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Serum X-Linked Inhibitor of Apoptosis Protein (XIAP) as a Biomarker for Predicting Recurrence of Low-Grade Renal Cell Carcinoma

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Abstract

The X-linked Inhibitor of Apoptosis protein (XIAP) has been associated with cell survival because it blocks caspase-mediated apoptosis. Prognostic significance of XIAP in renal cell cancer (RCC) have rarely been studied. To evaluate the usefulness of serum XIAP levels in RCC patients as a biomarker for predicting recurrence after surgery. B lood samples were obtained from 95 patients (71 males and 24 females; median age, 60.0 years {36-85}) with RCC before surgery (radical or partial nephrectomy). Blood samples were also collected from 55 healthy controls. The serum XIAP levels were measured by ELISA. .The cut-off value was calculated by ROC analysis. Recurrence-free survival was evaluated in all patients

The mean serum XIAP levels in RCC patients were higher than those of normal control individuals (239.8 pg/ml vs. 156.2 pg/ml, P<0.001). At a median follow-up of 48months (3-105 months), tumors with low serum XIAP showed significantly longer recurrence-free survival than those with high serum XIAP in the Grade 1-2 group (n=75) (P<0.05). Serum XIAP level is associated with recurrence and prognosis of RCC patients, especially in patients with the lower nuclear grade of 1 and 2. These results suggest that it may be used as a novel biomarker for predicting prognosis.

Keywords: Biomarker; Renal Cell Cancer; XIAP; Surgery; Serum

List of abbreviations: XIAP: X-linked inhibitor of apoptosis protein; RCC: renal cell cancer; IAP: inhibitor of apoptosis protein; TKI: tyrosine kinase inhibitor; mTOR: mammarian target of rapamycin; IFNα: interferon α; IL-2: interleukin-2; ELISA: enzyme-linked immunosorbent assay; CAIX: Carbonic anhydrase IX; ROC: receiver operating characterisric; RFS: recurrence free survival; CRISPR: clustered regularly interspaced short palindromic repeat

Introduction

Renal cell carcinoma (RCC) is the most common malignancy of the adult kidney. It is estimated that there were 36,000 new cases of RCC in the United States in 2006, with almost 13,000 deaths [1]. One-third of the patients experience local or distant tumor recurrence with radical nephrectomy for localized renal cell carcinoma [2].

The TNM system has served as a standard for predicting prognosis, but its predictive value is not accurate enough for localized cancer [3]. To date, some nomograms based on clinical and pathological parameters have been established for predicting prognosis [4,5]. The molecular markers based on individual tumor behavior should improve patient management after surgery.

Mitochondrial pathways are activated by physiological stress, including that induced by conventional cancer therapies. XIAP (X-linked inhibitor of apoptosis protein) is the most downstream inhibitor of apoptosis and is considered to be the most potent and ubiquitous caspase inhibitor among the members of the IAP (inhibitor of apoptosis protein) family. Eight human IAPs have been reported, namely, X-linked IAP (XIAP), cIAP1, cIAP2, survivin, NAIP, Apollon, Livin, and ILP-2 [6]. XIAP is the best characterized of the IAP family members in terms of its potent caspase inhibitory mechanisms and is considered the prototype of the IAP protein family [7,8]. The translation of XIAP is stimulated under different conditions of cellular stress [9] and the overexpression of XIAP can be an important event in cancer progression and resistance to treatment [10].

We have reported the expression and prognostic value of XIAP in human prostate cancer using prostate tissue microarrays [11]. However, the expression and prognostic significance of XIAP in renal cell cancer (RCC) have rarely been studied. There has been a report about XIAP on tissue studies, but there is no study about serum XIAP.

In this study, we examined the usefulness of the serum XIAP level as a prognostic marker in RCC patients.

Methods

Peripheral blood samples were obtained from 95 patients with primary RCC without metastasis prior to surgery between December 2000 and November 2008 with follow-up of 3 months or more. The tumors were resected by radical nephrectomy. The patient characteristics were showed in Table 1. The patients were histopathologically diagnosed with RCC and comprised 71 males and 24 females, ranging in age from 36 to 85 years (median age, 60.0). Their histological classifications and staging data according to the TNM classification system (International Union Against Cancer, 6th edition, 2002) were (n): T1: 67, T2: 4, T3: 12, T4: 1, N0: 88, M0: 88, Grade 1: 12, Grade 2: 63, Grade 3: 20. Cut-off value was calculated by ROC analysis. Blood samples were also collected from 55 healthy donors without a past history of cancer.

