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■ ORIGINAL ARTICLE ■

Transglutaminase 2 Expression and Its Prognostic Significance in Clear Cell Renal Cell Carcinoma

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Background: A few recent studies have demonstrated a possible role of transglutaminase 2 (TG2) in tumorigenesis or progression of renal cell carcinoma (RCC). The aim of this study was to examine TG2 expression and its clinicopathologic significance in a large number of human clear cell RCCs (CCRCCs). **Methods:** We analyzed 638 CCRCC patients who underwent partial or radical nephrectomy between 1995 and 2005. The expression of TG2 was determined by immunohistochemistry and categorized into four groups, according to staining intensity: negative (0), mild (1+), moderate (2+), and strong (3+). **Results:** TG2 staining intensity was negative in 8.5% of CCRCC (n=54), 1+ in 32.6% (n=208), 2+ in 50.5% (n=322), and 3+ in 8.5% (n=54). Strong TG2 expression was correlated with high Fuhrman nuclear grade (p=.011), high T category (p=.049), metastasis (p=.043) and male sex (p<.001) but not with N category.The survival analysis showed a significant association between strong TG2 expression and worse overall and cancer-specific survival (p=.027 and p=.010, respectively). On multivariate analysis, strong TG2 expression was a marginally significant prognostic indicator for Fuhrman nuclear grade and TNM staging (p=.054). **Conclusions:** Our study is the first to demonstrate the clinicopathologic significance of TG2 expression in a large number of human CCRCC samples. Strong TG2 expression was associated with high nuclear grade and poor prognosis.

Key Words: Transglutaminases; Carcinoma, renal cell; Prognosis

Renal cell carcinoma (RCC) is the most common renal malignant tumor in adults, accounting for approximately 90% of renal malignancies.[1] RCC is classified into several subtypes such as clear cell, chromophobe, papillary, collecting duct, and other rare subtypes. These subtypes show distinct clinical, pathological and molecular characteristics. Clear cell RCC (CCRCC) is the most common RCC and has a worse prognosis compared with other common histologic subtypes, such as chromophobe and papillary RCC. Approximately 25%–30% of patients are diagnosed with metastatic RCC at initial presentation,[2] and the disease will progress during the follow-up period in up to 50% of patients treated for localized RCC.[3] Prognostic indicators in CCRCC include clinical parameters and pathological factors, and many molecular prognostic markers have been suggested.[4]

Transglutaminases are a family of enzymes, that exist as cross-linked protein polymers and are resistant to proteolytic degradation.[5,6] Transglutaminase 2 (TG2) is ubiquitously expressed, and is a multifunctional molecule, that catalyzes calcium-dependent acyl-transfer activities, resulting in the formation of stable multiprotein complexes that are resistant to proteolysis. In addition, TG2 can also act as a calcium-independent GTPase, a protein disulfide isomerase, and a kinase.[7] In addition, cell surface TG2 is involved in cell adhesion via its tight interaction with fibronectin.[8,9]

TG2 is up-regulated and activated in some pathological conditions, including cancer, tissue fibrosis, and celiac disease.[10,11] Many previous studies have demonstrated the relationship between TG2 expression and some cancers, including breast cancer,[12,13] ovarian cancer,[14] pancreatic cancer,[15,16] lung cancer,[17] and melanoma.[18] TG2 may be involved in cancer progression or metastasis via regulation of tumor cell growth, tumor survival, tumor cell–extracellular matrix (ECM) interaction, or epithelial-

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mesenchymal transition.[19,20]

A few recent studies showed that TG2 expression was increased in RCC,[21] and up-regulation of TG2 was associated with a decrease in tumor necrosis in RCC samples.[22] Furthermore a few studies have shown a relationship between TG2 and von Hippel–Lindau (*VHL*) gene expression in RCC cell lines.[23,24] However, the prognostic significance of TG2 expression in CCRCC has not been reported.

In this study, we investigated the TG2 expression in 638 CCRCC samples using immunohistochemistry and evaluated the clinicopathologic prognostic significance of TG2 expression.

MATERIAL AND METHODS

Patients and tumor specimens

We analyzed samples from 638 CCRCC patients who had undergone partial or radical nephrectomy at Seoul National University Hospital in Seoul, Korea between 1995 and 2005. The Seoul National University Hospital Institutional Review Board approved this study. All the clinicopathologic data were obtained from medical records, pathology reports and the review of hematoxylin and eosin (H&E)–stained slides. H&E slides were reviewed for tumor staging and nuclear grade. The tumor stage was determined according to the pTNM staging guidline published by the 2010 American Joint Committee on Cancer (AJCC),[25] and nuclear grading was performed based on the Fuhrman nuclear grading scale.[26] The recurrence or metastasis of CCRCC was determined by the clinical, radiological and pathological parameters. Confirmation of patients' deaths was obtained from medical records or death certificates.

