Crosstalk between the Androgen Receptor and β-Catenin in Castrate-Resistant Prostate Cancer

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Abstract

The androgen-signaling pathway plays an important role in the development and hormonal progression of prostate cancer to the castrate-resistant stage (also called androgen-independent or hormone refractory). The Wnt pathway and β-catenin contribute to prostate biology and pathology. Here application of Affymetrix GeneChip analysis revealed the genomic similarity of the LNCaP hollow fiber model to clinical samples and identified genes with differential expression during hormonal progression. The fiber model samples clustered according to the expression profile of androgen-regulated genes to provide genomic evidence for the reactivation of the AR signaling pathway in castrate-resistant prostate cancer. Pathway-based characterization of gene expression identified activation of the Wnt pathway. Together with the increased expression of AR and β-catenin, there was increased nuclear colocalization and interaction of endogenous AR and β-catenin in castrate-resistant prostate cancer from castrated mice. Surprisingly, no interaction or colocalization of AR and β-catenin could be detected in xenografts from noncastrated mice. These studies provide the first in vivo evidence to support aberrant activation of the AR through the Wnt/β-catenin signaling pathway during progression of prostate cancer to the terminal castrate-resistant stage. [Cancer Res 2008;68(23):9918–27]

Introduction

Androgen ablation is currently the most effective systematic therapy available for prostate cancer patients with metastatic disease. However, this therapy is palliative, and after an initial response to androgen ablation, most tumors eventually begin to grow in the absence of testicular androgens to form castrate-resistant disease (1). Molecular mechanisms underlying hormonal progression to the castrate-resistant stage remain unknown. One mechanism suspected to play a role is the transcriptional activity of the androgen receptor (AR). The AR is a ligand-dependent transcription factor that is a member of the steroid receptor family. Ligand-activated AR, complexed with coactivator proteins and general transcription factors, binds to androgen response elements located in the promoter and enhancer regions to activate or repress the transcription of specific target genes suspected to be involved in proliferation.

One important coregulator of the AR is β-catenin, which interacts with the AR in response to androgen to increase the transcriptional activity of the AR in cells maintained as a monolayer (2, 3). Levels of β-catenin and AR are both increased in castrate-resistant prostate cancer. Mutated forms of β-catenin, which can result in a stabilized protein, have also been detected in prostate cancer (4, 5). β-Catenin has dual functions that involve both cell adhesion and signal transduction in response to Wnt ligands (6, 7). The cellular localization of β-catenin is important for its function. Consistent with its role in cell adhesion, β-catenin is predominantly detected at the cell membrane where it interacts with E-cadherin and α-catenin (8). In response to Wnt ligands, β-catenin can also be detected in the nucleus and cytoplasm where it can interact with T-cell factor (TCF)/lymphoid enhancer factor (LEF) transcription factors to initiate transcription of target genes such as c-myc and cyclin D1 (9–11). β-Catenin activity is regulated by phosphorylation followed by degradation. This process involves interaction with glycogen synthase kinase 3β (GSK3β), axin, and adenomatous polyposis coli. Phosphorylation of β-catenin by CK1ε and GSK3β on serine and threonine residues targets it for degradation by the ubiquitin proteasome pathway (12). Degradation of β-catenin is inhibited by Wnt signaling, which results in elevation of levels of β-catenin in the nucleus and interaction with TCF/LEF transcription factors to regulate expression of target genes (9, 13).

Crosstalk between the Wnt and AR pathways occurs at several levels: (a) Wnt ligands can transactivate the AR (14); (b) β-catenin interacts with the AR to increase its transcriptional activity as measured by androgen-induced reporter gene constructs (2, 3, 15–17); (c) GSK3β negatively regulates AR-mediated transcription (18–20); (d) competition for β-catenin can occur between AR and TCF/LEF (21); and (e) the TCF/LEF target gene, cyclin D1, can interact with the AR to inhibit AR transcriptional activity (22–24).

In the present study, we applied Affymetrix GeneChip (Human Genome U133 plus 2) combined with the LNCaP xenograft and hollow fiber models to identify global changes in gene expression associated with castrate-resistant prostate cancer. One pathway identified to be activated in castrate-resistant samples was the Wnt/β-catenin signaling pathway. Colocalization and interaction of AR and β-catenin were detected for the first time in vivo in castrate-resistant tumors, but surprisingly, not from tumors harvested from noncastrated mice. These data suggest a role for β-catenin interaction with the AR in the progression of prostate cancer to the terminal castrate-resistant stage.