The following data were available: age at diagnosis, histological type, TNM stage, local and systemic therapy, and recurrence-free and overall survival. No tyrosine kinase inhibitor (TKI), mammarian target of rapamycin (mTOR) inhibitor, or immunotherapy (interferon α (IFNα) alone or with interleukin-2 (IL-2)) was administered before recurrence.

The study was approved by the Ethics Committee of Kyoto Prefectural University of Medicine (Approval No. RB-MR-C-2). All subjects have given written informed consent for participation in the study according to Declaration of Helsinki.

Measurement of the level of serum XIAP

Serum was separated by centrifugation of the blood samples and was stored at -80˚C for future enzyme-linked immunosorbent assay (ELISA).

A sandwich ELISA was performed in accordance with the manufacturer's instructions (Invitrogen Corp., Carlsbad, CA, USA) in order to determine XIAP levels in the sera of RCC patients and healthy controls. The XIAP concentration measurements were calibrated against titration curves generated using reference standards. Repeated measurements yielded consistent results.

Table 1: Patients demographics and tumor characteristics

Number	95
Age (range)	61.5 (36-85)
Gender	
Male	71
Female	24
Clinical T stage	
1	73
2	5
3	16
4	1
Histological subtype	
Clear cell	84
Non clear cell	11
Nuclear grade	
1	12
2	63
3	20
Microscopic venous invasion	
+	19
-	76

Statistical methods

The associations between clinicopathological characteristics and serum XIAP were examined by the $\chi 2$ test and *t*-test. Recurrence free survival (RFS) according to serum XIAP was analyzed using Kaplan–Meier curves and the differences of PFS in each category were assessed by the log-rank test. The Cox proportional hazards model was used for both univariate and multivariate analyses. Test results were considered significant at $P < 0.05$. Cut-off value of XIAP was calculated from receiver operating characteristic (ROC) curve analysis. All analyses were performed using JMP 10.0.2 (SAS institute Japan Ltd, Tokyo, Japan.

Results

Serum XIAP concentration in RCC patients

ELISA was used to measure XIAP in sera obtained from healthy controls (n=55) and patients with RCC (n=95). The data was showed in Table 2. The mean serum XIAP level (mean ± SE) in patients with RCC (239.8 ± 28.6 pg/ml) was higher than in healthy controls (156.2 ± 41.1 pg/ml) ($p<0.001$).

We examined the level of XIAP with respect to the stage. In the TI-T2 and T3-4 patients, the mean XIAP levels were 220 ± 24.1 and 318.5 ± 120.8 pg/mL, respectively. There were no significant differences. We also investigated it with respect to the grade. In the Grade 1 and 2 patients, the mean XIAP levels were 292.0 ± 136.7 and 237.7 ± 27.3 pg/mL, respectively. In the G3 patients, the mean XIAP level (161.6 ± 65.0 pg/mL) was higher than in the normal controls, but lower than in the G1/2 patients. There was no significant difference in the grade. High XIAP expression was not directly associated with tumor grade (Table. 2).

Table 2: Correlation analysis of serum XIAP with clinicopathological characteristics

	Serum XIAP value (mean±SE) (pg/ml)	Significance
normal (n=55)	156.2 ± 41.1	
RCC (n=95)	228.8 ±26.6	p<0.001
Tumor stage		
T1-2 (n=78)	216.2±24.1	
T3-4 (n=17)	285.4±97.5	p=0.5113
Tumor grade		
G1(n=10)	315.8±147.5	
G2 (n=65)	230.6±25.5	p=0.5811
G3 (n=20)	156.2±56.7	p=0.3311

Serum XIAP concentration and cancer recurrence

We next examined the association of serum XIAP concentration with tumor recurrence following radical nephrectomy. The cases were then categorized into two groups by the optimal cut-off point determined in ROC analysis of value of serum XIAP. On the basis of the ROC curve, the cut-off value was established as 130. Univariate and multivariative analysis of clinico- and histopathological findings including serum XIAP of all cases are shown in Table 3. Microscopic venous invasion (V (+)) was correlated with RFS (p<0.0001). Serum XIAP was not correlated with RFS (p=0.6056)