Tissue microarray and immunohistochemistry

Following a review of the tumor slides, a representative area from each tumor block was selected and utilized to construct tissue microarrays (TMA) using a trephine apparatus (Superbiochips Laboratories, Seoul, Korea).

Immunohistochemical staining was performed on 4-μm-thick sections taken from the TMA slides using the Bond-Max Autostainer (Leica Microsystems, Bannockburn, IL, USA). Polyclonal rabbit anti-TG2 antibody (Neomarkers, Fremont, CA, USA) was diluted 1:100. After heat-induced antigen retrieval, primary antibodies were incubated with the samples for 15 minutes. The binding of the primary antibody was detected using the Bond Polymer Refine Detection kit (Leica Microsystems) according to the manufacturer's instructions. TG2 immunoreactivity was classified into four categories according to staining intensity: negative (0), weak (1+), moderate (2+), and strong (3+) expression. Constant TG2 staining on endothelial cells was regarded as an internal positive control.

Statistical analysis

SPSS ver. 21 (IBM Co., Armonk, NY, USA) was used for statistical analysis. The association between clinicopathologic findings and TG expression was analyzed using the linear by linear association. Overall survival and cancer-specific survival were analyzed using the Kaplan-Meier method supported by the log-rank test. The overall survival was defined as the time interval from primary radical or partial nephrectomy to last follow-up or patients' death. The cancer-specific survival was defined as the time interval from primary radical or partial nephrectomy to last follow-up or cancer-related death and was recorded at final follow-up visit as alive or dead from an unrelated cause. The Cox regression model was used for multivariate analysis. All statistical analyses were 2-tailed, and a $p < .05$ was regarded as statistically significant.

RESULTS

Clinicopathologic characteristics

The study population included 473 men and 165 women. The ages of the patient population ranged from 24 to 84 years with a mean age of 56.0 years (mean±SD, 56.02±11.53 years). The measured tumors ranged in size from 0.8 to 22 cm with a mean size of 5.6 cm (mean±SD, 5.63±3.40 cm). Patient follow-up times ranged from 2 to 223 months with an average follow-up period of 85.7 months (mean±SD, 85.71± 48.46 months). At the time of surgery, lymph node metastases were found in 18 cases (2.8%), and distant metastases were found in 53 cases (8.3%). Four hundred and eight cases were classified as pTNM stage I (63.6%), 82 as stage II (12.8%), 91 as stage III (14.2%), and 57 as stage IV (8.9%). According to Fuhrman nuclear grade, 49 cases were classified as grade 1 (7.7%), 291 as grade 2 (45.6%), 235 as grade 3 (36.8%), and 63 as grade 4 (9.9%).

TG2 expression in CCRCC

In the non-neoplastic renal parenchyme, TG2 was faintly expressed, only focally. In CCRCC, TG2 was diffusely expressed in the cytoplasm of tumor cells in most cases (positive in 584 CCRCCs, 91.5%), but the staining intensity was variable. TG2 expression showed a cytoplasmic or membranous pattern. The evaluation of the staining intensity revealed that 54 (8.5%) were negative, 208 showed weak TG2 expression (32.6%), 322

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showed moderate TG2 expression (50.5%), and 54 (8.5%) showed strong TG2 expression (Fig. 1).

The relationship between TG2 expression and pathologic parameters is shown in Table 1. Strong TG2 expression was correlated with high Fuhrman nuclear grade (p = .011), high T category (p = .049), metastasis (p = .043) and male sex (p < .001) but not with N category.

TG2 expression and survival analysis

Overall and cancer-specific survival times were not significantly different between groups showing TG2 staining intensities of 0, 1+, and 2+ (p = .872 and p = .816, respectively). In contrast, the strong (3+) TG2 expression grouphad significantly shorter overall and cancer specific survival periods compared with groups showing staining intensities 0, 1+, or 2+ (p = .027 and p = .010, respectively) (Table 2, Fig. 2). Upon multivariate analysis, strong TG2 expression was shown to be a marginal independent prognostic indicator of cancer-specific survival for Fuhrman nuclear grade and TNM staging (p = .054). However, these analyses were not statistically significant for overall survival (p = .154) (Table 3).

