Overexpression of XIAP expression in renal cell carcinoma predicts a worse prognosis

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Abstract. X-linked inhibitor of apoptosis protein (XIAP) is the most potent caspase-inhibitory IAP family member and a negative regulator of various apoptotic stimuli. Thus, XIAP overexpression in cancer cells may select for tumor cell survival following various cytotoxic therapeutic modalities. The anatomical staging system in renal cell carcinoma (RCC) currently provides good prognostic information, albeit insufficient. We hypothesize that overexpression of XIAP in RCC may serve as a molecular prognostic marker in RCC and improve the staging of RCC. This study examined the protein level of XIAP in lysates from surgical specimens of 109 patients with RCC and 109 normal kidney specimens from the same patients. The level of XIAP expression was quantified by Western blot analysis using non-fixed fresh frozen tissues of RCCs and normal kidneys. Results indicated that the mean level of XIAP expression was higher in RCC compared to autologous normal kidney, and the XIAP expression level in 38/109 (35%) of RCC was more than 2-fold greater than that in normal kidney tissue. In Stage I/II RCC, the mean XIAP expression level was almost identical to that detected in normal kidney, whereas XIAP expression in Stage III/IV was 2.5-fold higher than that in Stage I/II RCC. Levels of XIAP expression also correlated with the grade of RCC. Patients with RCC with low XIAP expression had a longer postoperative disease-specific survival as compared to those with high expression in the 5-year follow-up. The suggested role of XIAP in the regulation of resistance in apoptosis was examined *in vitro* following treatment of RCC cell lines with XIAP antisense oligonucleotide and the cells were sensitized to both Fas-mediated and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-mediated apoptosis. The present study demonstrates at the protein level that XIAP is overexpressed in RCC, and that high XIAP expression in RCC predicted a worse prognosis. In addition, XIAP antisense oligonucleotide sensitized RCC to Fas/TRAIL-induced apoptosis. These results suggest that XIAP expression in RCC may be used as a prognostic parameter, and that downregulation or inhibition of XIAP expression in RCC may reverse immune resistance.

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Abbreviations: BIR, baculoviral inhibitor of apoptosis protein repeat; DISC, death inducing signaling complex; DIABLO, direct inhibitor of apoptosis protein-binding protein with low pl; IAP, inhibitor of apoptosis protein; mAb, monoclonal antibody; MTT, microculture tetrazolium dye; RCC, renal cell carcinoma; Smac, second mitochondria-derived activator of caspase; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; XIAP, X-linked inhibitor of apoptosis protein

Key words: XIAP, RCC, prognosis, apoptosis, TRAIL, Fas

Introduction

Renal cell carcinoma (RCC) accounts for approximately 2% of all cancer cases worldwide (1). Metastatic disease is often present at the time of diagnosis of RCC and its poor response to chemotherapy and radiotherapy determines its poor prognosis (2). Immunotherapy is relatively effective for RCC, however, the response rate is approximately 20% (3). Therefore, new therapeutic approaches are necessary for these patients with metastatic RCC.

The aggressive stage of cancer is characterized by the appearance of apoptosis-resistant cells as a result of various genetic mutations and overexpression of anti-apoptotic factors. Apoptosis can be achieved by a number of ligand receptor families, commonly called death receptors and also by various drugs or stress-induced stimuli. Death receptors bind to their respective ligands and form the death inducing signaling

complex (DISC) that is followed by a cascade mediating the apoptotic cellular events (4). The key executor of the apoptotic pathway is a group of cysteine proteases known as caspases that are present in the cytosol as inactive zymogens and are proteolytically activated by the appropriate apoptogenic agents (5). The initiator caspases become activated by association of the signaling complex of death receptors or with the apoptosome in the cytosol and trigger the activation of effector caspases. In type I cells, ligand binding to death receptors causes strong activation of caspase-8 which leads to processing and activation of caspase-3 and the subsequent induction of apoptosis. In type II cells, caspase-8 stimulation by DISC formation results in the cleavage of Bcl-2 family member BID. A fragment of BID is translocated into the mitochondria and induces the release of apoptogenic proteins, particularly cytocrome c, second mitochondria derived activator of caspase/direct inhibitor of apoptosis protein (IAP)-binding protein with low pl (Smac/DIABLO), and a serine protease HTR2/OMI. The latter are blockers of caspase inhibitory function of members of the IAP family (6). Cytosolic cytocrome c forms an apoptosome complex with pro-caspase-9 and Apaf-1, which in turn releases active caspase 9, and results in the activation of the executioner caspase-3 (7).

