Expression of X-Linked Inhibitor of Apoptosis Protein Is a Strong Predictor of Human Prostate Cancer Recurrence

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Abstract

Purpose: The X-linked inhibitor of apoptosis protein (XIAP) is associated with cell survival by blocking caspase-mediated apoptosis. We examined the expression patterns of XIAP with regard to human prostate cancer, predicting that XIAP status may predict cancer recurrence and/or clinical outcome.

Experimental Design: Immunohistochemistry was done on tissue microarrays constructed from 226 primary prostate cancer specimen. The protein expression distribution was examined across the spectrum of epithelial tissues and its association with standard clinicopathologic covariates and tumor recurrence was examined in 192 outcome-informative patients.

Results: The mean XIAP expression was significantly higher in prostate cancer compared with prostatic intraepithelial neoplasia (PIN), normal, and benign prostatic hyperplasia. We observed that XIAP is an independent predictor of tumor recurrence in multivariate Cox proportional hazards analysis in all patients as well as after substratifying by Gleason score. Interestingly, patients with high XIAP levels had a much lower probability of tumor recurrence than those with lower XIAP expression. Even patients with high-grade tumors who had higher XIAP levels had a lower risk of recurrence compared with any patient whose tumors express lower XIAP.

Conclusions: XIAP is expressed at higher levels in prostate cancers compared with matched normal tissues. High XIAP expression is strongly associated with a reduced risk of tumor recurrence and is not directly associated with Gleason score, tumor stage, capsular involvement, or preoperative prostate-specific antigen status, suggesting that it is a novel prognosticator and a potential target for prostate cancer diagnosis and therapy. Significantly, these findings provide important and extensive validation of previous results.

Prostate cancer is the most frequently diagnosed malignancy and ranks second among all cancers in men, with an estimated 232,090 new diagnoses and 30,350 deaths in the United States in 2005 (1). Most prostate cancers are clinically localized or regional upon diagnosis, and patients enjoy a 5-year survival rate approaching 100%.[9] Nonetheless, as evidence of the slow but steady nature of this disease, 30% to 40% will experience prostate-specific antigen (PSA) recurrence within 10 years following definitive surgery or radiation treatment (2). Patients with high risk or advanced disease on staging workup, or who have recurred, historically receive treatment with exogenous or endogenous androgen ablation, sometimes supplemented with chemotherapy and/or radiation (3). Unfortunately, progression of tumor cells to therapy resistance inevitably ensues, leaving few alternatives to care. As a result, the median survival in advanced disease is only 18 to 20 months, with an overall survival of 24 to 36 months.

Apoptosis (programmed cell death) is an important mechanism in tissue development, homeostasis, and response to stress factors. It relies on a concerted and tightly balanced signaling pathway involving pro- and antiapoptotic proteins. Dysregulation of apoptosis is a major contributor to tumorigenesis (4, 5), tumor growth (6), progression (4), metastases (7), and resistance to conventional therapies (8).

The mitochondrial pathway is activated by physiologic stress, including that induced by conventional cancer therapies, and is activated by p53 after DNA damage, ultimately resulting in increased mitochondrial membrane permeability and release of

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[9] American Cancer Society, http://www.cancer.org.

a variety of apoptogenic proteins, most notably cytochrome *c*, SMAC/DIABLO, and HtrA2/Omi (9, 10). Cytosolic cytochrome *c* forms an apoptosome complex (11) with procaspase-9 and APAF1, which in turn releases active caspase-9. Like the extrinsic pathway, the intrinsic pathway converges on activation of caspase-3 (12). Tight regulation of caspase activation is required to prevent unchecked cell death. To this end, members of the inhibitors of apoptosis protein (IAP) family provide an intrinsic layer of antiapoptotic regulation. IAPs are an evolutionarily conserved protein family that functions to block cell death by binding to and inhibiting caspases (13, 14).

Eight human IAPs have been reported, namely, X-linked IAP (XIAP), cIAP1, cIAP2, survivin, NAIP, Apollon, Livin, and ILP-2 (15). The X-linked inhibitor of apoptosis, XIAP, is the best characterized of the IAP family members in terms of its potent caspase inhibitory mechanisms and is considered the prototype

Table 1. Relationship of XIAP protein expression with clinicopathologic parameters in prostate adenocarcinomas

