NOXA and PUMA Expression Add to Clinical Markers in Predicting Biochemical Recurrence of Prostate Cancer Patients in a Survival Tree Model

Jean-Simon Diallo,1 Abdulhadi Aldejmah,1,4 Abdelali Filali Mouhim,1 Benjamin Péant,1 Mona Alam Fahmy,1 Ismaël Hervé Koumakpayi,1 Kanishka Sircar,2 Louis R. Bégin,3 Jean-Simon Diallo,1 Abdulhadi Aldejmah,1,4 Abdelali Filali Mouhim,1 Benjamin Péant,1 Mona Alam Fahmy,1 Ismaël Hervé Koumakpayi,1 Kanishka Sircar,2 Louis R. Bégin,3 Jean-Simon Diallo,1 Abdulhadi Aldejmah,1,4 Abdelali Filali Mouhim,1 Benjamin Péant,1 Mona Alam Fahmy,1 Ismaël Hervé Koumakpayi,1 Kanishka Sircar,2 Louis R. Bégin,3 Jean-Simon Diallo,1 Abdulhadi Aldejmah,1,4 Abdelali Filali Mouhim,1 Benjamin Péant,1 Mona Alam Fahmy,1 Ismaël Hervé Koumakpayi,1 Kanishka Sircar,2 Louis R. Bégin,3

Abstract

Purpose: To assess the expression of proapoptotic NOXA and PUMA in prostate tissues and delineate their association with prostate cancer (PCa) recurrence.

Experimental Design: Normal, prostatic intraepithelial neoplasia (PIN), hormone-sensitive (HS) PCa, and hormone-refractory (HR) PCa tissues were used to build tissue microarrays encompassing a total of 135 patients. Two observers assessed the intensity of NOXA and PUMA immunohistochemical staining using a composite color scale. One hundred and eighty recursive partitioning and regression tree (RPART) models were generated to predict biochemical recurrence (BCR) within HS cancer patients using NOXA, PUMA, and clinical parameters. Models were then ranked according to the integrated Brier score (IBS).

Results: Increasing NOXA expression was associated with PCa progression, reaching the highest levels in HR PCa. Increased NOXA expression was observed in 68% of HS cancer patients and was predictive of BCR (LR = 8.64; P = 0.003). In contrast, PUMA expression was highest in HS cancer, and although 70% of HS cancer patients exhibited increased PUMA expression, PUMA alone could not predict the onset of BCR. Interestingly, the top-ranking RPART model generated [IBS = 0.107; 95% confidence interval (95% CI), 0.065–0.128] included surgical margin status and NOXA and PUMA expression, although recurrent prognostic classification schemes obtained in the top 10 models favored a survival tree model containing margin status, NOXA expression, and preoperative prostate-specific antigen (PSA) (IBS = 0.114; 95% CI, 0.069–0.142).

Conclusion: We conclude that NOXA and PUMA expression may be linked to PCa progression and propose further validation of a survival tree model including surgical margin status, NOXA expression, and preoperative PSA for predicting BCR.

Prostate cancer (PCa) remains a leading cause of cancer-related death in North American men (1). Although localized forms of the disease can often be successfully treated by surgery or radiotherapy, a significant proportion of patients having undergone such interventions are at risk of disease relapse. For this reason, considerable efforts have been made to discover new molecular markers that can accurately predict the onset of disease relapse and lead to better targeted and more effective treatment.

Androgen deprivation therapy is often used to treat recurrent PCa and can increase patient survival; however, this form of therapy eventually gives rise to androgen-independent PCa (or AIPCa; refs. 2, 3). Because the treatment of AIPCa remains palliative to date (4–6), much effort has been devoted to describing the molecular mechanisms associated with the transition of androgen-dependent PCa to an androgen-independent state. Many studies have established a role for androgen receptor (AR) signaling in this phenomenon (7, 8). However, increasing evidence suggests that other signaling pathways may also be important for progression to an androgen-independent state (9–14). At the convergence of many of these pathways, it has been suggested that PCa cells can...
become resistant to treatment-induced apoptosis through the up-regulation of antiapoptotic proteins such as BCL-2, BCL-X\(_L\), and MCL-1 (15, 16). Several studies have detected up-regulated BCL-2, BCL-X\(_L\), and MCL-1 expression in high-grade PCa tumors and in AIPCa (17–21).

