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Expression and co-localization of beta-catenin and lymphoid enhancing factor-1 in prostate cancer progression

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Abstract

The purpose of this study was to objectively investigate β-catenin and LEF1 abundance, subcellular localization, and co-localization across benign and staged prostate cancer (PCa) specimens. A tissue microarray containing tumor-adjacent histologically benign prostate tissue (BPT; n=48 patients), high-grade prostatic intraepithelial neoplasia (HGPIN; n=25), localized PCa (n=42), aggressive PCa (n=31), and metastases (n=22) was stained using multiplexed immunohistochemistry with antibodies towards E-cadherin, β-catenin, and LEF1. Multispectral imaging was used for quantitation, and protein expression and co-localization was evaluated across prostate cancer progression. Stromal nuclear β-catenin abundance was greater in HGPIN and PCa compared to BPT ($p<0.05$ for both), and epithelial nuclear β-catenin abundance was lower in metastatic PCa than BPT ($p<0.05$ for both). Epithelial and stromal nuclear LEF1 abundance was greater in HGPIN compared to BPT, while epithelial nuclear LEF1 was also greater in metastases. The proportion of epithelial and stromal nuclear double positive β-cat^{+}/LEF1^{+} cells was greater in HGPIN compared to BPT. Additionally, the proportion of epithelial β-

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cat^{+}/LEF1^{+} cells was greater in localized PCa and metastases compared to BPT. A significant amount of stromal cells were positive for LEF1 but not β-catenin. β-catenin and LEF1 abundance were negatively correlated in the epithelium ($p<0.0001$) but not the stroma ($p>0.05$). We conclude that β-catenin and LEF1 co-localization is increased in HGPIN and metastasis relative to BPT, suggesting a role for β-catenin-LEF1-mediated transcription in both malignant transformation and metastasis of PCa. Further, our results suggest that LEF1 abundance alone is not a reliable readout for β-catenin activity in prostate tissues.

Keywords

beta-catenin; LEF1; multispectral imaging; prostate cancer; metastasis; immunohistochemistry

INTRODUCTION

Prostate cancer (PCa) is the fifth most common cause of cancer-associated death in the United States, with over 220,000 new cases expected in 2015 [1]. Prostate growth is primarily driven by the signaling of androgens such as testosterone and dihydrotestosterone (DHT) through androgen receptor (AR) [2], and androgen ablation therapy has been standard of care for patients with metastatic PCa for decades [3]. Unfortunately, androgen ablation inevitably leads to castration-resistant prostate cancer (CRPC), the mechanism of which is not fully understood. Potential mechanisms include increased expression of AR variants [4], increased stability of full length AR [5], and secretion of extracellular factors that affect the stability and transcriptional activity of AR, including Wnt proteins [6, 7].

Canonical Wnt signals stabilize β-catenin, which serves as a component of the adherens junctions complex and also functions as a transcriptional co-activator [8]. Canonical Wnts reduce β-catenin phosphorylation and ubiquitination, leading to β-catenin accumulation. Within the nucleus, β-catenin associates with lymphoid-enhancing factor-1 (LEF1) and activates the transcription of genes containing LEF1/TCF binding sites, including the LEF1 gene [9]. Dysregulation of Wnt/β-catenin is implicated in multiple types of cancer due to the downstream products that result from β-catenin/LEF1 transcriptional activation [10, 11]. For example, mutations in the tumor suppressor APC promote colon cancer progression through disruption of cadherin-dependent cell adhesion, leading to increased β-catenin signaling [9, 12]. Further, there is evidence that β-catenin can drive ligand-independent activation of AR in prostate cells [13–15].

If and how β-catenin abundance and subcellular location changes during prostate cancer progression is highly debated. Some groups report that β-catenin abundance increases with advancing PCa stage [16, 17], while others reported the inverse relationship [18–20]. Previous studies are limited by semi-quantitative methods of immunohistochemical (IHC) quantitation, along with the known alterative effects of variations in IHC and fixation protocols [20]. Further, while it is known that β-catenin and LEF1 are transcriptional coactivators and form a complex within the nucleus, and that β-catenin co-activates LEF1 transcription, these proteins are rarely studied together in context of disease. It is largely unknown whether these proteins localize to the nucleus independent of each other and

whether their abundance is associated in prostate cancer. This is the first study, to our knowledge, to investigate the co-localization and expression of β-catenin and LEF1 in prostate cancer progression using an automated pathology platform.