The IAPs are endogenous caspase inhibitors (8). The X-linked IAP (XIAP) is considered the prototype of the IAP family and has been identified as a potent caspase inhibitor (9). The IAPs, originally described in baculovirus (10), function by binding to activated caspases and inhibiting their pro-apoptotic function. The IAPs contain baculovirus IAP repeat (BIR) domains. In addition to BIR domains, several IAPs also contain a RING domain which binds ubiquitin-conjugating enzymes that promote degradation of IAP-caspase complexes (11). Eight human IAPs have been reported, namely XIAP, cIAP1, cIAP2, Survivin, NIAP, Bruce, ML-IAP and ILP-2 (12). All IAPs except NIAP can bind and inhibit activated caspase-3 and 7. In addition, cIAP1, cIAP2 and XIAP can also inhibit the activity of caspase-9 (13).

XIAP is the best characterized member of the IAP family in terms of the caspase inhibitory mechanism. XIAP contains three BIR domains. BIR2 and flanking regions are responsible for binding and potently inhibiting caspase-3 and caspase-7, while BIR3 and flanking regions suppress caspase-9 (8,13). Thus, XIAP has the potential to inhibit active caspases, slow down the process at this step and inhibit apoptosis (14). These findings implied that overexpression of XIAP in tumor cells may render the cells resistant to apoptotic stimuli and may survive following therapy. XIAP is expressed in normal tissues, however, overexpression of XIAP has been demonstrated in several cancers including lung cancer and prostate cancer (15,16). In addition, down-regulation of XIAP sensitizes the resistant cancer cells to death receptor- or cytotoxic drug-induced apoptosis (17,18). These findings suggest that XIAP plays an important role in the regulation of apoptotic responses in cancer cells to both immune- and drug-mediated therapies. The present study was designed to investigate the level of XIAP expression in RCCs compared with its expression in autologous normal kidneys and determine its prognostic significance.

Patients and methods

Patients. Surgical specimens were obtained from 109 patients with RCC. These patients were selected randomly for this study. They included 81 male and 28 female patients, ranging in age from 19 to 83 years. Histologic diagnosis revealed that 99, 9 and 1 patients had clear cell carcinoma, papillary RCC and Bellini duct carcinoma, respectively. Their histologic classification and staging according to TNM classification (UICC, 6th edition, 2002) were: T1 (n=69), T2 (n=9), T3 (n=23), T4 (n=8); N0 (n=105), N1 (n=1), N2 (n=3); M0 (n=94), M1 (n=15); Stage I (n=63), Stage II (n=5), Stage III (n=17), Stage IV (n=24), and G1 (n=13), G2 (n=66), G3 (n=30), respectively. Normal kidney specimens were collected from the same 109 patients with RCC. The paired samples were histologically confirmed to be RCC and normal kidney. The specimens were stored frozen at -80˚C until use.

This study was performed after approval by a local Human Investigations Committee. Informed consent was obtained from each patient.

RCC cell lines. NC65, ACHN and Caki-1 human RCC cell lines (19,20) were maintained in monolayers on plastic dishes in RPMI-1640 medium (Gibco, Bio-cult, Glasgow, Scotland, UK) supplemented with 25 mM HEPES (Gibco), 2 mM L-glutamine (Gibco), 1% non-essential amino acid (Gibco), 100 U/ml penicillin (Gibco), 100 mg/ml streptomycin (Gibco) and 10% heat-inactivated fetal bovine serum (Gibco), hereafter referred to as complete medium.