	All patients	Mean XIAP expression (SE)	*P* (XIAP: continuous variable)*	Low XIAP intensity ≤1.8 (% of total)	High XIAP intensity >1.8 (% of total)	*P* (XIAP: dichotomized variable) †
Total cases (*N* = 192)		1.28 (0.041)		158 (82)	34 (18)	
Age at surgery						0.26 (NS)*
Median (range)	65 (46-76)			65 (46-76)	63.0 (50-75)	
Mean	63.8			64.0	63.0	
Gleason score			0.99 (NS)			0.31 (NS)
2-6	112 (58)	1.28 (0.055)		89 (56)	23 (68)	
7-10	80 (42)	1.27 (0.063)		69 (44)	11 (32)	
Pathology pT stage ‡			0.63 (NS)			0.21 (NS)
PT2-pT3a	158 (82)	1.28 (0.046)		127 (80)	31 (91)	
PT3b	34 (18)	1.24 (0.092)		31 (20)	3 (9)	
Lymph node status (*n* = 190)			0.47 (NS)			>0.99 (NS)
Positive	11 (6)	1.12 (0.202)		9 (6)	2 (6)	
Negative	179 (94)	1.29 (0.042)		147 (94)	32 (94)	
Surgical margins			0.36 (NS)			0.55 (NS)
Positive	62 (32)	1.22 (0.076)		53 (34)	9 (26)	
Negative	130 (68)	1.30 (0.049)		105 (66)	25 (74)	
Capsular involvement			0.016§			0.11 (NS)
No invasion	40 (21)	1.10 (0.094)		34 (21)	6 (18)	
Invasion	113 (59)	1.38 (0.052)		88 (56)	25 (73)	
Extension	39 (20)	1.16 (0.090)		36 (23)	3 (9)	
Organ confined‖			0.15 (NS)			0.15 (NS)
Yes	100 (52)	1.33 (0.058)		78 (49)	22 (65)	
No	92 (48)	1.22 (0.058)		80 (51)	12 (35)	
High risk¶ (*n* = 190)			0.62 (NS)			0.28 (NS)
Yes	38 (20)	1.24 (0.090)		34 (22)	4 (12)	
No	152 (80)	1.29 (0.046)		122 (78)	30 (88)	
PreOpPSA, ng/mL (*n* = 172)						0.80 (NS)*
Median (range)	9.2 (0.6-96.5)			9.8 (0.6-76.0)	8.9 (3.2-96.5)	
Mean	14.0			14.0	14.0	
<10	87 (51)	1.31 (0.063)	0.74 (NS)			0.48 (NS)
≥10	85 (49)	1.31 (0.061)				
Recurrence**			0.082 (NS) ††			0.0010 ††
Yes	69 (36)	1.18 (0.059)		67 (42)	2 (6)	
No	123 (64)	1.33 (0.055)		91 (58)	32 (94)	
Overall follow-up ‡‡ (mo)						
Median (range)	78.5 (0.1-182.0)			74.0 (0.1-182.0)	88.5 (6.0-152.0)	
Mean	74.5			72.4	84.2	0.085 (NS)*
Total follow-up§§ (mo)						
Median (range)	48.5 (0.1-163.0)			41.0 (0.1-163.0)	87.0 (6.0-152.0)	
Mean	52.3			46.1	81.3	<0.0001*

**P* value was determined by the Mann-Whitney *U* test unless otherwise specified.
† *P* value was determined by the Pearson χ^2 test with Yates continuity correction unless otherwise specified.
‡ pT3b indicates seminal vesicle invasion. There are no pT4 cases.
§ *P* value was determined by the Kruskal-Wallis test. With capsular involvement as a continuous variable, *P* = 0.45 using the Spearman correlation corrected for ties.
‖ No capsular extension and/or seminal vesicle and/or lymph node involvement. Margins are negative.
¶ High-risk seminal vesicle and/or nodal positivity.
** Recurrence PSA elevation raising >0.2 ng/mL status post-radical prostatectomy.
†† XIAP mean intensity association with recurrence by logistic regression of continuous data; (*P* = 0.082; 0.63; 95% confidence interval, 0.37-1.06), and of dichotomized data (*P* = 0.0010; 11.78; 95% confidence interval, 2.73-50.88). XIAP expression was the independent variable.
‡‡ Overall follow-up time from primary surgery to last PSA follow-up.
§§ Total follow-up time to recurrence to last follow-up in nonrecurrence.

of the IAP protein family (14, 16, 17). Abundant XIAP protein expression has been reported in a number of human cancers, including leukemia (18, 19), lymphoma (20), and tumors derived from prostate (4, 7, 21, 22), colon (23), lung (24, 25), cervical (26), bladder (4), hepatocellular (27), and vascular cells (28).

Here, we report that XIAP is elevated in prostate cancer and prostatic intraepithelial neoplasia (PIN) and is an independent predictor of cancer recurrence. Significantly, our results validate and greatly expand upon results by Krajewska et al. (4), showing a similar pattern. This finding provides further evidence that XIAP expression produces a counterintuitive direct association between expression and favorable clinical outcome implicating an as-yet undetermined set of coregulated mechanisms in this disease model. Nonetheless, the strong associations of XIAP expression to prostate cancer recurrence identifies it as a key molecule for targeted therapeutic investigation.