Table 3: Cox regression hazards model analysis in all RCC patients

Univaritate				
Parameters	**Criteria**	**Hazard ratio**	**CI of HR 95%**	**Significance**
Age	vs <60 60≤	1.75337	0.75483-4.257487	p=0.1918
Sex	M vs F	1.618086	0.63031734-5.6042534	p=0.361
Stage	vs 3,4 1,2	0.239731	0.103084-0.582689	p=0.0023
Grade	vs 3 1.2	1.291116	0.921548-1.57078	p=0.1116
v factor	(-) v (+) vs v	9.212172	3.934812-22.53133	p<0.0001
Serum XIAP	L vs H	0.800736	0.335802-1.866751	p=0.6056
Multivariate				
Parameters	Criteria	Hazard ratio	CI of HR 95%	Significance
Stage	vs 3,4 1,2	0.442053	0.179094-1.146613	p=0.0909
Grade	vs 3 1.2	1.117405	0.866443-1.335549	p=0.3296
v factor	(-)v(+) vs v	7.131416	2.874289-18.19821	p<0.0001

Then, we analyzed the patients of grade 1-2. The patient characteristics of grade 1-2 were showed in Table 4. The patients were histopathologically diagnosed with G1-2 RCC and comprised 57 males and 18 females, ranging in age from 37 to 81 years (median age, 60.0). Their histological classifications and staging data according to the TNM classification system (International Union Against Cancer, 6th edition, 2002) were (n): T1: 65, T2: 4, T3: 8, T4: 0, N0: 75, M0: 75, Grade 1: 12, Grade 2: 63. Univariate and multivariative analysis of clinico- and histopathological findings including serum XIAP of G1-2 patients were shown (Table 4). Microscopic venous invasion (V (+)) was correlated with RFS (p<0.0001). Also, Serum XIAP was correlated with RFS (p=0.0041).

Table 4: Cox regression hazards model analysis in grade 1-2 RCC patients

Univaritate				
Parameters	**Criteria**	**Hazard ratio**	**95% CI of HR**	**Significance**
Age	≥60 vs <60	1.010572	0601055-1.684427	p=0.9679
Sex	M vs F	1.4113147	0.4309298-6.301306	p=0.5904
Stage	1,2 vs 3,4	0.294361	0.089938-1.313672	p=0.0995
v factor	v (+) vs v (-)	0.131296	0.043282-0.410747	p=0.0009
Serum XIAP	L vs H	0.307582	0.06836-1.017535	p=0.0123

Multivariate				
Parameters	**Criteria**	**Hazard ratio**	**95% CI of HR**	**Significance**
v factor	v(+) vs v(-)	0.078209	0.021376-0.271072	p=0.0001
Serum XIAP	L vs H	1.002249	1.000828-1.003429	p=0.0041

The G1-2 patients were divided into two groups based on the cut-of value. There was a significant difference for them in the RFS for them. RCC with low serum XIAP showed significantly longer RFS than those with high serum XIAP in the Grade 1-2 group (P<0.05) (Figure 1).

