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Overexpression of IL-32 is a novel prognostic factor in patients with localized clear cell renal cell carcinoma

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Abstract. Interleukin-32 (IL-32) is a proinflammatory cytokine that acts as a significant pathogenetic factor in various diseases and malignancies. However, the clinical effect of IL-32 expression in renal cell carcinoma (RCC) has not previously been investigated. The aim of the present study was to examine the significance of IL-32 overexpression in localized clear cell RCC (CCRCC). We examined 112 patients with localized CCRCC who underwent nephrectomy. The clinicopathological data were obtained by retrospective review and the expression levels of IL-32 were studied by immunohistochemistry. Tumors were classified according to staining intensity (0, no staining intensity; 1, weak; 2, intermediate; 3, strong). The cases with staining intensities from 0 to 2 comprised the IL-32 low-expression group (LEG), whereas those with a staining intensity of 3 comprised the IL-32 high-expression group (HEG). Correlations between IL-32 expression and clinicopathological parameters were determined. Staining intensities were determined for all cases as follows: 26 cases (23.2%) (score 0), 43 cases (38.4%) (score 1), 31 cases (27.7%) (score 2) and 12 cases (10.7%) (score 3). IL-32 HEG exhibited a higher recurrence rate compared to the IL-32 LEG (50 vs. 13%, P=0.001). For survival rates, the 5-year recurrence-free survival (RFS), disease-specific survival (DSS) and overall survival (OS) rates were lower in the IL-32 HEG group compared with the IL-32 LEG group (RFS, P=0.001; DSS, P<0.001; OS, P=0.026, respectively). Univariate analyses revealed that Fuhrman nuclear grade and a high IL-32 expression were significant prognostic factors for predicting RFS, DSS and OS in CCRCC, whereas multivariate analyses indicated that Fuhrman nuclear grade and high IL-32 expression were still independent risk factors. In conclusion, IL-32 overexpression was associated with high recurrence rates and low RFS, DSS and OS, indicating that it may be a novel prognostic factor for predicting outcomes in patients with CCRCC.

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Introduction

Renal cell carcinoma (RCC) is the most common primary malignant neoplasm of the kidney and one of the most fatal genitourinary malignancies. The incidence is consistently 3% of all malignant neoplasms and the lethality from RCC accounts for 4% of all deaths from malignancies (1). Even following curative nephrectomy, approximately 20% of RCC patients experience disease recurrence within 5 years (2). Following metastasis, RCC exhibits markedly poor prognosis, although immunotherapy and several targeted agents improve the survival in certain populations (3). It is therefore crucial to predict which patients are likely to develop disease recurrence after surgery for localized RCC.

The prognostic factors of non-metastatic RCC have been studied using various methods. Traditionally, the tumor-node-metastasis (TNM) stage, Fuhrman nuclear grade, Eastern Cooperative Oncology Group performance status (ECOG PS), and tumor necrosis were determined to be classic clinicopathologic prognostic factors (4). In molecular studies, biomarkers such as vascular endothelial growth factor (VEGF) (5), p53 mutation (6), hypoxia-inducible factor 1 α (7), and C-reactive protein (8) were identified as effective prognostic factors. In genetics, the Von-Hippel-Lindau (VHL) gene was thought to be associated with a more favorable prognosis than the sporadic form (9). However, the majority of molecular and genetic factors do not have notable impact significance in RCC.

Interleukin-32 (IL-32), previously denoted as natural killer cell transcript 4 (NK4), was first identified as a transcript in IL-2-activated natural killer (NK) cells and T lymphocytes in 1992 by Dahl *et al* (10). IL-32 is now recognized as a new

proinflammatory cytokine produced by epithelial cells and monocytes, as well as NK cells and T lymphocytes (11). The encoding gene of IL-32 is located at chromosome 16p13.3 and six splice variants have been reported: IL-32α, IL-32β, IL-32γ, IL-32δ, IL-32ε, and IL-32ξ (12). One study identified the specific receptor of IL-32 as proteinase-3 (13). The exact function of IL-32 is not clear but it has been implicated in inflammatory disorders such as rheumatoid arthritis (14), *Mycobacterium tuberculosis* infection (15), and inflammatory bowel disease (16). Recently, there has been a focus on the expression of IL-32 in human malignancies such as stomach cancer (17), lung cancer (18), pancreatic cancer (19), hematopoietic malignancies (20,21), and uterine cervical cancer (22). There are no previous studies about the expression of IL-32 in RCC. The aim of the present study was to investigate IL-32 expression in CCRCC tissue and reveal the novel value of IL-32 as a prognostic factor.

