β-Catenin-related Anomalies in Apoptosis-resistant and Hormone-refractory Prostate Cancer Cells

Alexandre de la Taille, Mark A. Rubin, Min-Wei Chen, Francis Vacherot, Sixtina Gil-Diez de Medina, Martin Burchardt, Ralph Buttyan, and Dominique Chopin


ABSTRACT

Purpose: β-Catenin is a critical end component of the wnt signaling pathway that regulates cell growth, apoptosis, and migratory behavior in response to intercellular adhesion molecules. The aim of this study was to evaluate abnormalities of β-catenin protein expression, subcellular localization, and activity in an in vitro model of acquired apoptosis-resistance in cultured PC cells and in primary human prostate cancers (PrCa).

Experimental Design: Apoptosis-resistant human prostate cancer cell line variants were derived from parental LNCaP cells by repeated brief exposure to apoptotic stimuli. The derivative and parental cells were analyzed for β-catenin expression and intracellular localization using cell fractionation and Western blotting procedures. Endogenous transcriptional activity from the TCF/LEF-1 response element was also studied in these variants after transfection with a β-catenin sensitive reporter plasmid. Finally, β-catenin protein expression and intracellular localization were evaluated on 212 patients [122 localized PrCa and 90 hormone-refractory (HRPC) PrCa specimens by immunohistochemistry].

Results: Western blot analysis showed that the intracellular partitioning of β-catenin was shifted from the membrane fraction in parental cells to the cytoplasmic/nuclear fractions of the apoptosis-resistant cell lines. Coordinate, transcriptional activity from a TCF/LEF-promoted reporter plasmid was increased significantly in the apoptosis-resistant lines. In the primary prostate tumors analyzed, cytoplasmic and/or nuclear β-catenin expression was correlated statistically with the HRPC status and Gleason score. In the group of localized PrCa, abnormal β-catenin expression tended to be associated with a higher Gleason score and with pT3 disease. No mutation was found in patients with HRPC and abnormal β-catenin expression.

Conclusion: These data suggest that anomalies of β-catenin expression occur in PrCa and that these anomalies are associated with disease progression, especially to the therapeutic-resistant state.

INTRODUCTION

Prostate cancer is one of the most commonly diagnosed malignancies in males of Western countries. The high frequency with which this cancer occurs and its propensity to affect the quality of life during aging has only recently made it a focus for cancer-related biomedical research. Whereas there has been some progress in identifying potential genetic loci associated with familial forms of this disease (1, 2), the basic molecular pathology underlying prostate cancer development and progression remains poorly described. One aspect of prostate cancer that has been more thoroughly investigated is the tendency of advanced disease to develop resistance to therapeutic agents, including both hormonal and standard cytotoxic agents (radiation and chemotherapy). Numerous studies have identified the possibility that therapeutic resistance of prostate cancer cells is a complex phenotype that can be acquired either through non-hormonal stimulation of the androgen signaling process or through perturbations of the apoptotic regulatory systems of the prostate cancer cell (3–7). Given the recent evidence that wnt signaling through the β-catenin protein is one means to induce a state of apoptosis resistance (8–10) and that defects in the β-catenin post-translational processing pathway are common in several other human solid tumor systems (melanoma, colon, ovarian, and endometrial cancer, and hepatocellular carcinoma; Ref. 11–17), we undertook a specific study of prostate cancer to evaluate the extent to which this protein might play any role in prostate cancer development or progression to therapeutic resistance (18).