DISCUSSION

In this study, we investigated the expression level and clinicopathologic significance of TG2 in CCRCC. TG2 was diffusely expressed in most CCRCCs (584/638, 91.5%). Strong TG2 expression in CCRCC was associated with high Fuhrman nuclear grade, but was not associated with TNM stage. In addition, strong expression of TG2 was associated with significantly worse cancer-specific survival in univariate analysis. A multivariate analysis also showed a marginally significant decrease in cancer-specific survival. To our knowledge, this is the first study to elucidate the prognostic significance of TG2 expression in CCRCC.

Previous studies using cancer cell lines or nude mice have

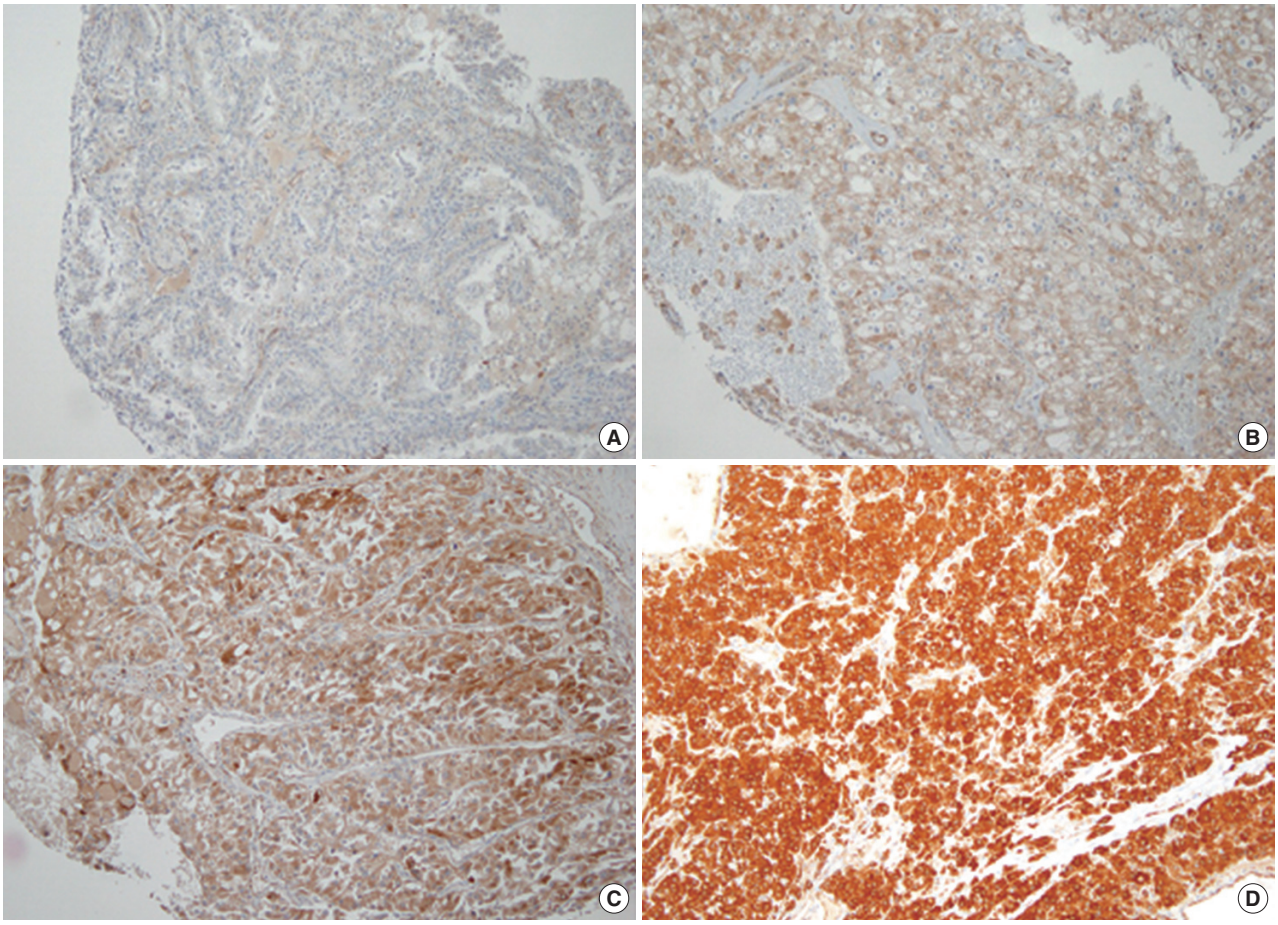

Fig. 1. Immunohistochemical analysis of the intensity of transglutaminase 2 expression in clear cell renal cell carcinoma showing 0 (A), 1+ (B), 2+ (C), and 3+ (D).

