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Clinicopathological Significance of MTUS1 Expression in Patients With Renal Cell Carcinoma

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Abstract. *Background/Aim: Microtubule-associated tumor suppressor 1 (MTUS1) is a novel tumor suppressor involved in proliferation and migration, and down-regulation of MTUS1 is associated with the poor prognosis of several cancers. We evaluated the clinicopathological significance of MTUS1 expression in renal cell carcinoma (RCC). Patients and Methods: We assessed MTUS1 expression by immunohistochemical staining of tissue microarrays from 249 cases of RCC. We analyzed the correlation of MTUS1 expression and clinicopathological characteristics. Additionally, we used public databases and performed bioinformatics analysis. Results: We investigated The Cancer Genome Atlas databases and identified that MTUS1 mRNA expression was significantly lower in RCC tissues than in normal tissues. Loss of MTUS1 expression was correlated with high WHO/ISUP nuclear grade, lymphovascular invasion, renal vein thrombus, and high pT stage in patients with RCC. Although there was no statistically significant correlation between MTUS1 expression and patients' prognosis in our cohort, MTUS1 overexpression was significantly correlated with a favorable prognosis in public data. Conclusion: Loss of MTUS1 expression in RCC might be a potential biomarker for predicting clinical outcome.*

Renal cell carcinoma (RCC) is the 8th most common malignancy, and occurs in about 4,500 patients and causes 950 deaths every year in Korea (1). Complete surgical excision remains the gold standard for managing patients with localized disease (2, 3). The survival rate is high for patients with localized RCC; however, the 5-year survival rate of patients with stage IV disease is less than 10% (3). The main pathologic prognostic parameters according to World Health Organization (WHO)/International Society of Urological Pathology (ISUP) include grade, histologic type, lymphovascular invasion, necrosis, sarcomatoid change, and tumor stage (4). Recently, with improved knowledge of tumor molecular genetics, antiangiogenic drugs targeting vascular endothelial growth factor (VEGF) receptor have been shown to improve the disease-free survival rate; however, it is still difficult to predict cancer progression and prognosis (5, 6). Therefore, additional reliable biomarkers are needed to predict prognosis and to provide appropriate patient management.

Microtubule-associated tumor suppressor 1 gene (*MTUS1*), also known as a mitochondrial tumor suppressor gene 1, is localized to chromosome 8p22 and comprises 17 exons (7, 8). *MTUS1* is a tumor suppressor gene that is down-regulated in several types of cancers, including bladder, breast, colon, gallbladder, head-and-neck, lung, ovary, stomach, and salivary gland cancers (9-21). Although the tumor suppressor function of *MTUS1* has been identified, the expression of MTUS1 and its prognostic significance in RCC remains unclear.

The aim of this study was to discover the prognostic significance of MTUS1 expression in patients with RCC. Thus, we evaluated the clinical significance of MTUS1 expression in patients with RCC by combining immunohistochemical (IHC) staining and bioinformatics.

Patients and Methods

Bioinformatics analysis. We downloaded 883 cases from The Cancer Genome Atlas (TCGA) kidney tumor datasets in the Genomic Data Commons (GDC) data portal, including transcriptome profiling and clinical information (22). The mRNA levels of MTUS1 in RCC and normal kidney tissues were

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determined. To select the best separation fragments per kilobase million (FPKM) value for grouping the patients with significant differences in survival, we performed a log-rank test for FPKM from the 20th to 80th percentile as descripted in Human Protein Atlas. We selected the FPKM with the lowest log-rank *p*-value and performed Kaplan–Meier survival analysis.

