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THE PROGNOSTIC VALUE OF SURVIVIN IN LOCALLY ADVANCED PROSTATE CANCER: A STUDY BASED ON RTOG 8610

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Abstract

Purpose—We examined the prognostic value of nuclear and cytoplasmic Survivin expression in men with locally advanced prostate cancer who were enrolled in Radiation Therapy Oncology Group (RTOG) protocol 8610.

Methods and Materials—RTOG 8610 was a phase III randomized study comparing the effect of radiotherapy (RT) plus short-term androgen deprivation (STAD) with RT alone. Of the 456 eligible cases, 68 patients had suitably-stained tumor material for nuclear Survivin analysis and 65 patients for cytoplasmic Survivin.

Results—Compared to patients with nuclear Survivin intensity scores ≤ 191.2 , those with intensity scores > 191.2 had significantly improved prostate cancer survival (hazard ratio (HR) = 0.45, 95% confidence intervals (CI) = 0.20–1.00, $p = 0.0452$). On multivariate analysis, nuclear Survivin intensity scores > 191.2 were significantly associated with improved overall survival (HR = 0.46, 95% CI = 0.25–0.86, $p = 0.0156$) and prostate cancer survival (HR = 0.36, 95% CI = 0.16–0.84, $p = 0.0173$). On univariate analysis, compared to patients with cytoplasmic Survivin integrated optical density ≤ 82.7 , those with integrated optical density > 82.7 showed a significantly increased risk of local progression (HR = 2.49, 95% CI = 1.03–6.01, $p = 0.0421$).

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Conclusions—Nuclear overexpression of Survivin was associated with improved overall and prostate cancer survivals on multivariate analysis, while cytoplasmic overexpression of Survivin was associated with increased rate of local progression on univariate analysis in patients with locally advanced prostate cancer treated on RTOG 8610. Our results may reflect the different functions of Survivin and its splice variants, which are known to exist in distinct subcellular compartments.

Keywords

Survivin; Prostate cancer; Radiotherapy; Prognostic factor

INTRODUCTION

A growing body of evidence indicates that Survivin is a unique member of the inhibitor of apoptosis (IAP) family. First, Survivin is structurally different from other IAPs in that it contains only one copy of the Baculovirus IAP Repeat (BIR) domain, and an extended COON-terminal α -helix, but no RING finger or other identifiable domain¹. Second, unlike the other IAPs, Survivin possesses a sharp differential expression in cancer versus normal tissues². Survivin is strongly expressed in embryonic and fetal organs^{3, 4}, but is undetectable in most terminally differentiated tissues¹. Moreover, it has been described to be selectively expressed in most common human neoplasms^{5–12}, including prostate cancer^{13–17}. Its expression in cancer cells is associated with cancer progression and prognosis. Third, Survivin seems to exist in two subcellular pools: cytoplasmic and nuclear¹⁸. Consistent with the multiple subcellular localizations, Survivin is involved in both regulation of cell death and control of cell division¹⁹. Fourth, four splice variants of human Survivin have been identified: Survivin-2 α , Survivin-2 β , Survivin-3 β , and Survivin- δ Ex3^{20–25}. The wild type Survivin contains 3 introns and 4 exons. Survivin-2 α and Survivin-3 β are predicted to be truncated forms with Survivin-2 β possessing an additional alternative exon 2 and Survivin- δ Ex3 omitting exon 3. The corresponding proteins, which display marked changes in their structure at the level of the BIR domain, are characterized by a different anti-apoptotic potential.

Survivin is a potential target in prostate cancer therapy. Survivin is not normally expressed in prostatic secretory epithelial cells, but is strongly expressed in prostate cancer cells¹⁵. Furthermore, Survivin has been associated with established features of biologically aggressive prostate carcinoma, such as higher Gleason scores and metastases to regional lymph nodes^{16, 17, 26}. Our laboratory has identified Survivin as a potentially important mediator of resistance to chemotherapy and antiandrogen therapy in human prostate cancer. Using human prostate cell lines and xenograft models, we have demonstrated that Survivin mediates paclitaxel-resistance in prostate cancer cells. The inhibition of Survivin sensitizes prostate cancer cells to paclitaxel-induced apoptosis through a caspase-dependant mechanism *in vitro* and *in vivo*¹³. Furthermore, our preclinical data indicates that the upregulation of Survivin plays a major role in resistance to antiandrogen therapy in prostate cancer cells. During androgen withdrawal, AR-independent upregulation of Survivin via IGFR1/AKT signaling confers resistance to antiandrogen therapy in prostate cancer cells¹⁴.

Given these important findings, we proceeded to evaluate Survivin as a prognostic marker in locally-advanced prostate cancer using specimens from Radiation Therapy Oncology Group (RTOG) protocol 8610. RTOG 8610 is a phase III prospective trial that randomly assigned patients with locally advanced prostate cancers (T2–T4) without evidence of distant metastasis to receive goserelin (3.6 mg) every 4 weeks and flutamide (250 mg) three times a day for 2 months before radiation therapy and during radiation therapy or radiation therapy alone²⁷. The study opened on April 15, 1987 and closed on June 1, 1991 with a total of 471 patients; 456 of the patients were analyzed (15 were excluded for the following reasons: 4 had no follow-up, 5 had too small tumor, 3 refused all treatment and follow-up, 1 had lung primary, 1 had

bone metastases, and 1 had benign disease). Results from the trial demonstrated a significant reduction in local progression and a prolongation of progression-free survival for the patients receiving neoadjuvant hormonal therapy. Given that Survivin isoforms can exist in distinct subcellular compartments and that isoform-specific antibodies have not demonstrated high reliability when used for immunohistochemistry in formalin-fixed tissues, we examined the prognostic value of both nuclear and cytoplasmic Survivin expression using an antibody sensitive to all Survivin isoforms in men with locally advanced prostate cancer enrolled in RTOG 8610.

