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TEK is a novel prognostic marker for clear cell renal cell carcinoma

M. HA[1], Y.R. SON[2], J. KIM[3], S.M. PARK[3], C.M. HONG[3], D. CHOI[4], W. KANG[4], J.H. KIM[4], K.J. LEE[4], D. PARK[5,6,7], M.E. HAN[1], S.O. OH[1], D. LEE[3], Y.H. KIM[8,9]

[1]Department of Anatomy, Pusan National University School of Medicine, Yangsan, Republic of Korea
[2]Department of Premedicine, Pusan National University School of Medicine, Yangsan, Republic of Korea
[3]Department of Convergence Medical Science, Pusan National University School of Medicine, Yangsan, Republic of Korea
[4]Division of Drug Process Development, New Drug Development Center, Osong Medical Innovation Foundation, Cheongju, Republic of Korea
[5]Department of Molecular Human Genetics, [6]Department of Pathology and Immunology, [7]Center for Skeletal Biology, Baylor College of Medicine, One Baylor Plaza, Houston, TX, USA
[8]Department of Anatomy and Department of Biomedical Informatics, Biomedical Research Institute, Pusan National University School of Medicine, Yangsan, Republic of Korea
[9]Biomedical Research Institute, Pusan National University Hospital, Busan, Republic of Korea

Mihyang Ha and Yu Ri Son contributed equally to this work

Abstract. – OBJECTIVE: Clear cell renal cell carcinoma (ccRCC) is the most common type of kidney cancer. However, effective therapeutics for ccRCC are lacking. Novel biomarkers could provide critical information when determining prognoses for patients with ccRCC. In this study, we sought to determine if the expression of receptor tyrosine kinase (TEK) could be a potential novel prognostic biomarker for ccRCC. TEK, originally identified as an endothelial cell-specific receptor, plays an important role in the modulation of vasculogenesis and remodeling. Altered TEK expression has been observed in tumor tissues (e.g., oral squamous cell carcinomas, leukemia) and breast, gastric and thyroid cancers. However, the role of TEK in ccRCC remains unknown.

PATIENTS AND METHODS: Differential TEK expression between non-metastatic (stage M0) and metastatic (stage M1) ccRCC patient cohorts was determined from The Cancer Genome Atlas (TCGA) and the International Cancer Genome Consortium (ICGC). Furthermore, TEK expression was assessed as a prognostic factor using the time-dependent area under the curve (AUC) of Uno's C-index, the AUC value of the receiver operating characteristics (ROC) at 5 years, Kaplan-Meier survival curves and multivariate analyses.

RESULTS: A Kaplan-Meier curve analysis revealed that the downregulation of TEK expression was associated with a poor prognosis for patients with ccRCC with good discrimination ($p<0.0001$ and $p=0.0044$ for the TGCA and ICGC cohorts, respectively). Analyses of C-indices and receiver operating characteristic AUC values further support this discriminative ability. Moreover, multivariate analyses showed the prognostic significance of TEK expression levels ($p<0.001$).

CONCLUSIONS: Although additional clinical investigations will be needed, our results suggest that TEK is a potential biomarker for ccRCC.

Key Words
Clear cell renal cell carcinoma, TCGA, ICGC, TEK.

Introduction

Renal cell carcinomas (RCCs) account for 5% of malignancies in the United States[1] and up to 90% of all kidney cancers[1,2]. RCC cases can mainly be categorized as clear cell (ccRCC), papillary, and chromophobe subtypes[3], with ccRCC being the most common type of RCC. The ccRCC cells easily invade local tissues and metastasize, and are resistant to currently available therapeutics[4]. Surgical treatment remains the most effective clinical therapy for ccRCC. In addition, patients with RCC typically respond poorly to radiation and conventional chemotherapy[5] and the efficacy of currently available therapeutics against renal cancer is unsatisfactory. Understanding the mechanisms underlying ccRCC pathogenesis will support the development of more effective therapeutic strategies, including new drugs and biomarkers.