Patients and tissue samples. The archived tissue samples from 249 patients with RCC, including 204 with clear cell type, 24 with papillary type, 13 with chromophobe type, and 8 patients with other types: 4 clear cell papillary RCC, 3 acquired cystic disease associated RCC, 1 collecting duct carcinoma. Clinicopathologic features, including age, sex, and other pathological parameters, namely histological diagnosis, and TNM stage, were retrieved from the electronic medical reports. Histological diagnosis was performed according to the 2016 WHO classification of tumors of the urinary system and male genital tract (4th edition) and TNM staging was used according to the 8th edition of the American Joint Committee on Cancer (AJCC) cancer staging system (23, 24). We graded ccRCC and papillary RCC according to 2013 WHO/ISUP grading system (23, 25). Chromophobe RCC was graded according to the published parameters (26). Follow-up information was retrieved from the electronic medical records. This study was approved by the Institutional Review Board of the Hanyang University Hospital (HYUH 2018-05-005), and the requirement for informed consent was waived.

Tissue microarray construction, immunohistochemical staining, and scoring. Cellular areas of the tumors on hematoxylin and eosin-(H&E) stained sections were chosen, and the corresponding areas were taken from the formalin-fixed paraffin embedded (FFPE) blocks for tissue microarray (TMA) construction. A 3.0-mm sized tissue core was obtained from each sample.

IHC staining was performed on 4-mm sections of TMA blocks using the fully automated slide preparation system Benchmark XT System (Ventana Medical Systems Inc, Tucson, AZ, USA), using a primary antibody against MTUS1 (1:100, Aviva, San Diego, CA, USA) according to the manufacturer's instructions. Assessment of MTUS1 expression was conducted using the H-score (27). Cells displaying strong either membranous or cytoplasmic staining for MTUS1 were scored as 3+, moderate staining as 2+, weak staining as 1+, and no staining as 0 (Figure 1). No case showed strong staining intensity. The percentage of cells at each staining intensity was then determined by naked eye, and finally, an H-score was calculated using the following formula: H-score = [1 × (% cells 1+) + 2 × (% cells 2+) + 3 × (% cells 3+)] (27). Patients were classified as having RCC that was negative (H-score < 5) or positive (H-score ≥5) for MTUS1 protein expression.

Statistical analysis. Comparisons of mRNA expression and survival analysis using bioinformatics were performed using the R program. Data from our cohort were analyzed using the Statistical Package for the Social Sciences (IBM, Armonk, NY, USA), version 23.0. The Chi-square test and Student's *t*-test were used to assess correlations among gene expression and clinicopathological factors. Kaplan–Meier plots were constructed to present the survival outcomes. For all statistical analyses, $p<0.05$ was considered statistically significant.

Results

The mRNA expressing pattern of MTUS1 on a public database. Public databases were selected to predict the expression levels of MTUS1 in RCC and normal kidney tissues. The mRNA expression of MTUS1 was significantly decreased in all types of RCC (Figure 2A) tissues, including clear cell RCC (Figure 2B), compared with normal tissues.

Correlation of clinicopathological parameters with MTUS1 expression in patients with renal cell carcinoma. A total of 249 cases were classified as either MTUS1 negative (n=161, 64.7%) or MTUS1 positive (n=88, 35.3%). We analyzed the association between MTUS1 expression and clinicopathological parameters of all types of RCC patients (Table I). Expression of MTUS1 was significantly associated with WHO/ISUP grade ($p=0.001$), lymphovascular invasion ($p=0.018$), renal vein tumor thrombus ($p=0.005$), and pT stage ($p=0.034$). However, no significant difference was found regarding age, sex, sinus fat invasion, perirenal soft tissue invasion, tumor necrosis, and sarcomatoid feature.

In particular, we evaluated the association between MTUS1 expression and clinicopathological features of clear cell RCC patients (Table II). The association between MTUS1 expression and clinicopathological factors was similar in all types of RCC. Lower expression of MTUS1 was significantly correlated with high WHO/ISUP grade ($p=0.031$), lymphovascular invasion ($p=0.012$), renal vein tumor thrombus ($p=0.015$), and high pT stage ($p=0.028$). Additionally, age was significantly correlated with MTUS1 expression ($p=0.025$).