In theory, enhanced resistance to apoptosis can also be achieved by the down-regulation of proapoptotic proteins (22). To date, few studies have looked at the expression of proapoptotic proteins in PCa. Thus far, most studies addressing this question have focused on BAX, a proapoptotic protein that elicits its effect at the level of the mitochondrial outer membrane where it promotes mitochondrial depolarization, a key event in the intrinsic apoptotic pathway. Although it is clear from several immunohistochemistry (IHC) studies that BAX is expressed in the large majority of tumors, the association between BAX expression and PCa progression remains uncertain (18, 23–26).

NOXA and PUMA are two BH3-only proapoptotic proteins that act upstream of BAX/BAK to promote mitochondrial depolarization. NOXA is essentially thought to sensitize cells to the action of activator BH3-only proapoptotic proteins by disrupting their interaction with antiapoptotic proteins. Recent evidence suggests that NOXA specifically disrupts the interaction of MCL-1 with activator BH3-only proteins BID, BIM, and PUMA (27). In turn, activator BH3-only proteins such as PUMA and BID interact with the H\(_\text{h}1\) helix of BAX to induce conformational changes leading to the permeation of the mitochondrial outer membrane (28).

To date, few IHC studies have looked at PUMA or NOXA expression in cancer. In melanoma, weak PUMA expression was linked to poor patient survival (29), particularly in patients also showing elevated levels of phosphorylated AKT (30). In colorectal cancer, no relationship with clinical outcome was found, although 29% of tumors overexpressed PUMA (as opposed to 4% showing decreased expression; ref. 31). Similarly, NOXA expression was increased in 16% of colorectal tumors but was not associated with disease outcome (32). To date, neither NOXA nor PUMA has been studied in relation to PCa progression and clinical outcome.

To assist in the process of prognostic marker discovery, increasingly powerful statistical methods are being developed and applied. Of these methods, survival trees are particularly attractive when looking at multiple markers within one or more signaling pathways. Survival tree algorithms are based on recursive partitioning of the covariate space (33, 34), and their graphical output facilitates the visualization of prognostic groups reflecting multimarker interactions. In this study, we looked at the expression of NOXA and PUMA using tissue microarrays containing normal prostate tissue, primary PCa, and its adjacent non-neoplastic tissue, as well as specimens of androgen-independent PCa, representing a total of 135 patients. We then used survival trees to evaluate the ability of NOXA and PUMA, alone or in combination with clinical markers, to predict the onset of biochemical recurrence (BCR) in patients presenting primary PCa.

### Table 1. Patient cohort characteristics

<table>
<thead>
<tr>
<th>Overall patient cohort</th>
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<tr>
<td>Age median (minimum-maximum)</td>
<td>Cancer-free patients</td>
<td>PCa patients</td>
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<tr>
<td>35 (15–45)</td>
<td>62 (49–70)</td>
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<tr>
<td>AIPCa patients</td>
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<table>
<thead>
<tr>
<th>Primary PCa patient cohort</th>
<th>Stage</th>
<th>Invasion</th>
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<tr>
<td>Stage 2</td>
<td>34</td>
<td>Extraprostatic extension</td>
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<tr>
<td>Stage 3</td>
<td>28</td>
<td>Lymph node metastasis</td>
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<tr>
<td>Seminal vesicle invasion</td>
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<td>&gt;10 ng</td>
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<tr>
<td>Positive</td>
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<tr>
<td>Deaths within follow-up period</td>
<td>9</td>
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</tr>
</tbody>
</table>