Table 1. Clinicopathological characteristics of patients with CCRCC and the associations with TG2 expression

Characteristic	Cases (n=638)	TG2 expression				p-value
		0 (n=54, 8.5%)	1+ (n=208, 32.6%)	2+ (n=322, 50.5%)	3+ (n=54, 8.5%)	
Sex						
Male	473	33	137	258	45	<.001
Female	165	21	71	64	9	
Age (yr)						
≤55	238	17	76	134	11	.779
>55	276	28	92	128	28	
Furman grade						
1, 2	340	32	118	172	18	.011
3, 4	298	22	90	150	36	
T category						
1, 2	511	47	172	251	41	.049
3, 4	127	7	36	71	13	
N category						
0 or x	620	53	201	316	50	.453
1	18	1	7	6	4	
M category						
0	585	53	194	289	49	.043
1	53	1	14	33	5	
Stage						
I, II	148	45	163	242	40	.147
III, IV		9	45	80	14	
Lung metastasis						
Absent	518	47	172	262	37	.031
Present	120	7	36	60	17	
Bone metastasis						
Absent	588	51	190	298	49	.767
present	50	3	18	24	5	

CCRCC, clear cell renal cell carcinoma; TG2, transglutaminase 2.

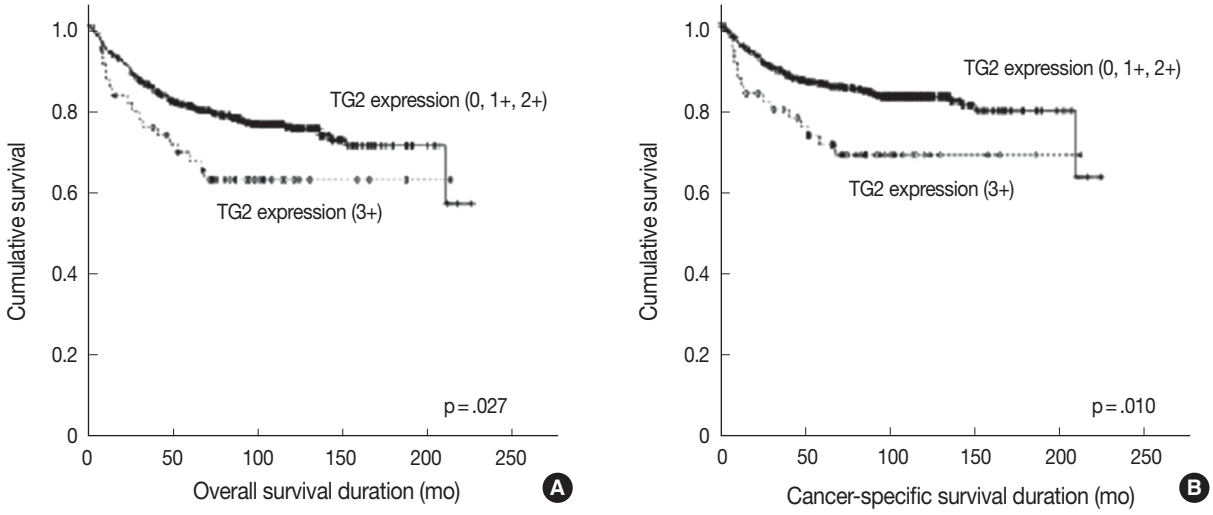

Fig. 2. Kaplan-Meier curves of overall survival (A) and cancer-specific survival (B) in 638 patients with clear cell renal cell carcinoma depending on the level of transglutaminase 2 (TG2) expression.

shown the role of TG2 in cancer cell growth, survival and metastasis.[13,16,18] In addition, there have been a few studies showing the clinicopathologic significance of TG2 expression in human cancer tissues. Overexpression of TG2 was associated with poor prognosis in ovarian cancer,[14] and high TG2 expression was associated with nodal metastasis and lymphovascular invasion in pancreatic ductal adenocarcinoma.[15] It has also been suggested that TG2 contributes to cancer progression and metastasis by

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Table 2. A univariate analysis of overall survival and cancer-specific survival in CCRCC patients (log-rank test)

Prognostic factor	Overall survival		Cancer-specific survival	
	Survival time (mean ± SE, mo)	p-value	Survival time (mean ± SE, mo)	p-value
TG2 expression				
0, 1, 2	171.4 ± 4.2	.027	184.7 ± 4.0	.010
3	143.1 ± 12.5		153.3 ± 12.1	
Nuclear grade				
1, 2	193.7 ± 4.1	< .001	209.0 ± 3.2	< .001
3, 4	139.2 ± 5.2		149.2 ± 5.0	
TNM stage				
I, II	186.5 ± 3.8	< .001	201.2 ± 3.3	< .001
III, IV	105.1 ± 8.7		111.6 ± 8.9	

CCRCC, clear cell renal cell carcinoma; SE: standard error, TG2, transglutaminase 2.