Survival analysis and MTUS1 expression in patients with renal cell carcinoma. Next, we proceeded to determine whether the expression of MTUS1 is associated with the outcome of RCC patients or not. We assessed the association between the expression of MTUS1 and the prognosis of RCC patients. As shown in Figure 3, MTUS1 expression showed no association with cancer-specific survival (CSS) and RFS in all types of RCC (Figure 3A and B) and in clear cell RCC (Figure 3C and D).

However, data from the TCGA showed that patients with high MTUS1 expression had a significantly favorable outcome compared with patients with low MTUS1 expression in all types of RCC (Figure 4A) and clear cell RCC (Figure 4B).

Discussion

In this study, we demonstrated that (1) MTUS1 expression was commonly down-regulated in RCC patients at the mRNA level compared with the normal control group in a public database, (2) negative MTUS1 expression was significantly correlated with poor prognostic factors, such as high WHO/ISUP grade, lymphovascular invasion, renal vein tumor thrombus, and high pT stage, and (3) RCC patients with MTUS1 overexpression had a significantly longer survival time than those with low expression in the TCGA database.

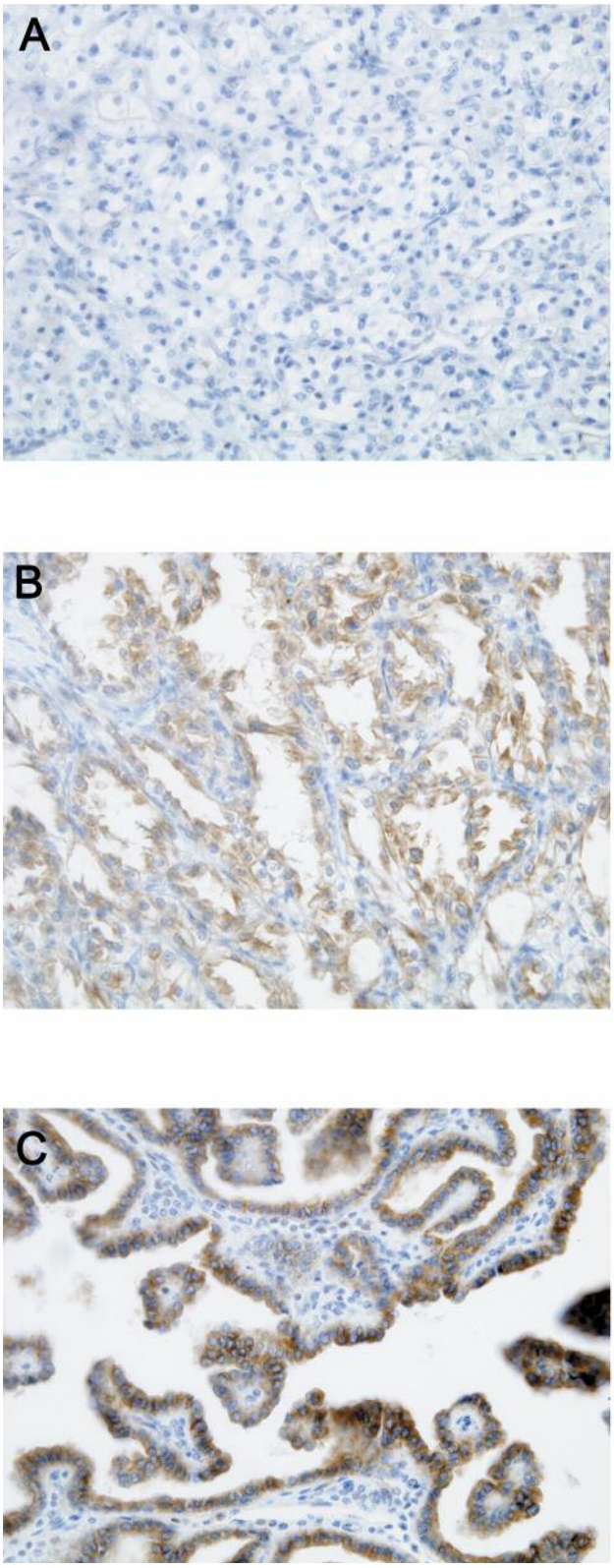

Figure 1. *Immunohistochemical staining for microtubule-associated tumor suppressor 1 (MTUS1) in renal cell carcinoma. (A) negative (0, ×400), (B) weak stain (+1, ×400), (C), moderate stain (+2, ×400).*