Since its discovery as a protein associated with the cytoplasmic domain of E-cadherin, β-catenin has been shown to be involved in two important functions: it has a crucial role in cell-cell adhesion in addition to a signaling role as an end component of the Wnt/wg signaling pathway. Whereas both functions are involved in the control of normal embryonic development, the latter function appears to be especially critical for its involvement in the development of solid tissue malignancies. As exemplified by our understanding of the role of β-catenin in colon cancer, abnormally high levels of this protein (in
conjunction with the TCF/LEF-1 transcription factor) in colon cancer cells transcriptionally up-regulates expression of cell growth-promoting gene products such as c-jun, c-myc, and cyclin D1, as well as inducing a state of apoptosis resistance (18, 19). The accumulation of β-catenin in colon cancer cells is generally associated with dysfunctions in its post-translational processing because of direct mutations in the β-catenin coding sequences that render its translation product refractory to an active degradation process or because of mutations within the APC gene, of which the product is one factor that controls the β-catenin degradation process (18, 20, 21). Any of these defects enable accumulation of β-catenin protein within the cytoplasm of the cancer cell and its translocation (in conjunction with TCF/LEF-1) to the nucleus where its effects on transcription are exerted (21, 22). Thus, not only are the expression levels of β-catenin perturbed in the colon cancer cell, its intracellular distribution is also altered significantly so that it is found in the cytoplasmic and nuclear fractions of the cancer cell in addition to its normal association with the membrane fraction via interaction with the classical cadherins (21).

To better evaluate the extent to which β-catenin action might be involved in prostate cancer progression to the therapeutic-resistant state, we analyzed a unique set of prostate cancer cell lines (LNCaP-TR3 and LNCaP-SSR), which have acquired an apoptotic-resistant phenotype as a result of repeated transient exposures of an apoptosis-sensitive human prostate cancer cell line, LNCaP, to apoptotic agents (23). Using the parental and progeny cell lines, and a large series of primary human prostate tumor specimens obtained from patients under treatment for prostate cancer, we evaluated the expression levels and intracellular distribution of the β-catenin protein to determine whether there might be any anomalies relevant to the development or progression of this common solid tumor system.

MATERIALS AND METHODS

Cell Lines. Parental LNCaP cells were obtained from the American Type Culture Collection. These cells are maintained in RPMI 1640 supplemented with 10% fetal bovine serum and antibiotics as described previously (23). Apoptosis-resistant derivatives of LNCaP cells were obtained after a sequential series of exposures of the parental cell line either to phorbol ester (10 nM TPA) or to serumless medium (serum starvation) for 24 h. These exposures are sufficient to induce apoptosis of between 80 and 85% of parental LNCaP cells (23). Surviving cells were transferred to the normal medium and were expanded to 80% confluence at which time they were again exposed to the apoptotic-inducing regimen. This was repeated for a total of five times and resulted in the derivation of two cell lines referred to as LNCaP-TR and LNCaP-SSR. These cell lines were assayed previously for their sensitivity both to the agent used in their selection as well as to the agent that was not used for their selection, and were found to be significantly resistant to both regimens (23). Likewise, the -TR and -SSR derivatives were found to have a hormone-resistant phenotype based on their ability to form tumors in castrated immune-deficient mice, in striking contrast to the parental LNCaP cell line (23).