Corresponding Authors: Dongjun Lee, MD; e-mail: lee.dongjun@pusan.ac.kr
Yun Hak Kim, MD; e-mail: yunhak10510@pusan.ac.kr

Angiogenesis is essential for development, wound healing, tumor growth and metastasis[6]. Several studies[7] have identified critical angiogenic pathways, including those involving the angiopoietin 1 (Ang1)-receptor tyrosine kinase (TEK), (i.e., Tie2) receptor family. TEK is a receptor tyrosine kinase that is expressed in endothelial cells[8-12]. The Ang1-TEK pathway controls vascular regeneration and stabilization[13,14], where Ang1 acts as a TEK agonist, and Angpt2 acts as either a TEK agonist or antagonist, depending on the context[13]. The prenatal deletion of TEK or Ang1 in mice leads to the impairment of cardiovascular development, with severe impairment occurring specifically in the absence of TEK[13,15,16], while the inhibition of Ang2 in mice causes defects in lymphatic maturation[17,18]. Dysregulated TEK expression has also been observed in several types of cancer, including oral squamous cell carcinoma, leukemia, breast, gastric and thyroid cancers[19-23]. These studies suggest that TEK expression could be of prognostic value for these types of cancer. In addition, while vascular endothelial growth factor (VEGF) is known to be highly expressed in ccRCC[24,25], the roles of many other angiogenic pathways, including those involving TEK, are unknown. In this work, we present the first analysis of TEK expression in patients with ccRCC in well-defined primary ccRCC cohorts from The Cancer Genome Atlas (TCGA)[26,27] and the International Cancer Genome Consortium (ICGC)[28]. Statistical analyses suggest that TEK expression could be an important prognostic marker for ccRCC.

Patients and Methods

Acquisition and Characteristics of Patient Data

Primary and processed data were downloaded from TCGA[26,27] and ICGC[28] (ICGC data portal, dcc.icgc.org) in March 2018. Specifically, mRNA expression data and clinical information were downloaded. Samples with any of the following were excluded from analysis: (1) "Not available" gene expression values and (2) insufficient survival information. As the TCGA and ICGC cohorts have already received Ethics Committee Approval, this study did not require additional approval. These processes were performed using R software version 3.5.0 (The R Foundation for Statistical Computing, 2018).

Wilcoxon Signed-Rank Test

Differences in the levels of TEK expression between stages M0 and M1 in both cohorts were identified by Wilcoxon signed-rank test using the "coin" package in R, as differences were not normally distributed.

Survival Analysis

Survival analyses were performed to predict overall survival (OS) using the following statistical methods: (1) Uno's C-index in the time-dependent area under the curve (AUC) analysis, (2) AUC values for receiver operating characteristics (ROC) at five years, and (3) Kaplan-Meier survival curves to evaluate the accuracy of discrimination as previously described[29,30]. These values were obtained using the "survival" and "survAUC" packages in R. The C-index is a global measure of the fitness of the survival model for continuous events in clinical studies[31-33]. For Kaplan-Meier analyses, we determined the optimal cut-off value with the maximal Uno's C-index by 5-fold cross-validation. We used univariate and multivariate Cox regression to compare the effects of TEK expression as a categorical value on prognosis and other clinical variables. We included clinical factors not associated with survival in the univariate and multivariate analyses. Statistical analyses were performed using R.

Results

Prognostic Value of TEK Expression for ccRCC

In total, 446 patients from TCGA and 91 patients from ICGC were included in this study[26-28]. Of the 446 patients in the TCGA cohort, 290 were male and 156 were female. Of the 91 patients in the ICGC cohort, 52 were male and 39 female. Patient information used in the present study is described in Table I.

To evaluate the prognostic value of TEK expression for ccRCC, we analyzed Kaplan-Meier curves of TEK gene expression with optimal cut-off values (Table II) and survival using data from the TCGA (Figure 1A, B and C) and ICGC cohorts (Figure 1D, E, and F). The group with low levels of TEK expression exhibited a significantly shorter survival duration than the group with high TEK expression in both the TCGA and ICGC cohorts (Figure 1). The prognostic value of TEK expression was further confirmed using multivariate analyses ($p<0.001$, Table IV).

Table I. Characteristics of patients from The Cancer Genome Atlas (TCGA) and International Cancer Genome Consortium (ICGC).