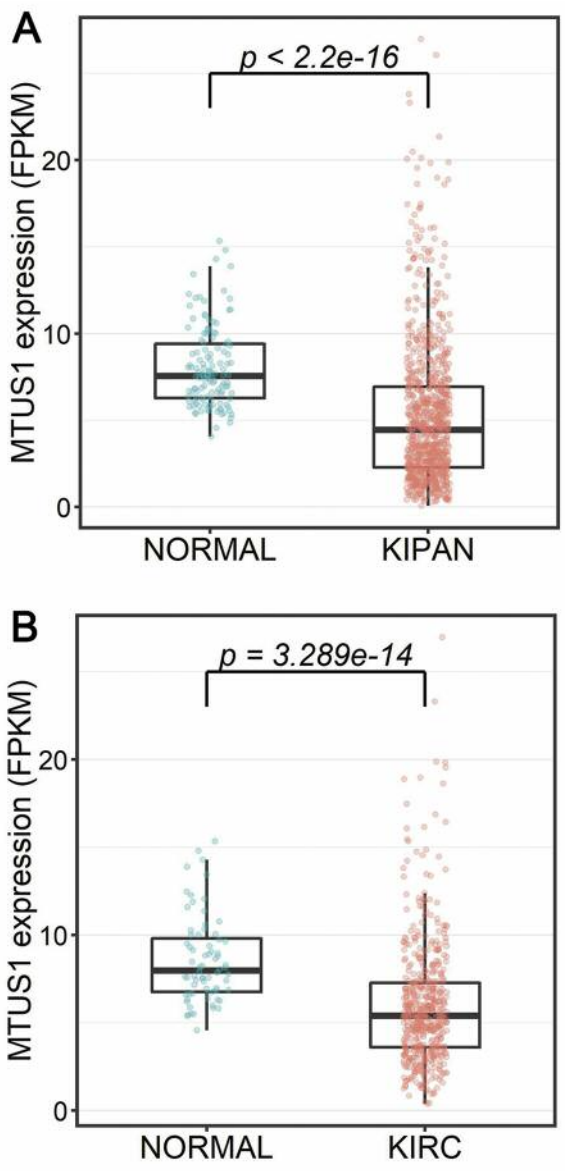

Figure 2. *mRNA expression of microtubule-associated tumor suppressor 1 (MTUS1) in renal cell carcinoma (RCC) and normal kidney determined by analyzing The Cancer Genome Atlas (TCGA) database. (A) all types of RCC, (B) clear cell RCC. *FPKM: fragments per kilobase million, KIPAN: Pan-Kidney cancer, KIRC: Clear cell renal cell carcinoma.*

Currently, VEGF-tyrosine kinase inhibitors (TKI) therapy or combination of interferon-alpha immunotherapy with the anti-VEGF monoclonal antibody bevacizumab are recommended as a first-line systemic therapy for patients with metastatic clear cell RCC (28, 29). These therapeutic agents have prolonged the survival of patients with metastatic disease, however, 20%-30% of patients derive no benefit from first-line VEGF-TKI therapy (30-32). In addition, the 5-year survival rate was still less than 10% in

Table I. *Summary of clinicopathological features according to microtubule-associated tumor suppressor 1 (MTUS1) expression in patients with all types of renal cell carcinoma (n=249).*