Subcellular Fractionation of Cell Lines, Protein Extraction, and Western Blot Analysis. Subcellular fractionated protein extracts were made from the parental LNCaP, LNCaP-SSR, and LNCaP-TR lines. After washing in ice-cold PBS, they were resuspended in 5 volume of buffer A [10 mM HEPES (pH 7.9), 1.5 mM MgCl2, and 0.5 mM DTT] and incubated for 10 min on ice. The cells were then lysed by homogenization with a Polytron tissue homogenizer (Brinkmann Instruments, Westbury, NY). The homogenate was centrifuged for 10 min at 3,500 rpm, and the supernatant was designated the cytoplasmic protein fraction. The pellet was resuspended in 0.11 volume of buffer B [0.15 mM HEPES (pH 7.9), 150 mM NaCl, 10% glycerol, 1% Triton X-100, 1.5 mM MgCl2, 1 mM EGTA, and 0.015 mg/ml aprotinin] and was centrifuged at 100,000 × g for 60 min. The supernatant of this extract (containing the membrane protein fraction) was dialyzed 5–8 h against buffer D (20 mM HEPES, 20% (v/v) glycerol, 0.1 mM KCl, 0.2 mM EDTA, 0.5 mM phenylmethylsulfonyl fluoride, and 0.5 mM DTT). The nuclear protein fraction was extracted as described by Dignam et al. (24). After washing cells in PBS, they were suspended in 5 packed cell pellet volumes of buffer A and allowed to stand 10 min on ice. The cells were collected by centrifugation and lysed in 2 volumes of buffer A by 10 strokes of a Kontes all-glass Dounce homogenizer. The homogenate was centrifuged for 10 min at 2,000 rpm. The pellet was resuspended in buffer A (1 ml) and was centrifuged at 25,000 × g for 20 min. The pellets (crude nuclei) were resuspended in buffer C (3 ml for 109 cells, 20 mM HEPES (pH 7.9), 25% (v/v) glycerol, 0.42 mM NaCl, 1.5 mM MgCl2, 0.2 mM EDTA, 0.5 mM phenylmethylsulfonyl fluoride, and 0.5 mM DTT) and were homogenized with the Polytron tissue homogenizer. The suspension was stirred gently for 30 min and centrifuged for 30 min at 25,000 × g. The resulting clear supernatant was dialyzed against 50 volumes of buffer D for 5 h. The dialysate was centrifuged at 25,000 × g for 20 min, and the supernatant was considered to be the nuclear protein fraction. For Western blotting, 50 μg aliquots of each protein fraction were coelectrophoresed on an 8% acrylamide SDS gel. After electrotransfer to nitrocellulose membranes, E-cadherin (Transduction Laboratories, Inc., Lexington, KY), β-catenin (Sigma Chemical Co., St. Louis, MO), β-catenin (Transduction Laboratories, Inc.) and nuclear lamin proteins (control for nuclear protein loading, lamin A/C antibody; Santa Cruz Biotechnology, Inc., Santa Cruz, CA) were detected by Western blotting in a similar manner to that described previously (23) using antibody dilutions as recommended by the manufacturer.

Assays for Transcriptional Response to a β-Catenin/Tcf Transcriptional Promoter Element. LNCaP cell variants were tested for β-catenin/Tcf transcriptional activity by transient transfection with the pTOP-Flash reporter plasmid (Upstate Biotechnology, Inc., Lake Placid, NY; Refs. 22, 25) in which the expression of luciferase is promoted by three tandem copies of the optimal Tcf-response element (CCTTGATC) adjacent to the minimal c-fos promoter. Control cultures were
transiently transfected with the pFOP-Flash reporter plasmid, containing all of the regulatory elements of the pTOP-Flash plasmid, except that the Tcf response motifs contain mutations (CCTTGGCC) that prevent its functional activation by Tcf. All of the transient transfections also contained an internal control reporter plasmid containing β-gal promoted by an SV40 promoter. Cells (2 × 10⁶, in triplicate cultures) were transfected with 10 μg of pTOP-Flash or pFOP-Flash in the presence of 1 μg of the β-gal reporter plasmid using a standard transfection protocol involving lipofectin (23). Four h after transfection, the transfection medium was replaced with RPMI 1640 containing 10% fetal bovine serum, and the incubation was continued in this medium for 48 h. Cells were then collected by scraping, pelleted and washed in ice cold PBS, and a cytosol was prepared by incubation in ice-cold radioimmunoprecipitation assay buffer followed by centrifugation at 10,000 × g to remove debris. The cytosol extracts were assayed for protein content using the Bio-Rad Protein Assay System. Luciferase and β-gal activity in the extracts were measured using the Luciferase Assay System (Promega, Inc., Madison, WI) or the Galacto-Light Assay System (CCTTGGCC) that prevent its functional activation by Tcf. All of the transient transfections also contained an internal control reporter plasmid containing β-gal promoted by an SV40 promoter. Cells (2 × 10⁶, in triplicate cultures) were transfected with 10 μg of pTOP-Flash or pFOP-Flash in the presence of 1 μg of the β-gal reporter plasmid using a standard transfection protocol involving lipofectin (23). Four h after transfection, the transfection medium was replaced with RPMI 1640 containing 10% fetal bovine serum, and the incubation was continued in this medium for 48 h. Cells were then collected by scraping, pelleted and washed in ice cold PBS, and a cytosol was prepared by incubation in ice-cold radioimmunoprecipitation assay buffer followed by centrifugation at 10,000 × g to remove debris. The cytosol extracts were assayed for protein content using the Bio-Rad Protein Assay System. Luciferase and β-gal activity in the extracts were measured using the Luciferase Assay System (Promega, Inc., Madison, WI) or the Galacto-Light Assay System (Promega, Inc., Madison, WI) or the Galacto-Light Assay System (Tropix, Inc., Bedford, MA). Activity in the assays was measured by a luminometer (TD 20/20; Turner Design, Inc.). Results of pTOP-Flash or pFOP-Flash transfections are reported as the ratio of luciferase to β-gal activity for each of the cell lines.