Materials and methods

Patients and tumor samples. We investigated 112 consecutive patients with CCRCC who underwent radical or partial nephrectomy for sporadic, localized (T1-3N0M0) RCC at the Chungnam National University Hospital, Korea, between 1999 and 2006. Clinicopathologic data of patients were obtained by reviewing medical records. ECOG performance status was assigned to each patient at the time of diagnosis. T classification was defined according to the 2002 American Joint Committee on Cancer criteria and the nuclear grade according to the Fuhrman grading system. Tumor samples were collected from the tissue blocks used for routine pathologic examination. All patients signed informed consent for therapy as well as for subsequent tissue studies, which had received prior approval by the local ethics committee.

Tissue microarray construction. Tissue microarrays were constructed from archival original formalin-fixed, paraffin-embedded tissue blocks from 112 cases of localized CCRCC. For each tumor, a representative tumor area was carefully selected from a hematoxylin and eosin-stained section of a donor block. Each case was represented by 2 cores of 2-mm cylinders from tumors that were punched using an automated tissue array (UNITMA, Seoul, Korea). Thus, tissue microarray blocks containing 224 cylinders were constructed.

Specimen preparation and immunohistochemistry. For immunohistochemistry, sections (3-μm) were cut from the recipient blocks and placed on 3-amino-propyltriethoxysilane-coated slides that were dried for 2 h prior to staining at 57˚C. Procedures were performed at room temperature, as recommended by the manufacturer. In brief, sections were dewaxed in xylene and rehydrated in graded alcohols. Sections were then washed in water prior to antigen retrieval, using a Dako PTLink machine (Dako, Glostrup, Denmark) with 10 mM sodium citrate buffer (pH 6.0) at 97˚C for 20 min. Sections were treated with 3% hydrogen peroxide for 10 min to block endogenous peroxidase and were then preincubated with a serum-free protein block solution (Dako, Carpinteria, CA, USA) for 20 min to eliminate background staining. A monoclonal mouse antibody against human IL-32 was used, as previously described (11). The slides were then incubated for 30 min with an EnVision anti-mouse (Dako, Denmark) polymer. Reaction products were visualized with diaminobenzidine plus substrate-chromogen solution applied for 5 min. The slides were counterstained with Meyer's hematoxylin and mounted. The sections were carefully rinsed with several changes of phosphate-buffered saline between each stage of the procedure. Negative controls were yielded by excluding the primary antibody or by using preimmune IgG1 to evaluate non-specific staining.

Evaluation of immunohistochemical staining. The results yielded by immunohistochemical staining were evaluated by two independent pathologists (J.M. Kim and H.J. Lee) who were blinded to the clinicopathological data of the patients. Immunohistochemical staining was classified according to a scoring method; tumors were classified into four grades based on the staining intensity (score 0, no staining intensity; score 1, weak staining intensity; score 2, intermediate staining intensity; score 3, strong staining intensity). In cases of heterogeneous staining within the samples, the respective higher score was selected when >50% of the cells exhibited higher staining intensity. For all patients, scores from two tumor cores from the same patient were averaged to obtain a mean score. The cases with staining intensity scores of 0, 1, and 2 comprised the IL-32 low-expression group (LEG), whereas those with score 3 staining intensity comprised the IL-32 high-expression group (HEG).

Statistical analysis. Pearson's Chi-square test was used to assess the associations between the expression of IL-32 and the clinicopathological parameters. To estimate the 5-year recurrence-free survival (5yr-RFS), 5-year disease-specific survival (5yr-DSS), and 5-year overall survival (5yr-OS) rates, we used the Kaplan-Meier method and log-rank test. The 5yr-RFS was measured from the date of nephrectomy to the date of recurrence or death from RCC (5yr-RFS). The 5yr-DSS was measured from the date of nephrectomy to the date of death from RCC only. The 5yr-OS was measured from the date of surgery to the date of death from any causes. To analyze the effect of the expression of IL-32 on RFS, DSS and OS, Cox's proportional hazards model was used. For all analyses, $P<0.05$ was considered statistically significant. Statistical analyses were conducted using the SPSS version 13.0 statistical software program (SPSS Inc., Chicago, IL, USA).

