67%)	38 (33%)	0.004
M1 (*n* = 21)	7 (33%)	14 (67%)	

N.S. = not significant.
[a] *P* values were calculated with Fisher's exact test.

Table 2
Univariate and multivariate Cox regression analysis of overall survival

	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value
pT stage				
pT1/2	1 (Ref.)		1 (Ref.)	
pT3/4	6.211 (3.309−12.34)	<0.001	3.754 (1.842−7.924)	0.003
pN stage				
pN0	1 (Ref.)		1 (Ref.)	
pN1	4.495 (2.209−8.562)	<0.001	2.655 (1.169−5.830)	0.012
pM stage				
pM0	1 (Ref.)		1 (Ref.)	
pM1	7.255 (3.825−13.39)	0.002	2.673 (1.244−5.639)	0.020
MAPT				
Negative	1 (Ref.)		1 (Ref.)	
Positive	0.370 (0.201−0.671)	< 0.001	0.516 (0.266−0.981)	0.043

CI = confidence interval; HR = hazard ratio; MAPT = microtubule-associated protein tau.

confirmed the efficiency of MAPT-AS1 knockdown by qRT-PCR (Fig. 3E). Western blotting showed that knockdown of MAPT-AS1 suppressed the expression of MAPT in 786-O cells (Fig. 3F). What is more, qRT-PCR showed that knockdown of MAPT suppressed the expression of MAPT-AS1 in 786-O cells (Fig. 3G). A recent study showed that MAPT-AS1 increased the stability of MAPT mRNA by forming RNA duplex [21]. To confirm that MAPT-AS1 and MAPT may form RNA duplex, we performed qRT-PCR assays on RNA from 786-O cells [24]. We used 18s ribosomal RNA, which was not affected by α-amanitin treatment as a control. qRT-PCR revealed that knockdown of MAPT-AS1 decreased the stability of MAPT mRNA (Fig. 3H), indicating that MAPT-AS1 and MAPT might form RNA duplex.

3.4. Clinical significance of MAPT-AS1 in ccRCC

To determine the clinical significance of MAPT-AS1 in ccRCC, we studied the expression of MAPT-AS1 in 41 ccRCC tissues and their corresponding normal kidney tissues by qRT-PCR. A RCC tissue/normal kidney tissue ratio >2.0 was considered to indicate significantly high expression. The expression of MAPT-AS1 was upregulated in 68% (28/41) of the ccRCC tissues compared with their corresponding normal kidney tissues ($P < 0.001$) (Fig. 4A). In this sample set, the expression of MAPT-AS1 was downregulated in high tumor stage ($P = 0.002$) (Fig. 4B) and metastatic stage ($P = 0.029$) (Fig. 4C). According to the TCGA-KIRC database, the expression of MAPT-AS1 was higher in ccRCC samples than that in non-neoplastic kidney samples (Supplementary Fig. 1A). Stage plot analysis showed that the expression of MAPT-AS1 was downregulated in accordance with the tumor stage (Supplementary Fig. 1B). A Kaplan-Meier analysis showed that low expression of MAPT-AS1 was associated with poor prognosis after nephrectomy in the TCGA-KIRC database (Fig. 4D). Additionally, we investigated the effect of the modulation of MAPT-AS1 on cell proliferation and invasion. MTT and invasion assay showed that knockdown of MAPT-AS1 promoted cell growth and invasion activity in 786-O cells (Fig. 4E and F).

3.5. Regulation of MAPT and MAPT-AS1 in ccRCC

Recent studies have reported the interaction between MAPT and p53 in Alzheimer's disease [25]. P53 induces the expression of phosphorylated MAPT in HEK293 cells [26]. Therefore, we analyze the interaction between MAPT and p53 in ccRCC. We generated p53 knockout cells using a CRISPR-P53 vector in 786-O cells. Western blotting showed that p53 expression was not detected in 786-O cells transfected with a CRISPR-P53 vector (Fig. 5A). Western blotting demonstrated that p53 knockout suppressed the expression of MAPT (Fig. 5A). qRT-PCR revealed that p53 knockout decreased the expression of MAPT-AS1 (Fig. 5B). These results suggest that p53 may regulate the expression of MAPT and MAPT-AS1 in ccRCC.