**Human Tissues.** To evaluate β-catenin expression in human specimens, immunohistochemistry was performed on 212 specimens containing prostate cancer. These specimens include 122 from patients initially diagnosed with presumed clinically localized prostate cancer (since 1993) who were treated by radical prostatectomy at the Columbia-Presbyterian Medical Center (New York, NY). Clinical follow-up data were available on all of these consecutive cases. The mean follow-up time was 3.6 years (range, 0.6–5.2 years). Twenty-six of these patients with clinically localized disease had received 3 months of hormonal neoadjuvant therapy. However, the vast majority of these specimens are presumed to contain hormone-sensitive prostate cancers. For patients who had received radical prostatectomy, PSA failure was defined as a rise of serum PSA >0.2 ng/ml on two consecutive occasions. The time of failure was measured from the date of surgery to the first of the two consecutive elevated serum PSA levels. All of the radical prostatectomy specimens were evaluated in a standard fashion. Specimens were inked before processing. SM from the apex and base were taken as shaved margins. These sections were considered positive if any ink was seen on tumor. Alternate sections were submitted from the apex to base. One pathologist (M. A. R.) reviewed all of the cases for this study. Extraprostatic extension was diagnosed if tumor was seen in the periprostatic soft tissue or was seen penetrating through a fibromuscular capsule coming out on the other side. The SVs were evaluated at the junction where they enter the prostate gland. All of the pelvic lymph nodes were evaluated for the presence of metastatic disease. All of the cases were assigned a GS. After review of each slide of the pathological specimens obtained from a given radical prostatectomy specimen, a fresh-cut slide from the block containing tissue with the highest density of tumor cells and the highest Gleason score was selected for immunohistochemical staining. Ninety additional cases of HRPC specimens were also obtained from transurethral resection of the prostate done for bladder obstruction (HRPC status was defined as patients having PSA progression despite complete androgen blockade therapy, a castrated level of testosterone, and follow-up patient testing for the antiandrogen withdrawal syndrome).

**Immunohistochemical Detection of β-Catenin in Human Prostate Cancer Specimens.** Standard avidin-biotin peroxidase immunohistochemical staining was performed on a 5-μm-thick section from paraffin embedded, formalin fixed tissue. The β-catenin antibody (Transduction Laboratories, Inc.) was used to identify β-catenin expression in prostate cancer cells. β-Catenin protein expression was evaluated for staining intensity and location in tumor cells (i.e., membranous, cytoplasmic, and/or nuclear). Each case was examined at low power first to identify areas of tumor and normal prostate. At ×200 and ×400 magnification, the staining of normal secretory epithelium was compared with the tumor cells in the specimen. Because normal secretory epithelium had a membrane-specific staining pattern, cancer cells exhibiting a similar quality staining were considered to have the “normal” pattern. Abnormal β-catenin expression was defined as a cytoplasmic and/or nuclear staining.

**Analysis for β-Catenin Mutations in Cell Lines and Hormone-refractory Prostate Cancer Specimens.** Eight tumor RNA samples from patients with HRPC disease and abnormal β-catenin expression were assessed for mutations in exon 3 of β-catenin (CTNNB1), the region of the gene in which mutations have been identified in prostate and in other human tumors (26). Exon 3 of β-catenin was amplified by PCR using primers bcat-fwd1 (5′-GCTGATTTGATGGAGTTGGA-3′) and bcat-rev1 (5′-GCTACTTGGTCTGTAGGGA-3′). Reactions contained 2 mM MgCl₂ and 1 unit of Taq polymerase. Cycling conditions included initial denaturation for 5 min at 94°C; 35 cycles of denaturation for 30 s at 94°C; 30 s annealing at 55°C; 30 s elongation at 72°C; followed by a final elongation step for 7 min at 72°C. Then reverse transcription-PCR products were sequenced directly.