Materials and Methods

Patients. The study cohort consisted of 226 randomly selected hormone-naive patients who underwent radical retropubic prostatectomy between 1984 and 1995 as previously described (29–31). All prostate tumors were staged according to the 1997 American Joint Committee on Cancer tumor-node-metastasis staging system (32) and histologically graded using the Gleason scoring system (33). All cases were of the histologic type "adenocarcinoma, conventional, not otherwise specified" (34). Of the 226, 192 were informative for both recurrence outcomes and marker expression data. Table 1 shows the clinicopathologic data for this cohort.

Prostate tissue microarray construction. Formalin-fixed, paraffin-embedded archival tumor specimens were obtained from the University of California at Los Angeles Department of Pathology under Institutional Review Board approval. Case material was reviewed for tissue array construction by a study pathologist (D.S.). At least three core tissue biopsies (each 0.6 mm in diameter) were taken from morphologically representative regions of each prostate tumor and precisely arrayed as previously described (28–30). Tumor samples were accompanied by matching benign (morphologically normal or hypertrophic) and *in situ* neoplastic lesions (PIN), when available. Case material was arrayed into three tissue microarray (TMA) blocks. For staining, sections (5 μm) were transferred to glass slides using an adhesive slide system (PSA-CS 4, Instrumedics Inc.) to support cohesion of the array elements.

Immunohistochemistry. Immunohistochemical staining was done using an affinity-purified polyclonal rabbit anti-human/mouse XIAP antibody (R&D Systems, Inc.; Immunogen: aa 244-263 of human XIAP). A standard two-step indirect avidin-biotin complex (ABC) method was used (Vector Laboratories) as previously described (29, 30). PC-3 cells were used as a positive staining control for XIAP and were prepared as previously described (29). As a negative assay control, pooled nonimmune rabbit immunoglobulin G was applied at the same concentration as the anti-XIAP antibody.

Scoring of immunohistochemistry. Semiquantitative assessment of antibody on the TMAs was done by a study pathologist (H.Y.) blinded to the clinopathologic variables. The TMA was spot checked by a second pathologist (D.B.S.) for consistency of scoring. The target tissue for scoring was the glandular prostatic epithelium; scoring of benign tissues did not include basal cells. Tissue spot histology and grading were confirmed on the counterstained study slides. XIAP cytoplasmic expression was scored using two measures, intensity on a 0 to 3 scale (0 = negative, 1 = weakly positive, 2 = moderately positive, 3 = strongly positive) and percentage of positively stained target cells (range, 0-100% positive) staining at each intensity. To better represent overall protein levels, we combined the frequency and the intensity measures into an integrated intensity using the following formula: (% staining at intensity 3) × 3] + [(% staining at intensity 2) × 2] + [(% staining at intensity 1) × 1/100. To represent expression within cases, the mean pooled integrated intensity of the invasive tumor spots was used.