Materials and Methods

Hollow fiber and s.c. xenograft models of prostate cancer. The LNCaP hollow fiber model of prostate cancer was done as described previously (25, 26). S.c. xenografts were prepared in male severe combined immunodeficient mice inoculated with about 1 × 10⁶ LNCaP cells suspended in 75 μL of RPMI 1640 (5% fetal bovine serum) with 75 μL of...
RNA isolation and microarray analysis. RNA was isolated and analyzed using microarrays as described previously (26). Briefly, total RNA was extracted from cells using Trizol (Invitrogen Life Technologies) according to the manufacturer’s protocol. RNA samples from cells were analyzed by Affymetrix GeneChip microarray. The syntheses of cDNA and biotinylated cRNA were done according to the protocols provided by the manufacturer (Affymetrix). Biotinylated fragmented cRNA probes were hybridized to the HGU133 plus2 GeneChips (Affymetrix). Hybridization was done at 45 °C for 16 h in a hybridization oven (Affymetrix). The GeneChips were automatically washed and stained with streptavidin-phycocerythrin conjugate in an Affymetrix GeneChip Fluidics Station. Fluorescence intensities were scanned with a GeneArray Scanner (Affymetrix). Hybridizations were carried out independently for each condition using three biological replicates.

Expression profile analysis. Comparative analyses between expression profiles for Affymetrix experiments were carried out using GeneSpring software version 7.2 (Silicon Genetics). The expression profiles from three animals were compared using two-way one-way ANOVA to identify genes that were differentially expressed across the three groups. The data set, GEO GDS1390, for clinical samples of androgen-dependent and castrate-resistant prostate cancer was downloaded from PubMed. For sample clustering, standard correlation was applied to measure the similarity of the expression pattern between different samples. Class prediction was done to calculate the similarity of the samples from the LNCaP hollow fiber model to the clinical samples of castrate-resistant prostate cancer.

Quantitative reverse transcription-PCR. Oligo(dT)–primed total RNAs (0.5 µg per sample) were reverse transcribed with SuperScript III (Invitrogen Life Technologies). An appropriate dilution of cDNA and gene-specific primers was combined with SYBR Green Supermix (Invitrogen) and amplified in ABI 7900 real-time PCR machine (Applied Biosystems). All quantitative PCR reactions were done in triplicate. The threshold cycle number (Ct) and expression values with SDs were calculated in Excel. Primer sequences for real-time PCRs are listed in Supplementary Table S1. Real-time amplification was done with initial denaturation at 95 °C for 2 min, followed by 40 cycles of two-step amplification (95 °C for 15 s, 55 °C for 30 s).

Whole-cell lysates and Western blot analyses. Protein from whole-cell lysate was isolated using Trizol according to the manufacturer’s protocol. Samples were stored at −80 °C until use. The antibodies used in these studies were obtained from various suppliers: AR (PG21, Upstate Biotechnology), PSA (C-19, Santa Cruz), β-catenin (Cell Signaling), phosho-Y142 β-catenin (Abcam), β-actin (Abcam), GSK3β (Abcam), CTNNBIP1 (Abcam), cyclin D1 (Abcam), p53 (Abcam), and TCF3 (Abcam).

Immunoprecipitation. Protein was extracted from LNCaP cells or xenograft tumor samples using Triton X-100 lysis buffer (150 mmol/L NaCl, 1% Triton X-100, 50 mmol/L Tris-HCl). The cell lysates of the xenografts were stripped of albumin by using the Montage Albumin Deplete Kit (Invitrogen). Protein was extracted from cells using Trizol (Invitrogen Life Technologies) according to the manufacturer’s protocol. RNA samples from cells were analyzed by Affymetrix GeneChip microarray. The syntheses of cDNA and biotinylated cRNA were done according to the protocols provided by the manufacturer (Affymetrix). Biotinylated fragmented cRNA probes were hybridized to the HGU133 plus2 GeneChips (Affymetrix). Hybridization was done at 45 °C for 16 h in a hybridization oven (Affymetrix). The GeneChips were automatically washed and stained with streptavidin-phycocerythrin conjugate in an Affymetrix GeneChip Fluidics Station. Fluorescence intensities were scanned with a GeneArray Scanner (Affymetrix). Hybridizations were carried out independently for each condition using three biological replicates.