**Statistical Analysis.** SPSS 9.0.1 (SPSS Inc.) and StatView 4.55 (Abacus Concepts, Inc., Berkeley, CA) were used for the statistical analysis. All of the tests were performed at the 0.05 significance level. Student test was used to compare two continuous variables. All of the data were summarized using method of Kaplan Meier. Comparisons between curves were carried out with the log-rank test.

**RESULTS**

**Altered Intracellular Compartmentalization of β-Catenin in Apoptosis-resistant Prostate Cell Lines Corresponds with Increased Endogenous Transcriptional Activity from the TCF/LEF-1 Promoter.** The parental LNCaP and the two apoptosis-resistant cell lines were fractionated into membrane, cytoplasmic, and nuclear fraction, and the individual fractions were extracted for protein. Extracts containing equal amounts of protein were electrophoresed by SDS-PAGE, and the proteins were electrotransferred to a nitrocellulose filter to make a Western blot. The Western blot was probed with antibodies that recognize E-cadherin or one of two cadherin-binding proteins, α-catenin or β-catenin. As
is shown in Fig. 1, E-cadherin was restricted to the membrane fractions of all of the lines. The two apoptosis-resistant lines appear to express lower levels of E-cadherin than the parental LNCaP cells. α-Catenin, a cell structural protein that anchors actin filaments, was also highly enriched in the membrane fractions as might be expected from its known association with cytoplasmic domains of the classical cadherins on the cell surface. This protein was equally present in each of the membrane extracts, but was also present in the cytoplasmic fractions. The β-catenin protein was restricted to the membrane fraction of the parental LNCaP cells only consistent with its affinity for cell surface cadherins. However, the apoptosis-resistant LNCaP-TR and -SSR variants demonstrated anomalous β-catenin protein in both the cytoplasmic and nuclear fractions of these cells.

The altered intracellular distribution of β-catenin that we detected in the apoptosis-resistant cell derivatives was also found to be associated with a coordinate increase in endogenous transcriptional activity from the TCF/LEF-1 transcriptional response element in these cells. When the variants were individually cotransfected with a luciferase reporter plasmid containing TCF/LEF-1 response elements (pTOP-Flash) and a β-gal reporter plasmid promoted by a simple cytomegalovirus promoter element, the ratio of luciferase:β-gal activity was found to be >2-fold greater than parental LNCaP in the -SSR line and >4-fold greater than LNCaP in the -TR line (Fig. 2A). Likewise, when we compared the expression of luciferase reporter in these lines after transfection with pTOP-Flash (promoted by the optimal TCF/LEF-1 response element) or pFOP-Flash (luciferase reporter promoted by mutated, inactive TCF/LEF-1 response elements) we found similar results; i.e., a >2-fold increased ratio of pTOP:pFOP luciferase activity in the LNCaP-SSR cells and a >2.5-fold increased ratio of pTOP:pFOP luciferase activity in the -TR cells (Fig. 2B). These results demonstrate that endogenous transcriptional activity promoted by the TCF/LEF-1 response element is significantly greater in the apoptosis-
resistant cell lines and support the concept that the β-catenin-regulated transcriptional pathway is more active in these cells.

Expression of β-Catenin in Human Prostate Cancer Specimens. Immunostaining for β-catenin was successful on all of the cases examined. Abnormal β-catenin expression was defined as cytoplasmic and/or nuclear staining (see Fig. 3). β-Catenin expression was abnormal (localized in cytoplasm and/or nucleus) in 29% of the 212 specimens. Interestingly, abnormal β-catenin expression was found in 23% of prostate cancers obtained from radical prostatectomy specimens, most of which are presumed to be hormonally sensitive. This proportion was significantly lower than what was observed in specimens from patients with HRPC (38%), and the difference was statistically significant (P = 0.042). We also observed that abnormal β-catenin expression was correlated with Gleason score (see Fig. 4).