4. Discussion

Cancer-specific survival rates for localized ccRCC are estimated to be approximately 80% at 10 years after nephrectomy [27]. Around 30% to 40% of patients with high-risk features have disease recurrence that is generally life threatening [27]. Adjuvant therapy would offer an effective anticancer systemic treatment to suppress micrometastasis and improve the cancer survival rate. A recent

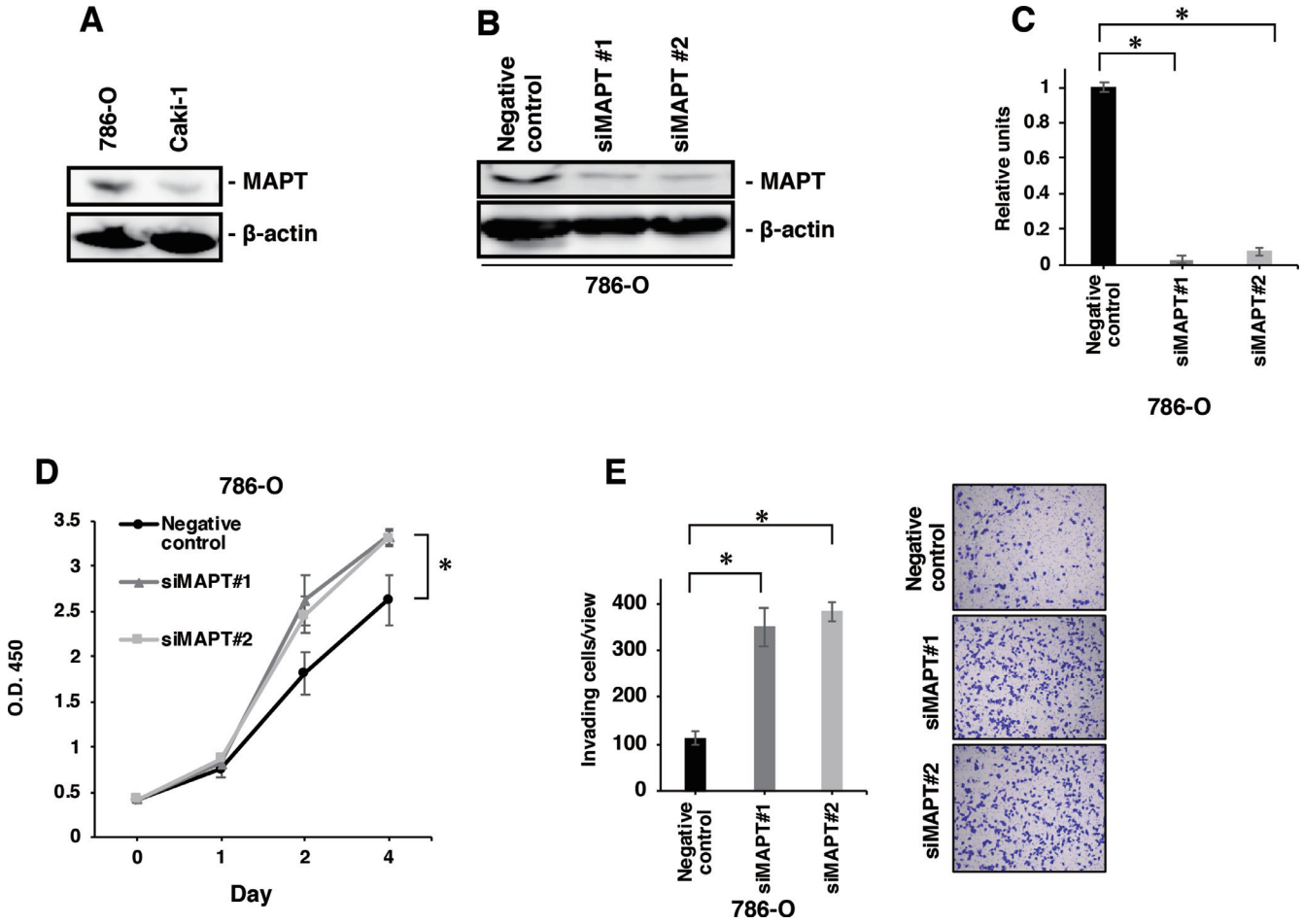

Fig. 2. The effect of MAPT knockdown on cell proliferation and invasion in clear cell renal cell carcinoma (ccRCC) cell lines. (A) Western blotting of MAPT in 786-O and Caki-1 cells. β-actin was used as a loading control. (B) Western blotting of MAPT in 786-O cells transfected with MAPT or negative control siRNAs. β-actin was used as a loading control. (C) qRT-PCR of MAPT in 786-O cells transfected with MAPT or negative control siRNAs. The results are expressed as the mean and S.D. of triplicate measurements. $^*P < 0.01$. (D) Cell growth assay in 786-O cells transfected with MAPT or negative control siRNAs. MTT assays assessed cell growth at 1, 2, and 4 days after seeding on 96-well plates. Bars and error bars indicate the mean and S.D., respectively, of 3 independent experiments. $^*P < 0.01$. (E) Cell invasion assay in 786-O cells transfected with MAPT or negative control siRNAs. Cell invasion was assessed using a modified Boyden chamber assay. Bars and error bars indicate the mean and S.D., respectively, of 3 independent experiments. $^*P < 0.01$. The representative images of the invasion assay.