METHODS

Tissue microarray and immunohistochemistry

The University of Wisconsin Institutional Review Board (IRB number M-007-110-CP003) approved retrospective review of patient and tumor characteristics. A Manual Tissue Arrayer (Beecher Instruments, Sun Prairie, WI; model MTA-1) was used to construct a tissue microarray (TMA) containing human prostate samples, as previously described [21–23]. The TMA contains 0.6mm cores arranged 0.8mm center to center and includes 96 cores (in duplicate from 48 patients) of tumor-adjacent normal prostate tissue (BPT), 50 cores of high-grade prostatic intraepithelial neoplasia (HGPIN; 25 patients), 84 cores of localized PCa (42 patients), 62 cores of aggressive PCa (31 patients) and 44 cores of mixed lymph node and distant metastatic tissue (22 patients). Localized PCa was defined as stage pT1 or pT2, and aggressive PCa was defined as pT3 or pT4 disease. Diagnosis of each core was confirmed every 10 sections by a board-certified genitourinary pathologist (WH).

Immunohistochemistry (IHC) using antibodies for β-catenin, LEF1, and E-cadherin was performed [23], with E-cadherin serving as a plasma membrane marker. Tissues were stained using rabbit monoclonal anti-LEF1 (Cell Signaling Technology, Beverly, MA; 1:150 in Renoir Red [Biocare, Concord, CA]) and goat anti-rabbit Mach 2 HRP-Polymer (Biocare) was used as a secondary antibody. Next, mouse monoclonal anti-β-catenin (BD Transduction Laboratories; San Jose, CA; 1:200 in Biocare Renoir Red) was applied and goat anti-mouse Mach 2 HRP-Polymer (Biocare) was used as a secondary antibody. Monoclonal mouse anti-E-cadherin (Dako, Carpinteria, CA; 1:150 in Biocare Renoir Red) was added to slides and goat anti-mouse Mach 2 HRP-Polymer secondary antibody was used. β-catenin was detected with Bajoran Purple chromogen (Biocare) and DAB (Biocare) chromogen was used to detect LEF1. Deep Space Black (Biocare) was used to detect E-cadherin.

Image Analysis

Quantitation of IHC staining was performed as previously described [23–26]. Briefly, the TMA slide was loaded onto the Vectra slide scanner (PerkinElmer, Waltham, MA). A scanning protocol was created, and the 20× objective lens was used to acquire 8-bit Nuance multispectral image cubes (0.5μm/pixel). Nuance software (PerkinElmer) was used to create spectral libraries from four control slides containing only hematoxylin, Deep Space Black, DAB, or Bajoran Purple. This spectral library was used for separation of chromogens on multi-chromogenic slides. inForm software (PerkinElmer) was used for tissue and cell segmentation, and an algorithm of differentiation was created using 18% of image cubes, assuring 97% segmentation accuracy [24]. E-cadherin was used to mark both the epithelium and membrane compartments. The algorithm was applied to all images and β-catenin and LEF1 were quantified in all tissue and cellular compartments. The mean optical density (OD) was calculated for each core, which gives the average density of staining per cell for each chromogen. The average mean OD or positivity of duplicate cores was used for

analysis if staining was sufficient for analysis. Cores with <5% epithelium or significant folding were eliminated from analysis.

Positivity and co-localization data was generated through thresholding of mean optical density values for each protein. A threshold of 0.1 was used for both β-catenin and LEF1 expression. Final output of protein expression from inForm software included total positivity, double positivity (β-catenin^{+}/LEF1^{+}), double negativity (β-catenin^{-}/LEF1^{-}) and single positivity (β-catenin^{+}/LEF1^{-} and β-catenin^{-}/LEF1^{+}), where output reflects the proportion of cells within a particular bin (single positive, double positive, or double negative) for each sample.

Statistical analysis

One-way analysis of variance (ANOVA) was used to investigate differences in protein levels between normal prostate, HGPIN, PCa, and metastasis samples. Linear regression was used to correlate β-catenin and LEF1 levels in each subcellular compartment. A p-value <0.05 was considered significant in all analyses. GraphPad Prism (GraphPad Software, Inc., La Jolla, Ca) was used for all statistical analysis.

RESULTS

Total and tissue compartment-specific protein levels of β-catenin and LEF1 in prostate samples

β-catenin and LEF1 staining were detected in prostate epithelial and stromal cell nuclei, cytoplasm, and membranes (Figure 1). Due to significant folding or low percentage of epithelium, 16/336 (4.8%) cores were eliminated from analysis. Total β-catenin staining did not differ among groups ($p>0.05$ for all; Figure 2A). Total LEF1 staining was greater in HGPIN compared to other groups ($p<0.0001$; Figure 2B).

