

The Usefulness of Serum X-linked Inhibitor of Apoptosis Protein (XIAP) for Predicting Recurrence of Low-Grade Renal Cell Carcinoma

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Abstract

Background: The X-linked Inhibitor of Apoptosis Protein (XIAP) has been associated with cell survival because it blocks caspase-mediated apoptosis. The expression of XIAP and its prognostic significance in Renal Cell Cancer (RCC) have rarely been studied.

Objective: To evaluate the usefulness of serum XIAP levels in RCC patients as a biomarker for predicting recurrence after surgery.

Methods: Peripheral blood samples were obtained from 88 patients (67 males and 21 females; median age, 60.0 years) with RCC before surgery. All of the patients underwent radical or partial nephrectomy. Blood samples were also collected from 52 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.

Results: The mean serum XIAP levels in patients with RCC 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 48 months (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 ($n=75$) ($P < 0.05$).

Conclusion: 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

Abbreviations: XIAP: X-linked Inhibitor of Apoptosis Protein; RCC: Renal Cell Cancer; IAP: Inhibitor of Apoptosis Protein; TKI: Tyrosine Kinase Inhibitor; mTOR: Mammalian Target of Rapamycin; IFN α : Interferon α ; IL-2: Interleukin-2; ELISA: Enzyme-Linked Immunosorbent Assay; CAIX: Carbonic Anhydrase IX; ROC: Receiver Operator Curve; 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. Here, we report the serum XIAP level in RCC patients and healthy individuals and evaluate its significance as a biomarker. The strong association of XIAP expression with renal cell cancer recurrence identifies it as a key molecule for targeted therapeutic investigation.

However, the expression and prognostic significance of XIAP in RCC have rarely been studied. 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 88 patients with primary RCC without metastasis prior to surgery between December 2000 and November 2008 with follow-up of 3 months or more. The

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Received May 19, 2017; **Accepted** May 28, 2017; **Published** June 05, 2017

Citation: Hongo F, Takaha N, Toiyama D, Ueda T, Ito-Ueda S, et al. (2017) The Usefulness of Serum X-linked Inhibitor of Apoptosis Protein (XIAP) for Predicting Recurrence of Low-Grade Renal Cell Carcinoma. J Biomol Res Ther 6: 152. doi:10.4172/2167-7956.1000152

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tumors were resected by radical nephrectomy. The patient characteristics were showed in Table 1. The patients were histopathologically diagnosed with RCC and comprised 67 males and 21 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: 67, T2: 4, T3: 12, T4: 1, N0: 88, M0: 88, Grade 1: 12, Grade 2: 65, Grade 3: 11. Cut-off value was calculated by ROC analysis. Blood samples were also collected from 52 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), Mammalian Target of Rapamycin (mTOR) inhibitor, or immunotherapy (interferon α (IFN α) 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. RBMR-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.

Statistical methods

The associations between clinicopathological characteristics and serum XIAP were examined by the χ^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 $P < 0.05$. All analyses were performed using JMP 10.0.2 (SAS institute Japan Ltd., Tokyo, Japan).

Number	88
Age (range)	60.0 (37-81)
Gender	
Male	67
Female	21
Clinical T stage	
1	69
2	5
3	13
4	1
Histological subtype	
Clear cell	81
Non clear cell	7
Nuclear grade	
1	12
2	63
3	13
Microscopic venous invasion	
+	15
-	73

Table 1: Patient's demographics and tumor characteristics.

Results

Serum XIAP concentrations in RCC patients

ELISA was used to measure XIAP in sera obtained from healthy controls (n=52) and patients with RCC (n=88). The data was showed in Table 2. The mean serum XIAP level (mean \pm SE) in patients with RCC (239.8 \pm 28.6 pg/ml) was higher than in healthy controls (156.2 \pm 41.1 pg/ml) ($p < 0.001$). We examined the level of XIAP with respect to the stage. In the T1-T2 and T3-4 patients, the mean XIAP levels were 220 \pm 24.1 and 318.5 \pm 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 \pm 136.7 and 237.7 \pm 27.3 pg/mL, respectively. In the G3 patients, the mean XIAP level (161.6 \pm 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 3).