MATERIALS AND METHODS

Study population

For this analysis, a subset of patients entered in RTOG 8610 who had sufficient pathologic material available was studied. Diagnostic material (from needle biopsies or transurethral resections) was reviewed centrally for 461 (98%) of the 471 patients, and the tumors were graded according to the Gleason criteria. Tissue blocks were requested from participating institutions (>100) at the time of central pathology review for all cases that were reviewed. During the early years of the study, prostate-specific antigen (PSA) evaluation on entry was not available. The protocol was later revised to include PSA determinations. Posttreatment PSA values during follow-up were available on all but 52 patients, and a total of 4,456 determinations were analyzed. The median number of PSA values per patient after initial treatment was 10.5. Pretreatment serum PSA determinations were available for less than 15% (10/68) of the eligible patients and hence PSA was not used as an outcome parameter in this study.

Immunohistochemical technique

Sections were deparaffinized and dehydrated. Microwave unmasking of antigens was performed for 75 minutes in Target Retrieval Solution (Dako, Carpinteria, CA, USA) at 93.0 °C followed by cooling for 20 minutes. Endogenous peroxide was subsequently blocked with peroxidase-blocking solution (Dako) for 15 minutes, followed by washing for 5 minutes with phosphate-buffered saline (PBS). The sections were incubated overnight at 4.0 °C with polyclonal anti-Survivin antibody (Novus Biologicals, Littleton, CO, USA) diluted by antibody diluent (Dako) at 1:300. They were then washed three times in PBS for 5 minutes each and incubated for 30 minutes with labeled-polymer conjugated second antibody (envision+ kit, Dako). They were washed and developed with 3,3'-diaminobenzidine tetrahydrochloride for 2 minutes, lightly counterstained with hematoxylin, dehydrated and mounted.

Formalin-fixed paraffin-embedded tumor samples were analyzed using the ACISTM (Clariant Chromavision Inc., San Juan Capistrano, CA, USA). The analysis was performed by Z.M. and L.-Y.K., who have performed image analysis on RTOG 8610 samples prior to this study after initially reviewing the cases with pathologists^{28–30}. The slides were scanned at 10× objective to give a pixel-to-tissue area ratio of 1:1 (1 pixel: 1 micron²). Using the Threshold Tool, brown and blue pixels were masked in each slide to derive the tumor tissue area and exclude all white areas. The analyses and threshold settings were performed separately for nuclear and cytoplasmic staining. Where possible, at least 6 regions of tumor cells were gated either by freehand around each tumor gland or by using a 40× circle if more than 90% of the region of interest contained tumor cells. Where there was heterogeneity in staining, the tumor area with the highest intensity of staining was chosen.

Nuclear Survivin mean index % and intensity score were calculated by the proprietary software. The Nuclear mean index % was the number of brown pixels divided by the total number of pixels (brown plus blue) in a gated region, taken as a percentage. The nuclear intensity score

was the average brown intensity derived from all brown pixels included in a gated region and is scored in arbitrary units using a grayscale of 0–255.

The integrated optical density of gated pixels and intensity score were used to quantify the cytoplasmic Survivin staining. The integrated optical density has been shown to accurately represent the amount of antigen present in an IHC sample³¹. As prostate tumor glands vary in size, the integrated optical density was standardized by dividing by the tumor tissue area. The cytoplasmic intensity score was the average brown intensity derived from all brown pixels included in a gated region and is scored in arbitrary units using a grayscale of 0–255.

Definition of end points

The event for overall survival was defined as death due to any cause. The following is considered an event in assessing prostate cancer survival: death documented as due to prostate cancer, death due to complications of treatment, or death from unknown causes with active malignancy (clinical disease relapse). Local failure was defined as an increase in tumor size of more than 50% by digital rectal exam for cases where complete tumor regression did not occur, or recurrence of a palpable nodule when there was complete regression or a positive biopsy of the prostate after two or more years of follow-up. Distant metastasis was defined as any clinical evidence of distant disease outside the pelvis.

Statistical analysis

The analysis was performed using the 456 eligible and assessable patients with follow-up. Of these patients, 68 had nuclear Survivin determinations and 65 had cytoplasmic Survivin determinations. Nuclear Survivin mean index % and Survivin intensity score were used for the nuclear Survivin analysis, while the integrated optical density and the intensity score for the cytoplasmic analysis. Each of these variables was modeled as a continuous variable as well as a dichotomized variable in Cox proportional hazards models to identify the impact on overall survival, prostate cancer survival, distant metastasis, and local progression.

Statistical comparisons to assess whether missing Survivin data were dependent on pre-treatment characteristics, treatment arm, or outcome were carried out using the Chi-square test and the Cox proportional hazards model. The five-year estimates for overall and prostate cancer survival were calculated using the Kaplan-Meier method³² and the cumulative incidence method³³. Finally, a multivariate Cox proportional hazards model was used to identify the impact of Survivin on overall survival, prostate cancer survival, distant metastasis, and local progression in the presence of other covariates. Correlation between cytoplasmic and nuclear Survivin scores were tested using Pearson correlation test statistics.

RESULTS

Patient characteristics

Of the 456 eligible and analyzable cases entered in RTOG 8610, 68 patients had adequate and suitably-stained tumor material for nuclear Survivin analysis. There were no statistically significant differences in the pretreatment characteristics (age combined Gleason score, clinical stage, and assigned treatment) of the 456 assessable patients with or without nuclear Survivin determinations (data not shown). The differences with the four end points between the two groups are shown in Table 1a. There were statistically significant differences in prostate cancer survival and distant metastasis between these two groups. This implied that the subset of cases with Survivin is not necessarily a random representation of the entire RTOG 8610 study population and may not be generalizable outside the subset of cases with Survivin data. The distribution of pretreatment characteristics by nuclear Survivin mean index and intensity score

are shown in Tables 1b and 1c, respectively. There were no statistically significant differences in the distribution of pretreatment characteristics between these two groups.