Measurement of the level of XIAP expression in RCC and normal kidney using Western blot and quantification image analysis. The presence of XIAP protein was determined in cell lysates by Western blot analysis as previously described (21). Briefly, 20 μg of the sample proteins was electrophoresed on 7.5% polyacrylamide gels in Tris-glycin buffer and transferred onto nitrocellulose membranes. The membrane was blocked for 30 min in blocking buffer (5% skim milk in 1% Tween-PBS) and probed with first antibody (anti-XIAP monoclonal antibody (mAb): R&D systems, Minneapolis, MN) for 1 h. After being washed, the membrane was incubated with peroxidase-conjugated goat anti-rabbit IgG and developed with the use of an enhanced chemiluminescence detection kit (Amersham Pharmacia Biotech, Piscataway, NJ). The relative expression of XIAP protein was determined with a chemiluminescence imaging system and quantified by image analysis (Gel Doc 2000: Bio-Rad, Osaka, Japan).

The NC65 cell line constitutively expressed XIAP and was used as internal standard to compare assays. All samples were analyzed at the same time. Repeated measurements yielded identical results. When the level of XIAP expression in RCC samples was >2-fold higher than that found in the autologous normal kidney, it was regarded as high expression. In contrast, when the level of XIAP expression was <2-fold, it was regarded as low expression.

Reagents. Phosphorothioate oligonucleotides (XIAP anti-sense oligonucleotide: 5'-CTG TTA AAA GTC ATC TTC TC-3', scrambled oligonucleotide: 5'-CTT GAT AGA ATC TAC TCT CT-3') were synthesized by ß-cyanoethyl-phospho-

roramidite chemistry using an automated DNA synthesizer (Applied Biosystems, Foster city, CA) (22). Deprotection and purification of phosphorothioate oligonucleotides were carried out according to the protocol described in the user manual. Phosphorothioate oligonucleotides were checked for purity by reverse-phase high-performance liquid chromatography.

Recombinant human tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and anti-Fas mAb (CH-11) were purchased from Peprotech, Rocky Hill, NJ and MBL Co. Ltd., Nagoya, Japan, respectively.

Cytotoxicity assay. Microculture tetrazolium dye (MTT) assay was used to determine tumor cell lysis as previously described (23,24). Briefly, 100 μl of target cell suspension ($2x10^4$ cells) was added to each well of 96-well flat-bottom microtiter plates (Corning Glass Works, Corning, NY), and each plate was incubated for 24 h at 37˚C in a humidified 5% CO_2 atmosphere. After incubation, the supernatant was aspirated and tumor cells were washed three times with RPMI medium, and 200 μl of drug solution or complete medium for control was distributed in the 96-well plates. Each plate was incubated for 72 h at 37˚C. Following incubation, 20 μl of MTT working solution (5 mg/ml, Sigma Chemical Co., St. Louis, MO) was added to each culture well and the cultures were incubated for 4 h at 37˚C in a humidified 5% CO_2 atmosphere. The culture medium was removed from the wells and replaced with 100 μl of isopropanol (Sigma Chemical Co.) supplemented with 0.05 N HCl. The absorbance of each well was measured with a microculture plate reader (Immunoreader, Japan Intermed Co. Ltd., Tokyo, Japan) at 540 nm. Percent cytotoxicity was calculated as follows: Percentage cytotoxicity = [1 - (absorbance of experimental wells/ absorbance of control wells)] x 100.

Statistical analysis. All determinations were made in triplicate. For statistical analysis, Student's t-test was used. Postoperative disease-specific survival was determined by the Kaplan-Meier method. The Cox-Mantel test was used to establish the statistical difference in survival between RCC patients with high and low XIAP expression. In addition, multivariate Cox proportional hazards risk analysis was also used. A $p \leq 0.05$ was considered significant.

Results

Expression levels of XIAP protein in lysates from RCC cell lines, RCCs and normal kidneys. The levels of XIAP protein expression in total cell lysates derived from RCC cell lines, RCC and normal kidney specimens were determined by Western blot analysis as described in Patients and methods. The NC65, ACHN, and Caki-1 RCC cell lines all expressed XIAP, although at different levels (Fig. 1). By comparison, NC65 expressed the highest level of XIAP, while ACHN expressed the lowest level.

The levels of XIAP expression were determined in lysates derived from 109 normal kidneys and 109 RCCs. Representative data of XIAP expression in RCCs and normal kidneys from the same patients are shown in Fig. 2. Clearly, XIAP expression was observed in all normal kidney specimens.