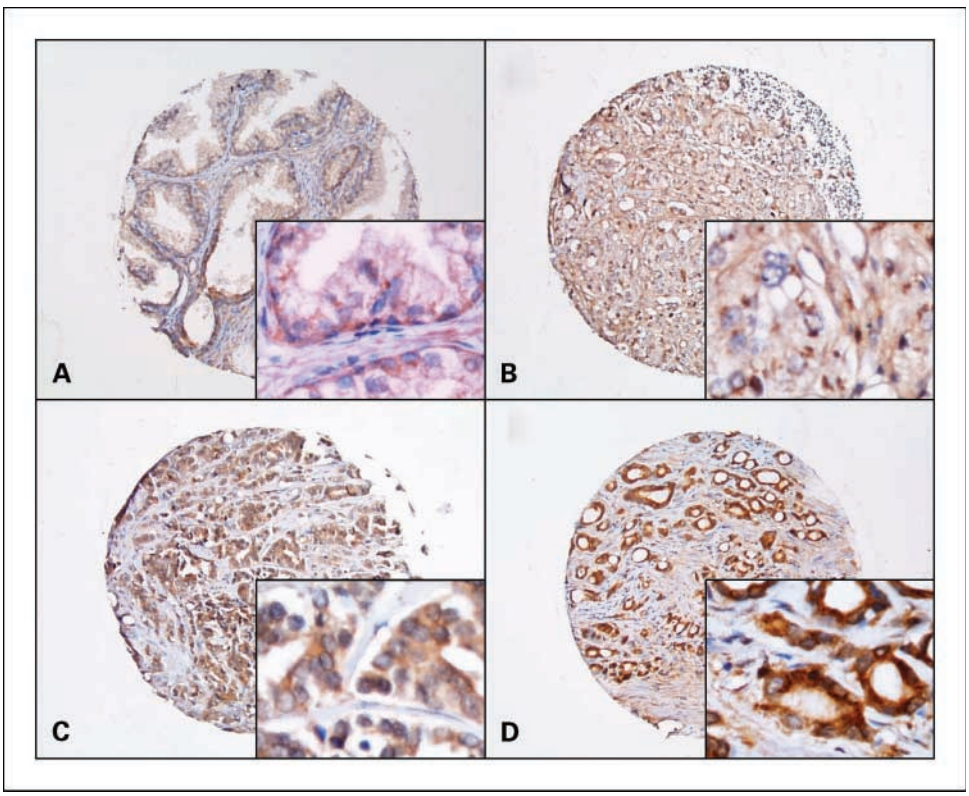

Fig. 1. XIAP protein expression in morphologically normal prostate and prostate cancer on tissue microarrays. Immunohistochemical staining for XIAP protein is seen on representative prostate tissue samples. *A,* normal tissue showing weak cytoplasmic epithelial staining of glandular cells. Staining in basal cells is frequently higher than that seen in glandular cells; scoring is from glandular cells. Invasive prostate cancers are shown demonstrating weak (*B*), moderate (*C*), and strong (*D*) cytoplasmic staining. Magnification, 100×, with 400× inserts.

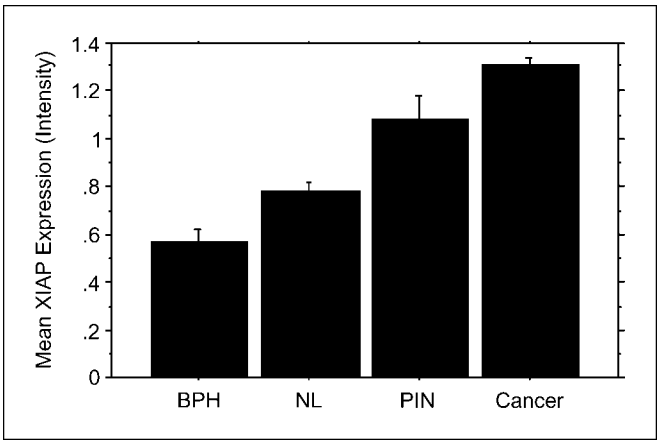

Fig. 2. XIAP protein expression distribution on the prostate tissue microarray stratified by histologic category. The intensity of XIAP protein expression in cells staining by immunohistochemistry as seen in 1,107 informative tissue microarray spots containing benign prostatic hyperplasia (*BPH*; $n = 122$), morphologically normal prostate (*NL*; $n = 252$), prostatic intraepithelial neoplasia (*PIN*; $n = 48$), and invasive prostate cancer (*Cancer*; $n = 685$) are shown as mean bar graphs. The mean XIAP expression was significantly higher in cancer (intensity = 1.32) compared with PIN (intensity = 1.08; $P = 0.019$), normal (intensity = 0.78; $P < 0.0001$), and BPH (intensity = 0.57; $P < 0.0001$). XIAP expression in PIN was significantly higher than normal ($P = 0.010$) and BPH ($P < 0.0001$), and expression in normal epithelium was significantly higher than that seen in BPH ($P = 0.0006$). The Mann-Whitney *U* test was used for two-group comparisons. Bars, 1 SE.

Statistical analysis. The Kruskal-Wallis and Mann-Whitney *U* tests were used to determine the significance of XIAP protein expression differences between categorical clinicopathologic prognostic variables. Associations of XIAP expression with continuous covariates were tested with the Spearman correlation. We used the Pearson χ^2 test to examine the association of dichotomized XIAP expression groups versus categorical variables. Recurrence was defined as a rising total PSA >0.2 ng/mL status post-prostatectomy, and time to recurrence was calculated from the date of the primary surgery. Patients without recurrence at last follow-up were censored. Kaplan-Meier plots were used to visualize recurrence-free time distributions, and the log-rank test was used to test for differences between them. We determined the optimal cut-point for dichotomized XIAP expression data using recursive partitioning, regression trees (rpart package), and plotting log-rank *P* values versus hazard ratios as previously described (35–37). An integrated intensity value of 1.8 gave a maximum hazard ratio and a minimal *P* value.

To assess which covariates associate with recurrence-free time, we fit both univariate and multivariate Cox proportional hazards regression models. The proportional hazards assumption was verified using Schoenfeld residuals (38). All *P* values were two-sided, and $P < 0.05$ was considered significant. All statistical analyses were done using R statistical software[10] and StatView version 5 (SAS Institute Inc.).

Results

XIAP protein expression in human prostate tissues. Using immunohistochemical techniques, we examined XIAP expression in human prostate tissue samples. Expression of XIAP in human prostate tissue was observed in the normal and malignant glandular epithelium, basal cells, and occasionally in stromal fibromuscular cells (Fig. 1). The human prostate cancer cell line, PC3, was used as a positive control for XIAP expression (data not shown). XIAP is typically expressed diffusely in the cytoplasm, but occasionally, discrete supranuclear staining in coarse clusters is additionally seen (Fig. 1).