Sixty-five patients had sufficient pathologic material available for cytoplasmic Survivin analysis. Table 2a illustrates the differences between patients with cytoplasmic Survivin data versus those without cytoplasmic Survivin data with regards to outcome. There were statistically significant differences in prostate cancer survival and distant metastasis between these two groups. Again, this indicated that the subset of cases with Survivin is not necessarily a random representation of the entire RTOG 8610 study population. Tables 2b and 2c stratify patient characteristics by cytoplasmic Survivin integrated optical density (cut point = 82.7) and intensity score (cut point = 128.2). There were no statistically significant differences in the distribution of pretreatment characteristics between these two groups.

The correlation between nuclear and cytoplasmic Survivin was assessed and found to be positive and statistically significant ($r = 0.40$, $p = 0.0012$ for nuclear mean index % vs. cytoplasmic integrated optical density; and $r = 0.42$, $p = 0.0005$ for nuclear vs. cytoplasmic intensity score). The positive correlation coefficients (r) indicated that a high nuclear Survivin is correlated with a high cytoplasmic Survivin. Scatter plot matrices were shown in Figure 1A). Examples of weak and strong Survivin immunostaining are illustrated in Figure 1B. Nuclear and cytoplasmic immunostaining were then correlated with clinical outcome.

Prognostic significance of nuclear Survivin

Univariate and multivariate analyses were performed to determine the relation of nuclear Survivin mean index % to the four end points. None of the analytical results showed a significant association between nuclear Survivin mean index % and any of the end points (data not shown).

Univariate analysis of nuclear Survivin staining stratified by intensity score, with a cut-point of 191.2, revealed a significant increase in prostate cancer survival for the patients with intensity score >191.2 (HR=0.45, 95% CI=0.20–1.00, $p = 0.0452$). Figure 2 demonstrates the Kaplan-Meier curves corresponding to this association. However, there were no other significant associations with either overall, local progression-free, or distant metastasis-free survivals, using nuclear Survivin intensity score >191.2 as a cut-point (data not shown). Of 68 patients with nuclear Survivin staining, 50 patients received salvage hormone therapy as “clinically indicated” for disease relapse. The protocol did not have guidelines as to type of salvage hormone therapy should be given or for when salvage hormone therapy should be initiated in relapsed patients³⁴. We examined the association between the nuclear Survivin intensity score and the five-year prostate cancer survival from the start of salvage hormone therapy. Compared to patients with Survivin intensity score ≤ 191.2 , those with intensity score >191.2 showed a significant increase in five-year prostate cancer survival (74.3% vs. 47.5%, $p = 0.010$).

The results of the multivariate analysis can be found in Table 3. For each end point, the model included Gleason grade, clinical stage, and assigned treatment. As many of these patients were entered prior to the PSA era, these values were not available for the multivariate model. Nuclear Survivin intensity score >191.2 remained significantly associated with improved prostate cancer survival ($p = 0.0173$). Moreover, patients with nuclear Survivin intensity score >191.2 showed a significantly improved overall survival ($p = 0.0156$).

Prognostic significance of cytoplasmic Survivin

The results of the univariate analysis of cytoplasmic Survivin can be found in Table 4. The univariate analysis revealed a significantly increased rate of local progression in patients with

higher integrated optical density of cytoplasmic Survivin immunostaining. Compared to those with integrated optical density ≤ 82.7 , those who had cytoplasmic integrated optical density > 82.7 showed a significantly increased risk of local progression (HR=2.49, 95% CI=1.03–6.01, $p=0.0421$). However, likely due to modest patient numbers, this association did not hold up on multivariate analysis (Table 5). The other word of caution here is that evaluation of local progression without a biopsy is inherently a subjective clinical endpoint in the setting of prostate cancer and must be viewed with caution. There is no significant association between cytoplasmic Survivin intensity score and any of the four end points on either univariate or multivariate analyses (data not shown).

DISCUSSION

RTOG 8610 is the first major, phase III randomized trial to test the hypothesis that short-term neoadjuvant androgen deprivation therapy (ADT) combined with radiotherapy (RT) would improve treatment outcomes compared with RT alone. Long-Term Results of this study suggests that the addition of 4 months of ADT to RT appears to have a dramatic impact on clinically meaningful end points in men with locally advanced disease with no statistically significant impact on the risk of fatal cardiac events³⁵. Now It is clear that long-term ADT + RT should be used in “high-risk” patients (clinical stage T2c-3, Gleason score 7–10, PSA > 20 ng/mL) and “low-risk” patients (clinical stage T1c-2a, Gleason score 2–6, PSA < 20 ng/mL) may benefit from short-term ADT + RT. Whether “intermediate risk” patients (clinical stage T2b, Gleason score 7, PSA 10–20 ng/mL) benefit from a combination of ADT and RT is not clear. Although the risk stratification system described by D’Amico *et al* is widely used and is relatively simple, it suffers from heterogeneity within the intermediate risk group³⁶. To address this heterogeneity, it is necessary to create additional subgroups using novel prognostic markers in order to correlate with survival end point. Based on our preclinical studies of Survivin in human prostate cancer cell models and the previous reports of Survivin as a molecular marker in other cancers, we hypothesized that Survivin expression may serve as a prognostic factor for prostate cancer patients and different subcellular localization of Survivin may alter its prognostic role. We tested these hypotheses using samples from men with locally advanced prostate cancer enrolled in RTOG 8610.