β-catenin and LEF1 abundance was next quantified separately in prostate stroma and epithelium. Epithelial β-catenin abundance did not differ among groups (Figure 2A). Stromal β-catenin was more abundant in localized PCa, aggressive PCa, and metastases than in HGPIN and BPT groups ($p<0.05$ for all). Epithelial LEF1 was more abundant in HGPIN ($p<0.0001$) and metastases ($p<0.05$) than in BPT and aggressive prostate cancer ($p>0.05$; Figure 2B). Stromal LEF1 was more abundant in HGPIN ($p<0.0001$) than in localized PCa, aggressive PCa, metastatic PCa, and BPT.

Cell compartment-specific levels of β-catenin and LEF1

β-catenin and LEF1 staining was then quantified within cell membranes, cytoplasm, and nuclei (Table 1). Mean optical density (OD) of nuclear β-catenin in the stroma was greater in all groups compared to BPT ($p<0.05$ for all). Epithelial nuclear β-catenin did not differ among BPT, HGPIN, localized PCa, and aggressive PCa groups, but was lower in the metastatic PCa group ($p<0.001$). Epithelial cytoplasmic and membrane associated β-catenin levels did not significantly differ among groups ($p>0.05$), while stromal cytoplasmic and membrane associated β-catenin levels were higher in localized PCa, aggressive PCa and metastatic PCa groups than in other groups ($p<0.05$ for all). Results were similar when

investigated as percentage of cells positive for β-catenin using thresholding of mean OD values (Figure 3A).

LEF1 mean OD was significantly elevated in HGPIN in all subcellular compartments in both the epithelium and cytoplasm compared to BPT ($p<0.001$ for all; Table 1). Epithelial nuclear LEF1 was higher in metastases than BPT ($p<0.01$). LEF1 protein abundance in membranes, cytoplasm and nuclei was similar among all other groups ($p>0.05$). LEF1 positivity was then investigated (Figure 3B). LEF1 positivity was significantly higher in HGPIN for all subcellular and tissue compartments than BPT ($p<0.001$ for all). Epithelial LEF1 was also higher in metastases in the nucleus, cytoplasm, and membrane than in BPT samples ($p<0.05$ for all). Nuclear LEF1 was elevated in localized PCa compared to BPT ($p<0.05$).

Co-localization of β-catenin and LEF1 in prostate tissues

Using positivity thresholds and inForm software (PerkinElmer), co-localization of β-catenin and LEF1 in prostate tissue and cell compartments was then investigated (Table 2). Double negative ($\beta\text{-cat}^-/\text{LEF1}^-$) epithelial and stromal cells were less prevalent in HGPIN than BPT. The proportion of $\beta\text{-cat}^-/\text{LEF1}^-$ cells was not significantly different in any other group or compartment. The proportion of epithelial double positive ($\beta\text{-cat}^+/\text{LEF1}^+$) cells was elevated in HGPIN, localized PCa, and metastases when the nuclear and cytoplasmic subcellular compartments were investigated. Membrane-specific $\beta\text{-cat}^+/\text{LEF1}^+$ cells were more common in HGPIN and metastases than BPT. Within the stromal compartment, only HGPIN samples displayed a higher proportion of $\beta\text{-cat}^+/\text{LEF1}^+$ cells than BPT.

The percentage of single positive ($\beta\text{-cat}^+/\text{LEF1}^-$ or $\beta\text{-cat}^-/\text{LEF1}^+$) cells was then evaluated. Epithelial-specific $\beta\text{-cat}^+/\text{LEF1}^-$ cells were less prevalent in HGPIN than BPT when the nucleus, cytoplasm, and membrane were investigated. The proportion of epithelial $\beta\text{-cat}^+/\text{LEF1}^-$ cells was also decreased in metastatic samples for the nucleus and cytoplasm but not the membrane compartment. Stromal, nucleus- and membrane-specific $\beta\text{-cat}^+/\text{LEF1}^-$ cells were found more often in localized and aggressive PCa than in BPT. The proportion of stromal, membrane-specific $\beta\text{-cat}^+/\text{LEF1}^-$ cells was also higher in metastases than BPT. The proportion of $\beta\text{-cat}^-/\text{LEF1}^+$ cells was higher in HGPIN for all subcellular and tissue compartments compared to BPT. Nucleus-specific $\beta\text{-cat}^-/\text{LEF1}^+$ cells were also found more often in epithelial cells from metastasis samples than BPT.

Subcellular relationship of β-catenin and LEF1 levels in prostate epithelium and stroma

The relationship between β-catenin and LEF1 abundance was investigated using linear regression with mean OD values. Within the epithelium, LEF1 and β-catenin abundance were negatively correlated between all subcellular compartments ($p<0.0001$ for all; Table 3A). Interestingly, within the stroma, there was no significant correlation between β-catenin and LEF1 abundance within any subcellular compartment ($p>0.05$ for all; Table 3B).