clinical study showed that adjuvant sunitinib therapy significantly improved disease-free survival in patients with locoregional RCC [28]. However, adjuvant therapy in ccRCC remains controversial [2]. To date, despite numerous efforts, there has been a lack of established biomarkers for adjuvant therapy in ccRCC [29]. A recent study showed that a genomic classifier based on the expression of 34 genes including MAPT, determined significant differences in outcomes in ccRCC by transcriptomic approaches [30]. In the present study, immunohistochemical analysis showed that low expression of MAPT was an independent prognostic indicator of overall survival after nephrectomy in a multivariate analysis. These findings suggest that immunohistochemical staining of MAPT would allow clinicians to select appropriate adjuvant therapy after nephrectomy better. Additionally, in the present study, low expression of MAPT-AS1 was associated with poor prognosis after nephrectomy in silico analysis. Several studies have found that integrated mRNA and lncRNA expression profiling are promising prognostic biomarkers in some cancers [31,32]. Collectively, these results indicate that MAPT and MAPT-AS1 would help to make promising prognostic biomarkers in ccRCC by integrating mRNA and lncRNA expression.

A bunch of studies have shown that MAPT overexpression is linked to resistance to taxane treatment in various types of cancer [9,33,34,35]. However, the involvement of MAPT in cell growth and invasion has not fully elucidated. A recent study has shown that knockdown of MAPT reduces cell growth in prostate cancer [36]. In neuroblastoma, MAPT overexpression promotes cell growth [15]. In our study, immunohistochemical analysis revealed that MAPT expression was inversely related to tumor grade and T, N, and M classification in ccRCC. Functional analysis showed that downregulation of MAPT-enhanced cell growth and invasion in 786-O cells. Collectively, these