The prognostic value of Survivin expression in prostate cancer has remained unclear. Most of the studies which have analyzed the prognostic significance of Survivin expression were either performed without accounting for the subcellular location of the protein, or reported Survivin location to be restricted to the cytoplasm of tumor cells^{16, 17, 26}. Our study for the first time separately describes the expression of Survivin protein in both the nuclear and cytoplasmic compartments of prostate cancer cells. In our analysis we identified that a different prognostic role is played by the nuclear and cytoplasmic Survivin expression. In particular, the nuclear expression of Survivin identified patients with improved overall and prostate cancer survival, while the cytoplasmic overexpression of Survivin was associated with increased rate of local progression. These findings have been confirmed by univariate and/or multivariate analysis of survival. In the multivariate analyses of nuclear and cytoplasmic Survivin, we used the independent variable “assigned treatment” to examine the risk of RT + hormone therapy as compared to RT alone. None of the analyses shown the significant difference between the two groups. However, among 50 patients who received salvage hormone therapy as “clinically indicated” for disease relapse, those with nuclear Survivin intensity score > 191.2 showed a significant increase in five-year prostate cancer survival compared to patients with Survivin intensity score ≤ 191.2 (74.3% vs. 47.5%, $p=0.010$), indicating that this subgroup of relapsed patients with higher nuclear Survivin expression may benefit from salvage hormone therapy.

Although growing evidence suggests that Survivin expression in cancer cells may represent an important prognostic marker to predict disease outcome, current reports are inconsistent

and propose opposing conclusions. Cytoplasmic Survivin immunoreactivity has been observed in the vast majority of human tumors and it has been consistently associated with poor prognosis¹⁹. Nuclear staining has been linked with a favorable prognosis in patients with gastric carcinoma³⁷, breast carcinoma³⁸, osteosarcoma³⁹, bladder mucosa and transitional cell carcinoma⁴⁰, and pancreatic cancer⁴¹. In contrast, studies in hepatocellular carcinoma⁴²,⁴³, esophageal malignancies⁴⁴, and mantle cell lymphoma⁴⁵ have shown nuclear Survivin to be associated with poor survival. In addition, Survivin has been detected in the nuclei of non-small-cell lung cancer⁴⁶ and ovarian cancer⁴⁷ from clinical samples, without any significant relation with clinical outcome. The results of these studies have contributed to the confusion regarding the role of nuclear expression of Survivin. The prognostic difference for nuclear Survivin among these studies is likely tumor-specific.

It has been demonstrated that Survivin exists in two distinct subcellular pools: cytoplasmic and nuclear¹⁸. The two Survivin pools are immunochemically distinct and independently modulated during cell cycle progression. Survivin has been described both as a chromosomal passenger protein in the nucleus and as a microtubule-associated protein in cytoplasm. In the nucleus, Survivin interacts with aurora kinase B and INCENP to complete mitosis⁴⁸, suggesting that strong nuclear staining of Survivin may represent increased mitotic events. Conversely, in the cytoplasm, Survivin blocks intrinsic and extrinsic apoptosis by directly inhibiting caspase-3 and -7, as well as interfering with caspase-9⁴⁹. This may partly explain the different prognostic implications of cytoplasmic and nuclear Survivin. The distinct roles of Survivin may also be due in part to splice variations. Survivin has four splice variants with different subcellular localizations: Survivin-2 α , Survivin-2 β , Survivin-3 β , and Survivin- δ Ex3^{20–25}. Survivin- δ Ex3 is localized preferentially in the nucleus, whereas the wild type Survivin and Survivin 2 β isoforms are found in the cytoplasm. Survivin-2 β and Survivin- δ Ex3 may antagonize wild type Survivin by forming heterodimers with reduced antiapoptotic potential, and acting as naturally occurring antagonists of Survivin^{24, 50}. Different isoforms of Survivin may interact, and the subcellular location ratio of the protein may contribute to the complex regulation of apoptosis and cell division according to these studies. Because the antibody we used in the immunohistochemical staining recognizes all these variants, it is not surprising that the intracellular location of Survivin has different prognostic implications in prostate cancer.

In summary, the present study is the first report to establish the prognostic relevance of the Survivin expression in prostate cancer in relation to its cellular distribution. The nuclear expression of Survivin is associated with improved overall and prostate cancer survival, while the cytoplasmic overexpression of Survivin is associated with increased rate of local progression in patients with locally advanced prostate cancer treated on RTOG 8610. Our data may reflect the divergent functions of Survivin isoforms, which exhibit distinct subcellular localizations. This study represents a retrospective correlative analysis performed on a prospective phase III study. The majority of patients entered this trial prior to the PSA era, and the extent of disease at entry is much more advanced than in the patients we see now who are being diagnosed in the second decade of the PSA era. Our data should be viewed with some caution because of the small dataset, low statistical power and potential cutpoint bias. A larger independent cohort of men with earlier-stage prostate cancer treated with radiotherapy should be investigated to clarify the potential of Survivin in prostate cancer prognosis.