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Double immunofluorescence microscopy. Tissue sections (5 µm) were blocked in immunohistochemistry solution (Immunovision Technologies) and immunostained with an anti-AR antibody (AB441, Santa Cruz; 1:10) at 4 °C overnight, then with anti–β-catenin antibody (Cell Signaling; 1:20) at 4 °C overnight. For immunofluorescence staining, sections were incubated with FITC-labeled anti-rabbit IgG for β-catenin and tetramethylrhodamine isothiocyanate–labeled anti-mouse IgG for AR (both 1:200) for 30 min. The immunofluorescence staining of proteins was detected using a Zeiss Axioplan-2 fluorescence microscope (Zeiss).

Results

The LNCaP hollow fiber model correlates to clinical hormonal progression of prostate cancer. The LNCaP hollow fiber model provides a reproducible method to obtain human prostate cancer cells that are free from host contamination from in vivo studies (25). A strength of this model is that it allows analyses of matched samples from the same animal at different time points due to the noninvasive procedures to harvest the fibers. Here we apply this model to obtain protein and RNA from LNCaP cells harvested from castrated mice during the different stages of progression to assess changes in gene expression and identify pathways associated with castrate-resistant growth. Consistent with previous reports, LNCaP cells maintained in hollow fibers progressed to castrate-resistance after castration of the host as indicatNormalizedized by serum PSA levels (Fig. 1A). The removal of fibers from the animals caused further reduction in serum PSA due to the removal of tumor cells. Serum PSA levels are correlated to tumor burden (27). At least a 3-fold increase in serum PSA over the nadir level was still observed in spite of removal of fibers to indicate castrate-resistant reexpression of PSA at 31 days after castration. Analyses of levels of PSA mRNA and protein (Fig. 1A) from LNCaP cells harvested from fibers immediately before castration (intact or androgen dependent; AD), after castration at day 10 (androgen ablation; AA), or 31 days after castration when serum PSA becomes elevated again (castrate resistant or androgen independent; AI) were consistent with hormonal progression in response to castration. PSA mRNA and protein were reduced by ∼80% in response to castration (AA) as compared with levels from samples harvested from intact mice (AD). Levels of expression of PSA mRNA and protein returned to pre-castration levels in castrate-resistant samples (compare levels in AD and AI in Fig. 1A). These samples were next used for Affymetrix analysis of changes in the transcriptome during hormonal progression, and expression profiles compared with Affymetrix data available for clinical samples of prostate cancer.

To confirm the similarity of the castrate-resistant samples harvested from the hollow fiber model to clinical samples, we screened a group of the most significantly differentially expressed genes in the clinical samples composed of both androgen-dependent and castrate-resistant prostate cancers (28). There were 79 genes identified to be significantly differentially expressed between castrate-resistant and androgen-dependent clinical samples with P < 0.001 (Supplementary Table S2). A hierarchical two-dimensional clustering algorithm based on similarity of expression patterns was applied to these 79 genes. Comparison of expression profiles showed a tendency to subgroup the castrate-resistant samples from the fiber model together with the clinical castrate-resistant prostate cancers, whereas the androgen-dependent samples from the fiber model clustered together with the clinical androgen-dependent prostate cancers (Fig. 1B). The class prediction analysis revealed that the androgen ablation and castrate-resistant samples from the fiber model have positive similarity scores to the clinical castrate-resistant prostate cancers, but castrate-resistant samples have significantly more similarity scores to the clinical castrate-resistant prostate cancers, whereas the androgen-dependent samples compared with Affymetrix data available for clinical samples of prostate cancer.