[10] http://www.r-project.org/

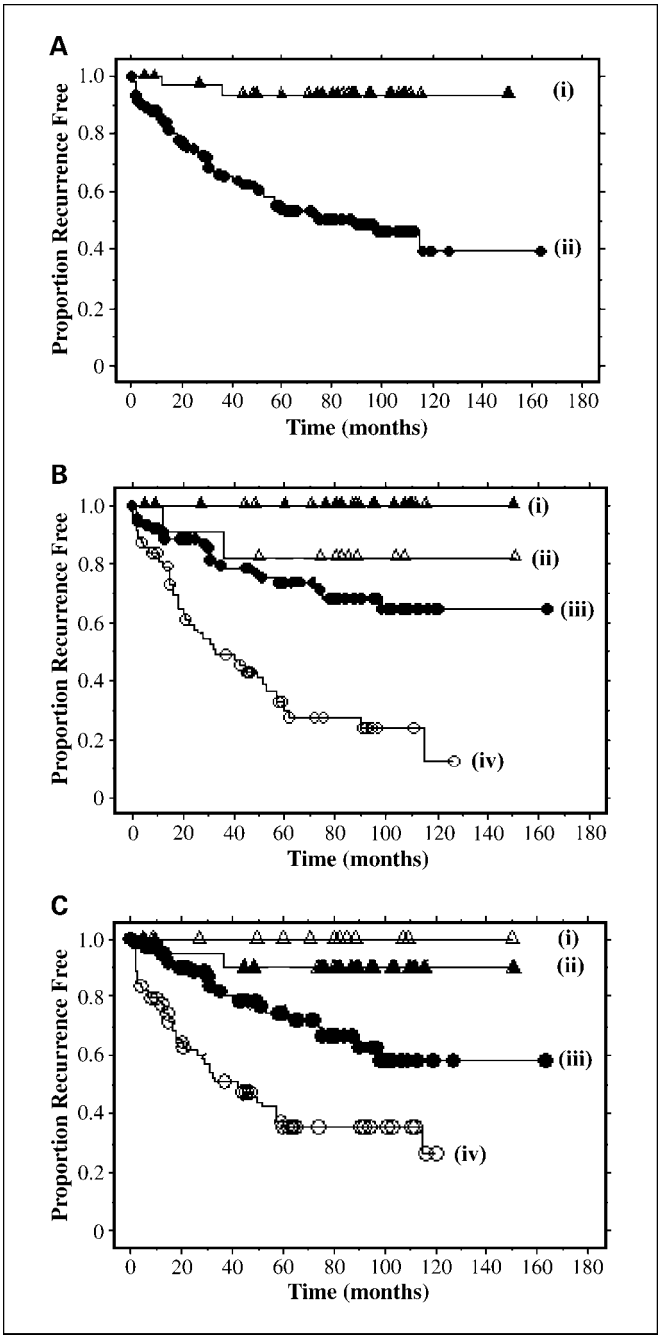

Fig. 3. Kaplan-Meier curves for time to prostate cancer recurrence. The high cytoplasmic XIAP expression phenotype is consistently associated with a lower risk of developing recurrent prostate cancer. For all figures, XIAP expression intensities of >1.8 and ≤1.8 are considered high and low XIAP, respectively. *A,* Kaplan-Meier curves for time to tumor recurrence stratified by cytoplamsic XIAP protein expression status ($n = 192$ patients) are seen in all patients. (*i*) High XIAP expression ($n = 34$); (*ii*) low XIAP expression ($n = 158$). Log-rank $P < 0.0001$. *B,* Kaplan-Meier curves in patients stratified by tumor grade. Gleason scores of 7 to 10 and 2 to 6 are considered high and low grade, respectively. (*i*) High XIAP, low grade ($n = 23$); (*ii*) high XIAP, high grade ($n = 11$); (*iii*) low XIAP, low grade ($n = 89$); (*iv*) low XIAP, high grade ($n = 69$). Log-rank $P < 0.0032$ for (*ii*) versus (*iv*). Log-rank $P < 0.0001$ for (*iii*) versus (*iv*) and for (*i*) versus (*iv*). There is no statistically significant difference between (*ii*) and (*iii*). *C,* Kaplan-Meier curves in patients stratified by their disease being organ confined with negative surgical margins ("confined") versus "not confined" (capsular extension and/or seminal vesicle involvement and/or lymph node involvement). (*i*) High XIAP, not confined ($n = 12$); (*ii*) high XIAP, confined ($n = 22$); (*iii*) low XIAP, confined ($n = 78$); (*iv*) low XIAP, not confined ($n = 80$). Log-rank $P < 0.041$ for (*ii*) versus (*iii*). Log-rank $P < 0.0001$ for (*ii*) versus (*iv*), (*i*) versus (*iv*), and (*iii*) versus (*iv*). For all figures, censored times are marked by either circles or triangles.