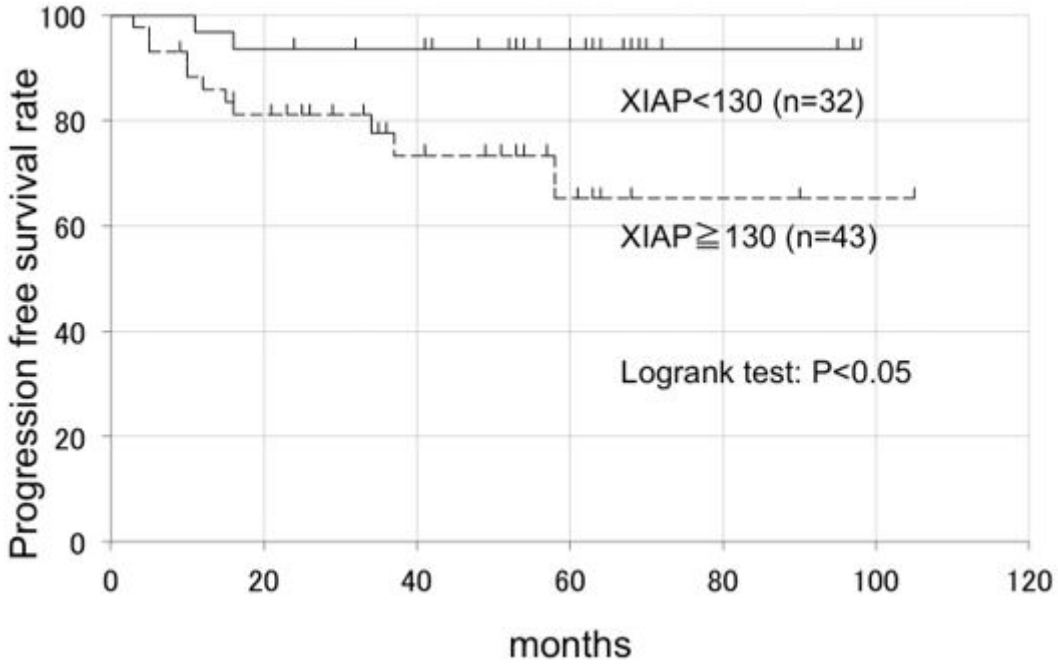

Figure 1: Among the RCC patients, we examined the relapse-free survival in the grade 1-2 group. It was significantly shortened in those with high serum XIAP levels

Discussion

This study demonstrated the significant increase of the serum XIAP levels in RCC patients in comparison to the normal individuals and evaluated its utility as a biomarker for predicting recurrence after surgery in 88 patients.

In RCC, prognostic markers predicting metastasis after surgery remains a major problem. Various clinical and pathological parameters such as tumor size, stage, grade and venous

infiltration have been studied to predict prognosis. Some molecular markers have considered as predictive or prognostic biomarkers. Carbonic anhydrase IX (CAIX) is one of the studied markers in RCC. High CAIX expression in metastatic cases was associated with better disease-specific survival [12], but this was not the case in non-metastatic cases. XIAP is associated with cell survival by blocking caspase-mediated apoptosis. XIAP protein expression has been reported in a number of human cancers, including leukemia [13], lymphoma [14], and tumors derived from prostate [15,16], colon [17], lung [18,19], cervical [20], hepatocellular [21], and vascular cells [22]. XIAP expression examined by tissue micro array was correlated with chemoresistance of primary chemotherapy, and identified as a prognostic marker for clear cell carcinoma of ovary [23].

It has also been shown that XIAP expression levels increased with the progression of RCC [24-26]. In addition, other studies reported that XIAP and Bcl-2 suppression was effective in inducing the apoptosis of RCC [27] and that down-regulation of XIAP expression could enhance the sensitivity of RCC cells to apoptosis [28].

The reports of serum XIAP as a predictive biomarker in cancer were rare. It has been shown that the median serum XIAP level of the patients and the control group showed no significant difference. There was no significant difference in progression-free survival (PFS) (p=0.432, respectively) and overall survival (OS) (p=0.989, respectively) [29]. On the other hand, in the study of the well-differentiated small intestine neuroendocrine tumors, XIAP and some other protein were verified as significant contributors to tumor classification [30].

In this study, we compared the serum XIAP level between normal controls and RCC patients. It was higher in the latter. This is consistent with the results of previous studies. We also investigated the serum XIAP level among RCC patients. It did not increase with malignancy. T classification is based on the tumor diameter, but it is also known that even a small diameter may cause infiltration or metastasis at an early stage. We considered that XIAP may be a new prognostic marker that is different from the tumor diameter. Furthermore, the results suggest that XIAP is a prognostic factor for relapse in grade 1-2 patients. Although XIAP inhibits apoptosis, it may not be involved in promoting metastasis or invasion. Indeed, a genome-wide CRISPR screening at different stages of tumor growth and metastasis in a mouse lung cancer model revealed that factors involving metastasis do not contribute significantly to tumor growth [31].

The usefulness of microscopic venous invasion [32] as a prognostic factor for RCC has been reported. On the other hand, relapse is sometimes detected even in early RCC patients, but no prognostic factor has been clarified. Microscopic venous invasion was a pathological factor examined on resected tumor samples after surgery, but serum XIAP was a parameter obtained before operation. Although there is needed for further study, serum XIAP might be a potential biomarker to determine the necessity of neoadjuvand therapy.

This study has limitations: the number of patients was limited, and the study design was a retrospective study. However, the results suggest that the serum XIAP level is useful for predicting the prognosis of RCC of lower nuclear grade.

In conclusion, the present study suggests that the serum XIAP level may be useful as a prognostic marker in RCC patients, especially in the lower nuclear grade of 1 and 2.

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