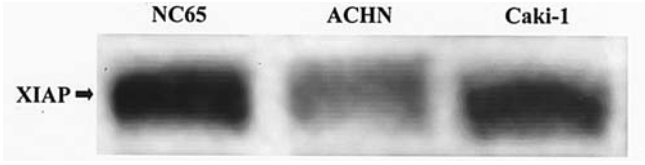

Figure 1. Expression of XIAP in RCC cell lines. XIAP expression in RCC cell lines (NC65, ACHN, Caki-1) was examined by Western blot analysis as described in Patients and methods.

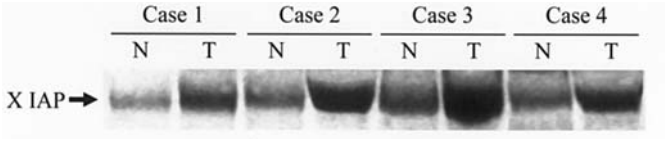

Figure 2. Expression of XIAP in RCCs and normal kidneys. XIAP expression in RCCs and normal kidneys was examined by Western blot analysis as described in Patients and methods. Cases 1-4 are representative cases. XIAP expression in RCCs was higher than that in normal kidneys. N, normal kidney, T, RCC.

Table I. The level of XIAP expression as a function of stage and grade in RCC compared to normal kidney.

RCC (stage/grade)	Ratio of XIAP expression level compared to normal kidney[a] (mean ± SE)
RCC (n=109)	1.61±0.09
Tumor stage	
I/II (n=70)	1.13±0.06
III/IV (n=39)	2.54±0.15[b]
Tumor grade	
1 (n=13)	1.14±0.15
2 (n=66)	1.33±0.08
3 (n=30)	2.51±0.21[c]

[a]Ratio of the level of XIAP expression in RCCs over normal kidneys was examined by Western blot analysis as described in Patients and methods. [b]$p<0.05$ vs. Stage I/II RCC. [c]$p<0.05$ vs. Grade 1, Grade 2 RCC.

Noteworthy, the XIAP expression in normal kidneys in patients with RCC was similar to that in patients with renal pelvic cancer or ureteral cancer (data not shown). By comparison, the level of XIAP expression in the majority of RCCs was higher than that in normal kidneys, as represented in Fig. 2. The ratios of XIAP expression in RCC over normal kidney were determined and the findings are summarized in Table I. The mean ratio of XIAP expression in RCC over normal kidney was 1.6. The mean ratio of XIAP expression in Stage I/II and Stage III/IV RCC compared to normal kidney was 1.1 and 2.5, respectively. The findings observed in the various stages were corroborated with Grades of RCC. Hence, the ratios of XIAP expression in Grade 1, Grade 2 and Grade 3

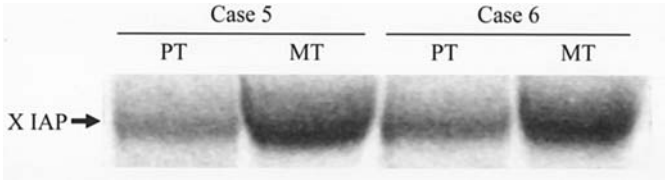

Figure 3. Expression of XIAP in primary and metastatic RCCs. XIAP expression in primary and metastatic RCCs was examined by Western blot analysis as described in Patients and methods. Cases 5 and 6 are representative cases. XIAP expression in metastatic RCCs was significantly higher than that in primary RCCs in these cases. Case 5, bone metastasis; Case 6, bone metastasis. PT, primary RCC; MT, metastatic RCC.