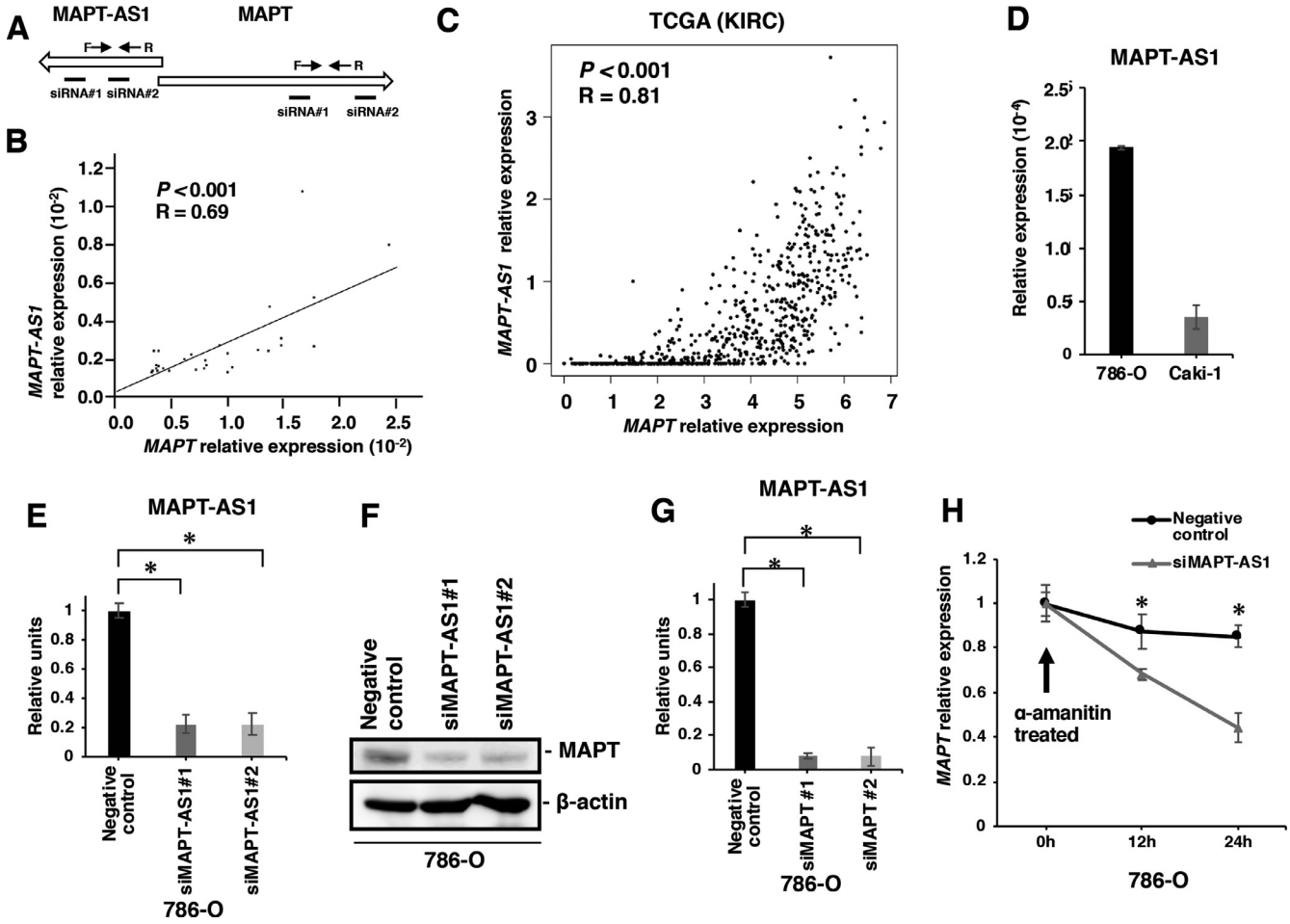

Fig. 3. Interaction between MAPT and MAPT-AS1 expression in clear cell renal cell carcinoma (ccRCC). (A) Schema of the genome location of MAPT and MAPT-AS1. F: forward primer, R: reverse primer (B) The correlation between the expression of MAPT and that of MAPT-AS1 in ccRCC. Spearman's correlation coefficients and *P* values are indicated. (C) The correlation between the expression of MAPT and that of MAPT-AS1 in ccRCC from the TCGA-KIRC database. Spearman's correlation coefficients and *P* values are indicated. (D) qRT-PCR of MAPT-AS1 expression in 786-O and Caki-1 cells. The results are expressed as the mean and S.D. of triplicate measurements. (E) qRT-PCR of MAPT-AS1 in 786-O cells transfected with MAPT-AS1 or negative control siRNAs. The results are expressed as the mean and S.D. of triplicate measurements. $*P < 0.01$. (F) Western blotting of MAPT in 786-O cells transfected with MAPT-AS1 or negative control siRNAs. β-actin was used as a loading control. (G) qRT-PCR of MAPT-AS1 in 786-O cells transfected with MAPT or negative control siRNAs. The results are expressed as the mean and S.D. of triplicate measurements. $*P < 0.01$. (H) Stability of MAPT mRNA over time was measured by qRT-PCR after treatment with α-amanitin (100 nM). 786-O cells were transfected with MAPT-AS1 or negative control siRNAs. The transfected cell lines were exposed to 100 nM α-amanitin for 12 or 24 hours. 18S RNA was used as a control. $*P < 0.01$.

findings indicate that the role of MAPT may depend on the cancer context, and MAPT may play a tumor-suppressive role in ccRCC.

MAPT-AS1 is the antisense transcript of MAPT. A recent study showed that MAPT-AS1 upregulated the stability of MAPT mRNA in breast cancer cells by forming RNA−RNA duplex [21]. Some recent reports demonstrated that the expression of a sense gene is regulated by forming RNA duplex between an antisense gene and a sense gene [37,38]. In the present study, we found a positive correlation between MAPT expression and MAPT-AS1 expression in ccRCC tissue. Additionally, knockdown of MAPT-AS1 markedly decreased the MAPT mRNA and protein levels. Meanwhile, knockdown of MAPT decreased the expression of MAPT-AS1. We further showed that MAPT-AS1 and MAPT may form an RNA−RNA duplex in ccRCC, which was consistent with previous findings [21]. Collectively, these results suggest that MAPT-AS1 stabilizes MAPT by forming an RNA−RNA duplex and then modulates MAPT expression at both the RNA and protein levels.