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References

1. Ambrosini G, Adida C, Altieri DC. A novel anti-apoptosis gene, survivin, expressed in cancer and lymphoma. *Nat Med* 1997;3:917–921. [PubMed: 9256286]
2. Salvesen GS, Duckett CS. IAP proteins: blocking the road to death's door. *Nat Rev Mol Cell Biol* 2002;3:401–410. [PubMed: 12042762]
3. Kobayashi K, Hatano M, Otaki M, et al. Expression of a murine homologue of the inhibitor of apoptosis protein is related to cell proliferation. *Proc Natl Acad Sci U S A* 1999;96:1457–1462. [PubMed: 9990045]
4. Adida C, Crotty PL, McGrath J, et al. Developmentally regulated expression of the novel cancer anti-apoptosis gene survivin in human and mouse differentiation. *Am J Pathol* 1998;152:43–49. [PubMed: 9422522]
5. Monzo M, Rosell R, Felip E, et al. A novel anti-apoptosis gene: Re-expression of survivin messenger RNA as a prognosis marker in non-small-cell lung cancers. *J Clin Oncol* 1999;17:2100–2104. [PubMed: 10561264]
6. Tanaka K, Iwamoto S, Gon G, et al. Expression of survivin and its relationship to loss of apoptosis in breast carcinomas. *Clin Cancer Res* 2000;6:127–134. [PubMed: 10656440]
7. Kawasaki H, Altieri DC, Lu CD, et al. Inhibition of apoptosis by survivin predicts shorter survival rates in colorectal cancer. *Cancer Res* 1998;58:5071–5074. [PubMed: 9823313]
8. Lu CD, Altieri DC, Tanigawa N. Expression of a novel antiapoptosis gene, survivin, correlated with tumor cell apoptosis and p53 accumulation in gastric carcinomas. *Cancer Res* 1998;58:1808–1812. [PubMed: 9581817]
9. Ikeguchi M, Ueta T, Yamane Y, et al. Inducible nitric oxide synthase and survivin messenger RNA expression in hepatocellular carcinoma. *Clin Cancer Res* 2002;8:3131–3136. [PubMed: 12374680]
10. Chakravarti A, Noll E, Black PM, et al. Quantitatively determined survivin expression levels are of prognostic value in human gliomas. *J Clin Oncol* 2002;20:1063–1068. [PubMed: 11844831]
11. Chakravarti A, Zhai GG, Zhang M, et al. Survivin enhances radiation resistance in primary human glioblastoma cells via caspase-independent mechanisms. *Oncogene* 2004;23:7494–7506. [PubMed: 15326475]
12. Grossman D, McNiff JM, Li F, et al. Expression and targeting of the apoptosis inhibitor, survivin, in human melanoma. *J Invest Dermatol* 1999;113:1076–1081. [PubMed: 10594755]
13. Zhang M, Mukherjee N, Bermudez RS, et al. Adenovirus-mediated inhibition of survivin expression sensitizes human prostate cancer cells to paclitaxel in vitro and in vivo. *Prostate* 2005;64:293–302. [PubMed: 15754318]
14. Zhang M, Latham DE, Delaney MA, et al. Survivin mediates resistance to antiandrogen therapy in prostate cancer. *Oncogene* 2005;24:2474–2482. [PubMed: 15735703]
15. McEleny KR, Watson RW, Coffey RN, et al. Inhibitors of apoptosis proteins in prostate cancer cell lines. *Prostate* 2002;51:133–140. [PubMed: 11948968]
16. Shariat SF, Lotan Y, Saboorian H, et al. Survivin expression is associated with features of biologically aggressive prostate carcinoma. *Cancer* 2004;100:751–757. [PubMed: 14770431]
17. Kishi H, Igawa M, Kikuno N, et al. Expression of the survivin gene in prostate cancer: correlation with clinicopathological characteristics, proliferative activity and apoptosis. *J Urol* 2004;171:1855–1860. [PubMed: 15076293]
18. Fortugno P, Wall NR, Giodini A, et al. Survivin exists in immunochemically distinct subcellular pools and is involved in spindle microtubule function. *J Cell Sci* 2002;115:575–585. [PubMed: 11861764]
19. Altieri DC. Validating survivin as a cancer therapeutic target. *Nat Rev Cancer* 2003;3:46–54. [PubMed: 12509766]
20. Badran A, Yoshida A, Ishikawa K, et al. Identification of a novel splice variant of the human anti-apoptosis gene survivin. *Biochem Biophys Res Commun* 2004;314:902–907. [PubMed: 14741722]
21. Mahotka C, Wenzel M, Springer E, et al. Survivin-deltaEx3 and survivin-2B: two novel splice variants of the apoptosis inhibitor survivin with different antiapoptotic properties. *Cancer Res* 1999;59:6097–6102. [PubMed: 10626797]
22. Mahotka C, Liebmann J, Wenzel M, et al. Differential subcellular localization of functionally divergent survivin splice variants. *Cell Death Differ* 2002;9:1334–1342. [PubMed: 12478470]