Table 2. Cox proportional hazards analysis for time to PSA recurrence

Variable	Univariate* (all patient†, *n* = 192)	Multivariate* (all patients‡, *n* = 172)		Univariate* (low Gleason§, *n* = 112)
		Continuous	Dichotomized	
Gleason score >7	<0.0001 3.70 (2.23-6.11)	0.0011 2.81 (1.51-5.24)	0.0014 2.80 (1.49-5.26)	NA
Seminal vesicle invasion (stage = pT3b)	<0.0001 4.10 (2.47-6.81)	0.0035 2.46 (1.35-4.51)	0.0032 2.46 (1.35-4.47)	0.0065 5.52 (1.61-18.89)
Capsular invasion	0.0038 1.73 (1.19-2.52)	0.019 1.67 (1.09-2.57)	0.036 1.55 (1.03-2.35)	0.014 2.21 (1.17-4.16)
Preoperative PSA	0.015 1.02 (1.00-1.03)††	0.60 1.00 (0.99-1.02)	0.70 1.00 (0.99-1.02)	0.024 1.04 (1.01-1.07)‡‡
XIAP intensity (continuous)‖‖	0.033 1.54 (1.04-2.29)	0.077 1.49 (0.96-2.33)	NA	0.028 2.20 (1.09-4.44)
XIAP intensity ≤1.8 (dichotomized)¶¶	0.0010 10.69 (2.61-43.73)	NA	0.0025 8.92 (2.16-38.86)	****

*P value; hazard ratio; (95% confidence interval) provided.
†64% of cases are censored.
‡67% of cases are censored.
§Gleason score 2 to 6; 79% of cases are censored.
‖Gleason score 2 to 6; 83% of cases are censored.
¶Gleason score 7 to 9 (no Gleason score 10 cases are present); 43% of cases are censored.
**High XIAP group has no events (all patients are censored).
††n = 172.
‡‡n = 103.
§§n = 69.
‖‖Pooled mean XIAP intensity. Used formula (3 − continuous XIAP intensity) to reverse hazard ratio to compare directly to other covariates. A high XIAP carries a reduced risk of recurrence.
¶¶Pooled mean XIAP intensity dichotomized: ≤1.8 (n = 158); >1.8 (n = 34).

Basal cells in normal glands are frequently stained more strongly than the glandular cells. Our scoring of benign epithelium was limited to these glandular cells.

We examined the XIAP expression distribution stratified by histologic category (Fig. 2). Notably, XIAP is elevated in prostate cancer versus matching benign tissues; this increase can be seen starting in PIN lesion. Regions of benign prostatic hyperplasia (BPH) showed the lowest expression. The intensity of XIAP staining are shown in Fig. 2. The mean XIAP expression was significantly higher in cancer (intensity = 1.32) compared with PIN (intensity = 1.08; P = 0.019), normal (intensity = 0.78; P < 0.0001), and BPH (intensity = 0.57; P < 0.0001). In addition, XIAP expression in PIN was significantly higher than normal (P = 0.010) and BPH (P < 0.0001), and expression in normal epithelium was significantly higher than that seen in BPH (P = 0.0006). We found no significant difference in XIAP expression when broken down by tumor grade or Gleason score (data not shown).

XIAP expression and cancer recurrence. We next examined the potential association XIAP protein expression with tumor recurrence following radical prostatectomy. Recurrence data were available for 192 XIAP-informative cases. Case-level expression was derived by pooling the mean integrated intensities of the spots as previously reported (38). Supervised survival tree analysis was applied to pooled data, and a dichotomized population was defined with an optimal cut-point of 1.8 mean integrated intensity representing individuals with higher versus lower XIAP expression. Specifically, an expression intensity of >1.8 was considered "Higher XIAP expression", and ≤1.8 was considered "Lower XIAP expression".

We examined the association of XIAP as either a continuous or dichotomized variable with established prognostic factors and found that expression of XIAP was associated with disease recurrence (Table 1). Figure 3A shows a Kaplan-Meier estimate of cancer recurrence-free time stratified by XIAP expression. Significantly, the median recurrence-free time was 75 months for cases with low XIAP, compared with >152 months for cases with high XIAP (P < 0.0001).

