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ABSTRACT

Objective

To assess the association of Survivin expression with clinicopathological features and biochemical recurrence (BCR) after radical prostatectomy (RP) in a large multi-institutional cohort.

Methods

Survivin expression was evaluated by immunohistochemistry on a tissue microarray of RP cores from 3117 patients. Survivin expression was considered altered when at least 10% of the tumor cells stained positive. Association of altered Survivin expression with BCR was evaluated using Cox proportional hazards regression models.

Results

Survivin expression was altered in 1330 (42.6%) patients. Altered expression was associated with higher Gleason score on RP (p=0.001), extracapsular extension (p=0.019), seminal vesicle invasion (p<0.001) and lymph node metastases (p=0.009). Median follow up was 38 months (IQR 21-66). Patients with altered Survivin had a shorter BCR free survival than those with normal expression (5-year BCR free survival estimates: 74.7% vs 79.0%, p=0.008). Altered Survivin, however did not retain its prognostic value when adjusted for the effect of established clinico-pathological factors (p=0.73). Subgroup analyses also showed no independent prognostic value of Survivin.

Conclusions

Survivin expression is commonly altered in RP patients. Altered Survivin expression is associated with clinicopathologic features of biologically and clinically aggressive PCa. Survivin expression was associated with BCR only in univariable analysis, limiting its value in daily clinical decision making.

Introduction:

With an estimated 220 800 new cases and 27 540 deaths in 2015, prostate cancer (PCa) is the most common noncutaneous malignancy in the USA[1]. Radical prostatectomy (RP) remains, with radiation therapy, the standard treatment for localized PCa providing durable cancer control [2]. However, almost one third of the patients will experience biochemical recurrence (BCR) after these treatments. Early identification of these patients could allow adjuvant therapy which may lower the recurrence rate [3]. To provide a personalized approach, risk assessment and predictive tools have been developed [4]. These models mostly rely on clinico-pathological features and fall short of optimal performance to date. Identification of new prognostic factors that could capture an individual's tumor potential could enhance current tools.

Survivin protein, a member of the inhibitors of apoptosis family, is implicated in the regulation of cell proliferation and apoptosis. Its expression has been extensively studied in various cancers making it a promising biomarker and, increasingly, target for therapy [5]. For example, in urothelial carcinoma of the bladder, Survivin has been shown to predict cancer recurrence and survival after radical cystectomy [6, 7]. To date, only two small single-center studies reported a prognostic value for Survivin in PCa[8, 9].

Therefore, as a part of the evaluation of any potential prognostic factor[10], our objective was to determine the value of Survivin as a prognostic marker for BCR in a large multi-institutional cohort of patients treated with RP.

Patient and methods:

Patient selection and data collection

All participating sites obtained an institutional-review-board approval for the study and provided institutional data sharing agreements prior to the initiation of the study. The initial study cohort comprised 3294 patients from eight European and North American centers with PCa treated with RP between 2000 and 2011, who had tumor specimens available. We excluded patients with preoperative PSA > 50 ng/ml, missing preoperative PSA, surgical margin status, lymph node status, and RP Gleason score from the analysis: 3117 patients were considered for analysis. No patient had metastases at the time of RP nor received preoperative radiotherapy, hormonal treatment, or chemotherapy.

Pathological evaluation and immunochemistry

All surgical specimens were processed according to standard pathologic procedures. Genito-urinary pathologists assigned pathologic stage and grade according to the 2007 American Joint Committee on Cancer (AJCC) tumor, node, and metastasis (TNM) staging system and International Society of Urological Pathology, respectively. Lymphatic tissue removed during RP was submitted for histological examination. Lymphovascular invasion (LVI) was defined as the presence of tumor cells or tumor emboli within endothelial spaces without affecting the underlying muscular wall. Tumor cells in contact with the inked surface of prostatectomy specimen defined positive surgical margins.