This study has some limitations. First, our data are based on retrospective analyses, which may have potential bias due to patient selection and changes in treatment strategies over time. As an example, the survival rate changed after tyrosine kinase inhibitor was introduced in ccRCC therapy. Therefore, the predictive value of MAPT and MAPT-AS1 needs to be validated in prospective studies. Second, we showed that knockout of p53 suppressed the expression of MAPT and MAPT-AS1. However, we did not analyze the effect of overexpression of p53 on the expression of MAPT and MAPT-AS1. In the future, we will surely analyze the interaction between p53, MAPT, and MAPT-AS1 in RCC.

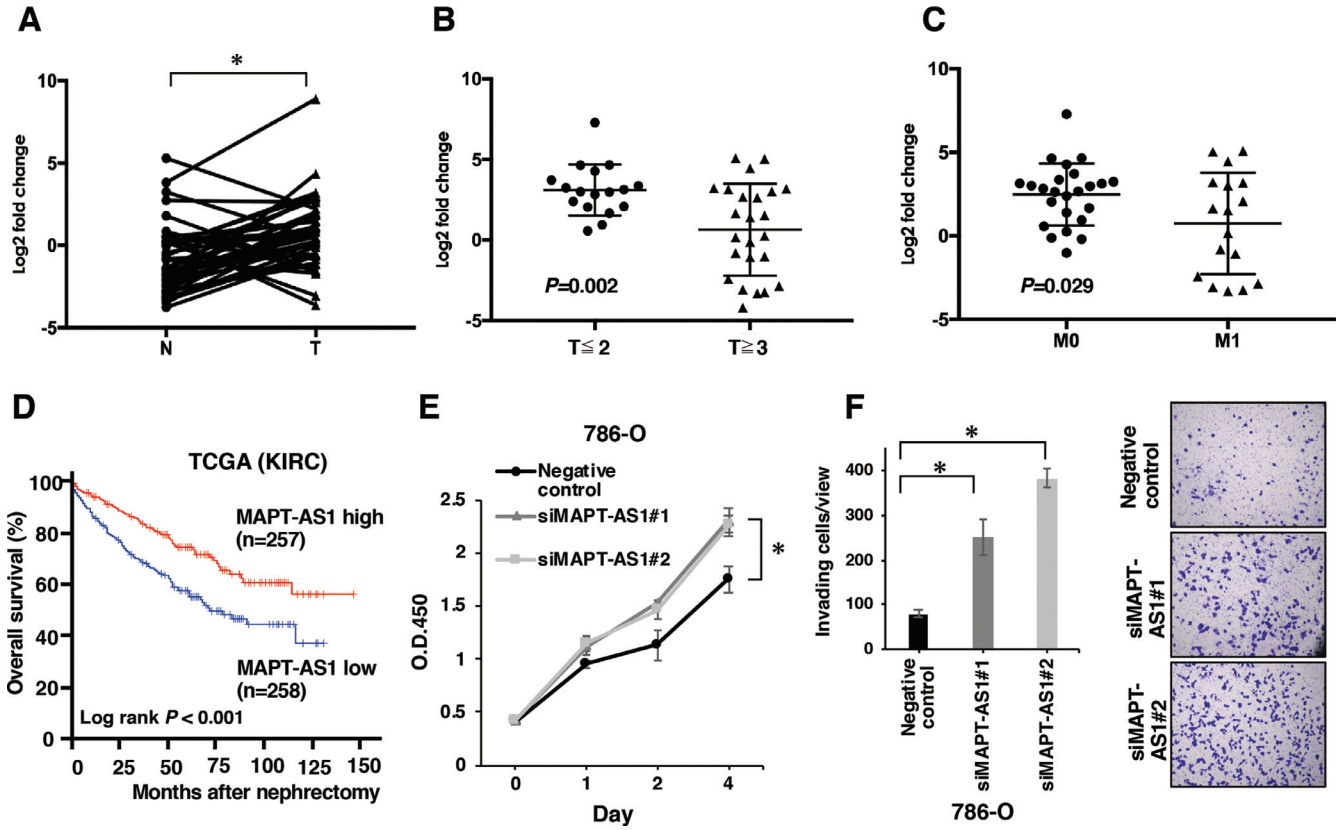

Fig. 4. The clinical significance of MAPT-AS1 in clear cell renal cell carcinoma (ccRCC). (A) The results of qRT-PCR analysis for the expression of MAPT-AS1 in 41 ccRCC tissues and corresponding normal kidney tissues. *$P < 0.01$. N: normal, T: tumor. (B) Scatter plot diagrams showing the association between the expression of MAPT and tumor stage (T). (C) Scatter plot diagrams showing the association between the expression of MAPT and metastatic stage (M). (D) A Kaplan-Meier plot of overall survival of ccRCC patients after nephrectomy from the TCGA dataset. (E) Cell growth assay in 786-O cells transfected with MAPT or negative control siRNAs. MTT assays assessed cell growth at 1, 2, and 4 days after seeding on 96-well plates. Bars and error bars indicate the mean and S.D., respectively, of 3 independent experiments. *$P < 0.01$. (F) Cell invasion assay in 786-O cells transfected with MAPT-AS1 or negative control siRNAs. Cell invasion was assessed using a modified Boyden chamber assay. Bars and error bars indicate the mean and S.D., respectively, of 3 independent experiments. *$P < 0.01$. The representative images of the invasion assay.

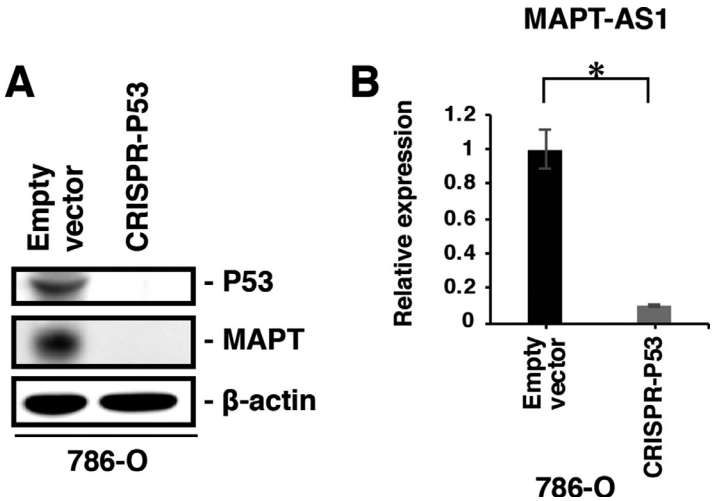