		TCGA (n = 446)	ICGC (n = 91)
Age (mean ± standard deviation)		60.62 ± 12.80	60.47 ± 10.03
M stage	**0**	376	82
	1	70	9
Sex	**Male**	290	52
	Female	156	39

Downregulation of TEK in the ccRCC Metastatic Stage

The expression of TEK was compared between patients with non-metastatic (stage M0) and metastatic (stage M1) ccRCC from the TCGA and ICGC cohorts. TEK expression in patients with non-metastatic (stage M0) ccRCC was notably higher than that in patients with metastatic (stage M1) ccRCC (Figure 2).

Table II. Optimal cutoff values for TEK expression in TCGA and ICGC cohorts.

	TCGA	ICGC
Optimal cutoff value	696.32	6.861

Table III. C-index and Area Under the Curve (AUC) values for TEK in stage M0 and stage M1 patients in the TCGA and ICGC cohorts.

Categories	C-index		AUC value at 5 years	
	TCGA	ICGC	TCGA	ICGC
All patients	0.691	0.676	0.685	0.580
M0	0.667	0.625	0.644	0.562
M1	0.637	0.839	0.724	0.750

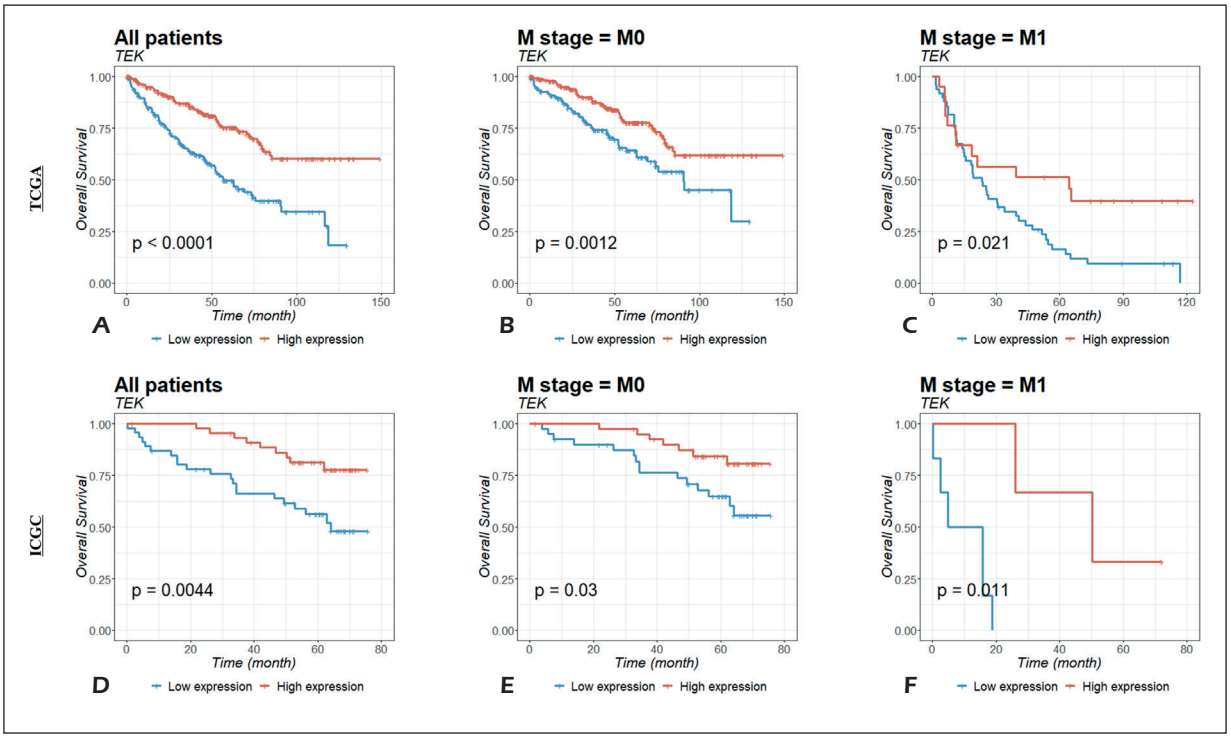

Figure 1. Kaplan-Meier estimates of survival for patients with ccRCC in the TCGA or ICGC cohorts according to TEK gene expression. The association between overall survival and TEK gene expression was examined in all patients **A**, **D**, stage M0 B, E, and stage M1 **C**, **F**. The *p*-value was calculated using a log-rank test and is indicated at the bottom left.