23. Mahotka C, Krieg T, Krieg A, et al. Distinct in vivo expression patterns of survivin splice variants in renal cell carcinomas. *Int J Cancer* 2002;100:30–36. [PubMed: 12115583]
24. Noton EA, Colnaghi R, Tate S, et al. Molecular analysis of survivin isoforms: evidence that alternatively spliced variants do not play a role in mitosis. *J Biol Chem* 2006;281:1286–1295. [PubMed: 16291752]
25. Caldas H, Jiang Y, Holloway MP, et al. Survivin splice variants regulate the balance between proliferation and cell death. *Oncogene* 2005;24:1994–2007. [PubMed: 15688031]
26. Kaur P, Kallakury BS, Sheehan CE, et al. Survivin and Bcl-2 expression in prostatic adenocarcinomas. *Arch Pathol Lab Med* 2004;128:39–43. [PubMed: 14692814]
27. Pilepich MV, Winter K, John MJ, et al. Phase III radiation therapy oncology group (RTOG) trial 86-10 of androgen deprivation adjuvant to definitive radiotherapy in locally advanced carcinoma of the prostate. *Int J Radiat Oncol Biol Phys* 2001;50:1243–1252. [PubMed: 11483335]
28. Khor LY, Desilvio M, Li R, et al. Bcl-2 and bax expression and prostate cancer outcome in men treated with radiotherapy in Radiation Therapy Oncology Group protocol 86-10. *Int J Radiat Oncol Biol Phys* 2006;66:25–30. [PubMed: 16814949]
29. Khor LY, Desilvio M, Al-Saleem T, et al. MDM2 as a predictor of prostate carcinoma outcome: an analysis of Radiation Therapy Oncology Group Protocol 8610. *Cancer* 2005;104:962–967. [PubMed: 16007688]
30. Torres-Roca JF, DeSilvio M, Mora LB, et al. Activated STAT3 as a correlate of distant metastasis in prostate cancer: a secondary analysis of Radiation Therapy Oncology Group 86-10. *Urology* 2007;69:505–509. [PubMed: 17382154]
31. Wicht H, Maronde E, Olcese J, et al. A semiquantitative image-analytical method for the recording of dose-response curves in immunocytochemical preparations. *J Histochem Cytochem* 1999;47:411–420. [PubMed: 10026243]
32. Kaplan EL, Meier P. Non parametric estimation from incomplete observations. *J Am stat assoc* 1958;53:457–816.
33. Gray RJ. A class of K-sample tests for comparing the cumulative incidence of a competing risk. *Ann Statist* 1988;16(16):1141–1154. 1141–1154.
34. Shipley WU, Desilvio M, Pilepich MV, et al. Early initiation of salvage hormone therapy influences survival in patients who failed initial radiation for locally advanced prostate cancer: A secondary analysis of RTOG protocol 86-10. *Int J Radiat Oncol Biol Phys* 2006;64:1162–1167. [PubMed: 16427211]
35. Roach M 3rd, Bae K, Speight J, et al. Short-term neoadjuvant androgen deprivation therapy and external-beam radiotherapy for locally advanced prostate cancer: long-term results of RTOG 8610. *J Clin Oncol* 2008;26:585–591. [PubMed: 18172188]
36. D'Amico AV. Radiation and hormonal therapy for locally advanced and clinically localized prostate cancer. *Urology* 2002;60:32–37. [PubMed: 12231043]discussion 37–38
37. Okada E, Murai Y, Matsui K, et al. Survivin expression in tumor cell nuclei is predictive of a favorable prognosis in gastric cancer patients. *Cancer Lett* 2001;163:109–116. [PubMed: 11163114]
38. Kennedy SM, O'Driscoll L, Purcell R, et al. Prognostic importance of survivin in breast cancer. *Br J Cancer* 2003;88:1077–1083. [PubMed: 12671708]
39. Trieb K, Lehner R, Stulnig T, et al. Survivin expression in human osteosarcoma is a marker for survival. *Eur J Surg Oncol* 2003;29:379–382. [PubMed: 12711293]
40. Lehner R, Lucia MS, Jarboe EA, et al. Immunohistochemical localization of the IAP protein survivin in bladder mucosa and transitional cell carcinoma. *Appl Immunohistochem Mol Morphol* 2002;10:134–138. [PubMed: 12051631]
41. Tonini G, Vincenzi B, Santini D, et al. Nuclear and cytoplasmic expression of survivin in 67 surgically resected pancreatic cancer patients. *Br J Cancer* 2005;92:2225–2232. [PubMed: 15928668]
42. Fields AC, Cotsonis G, Sexton D, et al. Survivin expression in hepatocellular carcinoma: correlation with proliferation, prognostic parameters, and outcome. *Mod Pathol* 2004;17:1378–1385. [PubMed: 15195112]
43. Ito T, Shiraki K, Sugimoto K, et al. Survivin promotes cell proliferation in human hepatocellular carcinoma. *Hepatology* 2000;31:1080–1085. [PubMed: 10796883]

44. Grabowski P, Kuhnel T, Muhr-Wilkenshoff F, et al. Prognostic value of nuclear survivin expression in oesophageal squamous cell carcinoma. *Br J Cancer* 2003;88:115–119. [PubMed: 12556969]
45. Martinez A, Bellosillo B, Bosch F, et al. Nuclear survivin expression in mantle cell lymphoma is associated with cell proliferation and survival. *Am J Pathol* 2004;164:501–510. [PubMed: 14742256]
46. Falleni M, Pellegrini C, Marchetti A, et al. Survivin gene expression in early-stage non-small cell lung cancer. *J Pathol* 2003;200:620–626. [PubMed: 12898598]
47. Ferrandina G, Legge F, Martinelli E, et al. Survivin expression in ovarian cancer and its correlation with clinico-pathological, surgical and apoptosis-related parameters. *Br J Cancer* 2005;92:271–277. [PubMed: 15655541]
48. Honda R, Korner R, Nigg EA. Exploring the functional interactions between Aurora B, INCENP, and survivin in mitosis. *Mol Biol Cell* 2003;14:3325–3341. [PubMed: 12925766]
49. Tamm I, Wang Y, Sausville E, et al. IAP-family protein survivin inhibits caspase activity and apoptosis induced by Fas (CD95), Bax, caspases, and anticancer drugs. *Cancer Res* 1998;58:5315–5320. [PubMed: 9850056]
50. Krieg A, Mahotka C, Krieg T, et al. Expression of different survivin variants in gastric carcinomas: first clues to a role of survivin-2B in tumour progression. *Br J Cancer* 2002;86:737–743. [PubMed: 11875736]

A.

Scatter Plot of Nuclear Mean Index % and Cytoplasmic Integrated Density

(Noted: Dotted lines are medians of nuclear mean index% and cytoplasmic integrated optical density respectively)

Scatter Plot of Nuclear and Cytoplasmic Survivin Intensity Score

(Noted: Dotted lines are medians of respective intensity score)

B.

Fig 1.

A. Scatter plot matrices of nuclear mean index % vs. cytoplasmic integrated optical density (upper) and nuclear vs. cytoplasmic intensity score (lower). B. Representative stained slides showing Survivin negative immunostaining (left) and Survivin positive immunostaining (right).

Fig 2.
Kaplan-Meier curve of prostate cancer survival by nuclear Survivin intensity score.