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 multivariate analysis of clinic-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 ($p = 0.0536$). 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 multivariate analysis of clinico-and histopathological findings including serum XIAP of G1-2 patients were

	Serum XIAP value (mean \pm SE) (pg/ml)	Significance
Normal (n=52)	156.2 \pm 41.1	-
RCC (n=88)	239.8 \pm 28.6	$p < 0.001$
Tumor stage		
T1-2 (n=74)	220.0 \pm 24.1	-
T3-4 (n=14)	318.5 \pm 120.8	$p = 0.4278$
Tumor grade		
G1 (n=12)	292.0 \pm 136.7	-
G2 (n=65)	237.7 \pm 27.3	$p = 0.5811$
G3 (n=13)	161.6 \pm 65.0	$p = 0.4022$

Table 2: Correlation analysis of serum XIAP with clinicopathological characteristics.

Univariate				
Parameters	Criteria	Hazard ratio	95% CI of HR	Significance
Age	≥ 60 vs. < 60	1.010572	0.601055-1.684427	$p = 0.9679$
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	$p = 0.0041$

Table 3: Cox regression hazards model analysis in grade 1-2 RCC patients.

Number	75
Age (range)	60.0 (37-81)
Gender	
Male	57
Female	18
Clinical T stage	
1	65
2	4
3	6
4	0
Histological subtype	
Clear cell	69
Non clear cell	6
Nuclear grade	
1	12
2	63
Microscopic venous invasion	
+	6
-	69

Table 4: Patients demographics and tumor characteristics in grade 1-2 RCC patients.

shown in 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). 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).

Discussion and Conclusion

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, another study reported that XIAP and Bcl-2 suppression was effective in inducing the apoptosis of RCC [27]. 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) [28]. On the other hand, in the study of the well-differentiated small

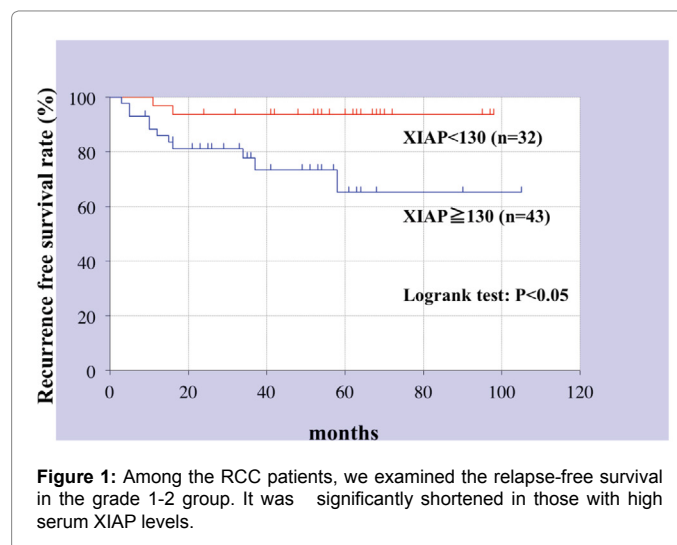


Figure 1: Among the RCC patients, we examined the relapse-free survival in the grade 1-2 group. It was significantly shortened in those with high serum XIAP levels.

intestine neuroendocrine tumors, XIAP and some other protein were verified as significant contributors to tumor classification [29].

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. This suggests that XIAP is closely associated with the relatively early proliferation of RCC, although it is not involved in infiltration or metastasis. 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 [30].

The usefulness of microscopic venous invasion as a prognostic factor for RCC has been reported [31]. 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 neoadjuvant 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.

Acknowledgements

The authors would like to thank Ms. Yukako Morioka for technical assistance.

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Citation: Hongo F, Takaha N, Toiyama D, Ueda T, Ito-Ueda S, et al. (2017) The Usefulness of Serum X-linked Inhibitor of Apoptosis Protein (XIAP) for Predicting Recurrence of Low-Grade Renal Cell Carcinoma. *J Biomol Res Ther* 6: 152. doi:[10.4172/2167-7956.1000152](https://doi.org/10.4172/2167-7956.1000152)

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