Concerning the group of patients with clinically localized prostate cancer, median age was 62.1 years (range, 43–73), and preoperative serum PSA was 11.2 ng/ml (range, 0.5–29.0). Final pathology revealed 87 patients had pT2 disease (71%) and 35 had pT3 (29%). Patient demographics were reported in Table 1. Patients with abnormal β-catenin expression had a slightly higher mean Gleason score compared with patients with normal β-catenin (6.46 versus 6.29; P = 0.42) and higher rate of pT3 disease (40% versus 25%; P = 0.15). After a mean follow-up of 3.6 years, 33 patients had a biochemical progression. As expected, PSA, Gleason score, tumor stage, and positive SMs were statistically associated with the highest biochemical progression survivals after radical prostatectomy (see Table 2). Biochemical recurrence survivals of patients with or without abnormal β-catenin expression were not different (38.4% versus 29.5%, Kaplan Meier analysis; P = 0.43); abnormal β-catenin expression was not correlated with a poor outcome in this selected group of patients with clinically localized prostate cancer who underwent a radical prostatectomy.

Ninety prostate specimens from patients with HRPC were included in this analysis. The mean age of these patients was 72.3 years (range, 68–84 years), mean PSA was 57.6 ng/ml (range, 6–250 ng/ml), and the mean Gleason score was 8. β-Catenin expression was found to be abnormal in 38.8% of these cases. Tissue specimens from 8 HRPC patients with abnormal β-catenin expression were analyzed for β-catenin mutations. None of them had a mutation in the exon 3.

**DISCUSSION**

The β-catenin protein was initially discovered based on its association with the cell membrane-bound cadherin complex. However, it was soon realized to be a major effector in a chain of complex signaling events referred to as the wnt/wg signaling pathway. This signaling pathway involves several proteins that...
have proven to be of great importance for our understanding of certain malignant processes including the cadherins, a-catenin, axin, glycogen synthase kinase-3β, and adenomatous polyposis coli (27). Many of these proteins function by regulating the ability of the β-catenin protein to survive a post-translational degradation processes in the cytoplasm and to enter the nucleus (in conjunction with the TCF/LEF-1 transcription factor). There it affects the transcription of a growing list of gene products that have the potential to affect cell growth and survival. Numerous human malignancies now demonstrate some potential for the involvement of β-catenin anomalies in their development and progression including hepatocarcinoma, melanoma, colon cancer, ovarian cancer, breast cancer, gastric cancer, and endometrial carcinoma. There have been some reports previously that might place prostate cancer among this group. These include: (a) reports of a small percentage (∼5%) of prostate cancers with mutations in the β-catenin gene (26, 28); (b) reports that the β-catenin synergizes transcriptional regulation from the androgen receptor in prostate cancer cells (29, 30) and; more recently, (c) immunohistochemical evidence that advanced prostate cancers can have β-catenin in the nucleus (12). Our work reported here continues to support a potential role for β-catenin in prostate cancer, especially for the progression of this disease to the therapeutic-resistant state.

This is evidenced by the drastic change in the intracellular localization of β-catenin in prostate cancer cell lines that have been selected for the phenotype of apoptosis resistance. These cell lines, LNCaP-TR and LNCaP-SSR, have significant levels of β-catenin protein in their cytoplasmic and nuclear fractions, in striking contrast to parental (apoptosis-sensitive) LNCaP cells in which β-catenin is restricted to the membrane fraction. This anomalous localization corresponds with significantly increased transcription potential from β-catenin/Tcf promoter elements in the apoptosis-resistant cells compared with the parental cells. There are several reports in the literature demonstrating that β-catenin transcriptional-promoting activity induces apoptosis resistance, although it is not yet clear the precise mechanism through which this is exerted (12, 31). We have also considered the possibility that the process used in selecting the apoptosis-resistant cells may have also selected for cells with mutations in the β-catenin gene, but our sequence analysis of the third exon of β-catenin expressed in these cell lines (LNCaP, -TR and -SSR derivatives) showed only the wild-type sequence. It is also of note that the apoptosis-resistant variants of the LNCaP were shown previously to have selective expression of a novel protocadherin-family gene that we have termed protocadherin-PC. This protocadherin-family gene is unique in that it appears to be cytoplasmically localized rather than membrane-bound like other members of the protocadherin gene family (32). Additionally, protocadherin-PC appears to retain a β-catenin binding site, and we have proposed that protocadherin-PC might have some role in the anomalous distribution of the β-catenin molecule in the apoptosis-resistant LNCaP cell variants (32).