Table 1

Table 1a. Missing vs. Determined Nuclear Survivin Data (n=456)				
Variable	Overall Survival	Prostate Cancer Survival	Local Progression	Distant Metastasis
Relative Risk [*] (95% C.I.) (Missing vs. Determined)	1.28(0.93,1.75)	1.68(1.10,2.59)	1.39(0.91,2.13)	1.51(1.06,2.15)
<i>p</i> -value [†]	0.13	0.02	0.13	0.02
Number of Events				
Missing (n=388)	212	92	118	150
Determined (n=68)	47	27	26	39

Table 1b. Patient Characteristics by Nuclear Survivin Mean Index % (n=68)			
Characteristics	≤74.1 (n=34)	>74.1 (n=34)	<i>p</i>-value[*]
Age			
<75	26 (76%)	25 (74%)	0.78
≥75	8 (24%)	9 (26%)	
Combined Gleason Score			
2–6	10 (29%)	4 (12%)	0.08
7–10	24 (71%)	29 (88%)	
Clinical Stage			
T2	7 (21%)	10 (29%)	0.40
T3	27 (79%)	24 (71%)	
Assigned Treatment			
RT Alone	19 (56%)	19 (56%)	>0.99
RT + Hormone Therapy	15 (44%)	15 (44%)	

Table 1c. Patient Characteristics by Nuclear Survivin Intensity Score (n=68)			
Characteristics	≤191.2 (n=34)	>191.2 (n=34)	<i>p</i>-value[*]
Age			
<75	26 (76%)	25 (74%)	0.78
≥75	8 (24%)	9 (26%)	
Combined Gleason Score			
2–6	10 (29%)	4 (12%)	0.08
7–10	24 (71%)	29 (88%)	
Clinical Stage			
T2	11 (32%)	6 (18%)	0.16
T3	23 (68%)	28 (82%)	
Assigned Treatment			
RT Alone	18 (53%)	20 (59%)	0.63

Table 1c. Patient Characteristics by Nuclear Survivin Intensity Score (n=68)

Characteristics	≤191.2 (n=34)	>191.2 (n=34)	p-value*
RT + Hormone Therapy	16 (47%)	14 (41%)	

* Relative Risk: A risk ratio of 1 indicates no difference between the two subgroups. The Survivin indicator was coded as 0: *Missing*, 1: *Determined*.

†
p -value from Chi-square test using the Cox proportional hazards model.

*
Chi-square statistics

*
Chi-square statistics

Table 2

Table 2a. Missing vs. Determined Cytoplasmic Survivin Data (n=456)				
Variable	Overall Survival	Prostate Cancer Survival	Local Progression	Distant Metastasis
Relative Risk [*] (95% C.I.)	1.32(0.96,1.82)	1.65(1.06,2.56)	1.27(0.81,1.98)	1.55(1.08,2.22)
(Missing vs. Determined)				
<i>p</i> -value [†]	0.0892	0.0263	0.3015	0.0169
Number of Events				
Missing (n=391)	214	94	121	152
Determined (n=65)	45	25	23	37

Table 2b. Pretreatment Characteristics by Cytoplasmic Survivin Integrated Optical Density (n=65)			
Characteristics	≤82.7 (n=33)	>82.7 (n=32)	<i>p</i> -value [*]
Age			
<75	24 (73%)	25 (78%)	0.6135
≥75	9 (27%)	7 (22%)	
Combined Gleason Score [†]			
2–6	6 (18%)	9 (29%)	0.3058
7–10	27 (82%)	22 (71%)	
Clinical Stage			
T2	10 (30%)	6 (19%)	0.2797
T3	23 (70%)	26 (81%)	
Assigned Treatment			
RT Alone	16 (48%)	22 (69%)	0.0974
RT + Hormone Therapy	17 (52%)	10 (31%)	

Table 2c. Pretreatment Characteristics by Cytoplasmic Survivin Intensity Score (n=65)			
Characteristics	≤128.2 (n=33)	>128.2 (n=32)	<i>p</i> -value [*]
Age			
<75	26 (79%)	23 (72%)	0.5177
≥75	7 (21%)	9 (28%)	
Combined Gleason Score [†]			
2–6	11 (33%)	4 (13%)	0.0538
7–10	22 (67%)	27 (87%)	
Clinical Stage			
T2	10 (30%)	6 (19%)	0.2797
T3	23 (70%)	26 (81%)	
Assigned Treatment			
RT Alone	18 (55%)	20 (63%)	0.5153

Table 2c. Pretreatment Characteristics by Cytoplasmic Survivin Intensity Score (n=65)

Characteristics	≤128.2 (n=33)	>128.2 (n=32)	p-value [*]
RT + Hormone Therapy	15 (45%)	12 (37%)	

* Relative Risk: A risk ratio of 1 indicates no difference between the two subgroups. The Survivin indicator was coded as 0: *Missing*, 1: *Determined*.

[†] p-value from Chi-square test using the Cox proportional hazards model.

* p-value is from Chi-square test

[†] 1 patient did not have combined Gleason score

* p-value is from Chi-square test

[†] 1 patient did not have combined Gleason score

Table 3
Multivariate Analysis of Nuclear Survivin Intensity Score

Patients with Determined SURVIVIN ACIS (n=67 [*])				
Endpoint	Covariate	Hazard Ratio ^{**}	95% CI [†]	p-value [‡]
Overall Survival (Wald stat = 10.3306, p=0.0352)	Nuclear Survivin Intensity Score:			
	≤ 191.2% vs. > 191.2%	0.46	0.25–0.86	0.0156
	Gleason Score:			
	2–6 vs. 7–10	2.43	1.06–5.55	0.0360
	Clinical Stage			
Prostate Cancer Survival (Wald stat = 8.5136, p=0.0745)	T2 vs. T3	1.32	0.64–2.73	0.4576
	Assigned Treatment			
	RT Alone vs. RT + Hormone Therapy	0.59	0.32–1.09	0.0932
	Nuclear Survivin Intensity Score:			
	≤191.2% vs. > 191.2%	0.36	0.16–0.84	0.0173
Distant Metastases (Wald stat = 5.3717, p=0.2512)	Gleason Score:			
	2–6 vs. 7–10	3.42	1.07–10.91	0.0379
	Clinical Stage			
	T2 vs. T3	0.72	0.32–1.65	0.4360
	Assigned Treatment			
Local Progression (Wald stat = 4.0541, p=0.3987)	RT Alone vs. RT + Hormone Therapy	0.78	0.35–1.74	0.5429
	Nuclear Survivin Intensity Score:			
	≤ 191.2% vs. > 191.2%	0.60	0.31–1.16	0.1273
	Gleason Score:			
	2–6 vs. 7–10	2.56	0.96–6.87	0.0613
	Clinical Stage			
	T2 vs. T3	0.85	0.42–1.72	0.6447
	Assigned Treatment			
	RT Alone vs. RT + Hormone Therapy	0.76	0.39–1.47	0.4087
	Nuclear Survivin Intensity Score:			
	≤191.2% vs. >191.2%	1.23	0.55–2.75	0.6209
	Gleason Score:			
	2–6 vs. 7–10	0.98	0.38–2.50	0.9580
	Clinical Stage			
	T2 vs. T3	1.00	0.39–2.55	0.9969
	Assigned Treatment			
	RT Alone vs. RT + Hormone Therapy	0.41	0.16–1.04	0.0614

