Reduced β-catenin immunostaining in prostate cancer and negatively associated with poorly differentiated Gleason grades

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Received [28\09\2021], Accepted [10\09\2021]

Abstract

Prostate cancer has few prognostic biomarkers, and differentiating between relapsing and non-relapsing tumour can be challenging clinically. This study aims to investigate the hypothesis that β-catenin might be a potential biomarker for prostate tumour and could distinguish between aggressive tumours requiring radical intervention and those that have a good prognosis. It is thought to be a potential biomarker that can predict the clinical progression and prognosis of different kinds of tumors. However, its role in prostate cancer remains unclear.β-catenin immunostaining has been evaluated by immunohistochemistry using two sources of patient samples. The tissue microarray group consists of 96 cases including normal, adjacent normal and malignant prostate tissue. The Bath cohort consists of 36 prostate samples, including normal and malignant prostate tissues. Immunohistochemistry showed nuclear and cytoplasmic β-catenin staining in normal and malignant prostate tissues. β-catenin expression is reduced significantly in prostate cancer and negatively associated with increasing Gleason grade. This reduction was not associated with clinical stage and biochemical relapse.
This preliminary data suggests that β-catenin may have a role in cancer development and/or aggressiveness and warrants further investigation to understand its function and establish if it could be a potential diagnostic biomarker for prostate cancer.

Keywords: Prostate cancer, β-catenin, IHC.

انخفاض التصبيغ المناعي لمعلم بيتا-كاتينين في سرطان البروستاتا وارتباطه سلبا مع مرحله تمييز جليسون السيئة لسرطان البروستاتا

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الخلاصة

يحتوي سرطان البروستاتا على عدد قليل من المؤشرات الحيوية التنبؤية، ويمكن أن يكون التفريق بين الورم المنتكس وغير الناكس تحديًا إكلينيكيًا. تهدف هذه الدراسة إلى التحقيق في الفرضية القائلة بأن بيتا-كاتينين قد يكون علامة بيولوجية محتملة لورم البروستاتا ويمكنه التمييز بين الأورام العدوانية التي تتطلب تدخلاً جذريًا وتلك التي لها تشخيص جيد. يُعتقد أنه علامة بيولوجية محتملة يمكنها التنبؤ بالتقدم السريري والتشخيص لأنواع مختلفة من الأورام. ومع ذلك، لا يزال دوره في سرطان البروستاتا غير واضح.تم تقييم التعبير النووي والهيولي (السايتوبلازمي) لمعلم بيتا-كاتينين بطريقة التصبيغ المناعي النسيجي الكيميائي باستخدام مصدرين لعينات المرضى. تتكون مجموعة المصفوفة الصغيرة (TMA) من ٩٦ عينه بعضها طبيعي والأخر خبيث. ما مجموعه باث فمكونه من ٢٦ عينه، بما في ذلك عينات من مرضى يعانون من تكرار المرض او عدم تكراره.أظهرت الدراسة ان هناك تعبير نووي وهيولي لمعلم بيتا كاتينين في أنسجة البروستاتا الطبيعية والخبيثة. انخفض هذا التعبير بشكل ملحوظ في الأنسجة السرطانية مقارنة بالأنسجة الطبيعية للبروستاتا. وكان لهذا الانخفاض ارتباطا سلبيا مع زيادة درجه جليسون. الا انه لم تسجل أي علاقة بينه وبين مراحل المرض السريرية والانتكاس البيوكيميائي.تشير هذه البيانات الأولية إلى أن بيتا-كاتينين قد يكون له دور في تطور السرطان و / أو العدوانية ويستدعي مزيدًا من التحقيق لفهم وظيفته وتحديد ما إذا كان يمكن أن يكون علامة بيولوجية تشخيصية محتملة لسرطان البروستاتا.

الكلمات المفتاحية: سرطان البروستاتا، المَعلمات، بيتا-كاتينين ، التصبيغ المناعي النسيجي الكيميائي.

Introduction

Prostate cancer (PCa) is an abnormal growth that usually begins in the prostate gland. It is a heterogeneous disease and represents the second most frequent malignancy and is the second highest cause of death in males after lung cancer [1,2]. Adenocarcinoma is the most common type of PCa which is found in more than 90% of Pca patients, and it originates from the glandular regions of the prostate gland [3,4]. A few diagnostic and prognostic biomarkers have been identified for PCa, including prostate-specific antigen (PSA) [5]. However, there is a need for more specific and/or sensitive biomarkers in PCa diagnosis and especially for measuring PCa prognosis, for example, to distinguish between relapsing and non-relapsing cases. The major goal of this study was to identify proteins that are differentially expressed between normal and malignant prostate tissues and/or between different Gleason grades, clinical stages and recurrence vs non-recurrence PCa. This is important to improve our understanding of the molecular basis of PCa formation and progression and potentially help in the development of future biomarkers.