DISCUSSION

Prostate cancer affects a substantial proportion of elderly men and is responsible for over 27,000 deaths per year [1]. The mechanism of PCa development likely involves the interplay of multiple different pathways, including sex hormone signaling, cellular differentiation

pathways, and epithelial-stromal paracrine interactions, among others. One protein that is postulated to be involved with prostate cancer development and progression is β-catenin, which is both a component of cadherin cell adhesion complexes and a nuclear transcriptional co-activator [8]. To date, the current literature on nuclear β-catenin expression in prostate cancer is conflicting [16–20]. This is the first study to investigate β-catenin and LEF1 expression and co-localization in prostate cancer progression using multispectral imaging.

Previous studies have shown an association between increased β-catenin expression and PCa progression [16, 17]. Contrarily, others have reported an association of decreased β-catenin with advanced PCa and prognosis [18–20]. Two limitations of previous studies are semi-quantitative methods of analyzing immunohistochemical staining and evaluation of β-catenin alone rather than in parallel with other signaling complex proteins. By using multispectral imaging with TMA analysis [23–26], we are able to quantify the average expression of each protein on a per cell basis using mean optical density values and co-localization using thresholding of mean optical densities. Our results indicate that total epithelial β-catenin expression does not substantially change as PCa progresses. While β-catenin expression remained fairly consistent, LEF1 expression was significantly increased in HGPIN and metastasis samples, resulting in a concordant increase in the proportion of double positive β-cat^{+}/LEF1^{+} cells. We conclude that LEF1/β-catenin-directed transcription is likely increased in both the early stages of prostate cancer development and metastatic disease.

Our results from quantifying prostate stromal β-catenin content are consistent with previous findings of minimal β-catenin expression in prostate stroma [17]. Indeed, less than 5% of cells in the stroma were positive for nuclear β-catenin in this study. Interestingly, a significant proportion of stromal cells were positive for LEF1 but not β-catenin, and the proportion of this cellular phenotype was substantially increased in HGPIN compared to BPT. This led us to hypothesize that LEF1 and β-catenin localize to the nucleus independent of each other within the stroma. We investigated this further through linear regression analysis with protein expression data from each TMA core and found that β-catenin and LEF1 were not correlated within the stroma. In the absence of appreciable β-catenin, LEF1 is thought to have little ability to initiate transcription in non-hematopoietic cells [27] but is still able act as a scaffold protein by binding the minor groove of DNA through its HMG domain, resulting in a 130 degree turn of the DNA [28]. Further, LEF1 is able to bind factors other than β-catenin such as ALY or c-Jun to form nuclear complexes that direct transcription [29]. Given the overall low percentage of double positive β-cat^{+}/LEF1^{+} stromal cells in all samples and lack of significant correlation of β-catenin and LEF1, we conclude that stromal LEF1 is likely acting through different pathways than the canonical β-catenin/LEF1-directed transcription pathway. Alternatively, these β-cat^{-}/LEF1^{+} cells may be immune cells, as there is evidence for β-catenin-independent LEF1 transcription in hematopoietic lineages [30], suggesting evidence for inflammatory reactions in PCa stroma.

Because LEF1 is a downstream transcriptional target of β-catenin [9], LEF1 activity is thought to represent canonical β-catenin signaling. Therefore, we hypothesized that LEF1 and β-catenin would be positively correlated in the epithelium. Interestingly, when considering all TMA samples together, we observed a significantly negative correlation between β-catenin and LEF1 expression in the epithelium. While the biological meaning of

this observation is unclear, co-localization analysis revealed an increase in β-cat$^+$/LEF1$^+$ double positive cells in HGPIN, localized prostate cancer, and metastases compared to BPT. These data indicate that LEF1 expression alone is not a reliable readout of β-catenin signaling, and other methods such as quantitation of β-cat$^+$/LEF1$^+$ co-localized cells is more likely to reflect canonical β-catenin signaling. The increase in double positive β-cat$^+$/LEF1$^+$ epithelial cells in both the early phase (HGPIN) and later, more aggressive phase (metastasis) of prostate cancer progression is also not biologically intuitive. Because β-catenin is expressed at a ubiquitously high level in the epithelium, it is possible that LEF1+ foci are more aggressive clones with a greater propensity to metastasize. Alternatively, β-catenin/LEF1 signaling may be important for malignant transformation and colonization of distal metastatic sites, but less so for local invasion. This study was not specifically designed to answer this question, but the mechanisms of the observed phenomenon herein are important areas of future research.

Limitations of this study include low sample size for some prostate tissue types on the TMA and the use of tumor-adjacent normal prostate tissue for comparison. Previous studies have demonstrated that Wnt signaling between stromal and epithelial cells occurs almost exclusively in immediately proximal glands [31, 32], but it is still possible that paracrine Wnt secretion from cancer-associated fibroblasts or other stromal constituents affected β-catenin expression in tumor-adjacent epithelium. The use of tumor-adjacent tissue is common practice due to the difficulty of obtaining truly normal prostate tissue. Future studies are needed which include autopsy normal prostate tissue to confirm the results of this study.