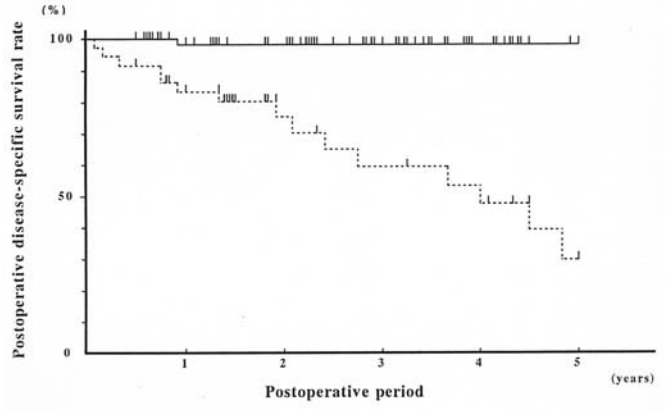

Figure 4. Relationship between XIAP expression and postoperative disease-specific survival in patients with RCC. Postoperative disease-specific survival of RCC patients undergoing radical nephrectomy was determined by the Kaplan-Meier method. Patients with RCC were divided into two groups, namely, those with low XIAP expression and those with high expression. Patients with RCC with low XIAP expression had a longer disease-specific survival as compared to those with high expression in the 5-year follow-up (P<0.01 by Cox-Mantel test). Solid lines, 71 patients with low XIAP expression; interrupted lines, 38 patients with high XIAP expression.

RCCs compared to normal kidney were 1.1, 1.3 and 2.5, respectively.

Preliminary experiments in two patients with metastatic RCC demonstrated that XIAP expression was significantly higher in metastatic RCC compared to that found in primary RCC (Fig. 3). The level of XIAP expression in clear cell RCC was similar to that in papillary RCC (data not shown).

Altogether, these data demonstrate that there was a significant increase of the level of XIAP expression in RCCs as compared to the level found in normal kidneys. Further, the levels of XIAP expression detected correlated with the stage of tumor progression and the increase of the histologic grade of RCC.

Correlation between the level of XIAP expression and post-operative disease-specific survival in patients with RCC. RCC patients undergoing radical nephrectomy were evaluated for the postoperative clinical course. The postoperative disease-specific survival was estimated by Kaplan-Meier analysis. Based on the analysis, patients with RCC were divided into two groups, namely, those with high XIAP expression (>2-fold increase of normal levels of XIAP expression) and those with low expression (<2-fold) as described in Patients and methods. Patients with RCC with low levels of XIAP expression had a longer disease-specific survival as compared to those with high expression in the 5-year follow-up (Fig. 4). Noteworthy, only one patient with RCC with low XIAP expression died during the course of this study, and the expression of XIAP was very high in metastatic tumor (representative case 6, Fig. 3). Multivariate analysis of the data showed that the level of XIAP expression was an independent prognostic factor in patients with RCC (p=0.0368). These findings suggest that the level of XIAP expression in RCC may be a prognostic indicator, and that, more specifically, low XIAP expression in RCC may be a good prognostic sign.

Table II. Enhancement of Fas-mediated cytotoxicity against NC65 cells by XIAP antisense oligonucleotide.

Concentration of XIAP antisense oligonucleotide (μg/ml)	% Cytotoxicity (mean ± SD)[a] Concentration of anti-Fas mAb (ng/ml)			
	0	10	100	1000
0	0	52.6±6.2	63.6±3.8	75.1±9.3
0.1	0.6±0.3	62.7±6.2[b]	71.2±7.4	78.0±8.7
1	1.5±0.3	68.4±7.2[b]	75.0±6.0[b]	82.1±7.2
10	2.9±0.2	70.3±6.4[b]	85.5±3.3[b]	92.7±1.8[b]

[a]The cytotoxic effect of anti-Fas mAb and XIAP antisense oligonucleotide used in combination on NC65 cells was assessed in a 3-day MTT assay. The results are expressed as the mean ± SD of 3 different experiments. [b]Values in the combination treatment are significantly higher than those achieved by treatment with anti-Fas mAb alone plus those with XIAP antisense oligonucleotide alone at p<0.05.

Table III. Enhancement of TRAIL-mediated cytotoxicity against NC65 cells by XIAP antisense oligonucleotide.