	Negative group (n=161) (%)	Positive group (n=88) (%)	*p*-Value
Age			
<60	84 (60.0)	56 (40.0)	0.081
≥60	77 (70.6)	33 (29.4)	
Gender			
Male	108 (62.8)	64 (37.2)	0.357
Female	53 (68.8)	24 (31.2)	
WHO/ISUP grade*			
1	11 (36.7)	19 (63.3)	**0.001**
2-4	140 (68.0)	66 (32.0)	
Lymphovascular invasion			
Absent	130 (61.6)	81 (38.4)	**0.018**
Present	31 (81.6)	7 (18.4)	
Renal vein tumor thrombus			
Absent	137 (61.7)	85 (38.3)	**0.005**
Present	24 (88.9)	3 (11.1)	
Sinus fat invasion			
Absent	147 (63.9)	83 (36.1)	0.392
Present	14 (73.7)	5 (26.3)	
Perirenal soft tissue invasion			
Absent	142 (64.3)	79 (35.9)	0.707
Present	19 (67.9)	9 (32.1)	
Tumor necrosis			
Absent	129 (62.3)	78 (37.7)	0.086
Present	32 (76.2)	10 (23.8)	
Sarcomatoid feature			
Absent	148 (64.1)	83 (35.9)	0.486
Present	13 (72.2)	5 (27.8)	
pT stage by 8th AJCC*			
1	114 (61.0)	73 (39.0)	**0.034**
2-4	47 (75.8)	15 (24.2)	

*AJCC: American Joint Committee on Cancer; *WHO/ISUP grade: World Health Organization/International Society of Urological Pathology grade for clear cell renal cell carcinoma and papillary renal cell carcinoma. Bold values show significance.

Table II. *Summary of clinicopathological features according to microtubule-associated tumor suppressor 1 (MTUS1) expression in patients with clear cell renal cell carcinoma (n=204).*

	Negative group (n=138) (%)	Positive group (n=66) (%)	*p*-Value
Age			
<60	69 (61.1)	44 (38.9)	**0.025**
≥60	69 (75.8)	22 (24.2)	
Gender			
Male	94 (65.7)	49 (34.3)	0.371
Female	44 (72.1)	17 (27.9)	
WHO/ISUP grade*			
1	11 (47.8)	12 (52.2)	**0.031**
2-4	127 (70.2)	54 (29.8)	
Lymphovascular invasion			
Absent	108 (63.9)	61 (36.1)	**0.012**
Present	30 (85.7)	5 (14.3)	
Renal vein tumor thrombus			
Absent	115 (64.6)	63 (35.4)	**0.015**
Present	23 (88.5)	3 (11.5)	
Sinus fat invasion			
Absent	125 (66.8)	62 (33.2)	0.417
Present	13 (76.5)	4 (23.5)	
Perirenal soft tissue invasion			
Absent	121 (67.2)	59 (32.8)	0.722
Present	17 (70.8)	7 (29.2)	
Tumor necrosis			
Absent	109 (64.9)	59 (35.1)	0.068
Present	29 (80.6)	7 (19.4)	
Sarcomatoid feature			
Absent	128 (67.7)	61 (32.3)	0.933
Present	10 (66.7)	5 (33.3)	
pT stage by 8th AJCC*			
1	95 (63.3)	55 (36.7)	**0.028**
2-4	43 (79.6)	11 (20.4)	

*AJCC: American Joint Committee on Cancer; *WHO/ISUP grade: World Health Organization/International Society of Urological Pathology grade for clear cell renal cell carcinoma and papillary renal cell carcinoma. Bold values show significance.

clear cell RCC patients with stage IV disease (3, 33). Important advances in the understanding of RCC molecular tumorigenesis has led to the development of molecular-targeted therapies (34). Agents targeting VEGF receptors, and mTOR are a major class of targeted drugs, and recently, immunotherapy using monoclonal antibodies disrupting the programmed cell death-1/programmed cell death-ligand 1 interaction has shown favorable activity in RCC (35, 36). However, it is worthwhile to identify new biomarkers to predict the prognosis of patients with RCC.