β-catenin is a multifunctional protein that plays an important role in cadherin-mediated adhesion and the Wnt signaling pathway [6,7]. Wnt/ β-catenin pathway dysregulation is found to be associated with many human diseases, including a range of cancers [8]. There are several publications that have studied β-catenin levels and localisation in PCa compared to normal prostate (NP) and/or Benign prostate hyperplasia (BPH), however, in many cases, these studies had different findings. It has been recently found that nuclear β-catenin localisation was increased significantly in PCa compared to BPH [9]. In contrast, other studies showed decreased nuclear staining or localisation of β-catenin significantly in PCa compared to BPH [10,11]. Another study with a small number of PCa samples (17) showed no significant staining for β-catenin in PCa tissue samples [7]. In addition, membranous and cytoplasmic β-catenin has been observed in normal and malignant prostate tissues. For example, a study reported that membranous β-catenin staining was reduced significantly in localised PCa compared to BPH, whereas, cytoplasmic β-catenin staining did not show a significant difference between benign and malignant prostate groups [10]. Membranous β-catenin staining was found to be reduced in PCa compared to BPH [7,12]. The published evidence is also complicated and often at least partially contradictory for PCa Gleason grades which are used to evaluate prognosis of men prostate cancer.

Nuclear β-catenin expression was found to be reduced significantly in PCa tissues with a high Gleason grade compared to those with a low grade [11]. Other studies found membranous staining of β-catenin in PCa significantly reduced with increasing Gleason grades [12,13,14]. In contrast, increased nuclear β-catenin staining was significantly associated with increasing Gleason grade [12]. The latter study, however, had a small number of PCa cases (17 cases). Other data showed no significant association between β-catenin staining patterns or nuclear localisation with Gleason grades [9,10]. Therefore, these studies came to different conclusions about the changes that occur in β-catenin expression and localisation in different Gleason grades.

There are a large number of published studies that describe the association between β-catenin expression and PCa stage. For example, nuclear β-catenin staining was found to be significantly increased in advanced PCa (T3-4) compared to localised disease (T3-4) [9,14]. In contrast, a small sample size study reported no association between nuclear or membranous β-catenin expression and PCa stage [12]. Finally, Horvath, *et al.* found that patients who had an advanced PCa had a significant reduction in nuclear β-catenin staining compared to those with a localised PCa [10]. This study had the largest cohort suggesting that the results are more likely to be reliable than some of the studies based on smaller cohorts.

Finally, in terms of a link to biochemical relapse, a reduction of nuclear β-catenin localisation has been suggested to be significantly associated with a higher risk of biochemical relapse [10]. In addition, Whitaker, *et al*. found the reduction of nuclear β-catenin localisation in hormone recurrent PCa compared to non-recurrent, but the data was not significant [11]. In contrast, there was no association between membranous β-catenin expression and PCa biochemical relapse [10,13].

Materials and methods

This retrospective study was covered by the National Health Service (NHS) ethical and research approval (REC reference: 13/WS/0153; IRAS project ID: 112241). In this study, two different sources of prostate tissue samples were used, including a Bath and a tissue microarray (TMA) cohorts. The Bath cohort consists of 26 paraffin-embedded blocks (FFPE) of PCa, including those from patients who had, and had not, undergone recurrence, and 10 normal

prostate (NP) tissue samples, were obtained from the histopathological laboratories of the Royal United Hospital (RUH), Bath city/ UK. The samples were collected between 1997 and 2018. TMA cohort (PR1921) had 96 cases, 80 of them were PCa, whereas, the rest were normal or normal tissues that were adjacent to the PCa, termed adjacent normal (8 cases for each). Each case was represented with two core tissue biopsies to form a total of 192 cores. This study also used normal Testis tissues as positive controls. The clinical data of the patients in both cohorts are shown in Table 1.

Table 1: Clinical data of prostate sample in both cohorts.

Clinical data		TMA cohort %	Bath cohort%
Number of samples	Normal	16	10
	Malignant	80	26
Age range	Normal	21-68	31-62
	Malignant	20-85	58-81
Primary Gleason grade	3	13 (16.25%)	15 (44.1%)
	4	46 (57.5%)	16 (47%)
	5	18 (23. 75)	0 (0%)
	ND	3 (2.5%)	3(8.9%)
T category	T1-T2	51 (63.8%)	19 (55.9%)
	T3-T4	28 (35%)	9 (26.5%)
	N/A	1 (1.2%)	6 (17.6%)
N category	N0	65 (81.2%)	24 (70.6%)
	N1	14 (17.5%)	3 (8.8%)
	ND	1(1.3%)	7 (20.6%)
M category	M0	64 (80%)	20 (58.8%)
	M1	15 (18.7%)	3 (8.8%)
	ND	1 (1.3%)	11(32.4%)
Biochemical recurrence status (at 5 years)	Non-Recurrent	N/A	13 (38.2%)
	Recurrent	N/A	19 (55.9%)
	N/A	N/A	2 (5.9%)

Immunohistochemistry

Immunohistochemistry (IHC) staining was carried out using anti-β-catenin rabbit polyclonal (Cell Signaling Technology, catalogue number 95625). 5um thick sections of prostate tissues were baked overnight at 37°C. Prior to IHC, deparaffinization and rehydration through graded ethanol series of decreasing ethanol concentration (100%, 95% & 70% respectively) for a minute each concentration was necessary to remove the paraffin from tissues and to rehydrate tissue samples, respectively. Tissues were then permeabilized with 0.5% Triton X-100 in phosphate buffer saline (PBS), subjected to heat-induced epitope retrieval in a citrate buffer, pH 6 with 0.05% Tween 20 for 30 minutes at 90°C, and allowed to cool to room temperature

for 20 minutes. Subsequently, the sections were incubated in 3% H_2O_2 (Dako peroxidase) at room temperature for 10 minutes, followed by rinsing gently three times with Phosphate buffer

saline (PBS) for 5 minutes each. After blocking for 30 minutes in 10% normal goat serum and 0.5% BSA in PBS, samples were treated with anti-ABCG2 antibody, dilution1:100 (Dako, Ely, UK) overnight at 4°C.