Cox proportional hazards analyses were done for established prognostic factors and time to PSA recurrence (Table 2). Of particular note is the strength of XIAP predictive power as a dichotomized variable, which was higher in all cases than the conventional prognosticators. Higher XIAP expression predicted a reduced risk of tumor recurrence both as a continuous (P = 0.033) and a dichotomized (P = 0.0010) variable in univariate analysis. The dichotomized XIAP remains highly significant in multivariate analysis in this category (P = 0.0025), as well as after substratifying by Gleason score (P = 0.010 for high-grade cases). Significantly, in patients with primary low-grade cancer, no individuals who had high levels of XIAP had tumor recurrence (n = 23). In contrast, 26% of individuals with low-grade cancer who had low levels of XIAP had tumor recurrence (n = 89). Figure 3B shows XIAP expression further substratified by Gleason score, and Fig. 3C shows XIAP expression further substratified by whether or not the tumor is organ confined. Significantly, higher XIAP portends a good outcome regardless of the grade or organ confinement status; patients with higher grade or non–organ-confined tumors with higher XIAP expression do better as a group than any patient whose tumors express

Table 2. Cox proportional hazards analysis for time to PSA recurrence (Cont'd)

Multivariate* (low Gleason‖, *n* = 103)		Univariate* (high Gleason¶, *n* = 80)	Multivariate* (high Gleason¶, *n* = 69)	
Continuous	**Dichotomized**		**Continuous**	**Dichotomized**
NA	NA	NA	NA	NA
0.037	**	0.0086	0.012	0.0089
4.07 (1.09-15.20)		2.21 (1.22-3.98)	2.36 (1.21-4.60)	2.45 (1.25-4.80)
0.0049	**	0.42	0.52	0.69
3.08 (1.41-6.73)		1.23 (0.75-2.04)	1.20 (0.69-2.09)	1.11 (0.66-1.86)
0.011	**	0.95	0.84	0.67
1.04 (1.01-1.08)		1.00 (0.98-1.02)§§	1.00 (0.98-1.02)	1.00 (0.98-1.02)
0.17	NA	0.25	0.19	NA
1.85 (0.77-4.43)		1.33 (0.82-2.17)	1.42 (0.84-2.41)	
NA	**	0.011	NA	0.010
		6.37 (1.54-26.43)		6.61 (1.57-27.89)

low XIAP, even those of low grade or that are organ confined (Fig. 3B and C).

Of note, the high predictive value of XIAP in the specific substrata described above generate subgroups in which 100% of the population was without tumor recurrence (Fig. 3B and C). Because of this, no Cox *P* values can be calculated in these statistical models. However, Table 3 shows how effectively XIAP stratification can isolate low-recurrence groups in all patient substrata examined. For example, in patients whose tumors were not organ confined ($n = 92$), 50% experienced disease recurrence. However, within this group, none of the 12 patients with high XIAP expression tumors experienced recurrence.

Discussion

The IAP family member XIAP is the strongest direct inhibitor of caspases and is therefore a significant downstream anti-apoptotic protein. Aberrant expression of XIAP has been implicated in the pathology of a number of human cancers; however, few large-scale *ex vivo* studies have been done, and fewer provide translational associations of XIAP expression levels to clinical outcomes.

In support of the role of XIAP as an apoptosis inhibitor, we find that the level of XIAP expression is higher overall in prostate cancer as compared with matched benign tissues, with an intermediate expression observed in PIN. These findings are in agreement with other studies, suggesting that XIAP helps to promote tumor cell survival. Pathologically elevated XIAP levels have been found in a number of hematologic (19, 20, 40–42), vascular (28), and epithelial (4, 23–25, 27) malignancies, as well as in most cell lines of the NCI-60 tumor screening panel (40, 43). Only rare exceptions to this pattern have been reported (26).

We further examined the potential association of XIAP expression with clinicopathologic parameters. Paradoxically, when dichotomized optimally, lower levels of XIAP expression were a strong predictor of recurrence, whereas higher expression strongly predicted a substantially reduced risk of recurrence. In fact, XIAP generated a larger hazard ratio (i.e., stronger predictive power) than those seen from conventional prognostic indicators, including Gleason score, tumor stage capsular invasion, and preoperative PSA. As demonstration of its predictive power, patients with high-grade metastatic tumors and high XIAP had a lower risk of recurrence than patients with low-grade nonmetastatic tumors and with low XIAP. Strikingly, no patients with low-grade tumors plus high XIAP levels had tumor recurrence. In contrast, more than 25% of patients with low XIAP expression experienced recurrences. Despite having a longer overall PSA follow-up, 94% of all patients with high XIAP expression were recurrence-free at the end of follow-up, versus 58% of patients with low XIAP tumors. These findings,

Table 3. Prostate cancer recurrence status in patient groups and substratified by XIAP protein expression category

Patient group	Total count (*n*)	Total % censored*	Low XIAP† % censored (count, *n*)	High XIAP† % censored (count, *n*)
All patients	192	64	58 (158)	94 (34)
Low grade‡	112	79	74 (89)	100 (23)
High grade	80	43	36 (69)	82 (11)
Organ confined§	100	77	73 (78)	91 (22)
Not confined	92	50	42 (80)	100 (12)