NOTE: Mean patient follow-up was 72 mo.
Whole cell extracts were collected after centrifugation in a Heraeus 2 mg/mL of aprotinin, leupeptin, and pepstatin on ice for 30 min. 0.5mmol/L phenylmethylsulfonyl fluoride, 0.2mmol/L orthovanadate, NaCl, 1 mmol/L EDTA, 1 mmol/L DTT, 1 mmol/L NaF, 0.5% NP40, cold lysis buffer [10 mmol/L Tris-HCl (pH, 7.4), 150 mmol/L NaCl, 1 mmol/L EDTA, 1 mmol/L DTT, 1 mmol/L NaF, 0.5% NP40, 0.5 mmol/L phenylmethylsulfonyl fluoride, 0.2 mmol/L orthovanadate, 2 mg/mL of aprotinin, leupeptin, and pepstatin] on ice for 30 min. Whole cell extracts were collected after centrifugation in a Heraeus Biofuge (13,000 rpm for 10 min at 4°C) and were immediately stored at -80°C. Protein concentration was measured by Bradford assays done using the LSAB 2 peroxidase system from DAKO Diagnostics Inc.

**Protein extraction.** Confluent LNCaP, 22Rv1, PC3, and DU145 cells were scraped and washed twice with cold PBS, and pellets were frozen at -80°C. Subsequently, whole cell extracts were done by applying cold lysis buffer [10 mmol/L Tris-HCl (pH, 7.4), 150 mmol/L NaCl, 1 mmol/L EDTA, 1 mmol/L DTT, 1 mmol/L NaF, 0.5% NP40, 0.5 mmol/L phenylmethylsulfonyl fluoride, 0.2 mmol/L orthovanadate, 2 mg/mL of aprotinin, leupeptin, and pepstatin] on ice for 30 min. Whole cell extracts were collected after centrifugation in a Heraeus Biofuge (13,000 rpm for 10 min at 4°C) and were immediately stored at -80°C. Protein concentration was measured by Bradford assays (Bio-Rad Laboratories Inc.) according to the manufacturer’s instructions.

**Western blot analysis.** For Western blot analysis, 50 µg of whole cell protein extract were resolved on a 12.5% polyacrylamide gel and then transferred onto polyvinylidene difluoride membranes (Millipore). Blots were blocked using 5% nonfat dry milk in PBS-Tween 0.05% buffer overnight at 4°C and probed using either a monoclonal antibody raised against recombinant glutathione S-transferase–tagged full-length NOXA (OP180, Calbiochem), polyclonal antibody raised against amino acids 2 to 16 of PUMA (PC686, Calbiochem) or actin B (ab6276-100, Abcam) for 1 h at room temperature in blocking buffer (1:500). Membranes were then incubated with secondary antibody conjugated to horseradish peroxidase (Amersham Life Sciences Inc.) in blocking buffer for 1 h at room temperature and developed with enhanced chemiluminescence (ECL) substrate (Amersham Life Sciences Inc.).

**Immunohistochemistry.** Samples were immunostained with either anti-NOXA antibody (OP180) at 50 ng/µl or anti-PUMA antibody (PC686) at 4 ng/µl diluted in PBS. Primary antibody detection was done using the LSAB 2 peroxidase system from DAKO Diagnostics Inc.

Staining was done as described previously (35, 38–40). Briefly, tissue samples were deparaffinized, rehydrated, and treated with 0.3% H2O2 to eliminate endogenous peroxidase activity. An antigen retrieval step was done using 10 mmol/L citrate buffer (pH, 6.0) applied for 17.5 min at 95°C. All following steps were done at room temperature. The sections were blocked with a protein-blocking serum-free reagent (DAKO) and incubated with primary antibody for 60 min, followed by a 20-min treatment with the secondary biotinylated antibody (DAKO), washed 5 min in PBS, and then incubated for 20 min with streptavidin-peroxidase (DAKO). Following an additional 5-min PBS wash, reaction products were developed with diaminobenzidine (DAKO) containing 0.3% H2O2 as a substrate for peroxidase. Nuclei were counterstained with Harris hematoxylin (Sigma-Aldrich). No nonspecific secondary antibody staining was observed when PBS was used instead of the primary antibody.