On the next day, immuno-detection was performed using the EnVision+ Kit (K400611-2 and K401011-2, Dako, Ely, UK) following the manufacturer's instructions with DAB exposure for 5 minutes. The sections were counterstained with Vector Hematoxylin solution (H3401, Vector Laboratories, Peterborough, UK) at room temperature for a minute to stain the nucleus of cells. Slides then were rinsed thoroughly with the running tap water for 3 minutes. To differentiate the hematoxylin stain, the slides were then soaked three times in 70% ethanol with 1% HCl. The slides were also immersed for a minute in an alkaline solution that was prepared by adding 1% ammonium hydroxide to 70% ethanol to restore the bluing stain of Haematoxylin. At this point, the staining steps were finished. After that, the slides were washed with two changes of different ethanol concentrations 95% and 100% for a minute. Slides were then washed twice with Histoclear for 2 minutes each. Next day, the slides were ready to examine under a light microscope (Nikon Eclipse E800) equipped with a Nikon digital camera (DS-U1 CCD).

For assessment of IHC staining, the whole sections were examined under a 20x objective to determine the nuclear and cytoplasmic expression of B-catenin staining in prostate tissues. The nuclear staining was scored using the H- score system. This score depends on the proportion of positive cells and staining intensity. To carry out the scoring, nuclei of epithelial cells were counted based on four DAB staining intensity categories (0: No nuclear staining 1: Weak nuclear staining, 2: Moderate nuclear staining and 3: Strong nuclear staining). After scoring, the H-score was calculated as the following formula:

H score= 3 x % of strongly nuclear staining + 2 x % of moderately nuclear staining + % of weakly nuclear staining.

The range of the H-score can vary between 0 and 300 [15]. The cytoplasmic staining was scored using a semi-quantitative scoring system as the following: the percentage of positive cells was scored as: (0: 0; 1: 1-25%; 2: 26-50%; 3:51-75%; and 4: 76-100%) and the intensity was graded as (0: negative, 1: weak, 2: moderate; and 3: strong). The final score represents the sum of the proportion and intensity scores, which ranged from 0 to 7 [16].

Statistical analysis

Statistical analysis was performed using GraphPad Prism version 6.00 for Windows, GraphPad Software, La Jolla California USA, www.graphpad.com, including mean, standard error and standard deviation values. Statistical analysis was carried out either using unpaired T-test and one-way A-NOVA with Tukey's multiple comparisons tests. Results were considered significant if the P. value was ≤ 0.05.

Results

A) Immunohistochemical staining of β-catenin in the normal and malignant prostate tissues from both cohorts

β-catenin staining was carried out using IHC on prostate tissue samples from two independent cohorts. The IHC results showed membranous, cytoplasmic and nuclear staining for β-catenin in normal and malignant prostate tissue from both cohorts, with variable levels of staining (Figure 1&2). Nuclear β-catenin staining was found in both normal and malignant prostate tissues with a variable level of staining, ranging from strong (Figure 1&2 (A& D), arrows), moderate (Figure 1, B&F; Figure 2, B, arrows), and weak in some cases (Figure 2. C& F, arrows). In addition, membranous β-catenin staining was also found in prostate tissue samples with variable levels of staining, ranging from strong (Figure 1 D, red arrowhead), moderate (Figure 1 C, red arrowhead) and weak (Figure 1 E, red arrowhead). The normal and malignant prostate tissues showed cytoplasmic β-catenin staining, with variable levels of staining between cases, ranging from strong (Figure 1 D; Figure 2 D&E, black arrowheads), moderate (Figure 1&2 A, black arrowheads) and weak (Figure 1&2 B, C, E & F&G, black arrowheads). β-catenin is expressed in Sertoli, spermatid and spermatozoa cells of normal testis tissues (17) and so this study used the normal testis as a positive control for β-catenin and as expected IHC staining showed nuclear β-catenin staining in the spermatid and spermatozoa cells of testis tissues (Figure 1 G, arrow). A negative control, with no primary antibody, showed no significant background staining in prostate tissue (Figure 1&2 H, arrows).