* 1 patient did not have Gleason score.

** Relative Risk: a risk ratio of 1 indicates no difference between the two subgroups (first category is the reference group).

[†] 95% confidence intervals of the relative risk

[‡] *p*-value from Chi-square test using the Cox proportional hazards model.

Table 4
Dichotomized Cytoplasmic Survivin Integrated Optical Density: Cut-point = 82.7

Endpoint	Survivin Cut-point	n	Failures	RR [*]	(95% CI)	p-value [†]	5-Year Rate [‡]	5-Year (95% CI)
Overall	≤82.7	33	22	R.L. [§]			78.8%	(60.6%, 89.3%)
Survival	>82.7	32	23	1.18	(0.66,2.13)	0.5791	68.8%	(49.7%, 81.8%)
Prostate Cancer	≤82.7	33	12	R.L.			84.6%	(66.9%, 93.3%)
Survival	>82.7	32	13	1.29	(0.58,2.83)	0.5333	78.7%	(58.6%, 89.8%)
Distant	≤82.7	33	20	R.L.			48.5%	(31.0%, 65.9%)
Metastases	>82.7	32	17	0.79	(0.41,1.51)	0.4691	44.9%	(26.7%, 63.1%)
Local	≤82.7	33	9	R.L.			9.1%	(0.0%, 19.1%)
Progression	>82.7	32	14	2.49	(1.03,6.01)	0.0421	37.5%	(20.3, 54.7%)

* RR (Relative risk): a risk ratio of 1 indicates no difference between the two subgroups. The cytoplasmic Survivin indicator was coded as 0: *cut-point*, 1:>*cut-point*

† p-value is from Chi-square test using the Cox proportional hazards model

‡ The five-year actuarial estimates for overall and prostate cancer survival were calculated using the Kaplan-Meier method and the cumulative incidence method was used to estimate the five-year local progression and distant metastasis failure rates

§ Reference level

Table 5
Multivariate Analysis of Cytoplasmic Survivin Integrated Optical Density

Patients with Determined SURVIVIN (n=64 [*])				
Endpoint	Covariate	Hazard Ratio ^{**}	95% CI [†]	p-value [‡]
Overall Survival (Wald stat = 4.2088, <i>p</i> =0.3785)	Cytoplasmic Survivin Integrated Optical Density			
	≤82.7% vs. > 82.7%	1.20	0.64–2.23	0.5688
	Gleason Score:			
	2–6 vs. 7–10	2.04	0.91–4.55	0.0817
	Clinical Stage			
	T2 vs. T3	1.30	0.62–2.74	0.4907
Prostate Cancer Survival (Wald stat = 3.5056, <i>p</i> =0.4770)	Assigned Treatment			
	≤RT Alone vs. RT + Hormone Therapy	0.79	0.41–1.51	0.4705
	Cytoplasmic Survivin Integrated Optical Density			
	≤82.7% vs. > 82.7%	1.46	0.65–3.26	0.3609
	Gleason Score:			
	2–6 vs. 7–10	2.36	0.77–7.21	0.1333
Distant Metastases (Wald stat = 3.4788, <i>p</i> =0.4811)	Clinical Stage			
	T2 vs. T3	0.74	0.31–1.74	0.4879
	Assigned Treatment			
	RT Alone vs. RT + Hormone Therapy	1.03	0.45–2.38	0.9445
	Cytoplasmic Survivin Integrated Optical Density			
	≤82.7% vs. > 82.7%	0.82	0.42–1.62	0.5746
Local Progression (Wald stat = 6.9845, <i>p</i> =0.1367)	Gleason Score:			
	2–6 vs. 7–10	2.32	0.86–6.23	0.0950
	Clinical Stage			
	T2 vs. T3	0.90	0.43–1.87	0.7669
	Assigned Treatment			
	RT Alone vs. RT + Hormone Therapy	0.88	0.44–1.74	0.7022
	Cytoplasmic Survivin Integrated Optical Density			
	≤82.7% vs. > 82.7%	2.31	0.94–5.68	0.0688
	Gleason Score:			
	2–6 vs. 7–10	1.16	0.45–3.00	0.7550
	Clinical Stage			
	T2 vs. T3	0.97	0.35–2.67	0.9510
	Assigned Treatment			

Patients with Determined SURVIVIN (n=64 [*])				
Endpoint	Covariate	Hazard Ratio ^{**}	95% CI [†]	p-value [‡]
	RT Alone vs. RT + Hormone Therapy	0.42	0.15–1.22	0.1103

* 1 patient did not have GS.

** Relative Risk: a risk ratio of 1 indicates no difference between the two subgroups (first category is the reference group).

† 95% confidence intervals of the relative risk

‡ P-value from Chi-square test using the Cox proportional hazards model.