**Scoring procedure.** For NOXA- and PUMA-stained tissues, digital pictures were taken of each core on an Olympus BX51 microscope using Q capture imaging software (Olympus). Two independent observers quantified epithelial staining intensity using a color scale (Fig. 2G) constructed from the various staining intensities observable in the digital pictures using the eyedropper tool in Adobe Photoshop 7.0. The observers assessed the percentage of epithelial cells representing each color of the scale (0-9), and an overall score was calculated from the sum of the products derived from the percentage (0-100%) multiplied by the scale value (0-9) for each core. Hence, all staining intensity values are on a continuous scale of 0 to 9. Notably, intraclass correlation coefficients (ICC: a measure of reliability between the two observers) were found to be excellent using this method (ICC > 0.75; ref. 41). In sharp contrast with initial estimates done using conventional microscopy methods (ICC < 0.5). Overall intensity values from each observer, obtained using the digital pictures, were then averaged and used for further statistical analyses.

**Statistics.** Mean staining intensities of cores from cancer-free patients, of non-neoplastic, PIN, and cancer cores from hormone-sensitive (HS) PCa patients as well as of cancer TURP cores from HR PCa patients were calculated. Kruskal-Wallis nonparametric tests were used to assess statistical significance of observed differences in mean staining intensity. All correlation coefficients were computed using Spearman’s nonparametric test. Cutoff determination and survival tree construction was done using the recursive partitioning and regression tree (RPART) libraries (33), which extends the classification and regression trees (CART) routine (34). Model accuracy was assessed using the integrated Brier score (IBS) for censored data (IBS; ref. 42). We used 200 bootstrap (43) samples to compute the 95% confidence interval (95% CI) on the IBS. Survival tree growth was controlled using the minimum splitting (minsplit) criterion implemented in RPART. This parameter controls the minimum number of observations that must exist in a node for a split to be attempted. For combination models including NOXA and/or PUMA, as well as combinations of the four clinical markers, all possible RPART models were generated using three different values of minsplit (20, 25, and 30). This generated 180 combinations corresponding to 69 different unique tree models, which were ranked according to IBS. Kruskal-Wallis, Spearman, and Kaplan-Meier analyses were done using Statistical Package for the Social Sciences (SPSS) version 11 (SPSS, Inc.). Tree building and the calculation of IBS were carried out in the R (version 2.4.0; ref. 44) system for statistical computing,6 using rpart and ipred packages, respectively.

**Results**

**NOXA and PUMA expression in PCa cell lines.** We used Western blotting on whole cell extracts to assess the expression of NOXA and PUMA proteins in PCa cell lines. As shown in

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**Fig. 1.** NOXA and PUMA expression in PCa cell lines. The Western blot was probed for NOXA, PUMA, and actin in whole cell extracts obtained from androgen-responsive (LNCaP; 22Rv1) and androgen-independent PCa cell lines. Because the anti-NOXA and anti-PUMA antibodies used here recognized their respective targets with little background, they were subsequently deemed adequate for IHC.
Fig. 1, the antibodies targeting NOXA and PUMA detected the expected ~6-kDa and 23-kDa bands (respectively) and revealed variable but apparent NOXA and PUMA expression in all PCa cell lines. For PUMA, cell line expression levels were found to be highest in 22Rv1 followed by LNCaP and PC3 cells, with DU145 exhibiting the lowest PUMA expression. The NOXA expression levels were highest in DU145 cells, followed by PC3 and 22Rv1 cells, with the lowest expression levels in LNCaP cells.