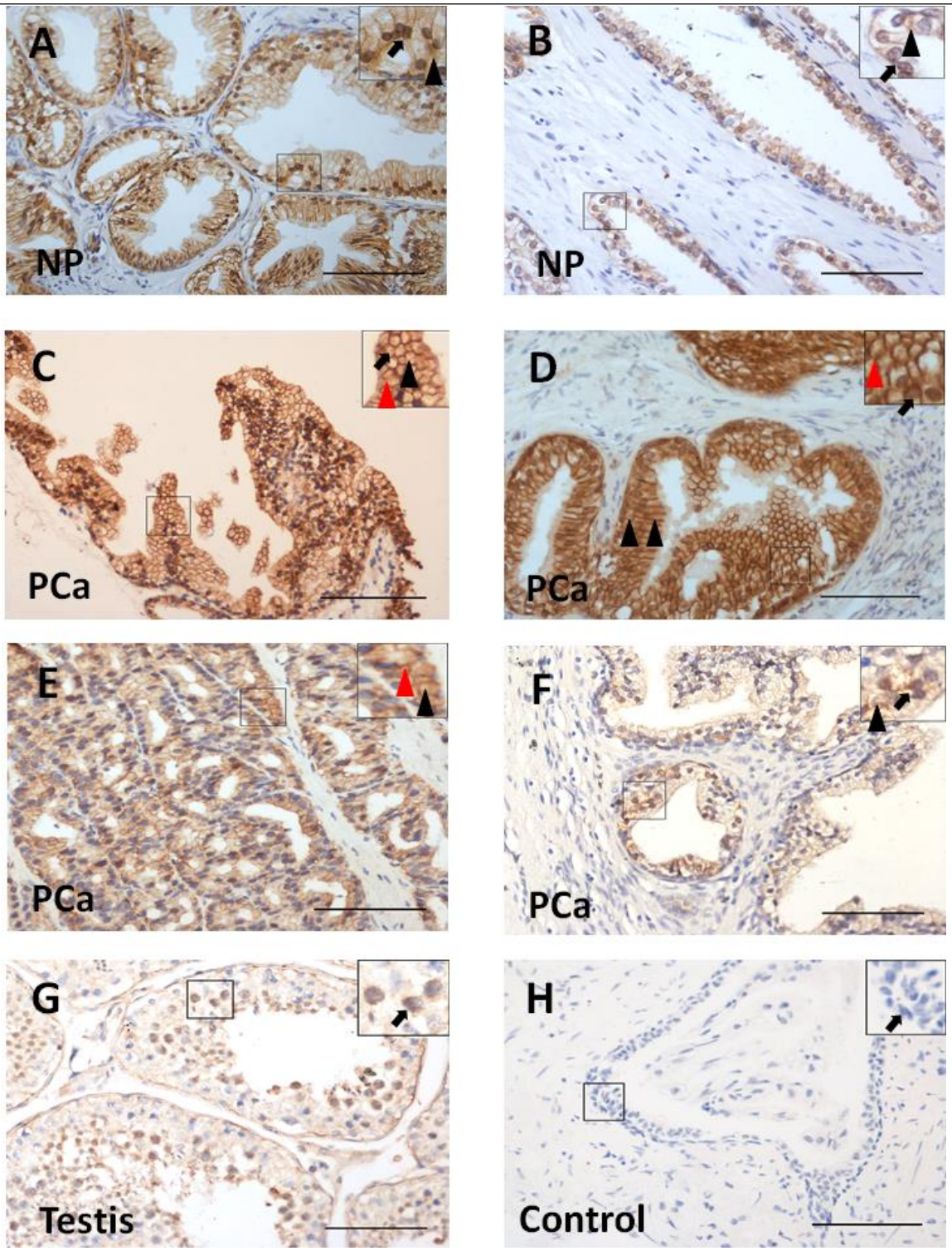

Figure 1: β-catenin staining in samples from the Bath cohort. β-catenin was stained heterogeneously in both normal and malignant tissues of the prostate. (A) Strong nuclear (Black arrow) and moderate cytoplasmic (Black arrowhead) β-catenin staining in NP. (B) Weak to moderate nuclear (Black arrow) and cytoplasmic (Black arrowhead) β-catenin staining in NP. (C) Weak cytoplasmic (Black arrowhead) and moderate membranous (Black arrow) β-catenin staining in PCa. (D) Strong nuclear (Black arrow), membranous (Red arrowhead) and cytoplasmic (Black arrowheads) β-catenin staining in PCa. (E) Weak membranous (Red arrowhead) and cytoplasmic (Black arrowhead) β-catenin staining in PCa. (F) Moderate nuclear (Black arrow) and weak cytoplasmic (Black arrowhead) β-catenin staining in PCa. (G) Nuclear β-catenin staining (Black arrowhead) in testis tissue. (H) Negative control (no primary antibody added) was free background staining (Black arrow) in PCa. Scale bars=100μm.