Results

Patient characteristics. The median age of the patients was 60 years (range 30-78), with a male predominance (male:female ratio of 2.4:1). ECOG 0 patients comprised 43.8% of the group and the remaining patients (56.3%) were in other ECOG performance-status groups (≥1). The tumor sizes varied from 1 to 15 cm at the largest diameter and the median size was 5 cm. Of the 112 patients, 41.9, 16.1 and 42.0% were found to have pT1, pT2 and pT3 primary tumors, respectively. The Fuhrman nuclear grade revealed grade 1, 2, 3 and 4 lesions in 15.2, 67.9, 14.3 and 2.7% of patients, respectively. Nineteen patients (17%) experienced recurrence following nephrectomy.

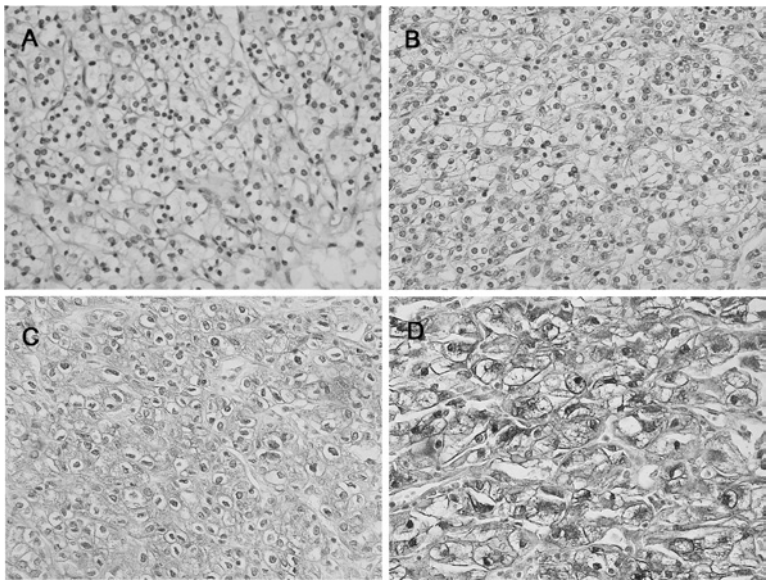

Figure 1. Representative photomicrographs of immunohistochemical staining for IL-32 in renal cell carcinoma tissues. (A) No staining intensity, (B) weak staining intensity, (C) intermediate staining intensity and (D) strong staining intensity (magnification, x400).

Immunohistochemical staining for IL-32 expression in CCRCC. In the majority of cases, the cytoplasm of the neoplastic cells stained positive for IL-32, and certain cells also stained in the nucleus and cytoplasmic membrane (Fig. 1). We estimated the level of IL-32 expression in the positively stained tumor cells using a staining intensity score of 0, 1, 2 or 3. In 26 (23.2%) of the 112 cases, the tumor cells did not exhibit IL-32 expression and 43 cases (38.4%) exhibited weak positivity (staining intensity score 1). Intermediate staining was found in 31 cases (27.7%) (score 2) and 12 cases (10.7%) showed a marked staining intensity (score 3). We divided the cases into two groups: Cases with scores from 0 to 2 comprised the IL-32 low-expression group (LEG) and score 3 cases comprised the IL-32 high-expression group (HEG). Therefore, 100 cases were classified into the IL-32 LEG while 12 cases were classified into the IL-32 HEG (Table I).

Association between IL-32 expression and clinicopathological characteristics. Analysis of the association between IL-32 expression and clinicopathologic characteristics showed a significant difference in the recurrence rate (P=0.001) (Table I). In the IL-32 LEG, recurrence was found in 13 cases (13%) following nephrectomy, whereas recurrence was noted in 6 cases (50%) in the IL-32 HEG. No significant differences were observed in age, gender, ECOG PS, tumor size, T stage, and Fuhrman nuclear grade between the IL-32 LEG and IL-32 HEG groups.

Correlation between IL-32 expression and survival. To determine the benefits of IL-32 expression in CCRCC, we investigated the associations between IL-32 expression and RFS, DSS and OS. The 5-year RFS, DSS and OS rates were 83.0, 88.4 and 81.3%, respectively, for the whole study population. The IL-32 LEG was censored 1 and the HEG was censored 2. The 5yr-RFS rates of the IL-32 LEG and HEG groups were 87 and 50%, respectively (Fig. 2A; P=0.001), and the rates of 5yr-DSS for the IL-32 LEG and HEG

Table I. Correlation between the expression of IL-32 and clinicopathological parameters.