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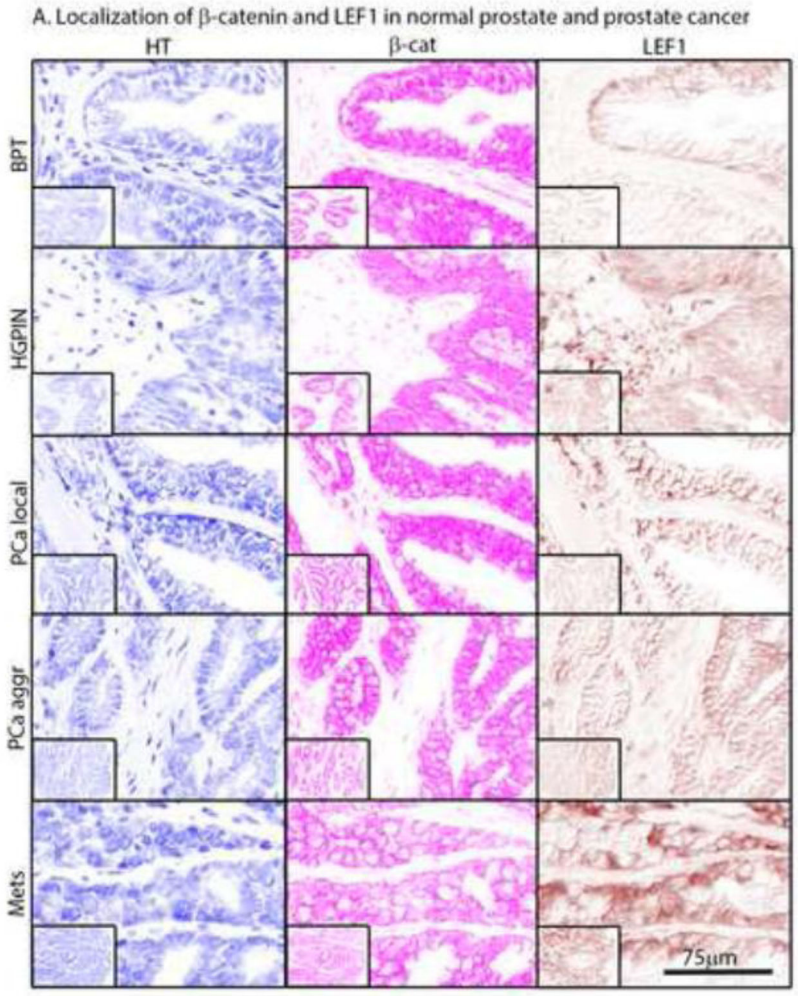

Figure 1.

Localization and expression of LEF1 and β-catenin in tumor adjacent histologically benign prostate tissue (BPT) and in prostate cancer (PCa). Multiplexed immunohistochemistry staining was unmixed using Nuance® software, resulting in separation of hematoxylin (left column), Bajoran purple (middle column), and 3,3′-Diaminobenzidine (right column) chromogens. Expression of β-catenin and LEF1 was quantified using inForm® software.

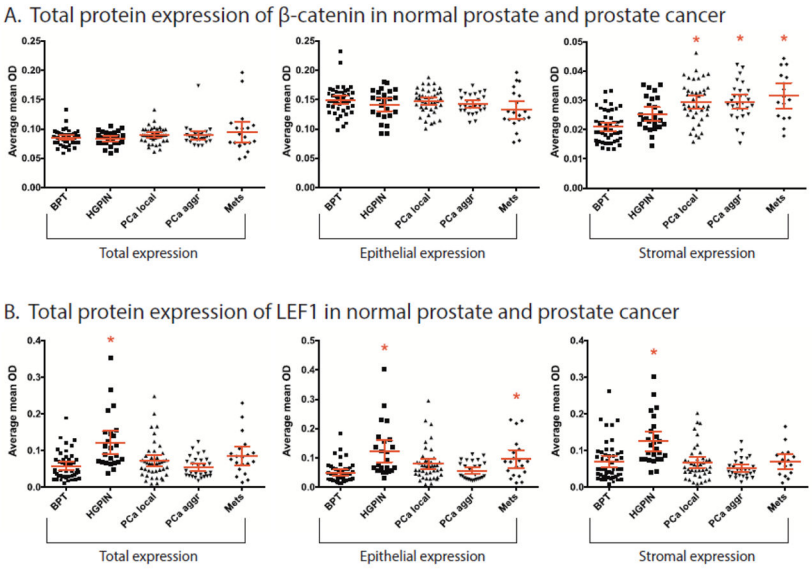

Figure 2.