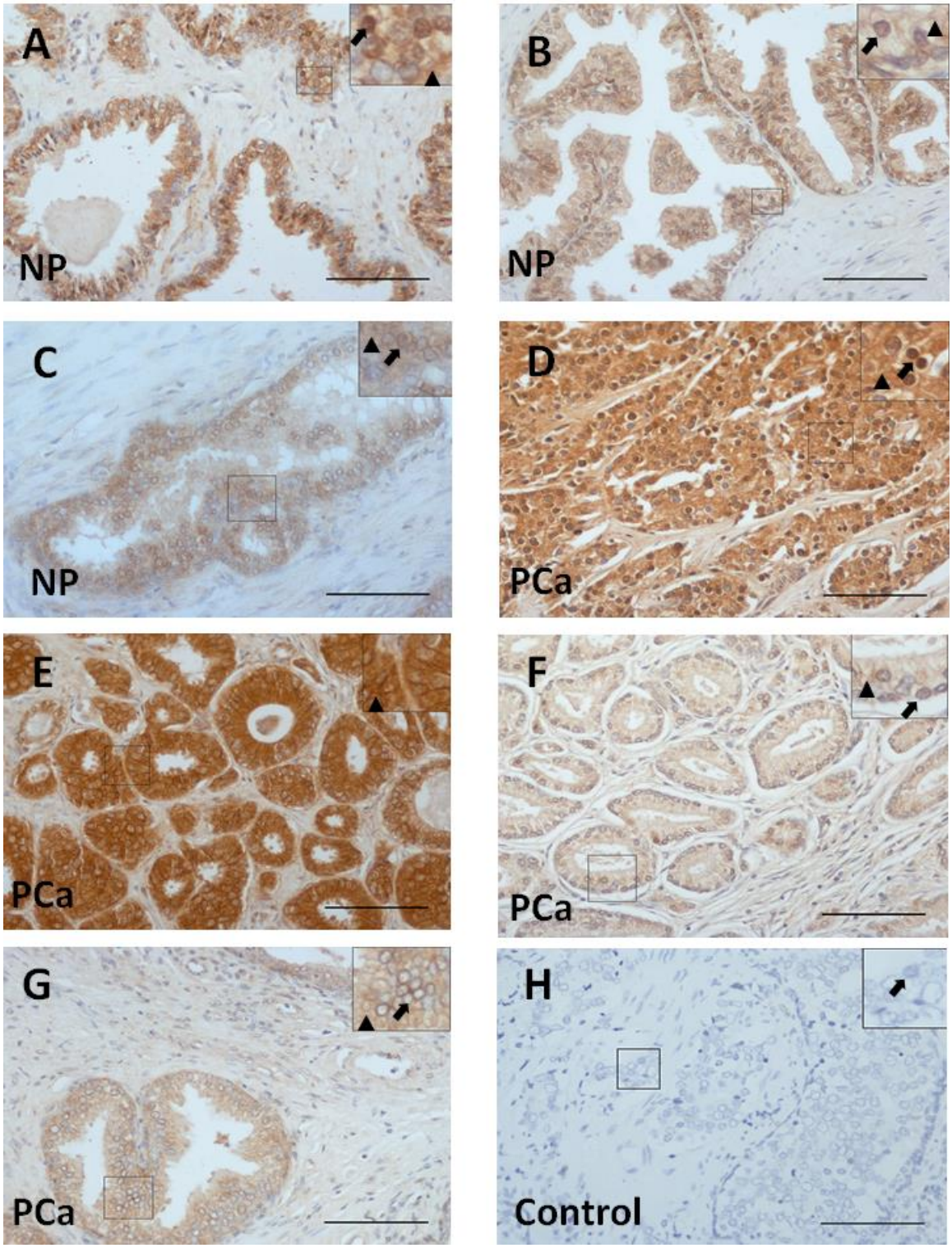

Figure 2: β-catenin staining in samples from the TMA cohort. β-catenin was stained heterogeneously in normal and malignant tissues of the prostate. (A) Strong nuclear (Black arrow) and moderate cytoplasmic (Black arrowhead) β-catenin staining in NP. (B) Moderate nuclear (Black arrow) and weak cytoplasmic (Black arrowhead) β-catenin staining in NP. (C) Weak nuclear (Black arrow) and cytoplasmic (Black arrowhead). β-catenin staining in NP. (D) Strong nuclear (Black arrow) and cytoplasmic (Black arrowhead) β-catenin staining in PCa. (E) Strong cytoplasmic β-catenin staining (Black arrowhead) in PCa. (F) Weak nuclear (Black arrow) and cytoplasmic (Black arrowhead) β-catenin staining in PCa. (G) Weak cytoplasmic (Black arrowhead) with some negative nuclear (Black arrow) β-catenin staining in PCa. (H) Negative control (no primary antibody added) showed no background staining (Black arrow) in PCa. Scale bars=100µm.

B) Association between β-catenin immunostaining and histopathological parameters of prostate cancer in both cohorts

Having carried out IHC staining on normal and malignant prostate samples from the Bath and TMA cohorts, the nuclear and cytoplasmic β-catenin staining was then quantified using H and proportion and intensity 1 scores, respectively and then compared to histopathological and clinical parameters of PCa using the clinical data available for both cohorts. It was decided not to study membranous staining as there were fewer links between changes in membranous expression and disease. This localisation could be examined in future.

Quantification of the IHC staining showed reduced nuclear β-catenin staining significantly in PCa compared to NP in the Bath and TMA cohorts (p= 0.0066 & <0.00001, respectively) (Figure 3 & 4 A & Table 1&2). Decreased nuclear β-catenin was also negatively associated with increasing primary Gleason grade in both cohorts (p= 0.0447& 0.0004, respectively). In the TMA cohort, analysis of data, using the multi-comparison Tukey's tests showed reduced nuclear β-catenin staining significantly when comparing PCa tissues with a primary Gleason grade 3 to those with a grade 4 (p=0.0254) or a grade 5 (p=0.0003). Nuclear β-catenin staining also showed a significant difference between primary Gleason grade 5 & 4 (p= 0.0494). nuclear β-catenin staining was not associated significantly with clinical stage T (T1-2 vs. T3-4) (p=0.2525) and biochemical relapse (p= 0.2808) (Table 3.3).

Cytoplasmic β-catenin staining was also reduced significantly in malignant prostate tissues compared to those with a normal nature in the TMA cohort (0.0412) (Figure 2 B& Table 2). Although, cytoplasmic β-catenin staining was also appeared to be decreased in the Bath PCa tissues, this result was not significant (p=0.1923) (Figure 3.4 B& Table 3.3). Reduction of cytoplasmic β-catenin staining was negatively associated with primary Gleason grade in both cohorts (p=0.0127, 0.0002, respectively). In the TMA cohort, analysis of data, using the multi-comparison Tukey's tests showed that cytoplasmic β-catenin staining was decreased significantly in PCa tissues with a primary Gleason grade 5 compared to those with a grade 3 (0.0004) or a grade 4 (p= 0.0030), but not between primary Gleason grade 3 and 4 (p=0.2284)

(Figure 4.2 C&D & Table 4.2). Both cohorts showed no significant association between cytoplasmic β-catenin staining and clinical stage (TNM) (Table 2&3), except cytoplasmic β-catenin staining showed a negative association with PCa metastasis in the TMA cohort (P=

0.0014) (Figure 4.2 F & Table 4.2). In the Bath cohort, cytoplasmic β-catenin staining was not associated significantly with biochemical relapse (p= 0.5135) (Table 3.3).