Survivin immunohistochemical staining was performed on tissue microarray slides in a single laboratory. Immunostaining was done on the DAKO Autostainer (DAKO

North America) using an anti-Survivin polyclonal rabbit antibody (Novus Biologicals) at a dilution of 1:100. We used bright-field microscopy imaging coupled with advanced color detection software (Automated Cellular Imaging System; Clarient) for staining assessment. No differentiation in the intensity or the localization of the staining was considered. Survivin expression was classified "altered" when more than 10% of the cells expressed Survivin, as previously described [7, 8]. This 10% cut-off was chosen according to a previous study that demonstrated 10% reactivity was an indicator for PSA recurrence after surgery[8].

Follow-up

Follow-up (FU) was performed according to institutional protocols in agreement with local guidelines at the time. Generally, patients were seen postoperatively quarterly for the first year, semiannually in the second year, and annually thereafter. BCR was defined as a PSA value >0.2 ng/ml on two consecutive visits. The date of BCR was attributed to the day of the first PSA. None of the patients received radiotherapy or hormone treatment before BCR.

Statistical analysis

Association of Survivin expression with categorical variables was assessed using χ^2 test. Differences in continuous variables were analyzed with Kruskal-Wallis tests. Kaplan–Meier method was used to generate BCR free survival curves. Survival curves were compared using the log-rank test. Univariable and multivariable Cox regression models addressed the association of Survivin with BCR after RP. All p values were two-sided, and statistical significance was defined as a $p<0.05$.

Statistical analyses were performed using Stata 11.0 statistical software (Stata Corp., College Station, TX, USA).

Results

Survivin expression was altered in 1330 (42.6%) patients. Clinico-pathological characteristics of overall cohort and associations with Survivin expression are shown in Table 1. Altered Survivin expression was associated with higher Gleason score on RP (p=0.001), extracapsular extension (p=0.019), seminal vesicle invasion (p<0.001), and lymph node metastases (p=0.009).

Within a median follow-up of 38 (IQR 21-66) months, 617 (19.8%) patients experimented BCR. Patients with altered Survivin expression have worse BCR free survival than those with normal expression of Survivin (p=0.008, Fig.1). Table 2 summarizes uni and multivariable Cox regression analyses. In univariable analyses, preoperative PSA, pathological Gleason sum, extracapsular extension, seminal vesicle invasion, surgical margin status, lymph node metastases, and altered Survivin expression were all significantly associated with BCR. In a model that adjusted for the effects of standard pathological features, Survivin did not retain its association with BCR. In subgroup analyses, Survivin did not reach association with BCR (Table 3).

Discussion

We assessed the association of Survivin expression with pathological features of PCa and its value for prognosticating BCR in a large and multi-institutional RP cohort.

We found that more than one third of the tumors in the studied cohort expressed Survivin. This expression was associated with established features of biologically and clinically aggressive PCa such as higher Gleason score on RP and lymph node metastasis. In various cancers, Survivin expression has been already associated with adverse pathological features[6, 11, 12]. Indeed, Survivin is known to regulate tumor cell proliferation[13] and apoptosis [5, 14]. Basic research has already accumulated evidence regarding the anti-apoptotic role of Survivin in PCa cell lines[15]. Kishi et al assessed the levels of mRNA Survivin in PCa specimens and reported that Survivin expression was associated with adverse pathological features such as higher pathological T stage, lymph node metastasis, vessel invasion, positive surgical margin and high Gleason score[16]. At the protein level, our results are in line with previous studies that reported a significant association between Survivin overexpression and high Gleason scores [8, 17].