	IL-32 expression				
	LEG (n=100)		HEG (n=12)		
Characteristic	No.	%	No.	%	P
Age (years)					
≤70	85	85.0	8	66.7	0.110
>70	15	15.0	4	33.3	
Gender					
Male	69	69.0	10	83.3	0.303
Female	31	31.0	2	16.7	
ECOG PS					
0	41	41.0	8	66.7	0.090
≥1	59	59.0	4	33.3	
Tumor size (cm)					
≤10	94	94.0	11	91.7	0.752
>10	6	6.0	1	8.3	
T stage					
T1	59	59.0	6	50.0	0.551
T2/3	41	41.0	6	50.0	
Fuhrman nuclear grade					
G1/2	83	83.0	10	83.3	0.977
G3/4	17	17.0	2	16.7	
Recurrence					
No	87	87.0	6	50.0	0.001
Yes	13	13.0	6	50.0	

ECOG, Eastern Cooperative Oncology Group; PS, performance status; LEG, low-expression group; HEG, high-expression group.

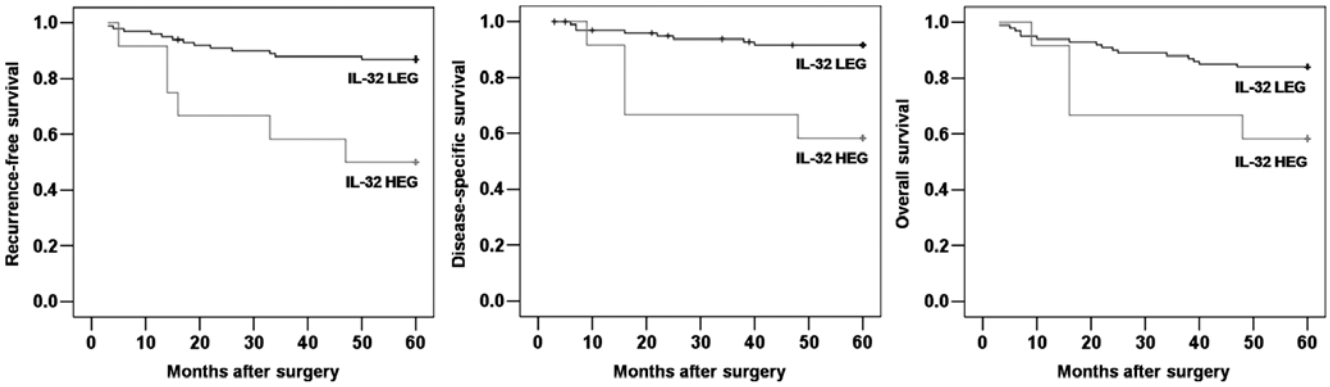

Figure 2. Correlation between IL-32 expression and survival rates in 112 patients with clear cell RCC. (A) 5yr-RFS (P=0.001), (B) 5yr-DFS (P<0.001) and (C) 5yr-OS (P=0.026).

groups were 92 and 58.3%, respectively (Fig. 2B; P<0.001). The 5yr-OS rates for the two groups were 84% and 58.3%, respectively (Fig. 2C; P=0.026). Therefore, the 5-year RFS, DSS, and OS of the IL-32 LEG group were significantly higher than those of the IL-32 HEG group.

To determine the clinical significance of various parameters that may affect tumor recurrence and survival in CCRCC, we performed univariate analyses (Table II). The Fuhrman nuclear grade and a high IL-32 expression were significant risk factors affecting RFS (P=0.009 and P=0.002, respectively). For DSS and OS, the Fuhrman nuclear grade (P=0.005 and P=0.026, respectively) and high IL-32 expression (P=0.002 and P=0.034, respectively) showed statistical significance.