Our immunohistochemical analysis of actual human prostate cancer specimens also supports the recent finding of Chesire et al. (12) that a fraction of prostate cancers have anomalous intracellular localization of β-catenin, consistent with an abnormality in the β-catenin processing pathway. This occurred with a frequency of ∼23% in a series of localized prostate cancers and with a frequency significantly higher (38.8%) in a group of hormone-refractory prostate cancer specimens. This observation is also consistent with the previous report of Chesire et al. (12, 28) in which they noted a higher frequency of nuclear β-catenin in advanced prostate cancer specimens. Again, this observation is consistent with the possibility that anomalous signaling through β-catenin in these cases is associated with the acquisition of an apoptosis-resistant tumor cell phenotype or therapeutic-resistance. Given our finding of a weak association between Gleason’s grade in primary tumors and abnormal β-catenin staining, it is also possible that the higher grades that are found consistently in hormone-refractory prostate cancers are the cause of this relationship. We consider it unlikely that this change in β-catenin localization in (hormone-refractory) prostate cancers is the result of widespread mutation of the β-catenin gene, because we were able to sequence β-catenin exon-3 from 8 of the hormone-refractory prostate cancer specimens with abnormal nuclear β-catenin accumulation, but these specimens also had only the wild-type sequence, supporting the previous demonstration of Chesire et al. (12) that this occurs infrequently in prostate cancers.

In summary, our results here support the idea that signaling through β-catenin plays some role in the progression of human prostate cancer, especially to the therapeutic-resistant state (acquisition of apoptosis-resistant phenotype and of HRPC status). This would place prostate cancer in a growing list of solid tissue malignancies of which the development or progression involves this critical protein. It also identifies the possibility that inhibitors of the β-catenin signaling pathway might have use in the therapeutics of hormone refractory prostate cancer.

### Table 2
Cumulative percentages for PSA recurrence using Kaplan-Meier analysis for patients treated with radical prostatectomy

<table>
<thead>
<tr>
<th>PSA recurrence</th>
<th>Cumulative percentage</th>
<th>( P^a )</th>
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<tbody>
<tr>
<td>Preoperative PSA</td>
<td></td>
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<tr>
<td>≥10 ng/ml</td>
<td>25.5%</td>
<td>0.008</td>
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<tr>
<td>&gt;10 ng/ml</td>
<td>44.2%</td>
<td></td>
</tr>
<tr>
<td>Stage</td>
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<td></td>
</tr>
<tr>
<td>pT2</td>
<td>18.4%</td>
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</tr>
<tr>
<td>pT3</td>
<td>68.7%</td>
<td>&lt;0.0001</td>
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<td>Extra prostatic extension</td>
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<td>19.6%</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>67.2%</td>
</tr>
<tr>
<td>SV invasion</td>
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<tr>
<td>No</td>
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<tr>
<td>Yes</td>
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<td>Positive surgical margins</td>
<td>No</td>
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<td></td>
<td>Yes</td>
<td>57.4%</td>
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<tr>
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</tr>
<tr>
<td>&lt;7</td>
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<tr>
<td>≥7</td>
<td>52.1%</td>
<td>&lt;0.0001</td>
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<tr>
<td>β-Catenin expression</td>
<td>Membrane</td>
<td>29.5%</td>
</tr>
<tr>
<td></td>
<td>Cytoplasm/nucleus</td>
<td>38.4%</td>
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\(^a\) Log-rank test.
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