NOXA expression in prostate tissue subtypes. To determine whether NOXA expression could be linked to PCa progression, we stained prostate tissue microarrays using the antibody recognizing NOXA (same as used in Fig. 1). In general, we found that this antibody stained the cytoplasm of epithelial cells (Fig. 2A, C, and E). In many normal cores from cancer-free patients and non-neoplastic cores found adjacent to cancer (hereby referred to as NA), we observed more intense staining in the basal cell layer of epithelial glands (Fig. 2A). To increase interobserver reliability and facilitate retrospective interpretation of the results obtained, we used a standard color scale (Fig. 2G) constructed from digital pictures of tissue cores as described in Materials and Methods. Overall, we found that cores taken from normal patients expressed significantly less NOXA than all other tissue subtypes obtained from PCa patients, including NA cores (Fig. 3A). We also observed a slight but statistically insignificant decrease in PIN as opposed to NA cores ($P = 0.09$). Although HS cancer tissues exhibited higher mean NOXA expression than both NA and PIN cores ($P < 0.001$), HR TURP specimens exhibited the highest mean NOXA staining (mean = 5.09; $P < 0.001$). Notably, in the subgroup of patients for which we had both NA and HS cores ($n = 51$), 68% exhibited increased NOXA expression in HS cores.

PUMA expression in prostate tissue subtypes. Similarly to what was observed with the anti-NOXA antibody, we found that the antibody targeted against PUMA (same as used in Fig. 1) generally stained the cytoplasm of epithelial cells (Fig. 2B, D, and F). Basal cell staining was also apparent in several normal prostate cores as well as in NA cores (Fig. 2B). PUMA staining was subsequently evaluated using the same method employed for NOXA, and mean core intensity was calculated for each core.

![Fig. 2](https://example.com/fig2.png)

**Fig. 2.** IHC staining of paraffin-embedded prostate tissues using anti-NOXA and anti-PUMA antibodies. **A** and **B**, normal prostate tissue probed for NOXA and PUMA, respectively. Note enhanced staining in the basal cell layer. **C** and **D**, HS prostate carcinomas probed for NOXA and PUMA, respectively. **E** and **F**, HR TURP specimens stained using anti-NOXA and anti-PUMA antibodies, respectively. **G**, color scale standard used for assessment of pictures obtained from tissue microarrays probed for NOXA and PUMA. Scale was constructed from several digital pictures evaluated in the study as described in Materials and Methods. Numerical values, associated intensity score.
primary splitter of the root node, we found that high NOXA expression (≥5.5 on a scale of 0-9) was associated with an earlier and more frequent onset of BCR (log rank or LR = 8.6; P = 0.003; Fig. 4A). On the other hand, PUMA expression alone was not significantly predictive of the onset of BCR (LR = 2.5; P = 0.114; Fig. 4B). Interestingly, including both NOXA and PUMA in the RPART model revealed that low PUMA expression was associated to more rapid progression toward BCR and specifically when NOXA expression was also low (LR = 15.6; P < 5 × 10⁻⁴; Fig. 4C, Table 2). In this model, patients exhibiting high NOXA (≥5.5) expression were most likely to quickly undergo BCR, with ~77% (10/13) of these patients having undergone relapse before 3 years. Within the group of patients expressing low levels of NOXA, low expression of PUMA (<6.6) was associated to earlier and more frequent onset of BCR, with close to 46% (16/35) of patients having relapsed within 3 years. In contrast, patients exhibiting both low NOXA and high PUMA infrequently underwent BCR, with only 14% (2/14) having undergone relapse at 3 years. Because of potential overfitting due to the application of cutoffs obtained by RPART from the same test data set, Kaplan-Meier plots and associated LR P values should be considered as purely descriptive measures because the survival outcomes were used to define the prognostic groups.