*Proportion of patients who reach the end of PSA follow-up without evidence of recurrence. Recurrence = PSA elevation raising >0.2 ng/mL status post–radical prostatectomy.
†Pooled mean XIAP intensity dichotomized: low ≤1.8; high >1.8 on a 0 to 3 scale.
‡Low grade = Gleason score of 2 to 6; high grade = Gleason score of 7 to 9 (there are no cases of Gleason 10 in this cohort).
§Organ confined = no capsular extension and/or seminal vesicle and/or lymph node involvement. Margins are negative.

coupled to the lack of direct association with any of the clinicopathologic variables tested, shows the independence XIAP and its widespread applicability as a prognostic indicator.

Our current study confirms the work of Krajewska et al., who also found that high levels of XIAP were associated with a reduced risk of recurrence in prostate cancer patients (4). The importance of independent validation for tumor biomarkers cannot be overemphasized because such verification is an absolute requirement for differentiating biomarkers, which have the potential to be meaningful clinical predictors from those that demonstrate merely idiosyncratic expression (44–46). In addition, such validation studies are also critical to minimize overfitting of statistical data. Therefore, that the predictive power of XIAP was observed in two separate and independent patient populations is highly significant.

The results shown here not only validate the findings of Krajewska et al., but it also extends their work (4). To our knowledge, our study is the largest study to date examining the association of XIAP protein to clinical outcomes. Moreover, the patient cohort for clinical outcomes in the aforementioned study (4) consisted of needle core biopsies from 64 $T_2N_0M_0$ radiation-treated patients. Here, we provide an expanded and unrelated patient population on tissue microarrays to include 192 informative patients with a spectrum of disease stages. The only other major difference between the two studies is that our results suggest that XIAP is an independent predictor of outcome, whereas Krajewska et al. found a significant inverse correlation of XIAP with preoperative PSA level; they offered this as a potential link to the positive outcome seen in high XIAP-expressing patients.

XIAP expression in other malignancies. The association of high XIAP expression with a positive clinical outcome is counterintuitive to expectations that IAPs promote tumor cell survival. Nevertheless, some recent studies of lung cancer have shown that increased levels of XIAP are associated with an improved prognosis (4, 47). For example, Ferriera et al. (47) found that higher levels of XIAP correlated with longer survival in early-stage non–small cell lung cancer (NSCLC) patients. Surprisingly, the same group found that XIAP was not associated with survival in advanced NSCLC (24).

Conversely, several studies have shown a negative association of XIAP levels to outcomes (cancer recurrence/remission and/or death) in other types malignancies. For example, XIAP expression was found in 95% of clear cell renal cell carcinomas (48). A significant increase was observed from well to poorly differentiated tumors. Tamm et al. and Carter et al. (18, 40–42) found that in patients with acute myelogenous leukemia, higher levels of XIAP correlated with a slightly shorter remission durations and a decreased survival time. Several other studies failed to find associations between XIAP levels and survival, including those focusing on colon (23), cervical (26), and bladder cancers (4); the latter two studies also noted a lack of association of XIAP with tumor grade and stage.

Potential mechanism of action. The observation that XIAP is elevated in primary prostate tumor cells, yet also high levels ultimately predict a lower probability of tumor recurrence, is intriguing. There are a number of possible explanations for these observations. XIAP has been reported to mediate cell cycle arrest via down-regulation of cyclins A and D1 and induction of cyclin-dependent kinase inhibitors p21Cip1/Waf1 and p27Kip1 (28). Thus, although XIAP may provide a selective antiapoptotic survival advantage, it may simultaneously impair the proliferation of cancer cells. It is possible that these two properties function with some degree of independence.

XIAP is itself regulated by antagonists such as SMAC/DIABLO, which is released from the mitochondria upon apototic stimuli (12, 49–51). Recent studies have shown that the relative proportion of XIAP compared with SMAC/DIABLO is the factor that dictates life versus death decisions. Therefore, it is possible that the high levels of XIAP expression seen in our study are counteracted by higher levels of anti-IAPs. We are currently exploring this possibility.

Finally, as is the case with all studies involving immunohistochemistry on archival paraffin-embedded sections, the overall activity of XIAP cannot be assessed. Whether XIAP functions differently in a progressing tumor cell and/or interacts with alternate target molecules in an evolving malignant cell is an intriguing possibility that warrants further study.

Malignant prostate cancer remains a disease with few useful outcome measures and no current consistently effective therapies. Therefore, informative biomarkers are urgently needed to guide patient surveillance and clinical intervention. This study reports the overexpression of XIAP in primary human prostate cancers and provides strong evidence for its beneficial prognostic association.

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