Total and tissue compartment-specific expression of β-catenin and LEF1. Total and epithelial-specific expression of β-catenin was not significantly different in PCa specimens compared to BPT, but stromal-specific β-catenin was significantly higher in all PCa groups ($p<0.05$; **A**). Total and compartment-specific expression of LEF1 was higher in HGPIN compared to BPT ($p<0.001$), but no changes in primary PCa samples were observed (**B**). Epithelial expression of LEF1 was also higher in metastases compared to BPT ($p<0.05$).

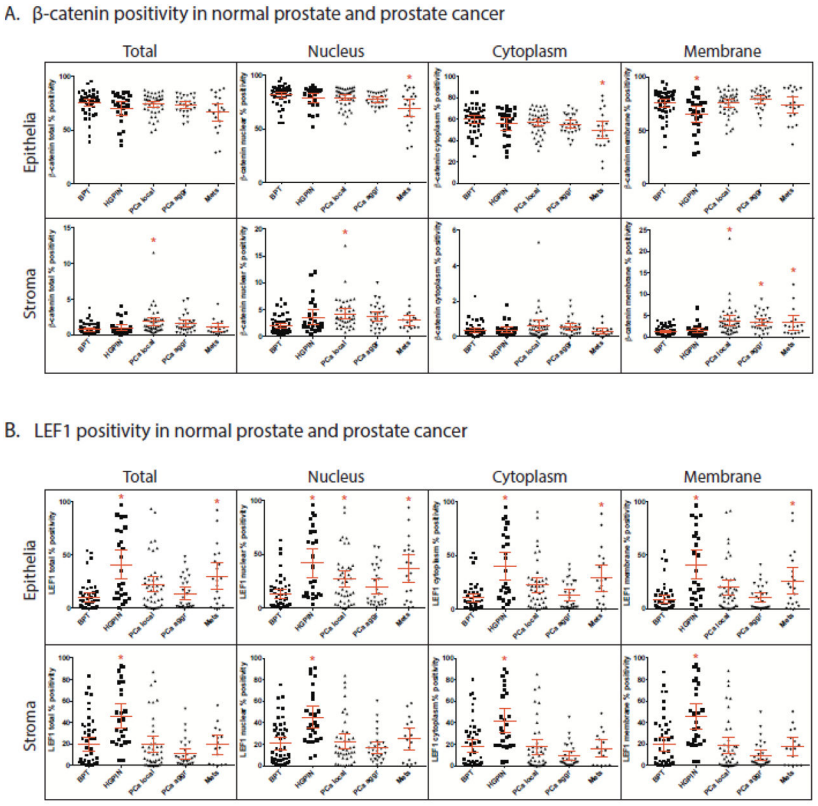

Figure 3.

Quantitative analysis of cell compartment specific β-catenin or LEF1 expression by percentage of positive cells within tissue. A lower threshold of 0.1 optical density units was applied and percent of positive cells was quantified using inForm® software. Nuclear and cytoplasmic positivity of β-catenin in the epithelia was significantly lower in metastases compared to BPT ($p<0.05$), and membrane-specific β-catenin was lower in HGPIN ($p<0.05$; **A**). Nuclear and total positivity of β-catenin in the stroma was higher in localized PCa ($p<0.05$), and elevated membrane-specific positivity of β-catenin in the stroma was observed in all malignant samples ($p<0.05$). LEF1 positivity was significantly higher in HGPIN ($p<0.001$) and metastases ($p<0.05$) in all cellular compartments in the epithelia compared to BPT (**B**). Elevated stromal LEF1 was associated with HGPIN ($p<0.001$) but not with malignant samples.

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Table 1

Expression of β-catenin and LEF1 in the epithelium and stroma of normal prostate tissue and prostate cancer specimens, mean OD (±SEM)