Fig. 5. The regulation of MAPT and MAPT-AS1 in clear cell renal cell carcinoma. (A) Western blotting of MAPT and P53 in 786-O cells transfected with an empty vector or a CRISPR-P53 vector. β-actin was used as a loading control. (B) qRT-PCR of MAPT-AS1 in 786-O cells transfected with an empty vector or a CRISPR-P53 vector. The results are expressed as the mean and S.D. of triplicate measurements. *$P < 0.01$.

In conclusion, our study revealed that low expression of MAPT and MAPT-AS1 was associated with poor prognosis in ccRCC. We also found a positive correlation between MAPT and MAPT-AS1. Knockdown of MAPT- and MAPT-AS1-enhanced cell growth and invasion in 786-O cells. MAPT and MAPT-AS1 may play tumor-suppressive roles and serve as promising potential biomarkers of prognosis after nephrectomy in ccRCC.

Ethics approval and consent to participate

Forty-one samples for qRT-PCR and 135 samples for immunohistochemistry were collected from patients at Hiroshima University Hospital. Written comprehensive approvals for basic or clinical research were obtained from each patient. This study was conducted in accordance with the Ethical Guidance for Human Genome/Gene Research of the Japanese Government. The Institutional Review Board of Hiroshima University Hospital approved this study (approval no. E-688).

Authors' contributions

Y.S., T.H., A.M., and W.Y. designed the study. K.G., T. B., and J.T. provided the patients' clinical information. Y. S., X.H., N.S., K.S., S.I., and N.O. performed experiments and acquired data. Y.S. and T.H. interpreted the results. Y. S. drafted the manuscript, and Y.S., T.H., A.M., and W.Y. edited it. All authors approved the final contents of the manuscript for journal submission and publication.

Conflicts of interest

The authors declare no conflicts of interest.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at https://doi.org/10.1016/j.urolonc.2020.02.010.

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