Global gene expression profiles of hormonal progression of prostate cancer. After confirmation of similarity between the hollow fiber model and the clinical samples at the transcriptome
Figure 1. The LNCaP hollow fiber model mimics hormonal progression of clinical prostate cancer. A, top, levels of serum PSA in at least three mice implanted with hollow fibers containing LNCaP cells after castration. Middle, levels of PSA mRNA measured by Affymetrix (line) and quantitative PCR (columns) using total RNA harvested from hollow fibers from animals at day 0, day 10, and day 31 shown in A. Fold change is based on levels of PSA mRNA normalized to levels of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA relative to levels of samples collected at day 0 (AD) and set at 1-fold. Samples are matched from the same animal at the different time points. AD signifies total RNA samples collected from animals immediately before castration at day 0 during growth in the presence of androgen. AA signifies RNA samples collected from animals 10 d after castration during androgen ablation. AI represents RNA samples collected at day 31 after castration when PSA becomes reexpressed signifying castrate-resistant growth. NE is the normalized expression. Bottom, Western blot analysis of levels of PSA protein normalized to β-actin protein using whole-cell lysates collected at the same time points as described above. Below is shown the densitometry analysis of PSA protein normalized to β-actin using matched samples from at least three different mice and set at 1-fold using the samples at day 0 (AD). B, unsupervised clustering analysis of Affymetrix data using RNA samples collected from the hollow fiber model (n = 9) with the clinical samples of prostate cancer (n = 20) according to the 79 genes identified to be most significantly differentially expressed in castrate-resistant versus androgen-dependent clinical samples, by standard correlation. The genes are represented by each row and the experimental samples are represented on each column. 10R, 11L, and 12R are the identities of the three mice bearing fibers containing LNCaP cells. The suffix (AD, AA, or AI) represents when the sample was collected from the animal as described above. The red box highlights the cluster for AI samples (clinical and from the fiber model). C, class prediction analysis to calculate the similarity of the fiber model samples to the clinical castrate-resistant prostate cancer samples according to the expression pattern of the same group of genes depending of stage of hormonal progression (e.g., AD, AA, and AI). Y-axis, margin scores to the castrate-resistant clinical samples. Columns, mean; bars, SD.
level, significance analysis of microarrays (29) was applied to identify genes differentially expressed across the different stages of hormonal progression. Using a two-way ANOVA ($P < 0.05$), 5,667 genes were measured as differentially expressed across the various time points (Fig. 2). These 5,667 genes were further clustered by standard correlation according to the expression pattern during the hormonal progression to castrate-resistant disease. Cluster 1 (represented by $\beta$-catenin) included 814 genes whose expression increased in castrate-resistant samples. Cluster 2 [represented by $\beta$-catenin (CTNNB1), and AR] included 1,143 genes whose expression increased after castration and further increased or remained elevated in castrate resistance. Cluster 3 (represented by MMP16 and Wnt5A) included 489 genes whose expression increased after castration but decreased in castrate resistance. Cluster 4 (represented by androgen-regulated genes Dic2 and FKB5, TP53, GSK3B, LEF1, and TCF3) included 1,540 genes whose expression decreased in response to castration and remained decreased in castrate resistance. Cluster 5 (represented by CSNK2B, CSNK1E, CCND1, MYC, and PLCB4) included 1,226 genes whose expression decreased only when the tumor progressed to castrate resistance. Cluster 6 (represented by androgen regulated genes KLK3, KLK2, ELL2, SOCS2, and RHOU) included 455 genes whose expression decreased in response to castration, but increased in castrate-resistant samples.

Figure 2. Genes differentially expressed during the hormonal progression of prostate cancer in the hollow fiber model. Unsupervised clustering analyses of the 5,667 genes according to their expression pattern during hormonal progression by standard correlation using the GeneSpring microarray analysis software. The genes are represented by each row and the experimental samples are represented on each column (in biological triplicates). Two-way ANOVA analysis of genes differentially expressed across all stages of progression ($P < 0.05$). Arrows, expression patterns of each cluster during hormonal progression from AD to AA with castration and finally to AI. For example, expression of CTNNBIP1 does not change from AD to AA, but increases when AI.
Hormonal progression of prostate cancer has been suggested to be at least partially due to the reactivation of AR after androgen ablation therapy (30). To characterize the status of the AR pathway in castrate-resistant prostate cancer, a hierarchical two-dimensional clustering algorithm that was based on similarity of expression patterns was applied to 1,092 genes previously identified to be differentially expressed in response to androgen in LNCaP cells maintained in cell culture (26). The genes are represented on each row (Fig. 3) and experimental samples are represented on each column. Comparison of expression profiles showed a strong tendency to subgroup the samples with respect to the stage of the tumors. This suggested that distinct and consistent differences in the levels of transcript occurred during hormonal progression. Of particular interest, hierarchical cluster analysis revealed that the expression patterns of androgen-regulated genes in castrate-resistant samples were more similar to that of the androgen-dependent than to androgen ablation samples. These data are consistent with reexpression of some androgen-regulated genes in castrate-resistant prostate cancer xenografts obtained from castrated hosts as previously observed (31).