Previous studies have shown that the expression levels of MTUS1 protein and mRNA were significantly decreased in tumor tissue samples compared with normal tissues in several types of malignant tumors, including bladder cancer, gallbladder carcinoma, oral squamous cell carcinoma, salivary adenoid cystic carcinoma, and stomach cancer (9, 10, 15, 19, 20, 21) and lower MTUS1 expression was associated with tumor cell proliferation, migration, poor prognostic factors, and an unfavorable clinical outcome in bladder, breast, colorectum, head-and-neck, stomach, and salivary gland cancers (9-21).

Dysregulation of microRNAs (miRNAs) is correlated with several mechanisms of tumorigenesis, such as proliferation, evading growth suppressors, apoptosis resistance, invasion, migration, metastasis, and angiogenesis of cancer cells (37, 38). Recently, several miRNAs have been reported to regulate MTUS1 expression in various cancers. Ozcan *et al.* have reported that MTUS1 mRNA expression is lower in colorectal cancer tissue than in normal colon tissue (39). In addition, they showed that miR-135b-5b, miR-373-3p, miR-

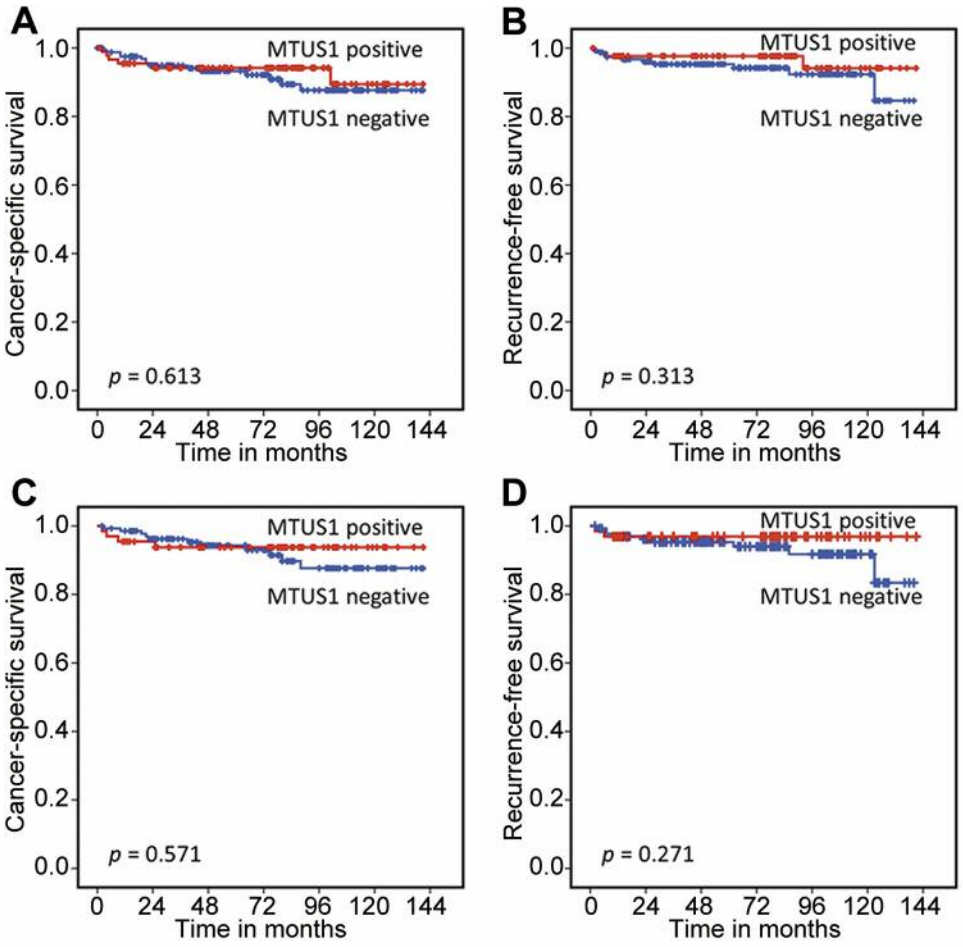

Figure 3. *Survival analyses according to microtubule-associated tumor suppressor 1 (MTUS1) expression in our cohort. Kaplan–Meier analysis for cancer-specific survival (CSS) and recurrence-free survival (RFS) in all types of renal cell carcinoma (RCC) (A: CSS, B: RFS) and clear cell RCC (C: CSS, D: RFS).*