To determine the independent prognostic factors, multivariate analyses were then performed using Cox's proportional hazards model (Table III). The model revealed that Fuhrman nuclear grade [hazard ratio (HR), 3.052; 95% confidence interval (95% CI), 1.075-8.664; P=0.036] and a high IL-32 expression (HR, 4.932; 95% CI, 1.781-13.657; P=0.009) were independent risk factors of RFS in CCRCC patients. With regard to predicting the DSS and OS, the Fuhrman nuclear grade and a high IL-32 expression were also significant as independent prognostic factors. For DSS, the Fuhrman nuclear grade HR was 4.688 (95% CI, 1.389 15.814; P=0.013) and the high IL-32 expression HR was 6.736 (95% CI, 2.01-22.563; P=0.002). For OS, the Fuhrman nuclear grade HR was 2.741 (95% CI, 1.056-7.114; P=0.038) and the high IL-32 expression HR was 2.992 (95% CI, 1.066-8.394; P=0.037). Moreover, the results revealed that a high expression of IL-32 in CCRCC was a more significant prognostic factor for predicting the RFS, DSS and OS than the Fuhrman nuclear grade (Table III).

Discussion

IL-32, initially denoted as NK4, was identified as a transcript produced by mitogen-activated T-cells and IL-2-activated NK-cells. Dahl *et al*, the first group to report NK4 in 1992, proposed that NK4 was a novel common product of activated NK and T cells that showed no homology with other sequences (10). Further studies of NK4 termed it IL-32 following the detection of cytokine-like gene expression in an IL-18-induced microarray (11). The IL-32 encoding gene locus has been identified at chromosome 16p13.3 and six isoforms, IL-32α, IL-32β, IL-32γ, IL-32δ, IL-32ε, and IL-32ξ, were identified by Kim *et al* and Chen *et al* (11,12). Moreover, several studies identified the production of IL-32 in other cell types, including epithelial cells and other monocytes, as well as the presence of IL-32 mRNA in Epstein-Barr virus-infected lymphoma, neuroblastoma, and hematopoietic progenitor cells (23-25).

IL-32 has been evaluated as an entity in a variety of diseases. Initially, the focus was on the expression of IL-32 in inflammatory diseases such as tuberculosis, rheumatoid arthritis, and inflammatory bowel disease. IL-32 pathways are reportedly involved in immune response. Initially, it was determined that IL-32 activated the NF-κB and p38MAPK signaling pathways (11). Netea *et al* demonstrated the synergetic effects of IL-32 with the nucleotide oligomerization domain (NOD) 1 and NOD 2 ligands through a caspase-1-dependent mechanism (26). Moreover, findings of recent studies have shown that IL-32 is involved in cancer pathogenesis. Elevated serum IL-32 levels were detected in stomach cancer patients and secreted IL-32 was increased in a K-562 lymphoblastic cell line as detected by ELISA and immunohistochemistry (17). Nishida *et al* demonstrated IL-32 expression in human pancreatic tissue and pancreatic cancer cell lines. These authors showed that although normal pancreatic duct cells exhibited weak positive staining, the staining intensity was markedly increased in chronic pancreatitis and strong in pancreatic cancer cells. This study was based on the hypothesis that chronic pancreatitis was a precancerous condition (19). Sorrentino *et al* studied the expression of IL-32 in human lung cancer (18). These authors demonstrated that malignancies expressed IL-32 with marked positive reactivity by immunohistochemical staining, with the exception of squamous cell carcinomas. They also focused on the relationship between inflammation and lung cancer and proposed that the proinflammatory cytokine IL-32 is involved in lung carcinogenesis (18). However, no previous study examined the clinicopathological significance between IL-32 expression and RCC.

RCC is the most common and lethal malignancy of the genitourinary tract cancers (1). Five subtypes are recognized: clear cell, papillary, chromophobe, collecting duct, and unclassified renal cell carcinoma (27). The most common subtype is CCRCC, which represents up to 82% of all renal carcinomas. Various prognostic factors, particularly in localized renal cell

Table II. Univariate analyses of clinicopathological parameters and IL-32 expression and their association with prognosis in clear cell renal cell carcinoma.

Variables	Hazard ratio	95% Confidence interval	P
Recurrence-free survival			
ECOG PS (≥1/0)	0.858	0.349-2.111	0.739
Tumor size (>10/≤10 cm)	3.440	1.000-11.836	0.050
Pathological T stage (T2-3/T1)	2.386	0.859-6.627	0.095
Fuhrman nuclear grade (G3-4/G1-2)	3.472	1.366-8.827	0.009
IL-32 expression (HEG/LEG)	4.694	1.179-12.384	0.002
Disease-specific survival			
ECOG PS (≥1/0)	0.895	0.301-2.664	0.842
Tumor size (>10/≤10 cm)	3.026	0.670-13.661	0.150
Pathological T stage (T2-3/T1)	2.803	0.771-10.189	0.117
Fuhrman nuclear grade (G3-4/G1-2)	4.737	1.590-14.117	0.005
IL-32 expression (HEG/LEG)	5.766	1.882-17.671	0.002
Overall survival			
ECOG PS (≥1/0)	0.840	0.357-1.977	0.689
Tumor size (>10/≤10 cm)	1.773	0.413-7.614	0.441
Pathological T stage (T2-3/T1)	1.688	0.681-4.185	0.258
Fuhrman nuclear grade (G3-4/G1-2)	2.799	1.128-6.943	0.026
IL-32 expression (HEG/LEG)	2.962	1.083-8.101	0.034