**Increased expression of AR and β-catenin in castrate-resistant prostate cancer.** Genes differentially expressed in castrate-resistant prostate cancer represented many biological categories; however, several of these genes have functional attributes that could contribute to castration-resistant growth. The most notable were the marked increase in levels of mRNA for AR (P = 0.04) and β-catenin (P = 2.45e−05; Fig. 4A) in the castrate-resistant samples measured by both Affymetrix and quantitative PCR. Western blot analysis confirmed increased levels of AR and β-catenin (Fig. 4B) proteins in castrate-resistant prostate cancer samples. These data are consistent with clinical data describing elevated expression of AR and β-catenin in hormone-refractory prostate cancer.

Cytoplasmic or nuclear localization of β-catenin is required to mediate Wnt signaling. For β-catenin to accumulate in the cytoplasm, it requires phosphorylation on its tyrosine residue (Y142) to decrease interaction with α-catenin (12, 32, 33). Therefore, levels of phosphorylated β-catenin were measured during the different stages of hormonal progression by Western blot analyses with an antibody against phospho-Y142 of β-catenin. No phosphorylation of Y142 could be detected in samples from intact mice (Fig. 4B). Androgen ablation increased phosphorylation of Y142-β-catenin to detectable levels, which were further increased with castrate resistance. These data imply that the cellular localization of β-catenin may be altered with hormonal progression of prostate cancer to the cytoplasmic or nuclear form to mediate signal transduction.

**Expression profile of members of the Wnt pathway in castrate-resistant prostate cancer.** β-Catenin is the mediator of the Wnt signal transduction pathway, and its activity is regulated by many Wnt pathway members. To investigate the status of the Wnt pathway in the hormonal progression of prostate cancer, we validated changes in the expression of some members of Wnt pathway during the prostate cancer progression as first indicated by Affymetrix data. Consistent with increased levels of Y142 phospho-β-catenin in castrate-resistant prostate cancer, expression of tyrosine kinase–associated YES1 and LYN that phosphorylate β-catenin (33) were increased with castrate resistance (Fig. 4C). The β-catenin activator WNT2B was also significantly increased with castrate resistance. Consistent with activation of the β-catenin pathway in castrate-resistant samples, the expression of β-catenin inhibitors such as CSNK2B, CSNK1E, GSK3B, TP53, WNT5A, and PLCB4 was decreased (Fig. 4C). The β-catenin downstream transcription factors, TCF3 and LEF, and expression of their target genes, MYC and CCND1, were significantly decreased, whereas expression of the transcription inhibitor CTNNBIP1 was increased in castrate-resistant prostate cancer. Consistent with the mRNA level, the protein level of CTNNBIP1 is increased whereas the levels

![Figure 3. Cluster analysis of the status of the androgen pathway in hormonal progression in the hollow fiber model.](image)

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of p53, TCF3, cyclin D1, and GSK3β are decreased in the castration-resistant samples (Fig. 4D). Together these data are consistent with the hypothesis of an increased cytoplasmic pool of β-catenin without increased activity of its downstream transcription factors (TCF and LEF).

Increased colocalization of AR and β-catenin in castrate-resistant prostate cancer. Activation of the Wnt pathway should induce the accumulation of β-catenin in cytoplasm, which would be expected to facilitate the activity of downstream transcription factors. Whereas we expect to see increased cytoplasmic or nuclear accumulation of β-catenin based on elevated levels of phospho-Y124-β-catenin, we in fact measured a decrease in its downstream transcription factors and target genes in castrate-resistant prostate cancer. One possible mechanism for why no concomitant increases in TCF/LEF target genes were measured may involve competition for β-catenin with the AR (21). To further explore for possible crosstalk between β-catenin and AR in castrate-resistant prostate cancer, we investigated the colocalization of AR and β-catenin by fluorescence microscopy using xenografts harvested before and after castration. Tissue sections were prepared from xenografts harvested the day of castration, at the PSA nadir, and when samples were castrate resistant and stained using antibodies against AR and β-catenin. The staining pattern for AR in xenografts harvested from noncastrated mice was strongly nuclear as expected, whereas β-catenin staining was predominately at cell borders (Fig. 5A). After castration, the AR was diffuse in the cytoplasm with little detected within the nucleus, whereas β-catenin was still mainly at the cell borders with little cytoplasm diffusion and low colocalization with AR. Castrate-resistant xenografts from castrated mice showed nuclear localization of β-catenin in cells that predominately colocalized with AR (arrowheads). Levels of β-catenin at cell borders were not observed to change in different stages of prostate cancer, whereas cytoplasmic levels increased with progression. These data suggest that colocalization of AR and β-catenin only occurs in vivo in castrate-resistant prostate cancer and, contrary to in vitro data, not in the presence of androgen in noncastrated mice.