**NOXA and PUMA expressions predict the onset of BCR in combination with clinical markers.** We next wondered whether NOXA and PUMA expression could help to predict BCR in combination with other clinicopathologic parameters such as preoperative PSA, Gleason score, pathologic stage, and resection margin status. We thus generated several RPART models using as input variables all possible combinations of the four clinical markers with NOXA and/or PUMA and ranked them according to the IBS, where lower IBS means greater accuracy. The top-ranking model stratified patients first on the basis of margin status (negative = good prognosis) and then on the basis of NOXA within negative margins (NOXA < 5.2 = good prognosis) and on the basis of PUMA within the positive margins (PUMA < 6.1 = good prognosis). Furthermore, PUMA also stratified negative margin patients expressing low levels of NOXA, with high PUMA expression (PUMA ≥ 6.1) being surprisingly associated with good prognosis (0/13 patients relapsed; Fig. 5A). This model had an IBS of 0.107 (95% CI, 0.063-0.128; Table 2). Interestingly, we found that 9 of the top 10 RPART models exhibited an initial stratification according to resection margin status followed by that of NOXA expression in negative margins (Fig. 5B). In contrast, only 3 of the top 10 models included PUMA. In 7 of the top 10 models, positive margin patients as well as negative margin patients with low NOXA expression could be further stratified by preoperative PSA and/or Gleason score where patients exhibiting high preoperative PSA or low Gleason had a better prognosis. Notably, in all of these seven models, the best prognostic group (negative margin, low NOXA expression, and low preoperative PSA or Gleason) did not relapse. In one of these models with an IBS of 0.114 (95% CI, 0.099-0.157; Table 2), 0/9 patients exhibiting negative margins, low NOXA expression, and low preoperative PSA (<6.5) relapsed in the best prognostic group (Fig. 5C). In contrast, the top model that included only margin status and preoperative PSA had an IBS of 0.135 (95% CI, 0.093-0.157; Table 2), where 3/20 patients relapsed in the best prognostic group. Of the RPART models composed exclusively

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**Fig. 3.** Average NOXA (A) and PUMA (B) expression in prostate tissue subtypes. Average was calculated over all the available cores in each subtype category. Normal, normal prostate tissue from autopsied patients (NOXA n = 94; PUMA n = 96 cores); NA, normal tissue found adjacent to cancer in radical prostatectomy specimens (n = 91 cores); PIN, PIN tissue obtained from radical prostatectomy (n = 43); HS cancer, HS cancer tissues obtained by radical prostatectomy (NOXA n = 225; PUMA n = 227); HR cancer, HR cancer tissue obtained from TURP specimens (n = 159). Two independent pathologists verified all core classifications. Error bars, SE. Associated nonparametric test. P values under 0.05 were considered significant.
of clinical markers, the top-ranking model was one that included margin status and Gleason score and had an IBS of 0.132 (95% CI, 0.090-0.160; Table 2).

Discussion

To our knowledge, this is the first study describing the expression of NOXA or PUMA in a cohort of patients representing various histopathologic subtypes of PCa. In our overall cohort of 135 patients, we found that mean NOXA expression increased gradually going from normal prostate cores to NA and PIN cores, followed by HS cancer cores, and finally, to HR cancer cores, the latter expressing the highest levels of NOXA (Fig. 2A). These data suggest that increasing NOXA expression may be associated to PCa progression. In contrast with what has been previously observed in colorectal cancer (32), we found that increased NOXA expression is a frequent occurrence in PCa (16% in colorectal cancer versus 68% of PCa patients). In addition, we found that NOXA expression is associated with clinical outcome, which was not observed in the colorectal cancer study. In contrast with NOXA, the association between PCa progression and PUMA seems to be more complex. Although we found that 70% of HS patients showed elevated PUMA (as opposed to 29% in melanoma; ref. 31), our data suggest that PUMA expression does not further increase in HR PCa.