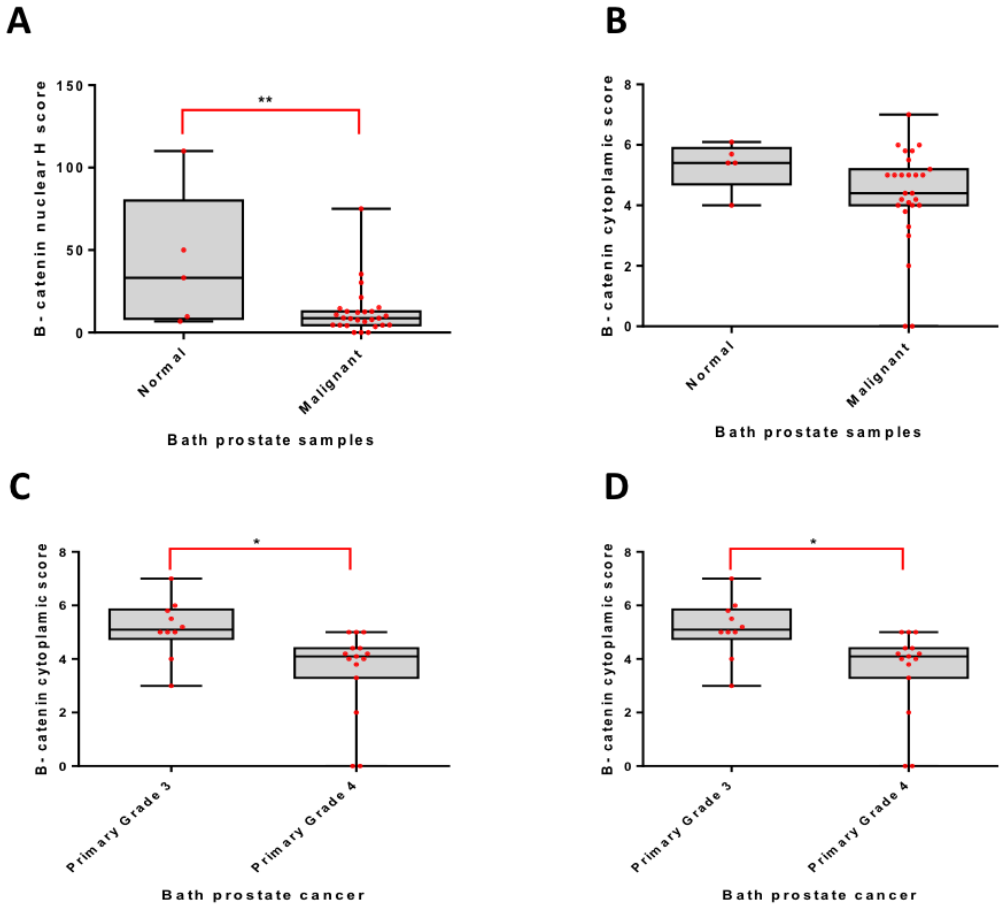

Figure (3) Quantification of nuclear and cytoplasmic β-catenin staining in both normal and malignant Bath prostate tissues. IHC staining of β-catenin was quantified in the Bath cohort using H and the proportion and intensity 1 score for nuclear and cytoplasmic IHC staining. (A) Nuclear β-catenin staining was significantly reduced in PCa compared with NP tissues (p=0.0066). (B) Cytoplasmic β-catenin staining was reduced but not significantly in PCa compared with the NP (P= 0.1923). (C) Nuclear β-catenin staining was significantly reduced in primary Gleason grade 4 compared with grade 3 (p= 0.0447). (D) Cytoplasmic β-catenin staining was also significantly reduced in primary Gleason grade 4 compared to grade 3 (p= 0.0127). The mean of five random fields was taken per prostate sample. Statistical significance was determined with an unpaired T-test for each set of conditions. NP (n=10), PCa (n=26), grade 3 (n=10) and grade 4 (n=15).

Table 2: Nuclear and cytoplasmic β-catenin staining results with clinical data

Comparison	Nuclear β-catenin staining		Cytoplasmic β-catenin staining	
	Results	p. value	Results	p. value
Normal vs malignant	Lower in malignant	0.0066	No statistically significant difference	0.1923
Primary Gleason grades (3 & 4)	Lower in high grade	0.0447	Lower in high grade	0.0127
Clinical Stage (T)	No statistically significant difference	0.2525	No statistically significant difference	0.9288
5 years Biochemical recurrence	No statistically significant difference	0. 2808	No statistically significant difference	0.5135