Table IV. Univariate and multivariate analysis of overall survival in each cohort.

Parameters	Univariate Cox regression				Multivariate Cox regression			
	p-value	Hazard ratio	95% confidence Interval		*p*-value	Hazard ratio	95% confidence Interval	
TCGA								
TEK	<0.001	0.398	0.286	0.554	<0.001	0.522	0.372	0.734
Age	<0.001	1.033	1.018	1.047	<0.001	1.035	1.020	1.051
M Stage (0 vs. 1)	<0.001	4.189	3.005	5.838	<0.001	3.916	2.775	5.528
Gender (Female vs. male)	0.333	0.850	0.612	1.181	0.569	0.904	0.640	1.278
ICGC								
TEK	0.007	0.339	0.155	0.740	< 0.001	0.243	0.105	0.562
Age	0.109	1.031	0.993	1.071	0.753	1.007	0.967	1.048
M Stage (0 vs. 1)	< 0.001	8.305	3.615	19.08	< 0.001	13.516	4.757	38.401
Gender (Female vs. male)	0.863	1.066	0.517	2.194	0.333	0.690	0.325	1.462

C-Index and AUC Values of TEK Expression

To assess the utility of TEK expression as a biomarker for ccRCC, we examined Uno's C-index in the time-dependent AUC analysis and AUC values at five years for the ROCs (Figure 3). TEK expression yielded high C-index values in the two independent cohorts (TCGA: 0.691, ICGC: 0.676; Figure 3A and Table III, respectively). The five-year ROC graphs revealed high AUC values for both the TCGA and ICGC cohorts (0.685 and 0.580; Figure 3B and Table III, respectively).

Discussion

Peters et al[34] have found that the Ang-TEK system is dysregulated in various types of cancers, where TEK and Ang2 have been shown to be upregulated in tumor vessel cells. While Ang1 overexpression has been detected in human breast cancer and squamous cell carcinoma cells[35,36], TEK expression in ccRCC remains uncharacterized. In the present work, we identified TEK expression as a prognostic factor for ccRCC, where TEK downregulation was associated with poor patient prognoses.

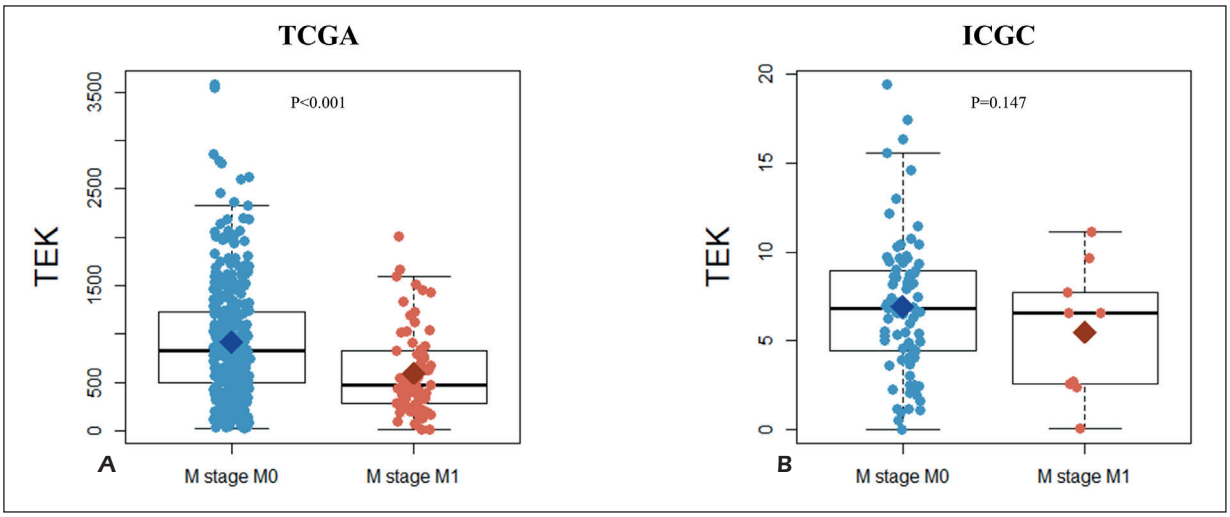

Figure 2. Comparison of TEK gene expression between patients with non-metastatic (stage M0) and metastatic (stage M1) ccRCC from the TCGA and ICGC cohorts. **A-B**, TEK expression in patients with ccRCC from **A**, TCGA and **B**, ICGC cohorts.