Increased interaction between AR and β-catenin in castrate-resistant prostate cancer. In cell culture, β-catenin interacts with the AR and acts as a coactivator to increase the transcriptional activity of the AR in response to androgen (2, 3, 15–17). To date, all studies have examined these interactions using cells maintained as a monolayer with no in vivo reports. Because β-catenin interacts with molecules involved in cell adherence, it would seem that cells maintained in a monolayer may not provide the optimal model to predict what may occur in a tumor or spheroid structure. Thus, here we provide the first in vivo study of interaction between AR and β-catenin. In addition, this is also the first study to attempt to show changes in this interaction during hormonal progression, something that cannot be accurately mimicked in vitro. To do this,

Figure 4. Expression of AR, β-catenin, and regulators of β-catenin in the hollow fiber model. A, left, levels of AR mRNA measured by Affymetrix (line) and quantitative PCR (columns) using total RNA harvested from the hollow fibers from animals at day 0, day 10, and day 31 shown in Fig. 1A. Fold change is based on levels of AR mRNA normalized to levels of GAPDH mRNA relative to levels of samples collected at day 0 (AD) and set at 1-fold. Right, levels of β-catenin mRNA in the fiber model measured by Affymetrix (line plot) and quantitative PCR (column plot). B, left, Western blot analysis of levels of AR protein normalized to β-actin protein using whole-cell lysates collected at the same time points as described above. Bottom, densitometry analysis of AR protein normalized to β-actin using matched samples from at least three different mice and set at 1-fold using the samples at day 0 (AD). Middle, Western blot analysis of levels of β-catenin protein using the same samples. Right, levels of phospho-Y124-β-catenin in the same samples. C, levels of mRNAs of genes known to be involved in regulating β-catenin in samples harvested from the hollow fiber model measured by Affymetrix (line plot) and quantitative PCR (column plot). AD, androgen dependent; AA, androgen ablation; AI, androgen independent (castrate resistant). Left Y-axis is the fold change detected by quantitative PCR; right Y-axis is the normalized expression (N.E.) for Affymetrix data. Columns, mean; bars, SD. D, Western blot analysis of protein levels of GSK3β, p53, TCF3, CTNNB1P1, and cyclin D1 using whole-cell lysates collected at the same time points as described above. β-Actin serves as the loading control.
endogenous complexes of AR and β-catenin were coimmunoprecipitated from LNCaP xenografts at various stages of hormonal progression. Xenografts were harvested at the day of castration (intact mice), at the PSA nadir (castrated), and when castrate resistant, and proteins were immunoprecipitated with antibodies directed to β-catenin or AR. The immunoprecipitated complexes were probed by Western blot analysis. Consistent with the lack of colocalization in the presence of androgen in noncastrated mice (Fig. 5A), no interaction between AR and β-catenin was detected in the presence of androgen (Fig. 5B). Importantly, these data showed increased levels of endogenous AR/β-catenin complex from in vivo samples harvested from castrate-resistant prostate cancer compared with androgen-dependent and androgen ablation tissues (Fig. 5B). These data are in agreement with the colocalization studies shown in Fig. 5A, and together they suggest that AR interacts with β-catenin in castrate-resistant prostate cancer.

Discussion

The Wnt/β-catenin pathway contributes to prostate biology and pathology. The AR is suspected to play an important role in castrate-resistant prostate cancer. These two pathways may crosstalk in castrate-resistant prostate cancer. The present studies investigated genes differentially expressed during the hormonal progression of prostate cancer and revealed the following: (a) The LNCaP hollow fiber model correlated with clinical hormonal progression of prostate cancer. (b) Hormonal progression of prostate cancer in the hollow fiber model was associated with differential expression of 5,667 genes. (c) The expression pattern of androgen-regulated genes in castrate-resistant prostate cancer was more similar to that obtained before castration compared with the tumors receiving androgen ablation at PSA nadir. (d) The expression of AR and β-catenin was increased in castrate-resistant prostate cancer. (e) Nuclear colocalization and protein-protein interaction between the endogenous AR and endogenous β-catenin were increased in vivo in castrate-resistant prostate cancer. (f) Deregulation of the Wnt pathway in castrate-resistant prostate cancer led to dissociation of β-catenin from the cell membrane but not to activation of its downstream TCF/LEF transcription factors.