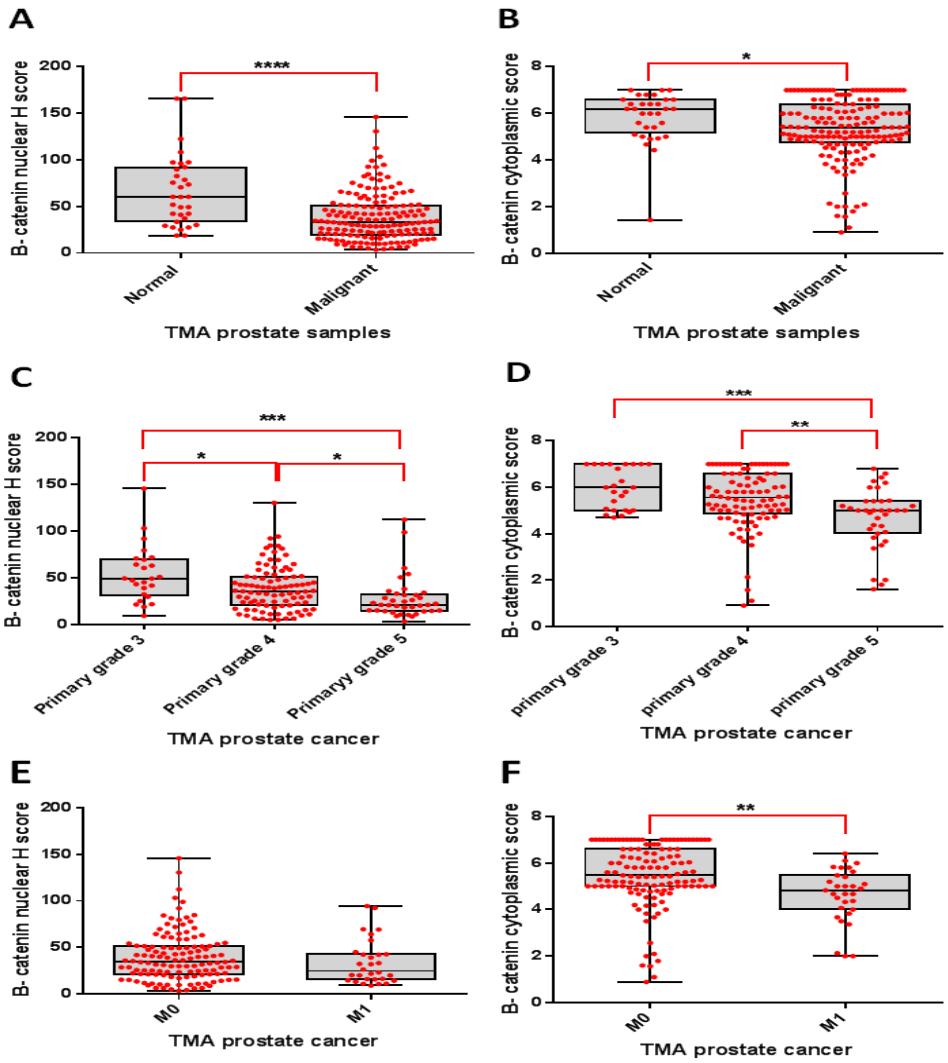

Figure 4: Quantification of nuclear and cytoplasmic β-catenin staining in both normal and malignant TMA prostate tissues. Immunohistochemical β-catenin staining was quantified in the TMA cohort using H and the proportion and intensity 1 scores for nuclear and cytoplasmic IHC staining respectively.

(A) Nuclear β-catenin staining was significantly reduced in PCa compared with NP tissues (p= < 0.0001). (B) A significant reduction of cytoplasmic β-catenin staining
was in the TMA malignant prostate tissues compared with NP (p= 0.0412). (C) Nuclear β-catenin staining showed a significant difference among primary Gleason grades (p= 0.0004). Multi comparison Tukey's tests showed a significant reduction in nuclear β-catenin staining when comparing grade 5 tissues to those with a grade 3 (p=0.0004) or grade 4 (p=0.0492). This reduction was significant between grades 4 and 3 (p=0.0254). (D) Cytoplasmic β-catenin staining showed a significant difference among Gleason grade groups (p= 0.0002). A multi comparison of Tukey's tests showed that cytoplasmic β-catenin staining was significantly reduced in grade 5 compared to grade 3 (p= 0.0004) or grade 4 (p=0.0030). There was no association between cytoplasmic β-catenin staining when comparing Gleason grades 4 and 3 (p= 0.2284). (E) Nuclear β-catenin staining was not associated with clinical stage M (P=0.2795). (F) Cytoplasmic β-catenin staining was negatively associated with metastasis (p=0.0014). Data represent the mean of five random images per case. Unpaired or one-way ANOVA tests were conducted to determine the statistical difference for each set of conditions. NP (n=16), PCa (n=80), grade 3 (n=13), grade 4 (n=46), grade 5 (n=18), M0 (n= 64) and M1 (n=15).

Table 3: Nuclear and cytoplasmic β-catenin staining results with clinical data.