Concentration of XIAP antisense oligonucleotide (μg/ml)	% Cytotoxicity (mean ± SD)[a] Concentration of TRAIL (ng/ml)			
	0	1	10	100
0	0	0.9±1.1	3.4±1.2	12.8±2.4
0.1	0.9±0.3	8.7±0.8	12.2±2.0	27.0±4.2[b]
1	1.5±1.2	15.2±1.6[b]	17.9±2.1[b]	31.0±3.2[b]
10	2.5±2.5	19.9±2.0[b]	22.8±3.2[b]	34.6±3.2[b]

[a]The cytotoxic effect of TRAIL and XIAP antisense oligonucleotide used in combination on NC65 cells was assessed in a 3-day MTT assay. The results are expressed as the mean ± SD of 3 different experiments. [b]Values in the combination treatment are significantly higher than those achieved by treatment with TRAIL alone plus those with XIAP antisense oligonucleotide alone at p<0.05.

XIAP regulates tumor cell sensitivity to Fas-mediated and TRAIL-mediated cytotoxicity. Since XIAP expression was upregulated in RCC, we then examined the effect of XIAP antisense oligonucleotide treatment of RCC cell lines upon tumor growth as well as Fas/TRAIL-induced cytotoxicity. XIAP antisense oligonucleotide had no direct effect on the growth of the NC65 RCC cell line (Tables II and III). However, treatment of NC65 cells with XIAP antisense oligonucleotide enhanced Fas-mediated cytotoxicity (Table II). When NC65 cells were treated with a combination of XIAP antisense oligonucleotide and TRAIL, significant potentiation of cytotoxicity and synergy were obtained (Table III). Treatment with XIAP antisense oligonucleotide also sensitized NC65 cells to TNF-α-mediated cytotoxicity (data not shown). In addition, when the ACHN cell line was used as a target, treatment with XIAP antisense oligonucleotide also potentiated Fas/TRAIL-induced cytotoxicity (data not shown).

These findings suggest that high expression of XIAP in RCC may be associated with immune-resistance, and that downregulation of XIAP expression may enhance Fas/TRAIL-mediated apoptosis in RCC.

Discussion

Tumor cells develop several mechanisms to evade cell death by apoptosis. This anti-apoptotic mechanism provides the tumor cells an advantage to escape both host-immune destruction as well as develop resistance to chemotherapeutic and radiation-induced cell death. Therefore, tumor cells that manage to resist apoptotic stimuli can progress and metastasize. Several mechanisms have been reported that underlie tumor cell escape from apoptosis-induced signaling and particularly perturbations in the main apoptotic signaling pathways, namely the death receptor type I and mitochondria type II pathways. Thus, gene products that regulate the apoptotic pathways have been shown to be de-regulated in various cancers and responsible, in large part, for resistance. Among the gene products that regulate apoptosis, the IAP family has been shown to play a major role in several cancers (4). For example, survivin has been shown to be overexpressed in many tumors (25,26) and has been also shown to be a prognostic marker (27,28). The expression and prognostic significance of XIAP, however, has been rarely studied.

Evidence is presented that XIAP expression was up-regulated in RCC, compared with normal kidney, and that the level of XIAP expression positively correlated with both the progression of the stage and the increase of the grade of RCC. Furthermore, this study shows that RCC patients with low XIAP expression had a longer disease-specific survival as compared to those with high expression in the 5-year follow-up. Although the data reported here corresponds to a small number of patients during a short-term follow-up, these findings suggest that XIAP in RCC may play an important role in regulating the malignant potential and apoptosis and may be of prognostic value in RCC.

Our findings here are in agreement with those reported recently by Ramp *et al* (29). These investigators used immunohistochemistry for their analysis, whereas we have examined total protein levels derived from fresh tumor lysates and normal kidneys by Western blot analysis. It is conceivable that detection of XIAP protein by immunohistochemistry may not be measuring the whole XIAP protein but only degraded XIAP products that are recognized by the antibody. The Western blot analysis quantified the XIAP expression levels based on expected molecular weight. In addition, Ramp *et al* primarily examined only clear cell RCC, whereas in our studies we have also analyzed papillary RCC which showed similar patterns to clear cell RCC. Comparable results to RCC were reported for the prognostic significance of XIAP in acute myeloid leukemia (30). Conflicting results have been reported for small cell lung carcinoma (15,31).