The LNCaP hollow fiber model correlates to clinical hormonal progression of prostate cancer. A number of animal models are available to investigate the mechanisms underlying the development and pathogenesis of the prostate cancer. We developed an in vivo model that encompasses the use of hollow fibers to obtain tumor cells that were free from contamination with host cells and to allow harvesting of multiple samples from an individual mouse at different stages of progression (25). LNCaP cells seeded in fibers that were s.c. implanted into the mice.
provided measurable levels of serum PSA that decreased by 90% to a nadir after castration, and subsequently, serum PSA increased within 4 to 5 weeks after castration signifying progression to androgen independence (Fig. 1A). Data from this fiber model for both serum PSA and levels of PSA mRNA and protein were consistent with those obtained with the LNCaP xenograft model (34). Condition clustering and the class prediction analysis provided support that the hollow fiber model mimics clinical samples at the level of global transcription during hormonal progression (Fig. 1B). These data provide the first evidence of similarity between the LNCaP hollow fiber model and the clinical scenario at the transcriptome level for hormonal progression.

Reactivation of the AR pathway in castrate-resistant prostate cancer. PSA is an example of an androgen-regulated gene that contains several well-characterized androgen response elements in the promoter and enhancer regions to which the AR binds to initiate transcription (35–37). The reexpression of PSA suggests the AR plays a role in castrate-resistant disease. Affymetrix GeneChip analysis identified changes in expression of 1,092 genes in response to androgen stimulation in LNCaP cells (26). Here we clustered the fiber model samples according to the expression profile of these androgen-regulated genes to investigate the status of the AR pathway during the hormonal progression of prostate cancer. Surprisingly, in spite of the similar androgen environment as the androgen ablation samples, the castrate-resistant prostate cancer samples shared a more common expression profile of androgen-regulated genes with the androgen-dependent samples before castration (Fig. 3). Specific examples include the expression of androgen-regulated genes such as KLK3, KLK2, ELL2, SOCS2, and RHOU that were reexpressed in castrate-resistant prostate cancer. Expression of genes that are known to be suppressed by androgen, such as MMP16 (26), were resuppressed in castrate-resistant prostate cancer (Fig. 2). Together these data suggest reactivation of the AR in castrate-resistant disease as previously suggested (38–40). Consistent with the reactivation of AR is the overexpression of AR. Levels of AR mRNA increased in androgen ablation samples (Fig. 4A), whereas levels of AR protein remained similar compared with the androgen-dependent samples (Fig. 4B). This was expected due to increased stability of the AR protein in the presence of androgen. One potential mechanism for reactivation of the AR in the absence of testicular androgen may involve changes in protein-protein interactions with coregulators in response to alternative signal transduction pathways. Some possible responsive signaling pathways and the AR interaction proteins have been recently reviewed (30) and include mitogen-activated protein kinase, Akt, protein kinase C, and protein kinase A. These pathways may directly modify the AR or indirectly activate AR by modification of coactivators. β-Catenin is such a coactivator of AR, which may be involved in the ligand-independent activation of AR. In the present study, we provide the first in vivo evidence of the relevance of the β-catenin pathway in the hormonal progression of prostate cancer.