These findings are somewhat reflected in what was observed in PCa cell lines using Western blots probing for NOXA and...
Fig. 5. Kaplan-Meier plots for RPART model including NOXA, PUMA, and clinical markers. A, top-ranking model obtained in the study (MGNXPU). IBS = 0.107 (95% CI, 0.065-0.128). Left, associated survival tree; numbers in circles represent MGNXPU groups depicted in the Kaplan-Meier plots (right). B, general survival tree structure determined from the top nine models ranked by IBS. Recurrent structures (bold line) were those present in all of the top nine ranking models. X and Y, node-splitting variables (discontinuous line) within the top nine models. In the top nine ranking models, splitting parameter X was either PUMA, Gleason, and preoperative PSA. One model exhibited no variable for X (no split). Splitting variable Y was either PUMA, Gleason, preoperative PSA, or NOXA. C, favored RPART model obtained from the top nine RPART models from this study (rank 6). IBS = 0.113 (95% CI, 0.065-0.142). Numbers within circles in the survival tree (left) represent groups depicted in the Kaplan-Meier plots (right). In (A) and (C), fractions below the colored circles, number of patients relapsed/number of patients in the group.
PUMA expression. In Fig. 1, we can see that taken together, the two androgen-insensitive cell lines PC3 and DU145 express relatively higher levels of NOXA as compared with androgen-responsive LNCaP and 22Rv1 cells. In contrast, LNCaP and 22Rv1 cells seem to exhibit higher levels of PUMA. These findings are somewhat surprising because NOXA and PUMA have been found to have more than one transcriptional regulator in common, including p53 and E2F1 (45). However, we found that within cores, there was a generally strong correlation between PUMA and NOXA expression (Spearman’s coefficient = 0.586; P < 10−6; data not shown). Altogether, these data may be indicative of the involvement of molecular pathways that lead to de-coupled PUMA/NOXA in HRPCa. Further investigation will be required to address this possibility.

To date, investigators have typically used linear Cox proportional hazard models to stratify patients’ risk with respect to the expression of molecular markers. However, Cox models neither handle complex interactions among prognostic factors efficiently nor take into account nonlinear effects (46, 47). To overcome these limitations, tree-based methods offer an attractive alternative to Cox models (48). In this study, we used survival trees to assess the ability of NOXA and PUMA to predict BCR alone and in combination with clinical markers. Although this method is increasingly used for immunohistochemical analyses in cancer, its specific application to PCa cohorts has been thus far limited and generally focused on existing clinical parameters (49–52). However, one PCa study has recently applied the survival tree method to assess the prognostic ability of α-methylacyl CoA racemase detected by IHC as was done here for PUMA and NOXA (53).

Assessing the predictive performance measure and model selection criteria for prognostic models remains a matter of debate. For survival tree-based methods, the IBS is currently thought to be the most appropriate index (42, 54). Using IBS, we determined that the most accurate model for predicting BCR in our cohort was one that included surgical margin status, NOXA, and PUMA expression (IBS = 0.107). Although this model was particularly good at predicting which patients would not undergo BCR (0/13 in the best prognostic group, Fig. 5A and B), it presented a complex behavior of PUMA wherein its effect on BCR onset in negative margins was opposite to that found in positive margins. Because PUMA expression alone could not significantly predict BCR as shown in Kaplan-Meier analyses (Fig. 4B), it is unclear whether PUMA expression truly holds valuable clinical information. On the other hand, NOXA was a significant predictor of BCR when assessed alone.

Conclusion

We conclude that NOXA and PUMA expression may be linked to PCa progression. We also suggest that the assessment of NOXA expression may be particularly useful for PCa prognosis because it may extend the ability of existing clinical markers to predict BCR. We believe that a survival tree model including NOXA, surgical margin status, and preoperative PSA status deserves external validation in a larger cohort.

Acknowledgments

We thank Dr. Armen Aprikian for allowing us to use some of the tissue specimens included in the tissue microarrays. We recognize the assistance of Jason Madore in the construction and cutting of the tissue microarrays. We are also grateful to Philippe O. Gannon, Dr. Laurent Lessard, and Dr. Cécile Le Page for critical review of the manuscript.

Table 2. Brier scores and associated 95% CI for selected RPART models

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<th>Rank</th>
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<td>0.065-0.128</td>
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<tr>
<td>NOXA + margin + PSA</td>
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<td>0.114</td>
<td>0.069-0.142</td>
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<td>0.132</td>
<td>0.09-0.157</td>
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<tr>
<td>Margin + PSA</td>
<td>31</td>
<td>0.135</td>
<td>0.093-0.157</td>
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<tr>
<td>NOXA + PUMA</td>
<td>59</td>
<td>0.155</td>
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NOTE: 95% CI were calculated from 200 bootstrapped samples.

Philippe O. Gannon, Dr. Laurent Lessard, and Dr. Cécile Le Page for critical review of the manuscript.

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References


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