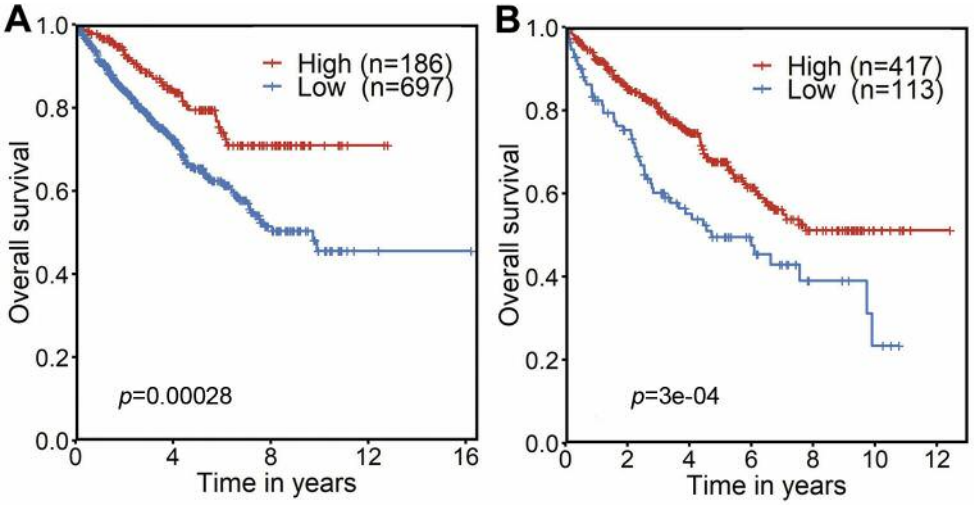

Figure 4. *Survival analysis according to microtubule-associated tumor suppressor 1 (MTUS1) expression level using The Cancer Genome Atlas (TCGA) database. Kaplan–Meier analysis for overall survival in patients with all types of renal cell carcinoma (RCC) (A) and clear cell RCC (B).*

183-5p, miR-142-5p, miR-200c-3p, and miR-19a-3p were highly expressed in colorectal cancer; these miRNAs might play an important role in colorectal carcinogenesis through MTUS1. Kara *et al*. have shown that MTUS1 expression was lower in breast cancer than in fibroadenoma, and they found 15 miRNA candidates for MTUS1 targeting using computational analysis (12). Of them, miR-183-5p was up-regulated in breast cancer. They, therefore, concluded that the MTUS1-miR-183-5p axis may play a significant role in breast cancer. Gu *et al*. have shown a significant decrease in MTUS1 protein and mRNA expression in lung cancer patients compared to normal controls. They also found that miR-19a/b synergistically suppressed MTUS1 expression to promote lung cancer cell proliferation and migration (17).

This study has some limitations. First, our cohort had relatively low-grade cancers and the follow-up period was not very long, and, therefore, overall cancer-specific death rate was lower. Second, the number of patient samples was small, particularly the number of patients with an advanced stage. Third, the detailed molecular mechanisms underlying the relationship between MTUS1 and RCC were not studied. Further studies using a larger sample size are required and this will be our next step to confirm the potential function of MTUS1 in RCC.

The present data provide new insights for the biological role of MTUS1 in patients with RCC. This study also suggests MTUS1 as a potential target in treating RCC.

Conflicts of Interest

The Authors declare no conflicts of interest regarding this study.

Authors' Contributions

Conceptualization: J Sim, and SJ Shin. Methodology: WC Wi, HY Park, K Jang, Formal analysis: J Sim. Data curation: SU Park, EU Yoon, S Bang, E Kim, SS Paik, SJ Shin. Investigation: J Sim, SJ Shin. Writing and original draft preparation: J Sim. Writing, review and editing: SJ Shin. Approval of final article: All Authors.

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