This association with several adverse pathological features in PCa is of great interest but does not ensure, however, that Survivin has any prognostic value. Nevertheless, prediction of recurrence and metastases spread remains one crucial issue in PCa. In the present study, we demonstrated that Survivin expression was associated with BCR in univariable analysis. However, when adjusted for standard prognostic factors, Survivin was no longer associated with impaired outcomes. These findings are similar to those previously observed in a smaller cohort[8]. In another study, Zhang et al distinguished nuclear and cytoplasmic expression of

Survivin in 68 and 65 patients, respectively[9]. These patients were part of the RTOG 8610 study cohort, and received either radiation therapy plus short term androgen deprivation or radiation therapy alone. Immunostainings were performed in PCa diagnostic needle biopsies or transurethral resections. In this study, only nuclear expression was independently associated with Cancer Specific Survival and Overall Survival. Surprisingly, nuclear expression did not predict distant metastasis however. Although the number of patients is low, this study supports the potential need for subcellular discrimination of Survivin expression, especially in PCa. Indeed, Survivin may be responsible for different functions according to its expression in the cell: promotion of cell proliferation in the nucleus and control of cell survival in the cytoplasm [18, 19]. Several studies have already reported the prognostic value of cytoplasmic-to-nuclear ratio of Survivin in breast cancer [5]. Nevertheless, these and our results highlight the limited value of easy and readily available immunohistochemical analysis of Survivin expression in Pca.

New methods to assess Survivin expression in malignancies have however been proposed. In breast cancer, several studies demonstrated Survivin expression in circulating tumor cells was associated with adverse clinicopathological features and may have prognostic relevance regarding recurrence[20, 21]. In bladder cancer, the detection of Survivin protein and mRNA in the urine has been also proposed as a diagnostic and prognostic marker[6, 22]. Finally, Survivin expression may also be assessed in the plasma and considered as a blood marker. Indeed, high Survivin serum level at diagnosis has been reported as a prognostic factor of poor outcomes in pancreatic and ovarian carcinomas [23, 24]. Khan et al. already demonstrated that, compared to healthy controls, levels of Survivin expression in plasma exosomes were higher in PCa patients[25]. These new methods to assess Survivin expression in

urine, plasma or circulating tumor cells are promising but warrant, however, further investigations to identify any prognostic value in PCa.

We have to mention several limitations regarding the present study. First limitation is inherent to any retrospective study. Second relates to the use of immunohistochemical techniques. These techniques may limit the reliability and reproducibility of the results since discrepancies exist regarding the antibodies, technical and scoring protocols. However, tissue micro-array, readily available staining protocols and automated scoring systems based on bright-field microscopy imaging coupled with advanced color detection software were used to limit any variation at each step of the process. Accordingly, we did not discriminate between nuclear and cytoplasmic Survivin expression. Finally, we only assess early BCR in this cohort with a short follow up. However, we assume that in PCa, early BCR should be considered a reliable endpoint associated with the risk of metastasis and cancer specific mortality [26].

Conclusion

Altered Survivin expression is associated with worse clinicopathologic features in PCa patients treated with RP. However, compared to standard post-operative features, altered Survivin expression is not an independent predictor of BCR after RP. Assessment of Survivin expression by standard immunostaining thus appears of limited value in clinical practice.

Ethical standards

This study has been approved by the appropriate ethics committee.

Conflict of interest

The authors declare that they have no conflict of interest.

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Table 1. Association of Survivin expression and clinicopathologic characteristics in 3117 patients treated with radical prostatectomy for prostate cancer.