	Total		***Nucleus***		***Cytoplasm***		***Membrane***	
β-catenin	**Epithelia**	**Stroma**	**Epithelia**	**Stroma**	**Epithelia**	**Stroma**	**Epithelia**	**Stroma**
BPT (n=48)	0.149 (±0.004)	0.021 (±0.001)	0.173 (±0.004)	0.034 (±0.001)	0.126 (±0.003)	0.011 (±0.001)	0.149 (±0.004)	0.018 (±0.001)
HGPIN (n=25)	0.141 (±0.005)	0.025 (±0.001)	0.172 (±0.004)	*b* 0.041 (±0.002)	0.120 (±0.005)	0.013 (±0.001)	0.132 (±0.006)	0.022 (±0.001)
PCa local (n=42)	0.147 (±0.003)	*c* 0.029 (±0.001)	0.172 (±0.004)	*c* 0.043 (±0.001)	0.121 (±0.003)	*c* 0.016 (±0.001)	0.149 (±0.003)	*c* 0.029 (±0.001)
PCa aggr (n=31)	0.143 (±0.003)	*c* 0.029 (±0.001)	0.160 (±0.003)	*c* 0.043 (±0.001)	0.116 (±0.003)	*c* 0.016 (±0.001)	0.152 (±0.003)	*c* 0.029 (±0.002)
Mets	0.132	*c* 0.032	*c* 0.142	*c* 0.044	0.110	*c* 0.019	0.145	*c* 0.032
(n=22)	(±0.007)	(±0.002)	(±0.009)	(±0.003)	(±0007)	(±0.001)	(±0.007)	(±0.003)
LEF1								
BPT (n=48)	0.050 (±0.005)	0.070 (±0.008)	0.056 (±0.006)	0.074 (±0.007)	0.050 (±0.005)	0.066 (±0.007)	0.046 (±0.005)	0.070 (±0.008)
HGPIN (n=25)	*c* 0.122 (±0.018)	0.125 (±0.013)	*c* 0.133 (±0.020)	*c* 0.131 (±0.013)	*c* 0.118 (±0.017)	*c* 0.117 (±0.013)	*c* 0.116 (±0.017)	*c* 0.127 (±0.014)
PCa local (n=42)	0.080 (±0.009)	0.067 (±0.007)	0.091 (±0.010)	0.075 (±0.008)	0.077 (±0.008)	0.062 (±0.007)	0.072 (±0.008)	0.065 (±0.008)
PCa aggr (n=31)	0.056 (±0.006)	0.052 (±0.005)	0.068 (±0.008)	0.063 (±0.006)	0.054 (±0.005)	0.046 (±0.004)	0.047 (±0.005)	0.047 (±0.005)
Mets (n=22)	*a* 0.095 (±0.015)	0.070 (±0.010)	*b* 0.118 (±0.019)	0.087 (±0.012)	0.089 (±0.013)	0.060 (±0.009)	0.079 (±0.013)	0.063 (±0.009)

a p<0.05,

b p<0.01,

c p<0.001

Abbreviations: optical density (OD), benign prostatic tissue (BPT), high-grade prostatic intraepithelial neoplasia (HGPIN), localized prostate cancer (PCa local), aggressive prostate cancer (PCa aggr), metastases (Mets)

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Table 2

Co-localization of β-catenin and LEF1 in the epithelia and stroma of normal prostate tissue and prostate cancer specimens, % (±SEM)