ECOG, Eastern Cooperative Oncology Group; PS, performance status; LEG, low-expression group; HEG, high-expression group.

Table III. Multivariate analyses of clinicopathological parameters and IL-32 expression and their association with prognosis in clear cell renal cell carcinoma.

Variables	Hazard ratio	95% confidence interval	P
Recurrence-free survival			
ECOG PS (≥1/0)	1.043	0.395-2.750	0.933
Tumor size (>10/≤10 cm)	1.620	0.396-6.625	0.502
Pathological T stage (T2-3/T1)	2.093	0.717-6.108	0.177
Fuhrman nuclear grade (G3-4/G1-2)	3.052	1.075-8.664	0.036
IL-32 expression (HEG/LEG)	4.932	1.781-13.657	0.009
Disease-specific survival			
ECOG PS (≥1/0)	1.244	0.384-4.037	0.842
Tumor size (>10/≤10 cm)	0.962	0.169-5.466	0.965
Pathological T stage (T2-3/T1)	2.780	0.697-11.084	0.147
Fuhrman nuclear grade (G3-4/G1-2)	4.688	1.389-15.814	0.013
IL-32 expression (HEG/LEG)	6.736	2.011-22.563	0.002
Overall survival			
ECOG PS (≥1/0)	0.945	0.390-2.291	0.901
Tumor size (>10/≤10 cm)	0.973	0.204-4.647	0.972
Pathological T stage (T2-3/T1)	1.549	0.605-3.964	0.361
Fuhrman nuclear grade (G3-4/G1-2)	2.741	1.056-7.114	0.038
IL-32 expression (HEG/LEG)	2.992	1.066-8.394	0.037

ECOG, Eastern Cooperative Oncology Group; PS, performance status; LEG, low-expression group; HEG, high-expression group.

carcinoma, have been identified. These factors are significant for appropriate treatment of the patient, providing more information to the patient, and producing treatment plans for new clinical trials. Classically, the prognostic factors of RCC are divided into three categories. Firstly, anatomical prognostic factors include tumor size; extra-renal fat invasion; invasion of main vessels such as the renal vein and inferior vena cava; and lymph node invasion. The tumor size was found to be a significant independent prognostic factor in a number of studies (28); however, there is some controversy in determining the cut-off value for tumor size. Extra-renal fat invasion and direct invasion of the ipsilateral adrenal gland of the RCC are also regarded as prognostic factors (29). Currently, these anatomical prognostic factors are included in the TNM classification as factors of T staging (30). Nodal invasion was confirmed to be a significant independent prognostic factor (28). However, the sub-classification of nodal invasion by the number of involved nodes is controversial (31). Secondly, histological prognostic factors include Fuhrman nuclear grade; RCC subtype; tumor necrosis; vascular invasion; and the presence of sarcomatoid features. Fuhrman grade is one of the mostly widely accepted prognostic factors, especially in the CCRCC subtype (32,33). CCRCC is more aggressive than papillary and chromophobe types of RCC (34). Patard *et al* maintain that the histological subtype was a less powerful prognostic factor than the stage of the RCC (35). Tumor necrosis is also a widely accepted prognostic factor in RCC (36). Finally, the clinical prognostic factors include ECOG performance status, patient symptoms, cachexia and anemia (37). Non-classical prognostic factors include certain molecular and genetic markers. The VHL tumor suppressor gene and HIF-1α are well known prognostic factors in RCC; however, some controversy remains regarding their value as prognostic factors (9). Several studies reported that VEGF and carbonic anhydrase 9 (CAIX) levels also have significance in predicting prognosis. VEGF expression is correlated with aggressive behavior and CAIX correlates with an improved prognosis (5). However, certain studies found no significant relationship between the level of CAIX and a more favorable prognosis (38). The positive reactivity of p53 and elevated serum CRP levels also have value as prognostic factors (6,8). However, the most reliable prognostic factor has not been identified until now.