β-Catenin has been reported to be a relatively specific coactivator of the AR, with only the vitamin D receptor of the steroid receptor family also showing some interaction (3). These studies are based on overexpression of β-catenin to increase the transcriptional activity of the AR measured by reporter gene constructs in response to androgen (2, 3). Chromatin immunoprecipitation assays detected β-catenin recruitment to the PSA promoter (41). In yeast cells exposed to dihydrotestosterone, repeat 6 of the armadillo repeats of β-catenin interacts with the AR ligand-binding domain (3). Interaction between β-catenin and the AR is reported to be dependent on androgen due to enhanced interaction on the addition of ligand (2, 3). These previous reports are based on studies using cells maintained in culture as a monolayer, which may not reflect in vivo conditions especially when considering the importance of adhesion molecules in modulating the function of β-catenin. Curiously, we did not detect interaction of β-catenin with the AR in vivo in xenografts harvested from noncastrated mice. Instead we detected an interaction between β-catenin and AR with hormonal progression in castrated mice (Fig. 5B), which was consistent with colocalization studies (Fig. 5A). These data are the first to show in vivo interactions between the AR and β-catenin and highlight potential discrepancies when extrapolating solely from in vitro experiments. The importance of validating endogenous complexes using in vivo physiologic or pathologic conditions is based on the following: (a) Protein-protein interactions are dependent on the concentrations of the proteins as well as the levels of stimulation, and thus overexpression of a protein by transfection may lead to false positives. (b) Overexpression or forced expression of proteins may cause aberrant cellular localization and/or inappropriate timing of expression if the expression of proteins is "normally" dependent on the cell cycle phase. (c) Protein modifications required for interactions may vary in different cells or under different cellular conditions. (d) Cell cultures cannot substitute the physiologic milieu of in vivo conditions, nor can cell cultures mimic accurately the hormonal progression that occurs in vivo in response to castration of the host. (e) The three-dimensional architecture of the xenograft and the potential effects on E-cadherin and other cell adhesion molecules may not be accurately represented using cells maintained in monolayer. These points are especially important when investigating protein-protein interactions of the AR activated by nonandrogenic pathways involving signal transduction pathways that may not be accurately mimicked in vitro.

E-cadherin mediates cell-cell contact and regulates the levels of β-catenin localized in the cytoplasm, with overexpression of E-cadherin causing redistribution of β-catenin to the cell membrane to reduce cytoplasmic and nuclear pools (3, 42). Loss of E-cadherin in castrate-resistant prostate cancer leads to increased levels of β-catenin in the cytoplasm (43–45). Consistent with our results shown here, expressions of both AR and β-catenin have been reported to be elevated in castrate-resistant prostate cancer (46, 47). Increased expression of β-catenin could be mediated through increased transcription (Fig. 4A) and/or stabilization by Y142-phosphorylation (Fig. 4B), decreased level of GSK3β, and interaction with AR (Fig. 5). Potential mechanisms of how β-catenin is able to enhance the transcriptional activity of the AR include facilitating the movement of the AR to the nucleus in response to androgen (15); modifying ligand requirement of the AR to use androstenediene and estradiol as agonists (2); and interactions with GRIP1, CARM1, p300, and FHL2 (reviewed in ref. 48).

Activity of the Wnt/β-catenin pathway in castrate-resistant prostate cancer. The Wnt pathway and its interaction with AR have been suspected to play important roles in prostate cancer (48–50). In Fig. 6 we illustrate changes in the expression levels of activators of β-catenin (Wnt2b and Yes1), which were increased (enlarged font size), whereas the inhibitors of β-catenin (GSK3β, Ck1, Ck2, p53, Wnt5a, and Pcl3) were decreased (reduced font size) in castrate-resistant prostate cancer compared with the androgen-dependent and/or androgen ablation samples as determined in...
these studies, these changes in expression would predict that β-catenin would dissociate from the cell membrane to facilitate its downstream transcription factors. However, expressions of TCF3 between TCF3/LEF and AR has been suggested (21). Increased levels of β-catenin combined with decreased TCF3/LEF would provide more β-catenin protein to be recruited by AR. Together these data suggest that an increased pool of β-catenin would be available for potential interaction with other transcription factors such as AR. Other players in the Wnt pathway may influence the transactivation of AR. These include GSK3β (18–20) and cyclin D1 (22–24), which interact with the AR to repress AR activity. Decreased expression of GSK3β and cyclin D1, as shown in this study, could contribute to the reactivation of the AR pathway in castrate-resistant prostate cancer.

Together these data provide a working model for a potential mechanism of progression to castrate-resistant prostate cancer that involves aberrant expression of members of the Wnt pathway, which promotes the interaction between β-catenin and AR and thereby increases the transactivation of the AR to initiate translocation of genes normally regulated by androgen. Aberrant activation of the AR through the Wnt/β-catenin signaling pathway may play a role in the progression of prostate cancer to the castrate-resistant state. Development of inhibitors that block protein-protein interactions between the AR and β-catenin may lead to viable therapies for this terminal stage of the disease.

Figure 6. Crosstalk between β-catenin and the AR in castrate-resistant prostate cancer. The schematic shows differentially expression of members of the Wnt pathway in castrate-resistant prostate cancer and the effect on AR transcriptional activity. See text for description.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References


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