The present study has shown that the level of XIAP expression in RCC predicted the clinical outcome. The precise reasons responsible for this relationship remain unclear at present. Since XIAP is an anti-apoptotic molecule, it is reasonable to assume that in spite of treatments, clones of cells which overexpress XIAP can grow more easily and rapidly than those which express XIAP at a low level. In addition, this study has shown that XIAP was overexpressed in metastatic RCC compared to primary RCC. Although more cases need to be examined to confirm it, these findings suggest that XIAP antagonists may provide a therapeutic means of preventing metastasis and growth of RCC.

Immunotherapy including interleukin-2 and interferon-α is relatively effective against metastatic RCC, and the overall response rate of immunotherapy and/or chemotherapy has gradually improved (3,32,33). However, the response rate is approximately 20%, and metastasis and recurrence of RCC still remain major problems in the therapy for RCC (3). Therefore, new therapeutic approaches are required for the patients. Enhanced XIAP expression in RCC compared to normal kidney identifies XIAP as a molecular therapeutic target. Our observation that downregulation of XIAP expression in RCC by antisense oligonucleotide resulted in high sensitivity to Fas/TRAIL-mediated killing may be of potential clinical importance in the management of patients with RCC. The endogenous level of XIAP expression in RCC may be too high to induce apoptosis. Thus, immunotherapy in combination with XIAP antagonists may be a promising strategy against RCC (17,18).

The role of XIAP in the regulation of tumor cell resistance to anticancer chemotherapeutic drugs and immunotherapy has been reported. The XIAP level determines tumor cell sensitivity to various drugs (34) and radiation (9). Also, XIAP levels regulate tumor cell sensitivity to Fas ligand- and TRAIL-induced apoptosis (35,36). The underlying mechanisms responsible for regulating overexpression of XIAP are not clear. Tumor cells have been shown to have high levels of constitutively activated NF-κB and NF-κB has been shown to be implicated in the transcriptional regulation of XIAP. XIAP is also regulated post-transcriptionally (9). XIAP contains a specific ring finger domain which has been shown to promote protein ubiquitination and auto-degradation in a proteasome-dependent manner (9). Smac/DIABLO accelerates XIAP auto-ubiquitination and self degradation and modulates protein expression levels. Noteworthy, we have recently reported in RCC that the expression of Smac/DIABLO is significantly decreased and is an independent prognostic factor for RCC (21). Thus, it is possible that the down-regulation of Smac/DIABLO contributed, in large part, to the stabilization and

high level of XIAP expression in RCC. Current studies are examining whether this correlation is found in the same patients.

Smac/DIABLO was recently identified as a protein that is released from mitochondria in response to apoptotic stimuli (37,38). Smac/DIABLO is able to bind to IAP family members, and XIAP is a predominant Smac/DIABLO binding protein. Smac/DIABLO binds to XIAP, displaces XIAP from caspase-9, promotes cleavage of effector caspases, and induces apoptosis (6,39). In addition, our previous study demonstrated that Smac/DIABLO expression was downregulated in RCC, and that no Smac/DIABLO expression in RCC predicted a worse prognosis (21). Therefore, the measurement of Smac/DIABLO expression as well as XIAP expression may be necessary for an accurate prediction of prognosis in RCC patients and an accurate evaluation of the efficacy of the therapy using XIAP antagonists in combination with immunotherapy.

Cancer therapy using TRAIL or anti-DR4/5 mAb is currently being investigated in clinical trials due to low toxicity to normal tissues (40,41). However, not all cancers respond to TRAIL, and resistance to apoptosis induced by TRAIL has been demonstrated to be overcome by anticancer agents (42,43), XIAP antisense oligonucleotide or small molecule antagonists of XIAP (17,18,22). Thus, analysis of the expression of XIAP in RCC may be helpful for determining therapeutic modalities such as TRAIL therapy and immunotherapy.

In conclusion, this study demonstrated that XIAP expression was upregulated in RCC, and that high XIAP expression was a poor prognostic sign. Furthermore, decreased XIAP expression by antisense oligonucleotide rendered resistant RCC cells sensitive to Fas/TRAIL-mediated cytotoxicity. These findings suggest that the assessment of XIAP expression may be useful in the management of RCC. Since XIAP expression could be used as a prognostic parameter in patients with RCC, an accurate prediction of prognosis may help select patients for more intensive surgical or immunotherapeutic approaches in combination with XIAP antagonists.

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