In this study, we demonstrated the value of IL-32 as a prognostic factor in CCRCC via the immunohistochemical staining of surgically resected human CCRCC tissue. Our data revealed that a high IL-32 expression significantly correlated with the recurrence rate. Additionally, the RFS, DSS and OS rates were significantly decreased in the IL-32 high-expression group compared with the IL-32 low-expression group. Based on these findings, we proposed IL-32 to be a potential prognostic factor for CCRCC. To identify the independent prognostic factors affecting the three different survival rates, univariate and multivariate analyses were performed. The Fuhrman nuclear grade and high IL-32 expression were identified as independent risk factors of RFS, DSS and OS in CCRCC patients. Our results revealed that IL-32 may have novel value as a prognostic factor in localized CCRCC patients undergoing nephrectomy. In our study, other classical prognostic factors of the CCRCC cases, such as tumor size, ECOG PS and pathological staging, did not correlate with the survival rates.

Sorrentino *et al*, who investigated IL-32 expression in premalignant and malignant lung samples including squamous and non-squamous cell carcinomas, also studied the correlation of IL-32 expression in tumor-infiltrating leukocytes (TIL) and tumor cells (TC) with microvessel density and 5-year survival rates. They found that IL-32 expression in TILs and TCs correlated with high microvessel density in tumors, and the 5-year survival rate was significantly shorter in the IL-32-positive group compared to the IL-32-negative group in all histotypes. These authors suggested that IL-32 expression was beneficial in predicting the prognosis of lung cancer, with the exception of squamous cell carcinoma, since the expression of IL-32 was more significant in metastatic malignancies (18). Results of the present study showed that a high expression of IL-32 in localized CCRCC cases correlated with shorter survival rates compared to cases with a low expression of IL-32. These findings suggest that IL-32 expression in patients with localized CCRCC indicates a poor outcome even after nephrectomy.

The pathogenic mechanism of IL-32 expression in human malignancy is not yet clear; however, certain authors suggested possible activity of IL-32 as a proinflammatory cytokine involved in precancerous inflammation in pancreas and lung cancers (18,19). Recently, Lee *et al* examined the mechanism by which the high-risk HPV-16 E7 oncogene induced IL-32 expression in cervical cancer cells. These authors investigated IL-32 expression on surgically resected human cervical cancer tissue. Normal cervical epithelium did not express IL-32, as shown by immunohistochemical staining. Their results also showed that COX-2 stimulated IL-32 in response to the HPV-16 E7 oncogene in cervical cancer cells. However, the overexpression of IL-32 inhibited the HPV-16 E7-mediated COX-2 activation pathway and, ultimately, exhibited anti-oncogenic effects (22). In the most recent study on IL-32 and human cancer, Oh *et al* studied the expression of IL-32 in colon cancer and malignant melanoma cells using xenograft nude mice inoculated with IL-32 γ-transfected malignant cells (39). They observed the inhibitory effect of IL-32 γ on the growth of both malignant cell types. This *in vitro* study revealed that the inhibitory effect of IL-32 γ was due to blockade of the NK-κB and STAT3 pathways involved in tumor cell growth. Moreover, IL-32 γ transgenic mice models showed a decreased expression of anti-apoptotic, cell proliferation, and tumor-producing genes such as cleaved *caspase-3* and *-9*, *bax*, *cyclin D*, *COX-2*, and *iNOS*, whereas the expression of their target apoptotic genes such as *BCL-2* and *X-chromosome inhibitor of apoptosis protein* was significantly increased (39). The findings of the two recent studies indicate that IL-32 has anticancer effects in human malignancies. In our study, CCRCC cases that stained strongly for IL-32 exhibited more aggressive behavior. As a result, IL-32 expression in surgically resected human malignancy tissue exhibits a potentially reactive host-immune response to the malignant cells. Thus, IL-32 expression was more intense in the more aggressive cases. Further studies are required to increase understanding of the role of IL-32 in carcinogenesis and cancer progression.

In conclusion, our findings indicate that IL-32 overexpression in localized CCRCC is a novel prognostic factor in surgically treated patients. Clinically, the analyses of IL-32 expression in surgically resected or biopsied specimens may

aid the determination of future treatment plans or prediction of patient prognosis. Additionally, further investigations are required to increase understanding of the pathogenesis of IL-32 in human cancers.

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