Table 3. A multivariate analysis of overall survival and cancer-specific survival in CCRCC patients (Cox proportional hazard model)

Prognostic factor		Overall survival		Cancer specific survival	
		HR (95% CI)	p-value	HR (95% CI)	p-value
TG2 expression	3 vs 0,1,2	0.665 (0.409–1.081)	.100	0.598 (0.350–1.020)	.059
Histologic grade	3,4 vs 1,2	0.472 (0.327–0.680)	< .001	0.295 (0.177–0.490)	< .001
TNM stage	III, IV vs I, II	0.190 (0.136–0.265)	< .001	0.107 (0.069–1.165)	< .001

CCRCC, clear cell renal cell carcinoma; HR, hazard ratio; CI, confidence interval; TG2, transglutaminase 2.

regulating cancer cell migration into the ECM, adhesion to endothelium, invasion, and angiogenesis.[20]

A few previous studies have described the relationship between TG2 and the *VHL* gene, a well-known gene altered in most CCRCCs.[27] Wykoff *et al*.[23] showed that TG2 is a novel target gene of *VHL* in RCC cell lines. Another study demonstrated that TG2 is a critical regulator of VHL.[24] These studies suggest a close relationship between TG2 and CCRCC. In addition to VHL, p53 was also depleted by TG2 in many RCC cell lines.[28] A recent study examining microRNA expression in RCC demonstrated that miR-1285, one of the microRNAs that are reduced in RCC specimens and cell lines, inhibited cancer cell proliferation, invasion and migration, as well as directly regulating TG2 expression.[21] Thus, previous studies indicate that TG2 may play a role in pathogenesis or progression of RCC. However, only few studies examined TG2 expression in human RCC samples. Erdem *et al*.[22] investigated TG2 mRNA expression in 95 primary RCC samples and found that TG2 expression alone was not associated with RCC subtype, nuclear grade, T classification or tumor size. Another study examined TG2 expression by immunohistochemistry in 70 RCC samples, finding an increased TG2 expression in RCC tissue compared to normal kidney tissue and a significant correlation between increased TG2 expression and high T classification.[21] Although these two previous studies showed the significance of TG2 expression in human RCC, in part, they also had some limitations such as relatively small sample sizes and no evaluation of the prognostic significance of TG2 expression.

We showed the clinicopathologic significance and prognostic implications of TG2 expression in a large number of CCRCCs. The majority of CCRCCs showed diffuse TG2 staining, but the staining intensity was variable. Only a small subset of CCRCCs showed strong TG2 expression (54/638, 6.5%) that correlated with significantly worse prognosis. The remaining cases showing negative to moderate TG2 expression revealed no significant association of TG2 staining intensity with prognosis.

Although the precise role of TG2 in the progression of CCRCC is not clear, previous studies have suggested some possible mechanisms. First, TG2 can regulate cell adhesion. Overexpression of TG2 in breast cancer cell lines has been reported to contribute to cancer cell invasion and metastasis through the interaction of TG2 with ECM components such as β integrins and fibronectin.[13,29]

In primary RCC samples, increased expression of TG2, along with β1 integrin and syndecan-4, was reported to be associated with metastasis.[22] Second, TG2 has been suggested to regulate apoptosis. Inhibition of TG2 was found to stabilize p53 expression, increasing apoptosis in RCC cell lines.[28] These results suggest that TG2 might enhance metastasis and tumor cell survival.

Our study also demonstrated that high TG2 expression is associated with tumor aggressiveness such as T category and metastasis. Our results are similar to those of previous studies showing an association between TG2 expression and metastasis or tumor

stage of RCC.[21,22] However, there were some differences between these previous studies and our study. First, the study sample was smaller than that of our study (70 and 95 in previous studies versus 638 in our study). Second, they did not separate the RCC subtypes, but we included CCRCC only.

In addition our study showed that strong TG2 expression was related to high nuclear grade of CCRCC. This result suggests that TG2 expression might be related to the differentiation of CCRCC.

In conclusion, our study is the first to demonstrate the clinicopathologic significance of TG2 expression in a large number of human CCRCC samples. Strong TG2 expression was related to tumor aggressiveness, high nuclear grade and poor prognosis.

Conflicts of Interest

No potential conflict of interest relevant to this article was reproted.

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