	All patients	Normal Survivin	Altered Survivin	p values
Total n (%)	3117	1789 (57.4)	1328 (42.6)	
Age				0.74
Median (range)	62 (58-67)	62 (58-67)	62 (58-67)	
Preoperative PSA				0.85
Median ng/ml(range)	7 (6-10)	7 (6-10)	7 (6-11)	
RP Gleason sum (n %)				0.001
6	1,094 (35.1)	679(38)	415 (31.2)	
7	1,821 (58.4)	1007 (56.2)	814 (61.3)	
8	133 (4.3)	69 (3.9)	64 (4.8)	
9	69 (2.2)	34 (1.9)	35 (2.6)	
Lymph node status n (%)				0.009
pN0	3,044 (97.7)	1758 (98.3)	1286 (96.8)	
pN+	73 (2.3)	31 (1.7)	42 (3.2)	
Lymphovascular invasion n (%)				0.29
no	2,793 (89.6)	1612 (90.1)	1181 (88.9)	
yes	324 (10.4)	177 (9.9)	147 (11.1)	
Extracapsular extension (n %)				0.019
no	2,292 (73.5)	1344 (75.1)	948 (71.4)	
yes	825 (26.5)	445 (24.9)	380 (28.6)	
Seminal vesicle invasion (n %)				<0.001
no	2,887 (92.6)	1684 (94.1)	1203 (90.6)	
yes	230 (7.4)	105 (5.9)	125 (9.4)	
Positive surgical margin (n %)				0.23
no	2,597 (83.3)	1503 (84)	1094 (82.4)	
yes	520 (16.7)	286 (16)	234 (17.6)	

RP: radical prostatectomy

Table 2 Univariable and multivariable Cox regression analyses for prediction of biochemical recurrence in 3117 patients treated with radical prostatectomy for prostate cancer with survivin status (overall analysis)

	Univariable			Multivariable, a		
	HR	95 % CI	p value	HR	95 % CI	p value
Preoperative PSA	1.06	1.05 - 1.07	<0.001	1.04	1.03 - 1.05	<0.001
RP Gleason sum	2.65	2.40 - 2.92	<0.001	1.61	1.42 - 1.82	<0.001
Extracapsular extension	4.59	3.91 - 5.38	<0.001	2.41	1.99 - 2.91	<0.001
Seminal vesicle invasion	6.48	5.38 - 7.80	<0.001	1.66	1.32 - 2.08	<0.001
Positive surgical margin	3.21	2.72 - 3.79	<0.001	1.96	1.65 - 2.33	<0.001
Lymph node metastasis	11.75	9.11 - 15.17	<0.001	2.33	1.71 - 3.18	<0.001
Survivin	1.24	1.06 - 1.45	0.008	1.03	0.88 - 1.21	0.73

CI confidence interval, HR hazard ratio, RP radical prostatectomy

a. Multivariable Cox regression adjusted for PSA value, RP Gleason score, lymph node involvement, positive surgical margins, extracapsular extension, seminal vesicle invasion, and survivin status.

Table 3 Multivariable Cox regression analyses for prediction of biochemical recurrence according to Survivin status in subgroups of patients treated with radical prostatectomy

Patient subgroup	Multivariable		
	HR	95 % CI	p value
No lymph node involvement, a	1.05	0.88 - 1.24	0.60
Lymph node involvement, a	0.90	0.52 - 1.55	0.71
Negative surgical margins, b	1.03	0.84 - 1.27	0.77
Positive surgical margins, b	1.04	0.79 - 1.35	0.82
Locally advanced disease (pN0 and Stage pT3a/b pN0), c	1.09	0.88 - 1.35	0.43
Localized disease (pN0 and no Stage pT3a/b pN0), c	1.06	0.83 - 1.34	0.62
RP Gleason sum < ou = 6, d	0.91	0.61 - 1.35	0.65
RP Gleason sum = 7, d	1.04	0.85 - 1.27	0.72
RP Gleason sum > ou = 8, d	1.20	0.82 - 1.74	0.35

CI confidence interval, HR hazard ratio, RP radical prostatectomy

a Multivariable Cox regression adjusted for PSA value, RP Gleason score, positive surgical margins, extracapsular extension, seminal vesicle invasion.
b Multivariable Cox regression adjusted for PSA value, RP Gleason score, lymph node involvement, extracapsular extension, seminal vesicle invasion
c Multivariable Cox regression adjusted for PSA value, RP Gleason score,
d Multivariable Cox regression adjusted for PSA value, lymph node involvement, positive surgical margins, extracapsular extension, seminal vesicle invasion

Figure 1. Biochemical recurrence free survival estimates according to the expression of Survivin expression in the cohort of 3117 patients treated with radical prostatectomy.