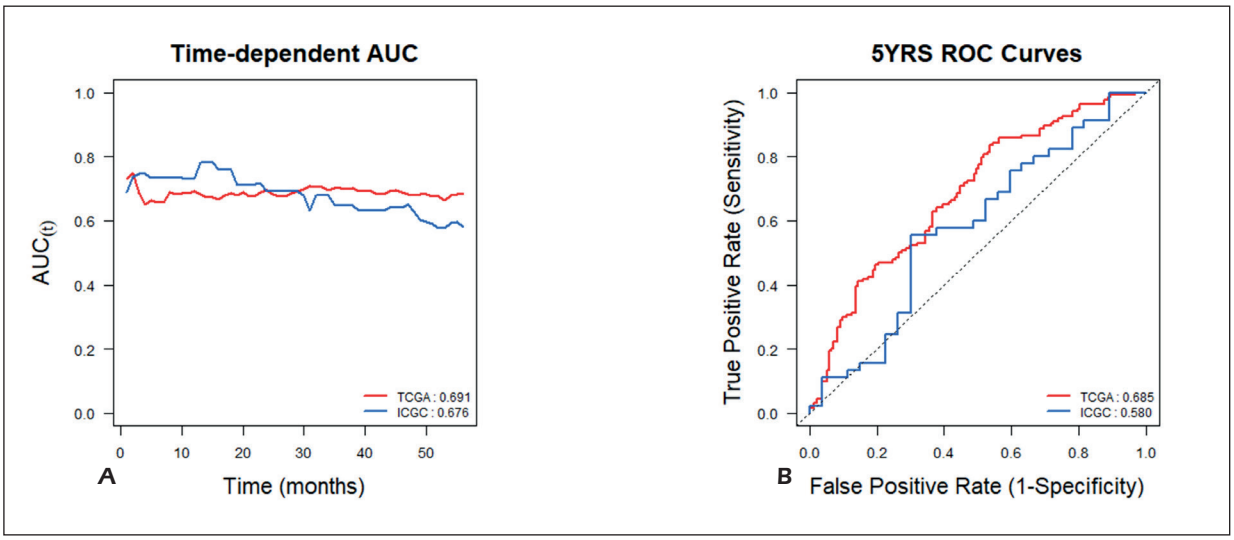

Figure 3. Time-dependent area under the curve (AUC) and receiver operating characteristic (ROC) curve at 5 years according to TEK gene expression for the TCGA and ICGC cohorts. **A**, Time-dependent AUC and **B**, ROC curve at 5 years for TCGA and ICGC cohorts according to TEK gene expression (red: TGCA, blue: ICGC). C-index values are described in the bottom right of panel A. AUC values at 5 years are indicated in the bottom right of panel B.

Patients with ccRCC can be divided into those with localized and advanced ccRCC. While there are many treatments options currently available for ccRCC, surgical treatment remains the most effective therapy for clinically localized ccRCC. The therapeutics available for ccRCC are lacking, especially drugs effective against localized ccRCC[37]. The current treatment for advanced ccRCC is VEGF-targeted therapy[38]. Most notably, VEGF expression results in the inactivation of the von Hippel-Lindau tumor suppressor (VHL) gene in most cases of ccRCC, and therefore VEGF is a particularly relevant therapeutic target for RCC[39]. Expression-based prognostic factors have been identified for many types of cancer; some of these factors have shown adequate efficacy in clinical settings[31,32,40-42].

Conclusions

The present work addressed the lack of available biomarkers to effectively predict the prognosis of patients with ccRCC. Based on our findings, we discovered a direct correlation between the levels of TEK expression and patient prognoses for both the ICGC and TCGA database cohorts. Despite the current limitations in studies based on TEK expression, we suggest that TEK is a potential prognostic gene for ccRCC.

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Author Contributions

Conception and design: YHK. Acquisition, analysis, interpretation of data, writing and review of the manuscript: DL and YHK. Study supervision: YHK. All authors have read and approved the final version of this manuscript.

Conflict of Interests

The authors declare that they have no conflict of interest.

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