	B-catenin⁻/LEF1⁻		***B-catenin⁺/LEF1⁺***		***B-catenin⁺/LEF1⁻***		***B-catenin⁻/LEF1⁺***	
TOTAL	**Epithelia**	**Stroma**	**Epithelia**	**Stroma**	**Epithelia**	**Stroma**	**Epithelia**	**Stroma**
BPT	20.92 (±1.49)	79.30 (±3.27)	5.96 (±1.29)	0.09 (±0.03)	68.88 (±2.23)	0.69 (±0.11)	4.25 (±1.19)	19.93 (±3.26)
HGPIN	^{b}13.55 (±1.18)	^{c}53.64 (±5.50)	^{c}24.47 (±3.65)	^{c}0.47 (±0.16)	^{c}45.65 (±5.83)	0.53 (±0.13)	^{c}16.33 (±3.80)	^{c}45.36 (±5.47)
PCa local	17.58 (±1.09)	78.88 (±3.68)	^{a}14.18 (±2.03)	0.16 (±0.04)	59.90 (±3.13)	^{b}1.70 (±0.30)	8.34 (±1.78)	19.26 (±3.71)
PCa aggr	21.48 (±1.23)	87.54 (±2.46)	9.07 (±1.89)	0.12 (±0.04)	64.50 (±2.94)	1.41 (±0.21)	4.95 (±1.11)	10.93 (±2.43)
Mets	21.14 (±2.83)	79.39 (±4.16)	^{a}18.00 (±4.23)	0.11 (±0.04)	^{b}48.59 (±5.61)	0.96 (±0.26)	12.27 (±3.00)	19.54 (±4.20)
NUCLEUS								
BPT	14.19 (±0.94)	76.95 (±2.97)	8.69 (±1.58)	0.58 (±0.15)	72.92 (±2.15)	1.48 (±0.21)	4.22 (±1.02)	21.00 (±2.89)
HGPIN	^{b}8.62 (±0.80)	^{c}52.20 (±4.98)	^{c}29.61 (±4.17)	^{c}2.41 (±0.62)	^{c}49.40 (±5.81)	1.33 (±0.27)	^{a}12.39 (±2.73)	^{c}44.08 (±4.72)
PCa local	12.74 (±0.91)	73.64 (±3.31)	^{a}19.18 (±2.36)	0.87 (±0.16)	^{a}59.95 (±3.27)	^{c}3.55 (±0.44)	8.14 (±1.52)	21.95 (±3.32)
PCa aggr	16.55 (±1.02)	79.52 (±2.58)	13.67 (±2.50)	0.49 (±0.12)	63.46 (±3.10)	^{b}3.19 (±0.37)	6.34 (±1.15)	16.81 (±2.59)
Mets	16.00 (±2.06)	71.45 (4.39)	^{a}21.85 (±4.54)	0.92 (±0.28)	^{c}47.31 (±5.50)	2.69 (±0.76)	^{b}14.85 (±3.46)	24.95 (±4.47)
CYTOPLASM								
BPT	34.95 (±1.77)	81.43 (±1.50)	5.12 (±1.04)	0.04 (±0.02)	54.72 (±2.07)	0.30 (±0.06)	5.22 (±1.31)	18.24 (±3.12)
HGPIN	^{b}24.27 (±1.90)	^{c}57.93 (±5.42)	^{c}19.96 (±2.70)	^{b}0.18 (±0.06)	^{c}35.52 (±4.77)	0.18 (±0.05)	^{c}20.25 (±4.36)	^{c}41.72 (±5.42)
PCa local	32.34 (±1.60)	82.00 (±3.55)	^{a}11.51 (±1.43)	0.05 (±0.02)	45.42 (±2.59)	0.59 (±0.13)	10.75 (±2.24)	17.37 (±3.57)
PCa aggr	39.22 (±1.55)	90.75 (±2.09)	7.01 (±1.36)	0.04 (±0.02)	47.92 (±2.56)	0.50 (±0.08)	5.86 (±1.24)	8.72 (±2.08)
Mets	36.08 (±3.63)	83.66 (±3.68)	^{b}14.63 (±3.65)	0.04 (±0.01)	^{c}35.23 (±4.70)	0.26 (±0.07)	14.06 (±3.26)	16.04 (±3.68)
MEMBRANE								
BPT	19.77 (±1.63)	79.10 (±3.41)	4.32 (±0.94)	0.13 (±0.05)	71.36 (±2.27)	1.11 (±0.14)	4.55 (±1.22)	19.66 (±3.36)
HGPIN	14.77 (±1.39)	^{c}53.29 (±5.55)	^{c}21.18 (±3.30)	^{b}0.53 (±0.18)	^{c}44.16 (±5.84)	0.99 (±0.18)	^{c}19.90 (±4.16)	^{c}45.19 (±5.55)
PCa local	16.37 (±1.14)	77.92 (±3.71)	11.80 (±2.10)	0.23 (±0.07)	63.70 (±3.26)	^{c}3.58 (±0.58)	8.14 (±1.67)	18.28 (±3.75)
PCa aggr	17.54 (±1.26)	87.17 (±2.31)	6.73 (±1.48)	0.11 (±0.04)	72.01 (±2.82)	^{b}3.40 (±0.41)	3.74 (±0.86)	9.33 (±2.33)
Mets	16.95 (±2.62)	79.22 (±3.90)	^{b}16.38 (±4.51)	0.21 (±0.07)	57.32 (±5.86)	^{a}3.20 (±0.77)	9.37 (±2.41)	17.38 (±3.96)

^{a}p<0.05,

[b] p<0.01,

[c] p<0.001

Abbreviations: benign prostatic tissue (BPT), high-grade prostatic intraepithelial neoplasia (HGPIN), localized prostate cancer (PCa local), aggressive prostate cancer (PCa aggr), metastases (Mets)

Table 3

Subcellular relationship of LEF1 and β-catenin expression in prostate epithelium (A) and stroma (B), Pearson r, p-value

		β-catenin					
		Epithelium			Stroma		
		Nucleus	Cytoplasm	Membrane	Nucleus	Cytoplasm	Membrane
LEF1	Nucleus	r=−0.524 p<0.0001	r=−0.446 p<0.0001	r=−0.500 p<0.0001	r=−0.004 p=0.95	r=−0.094 p=0.21	r=−0.026 p=0.72
	Cytoplasm	r=−0.475 p<0.0001	r=−0.423 p<0.0001	r=−0.506 p<0.0001	r=−0.033 p=0.66	r=−0.114 p=0.13	r=−0.057 p=0.44
	Membrane	r=−0.451 p<0.0001	r=−0.417 p<0.0001	r=−0.544 p<0.0001	r=−0.057 p=0.44	r=−0.130 p=0.08	r=−0.074 p=0.32