<table>
<tr><th rowspan="2">Comparison</th><th colspan="3">Nuclear β-catenin staining</th><th colspan="3">Cytoplasmic β-catenin staining</th></tr>
<tr><th colspan="2">Results</th><th>p. value</th><th colspan="2">Results</th><th>p. value</th></tr>
<tr><td>Normal vs malignant</td><td colspan="2">Lower in malignant</td><td>< 0.0001</td><td colspan="2">Lower in malignant</td><td>0.0412</td></tr>
<tr><td rowspan="4">Primary Gleason grades (3, 4 &5)</td><td rowspan="4">Lower in high Gleason grade</td><td>A nova test</td><td>0.0004</td><td rowspan="4">Lower in high Gleason grade</td><td>A nova test</td><td>0.0002</td></tr>
<tr><td>Grade 4 vs. Grade 3</td><td>0.0254</td><td>Grade 4 vs. Grade 3</td><td>0.2284</td></tr>
<tr><td>Grade 5 vs. Grade 3</td><td>0.0003</td><td>Grade 5 vs. Grade 3</td><td>0.0004</td></tr>
<tr><td>Grade 5 vs. Grade 4</td><td>0.0492</td><td>Grade 5 vs. Grade 4</td><td>0.0030</td></tr>
<tr><td>Stage (T)</td><td colspan="2">No statistically significant difference</td><td>0.9434</td><td colspan="2">No statistically significant difference</td><td>0.4809</td></tr>
<tr><td>Stage (M)</td><td colspan="2">No statistically significant difference</td><td>0.275</td><td colspan="2">No statistically significant difference</td><td>0.084</td></tr>
<tr><td>Stage (N)</td><td colspan="2">No statistically significant difference</td><td>0.2795</td><td colspan="2">Lower in PCa with metastasis</td><td>0.0014</td></tr>
</table>

In summary, nuclear and cytoplasmic β-catenin staining was reduced significantly in PCa and was negatively associated with increasing primary Gleason grade. β-catenin staining was not associated significanlty with clinical stage and biochemical relapse.

Discussion

Previous studies have reported contradictory β-catenin results in prostate tissue samples. In the current study, a reduction of nuclear β-catenin staining was observed in PCa compared to NP tissues from both cohorts. This data is supported by previous studies with a large cohort [10,11] but was inconsistent with Ipekci, *et al* data which is reported increased nuclear β-catenin staining in PCa compared to BPH [9]. In addition, cytoplasmic β-catenin staining was

decreased in PCa compared to NP tissues in the TMA cohort, but this reduction was not significant in the Bath cohort, perhaps due to the small sample size of this cohort. The cytoplasmic data was consistent with a previous finding [9] but was not supported by other previous literature [10,18]. The different outcomes of the literature may become due to using different antibodies and/or different scoring systems. Taken together, the results suggest that loss of β-catenin (nuclear and cytoplasmic) might be an early event in PCa formation.

Nuclear and cytoplasmic β-catenin staining was negatively associated with increasing primary Gleason grade in both cohorts. This data largely agreed with the previous findings [8,12,13,14]. In contrast, this was not supported by other data [9,10] which showed no significant association between β-catenin staining and Gleason grade. The use of different scoring systems may explain this difference. The nuclear data is not supported by Jaggi *et al*. study which demonstrated increased nuclear β-catenin localisation with increasing Gleason grade [12]. However, Jaggi *et al*. study had a small number of PCa cases [17] as well as used a different scoring system. Taken together, our results and previous studies suggest that β-catenin may play a role in PCa progression and downregulation of β-catenin may be considered to be a worse indicator for PCa aggressiveness.

In this study, nuclear and cytoplasmic β-catenin staining was not associated with the clinical stage except that there was a negative association between cytoplasmic staining and PCa metastasis in the TMA cohort. The association between cytoplasmic β-catenin and metastasis was consistent with [10,12] but inconsistent with other previous studies [9,14]. In summary, some of the current data suggest that β-catenin reduction may play a role in metastasis, while other studies contradict this conclusion.

There was also no association between β-catenin staining and risk of biochemical recurrence in the Bath cohort data. This was consistent with some previous reports [11,12,13], but inconsistent with the Horvath *et al* report that found PCa patients with less than 10% positive nuclear β-catenin staining had decreased biochemical relapse-free survival [10]. Therefore, the majority of evidence suggests there is no association between β-catenin staining and risk of biochemical recurrence.

Previous studies have proposed different functional roles for β-catenin in cancer, including PCa [19,20]. However, this section will focus on a couple of possible mechanisms for β-catenin which would be consistent with its role in PCa based on the expression data described above. Increased β-catenin levels can induce apoptosis in both normal and malignant cells independently of TCF transcriptional activity and/ or cell cycle/apoptosis regulators, including

p53 and transcription factor E2F1(alternatively named retinoblastoma-associated protein 1 or retinoblastoma-binding protein 3) [21], suggesting that β-catenin might interact with other proteins through a potential death domain to induce apoptosis. Decreased β-catenin might then protect cells from apoptosis. Other studies have reported that β-catenin can bind to the transcription factor TCF/LEF, which is located in the nucleus, and/or p300 (Histone acetyltransferase p300) to form a β-catenin/TCF/ P300 complex which plays a role to induce differentiation [19,20], suggesting reduction of β-catenin in cells may lead to reduced differentiation and promotion of PCa.

Taken together, it appears that reduced β-catenin signalling is involved in PCa tumorigenesis and decreased β-catenin levels may be a promising marker of a worse prognosis in localised PCa. However, further study is needed to confirm the staining patterns of β-catenin in prostate tissue samples, using either a second independent antibody and/or mRNA probe (RNAscope®) which can detect the mRNA level in prostate tissues from both cohorts. Finally, it will be very interesting to address the functional role of β-catenin in PCa formation and progression, using tissue culture.

Acknowledgements

The authors are grateful to the University of Thi-Qar for PhD scholarship funding of D.A. Alghezi. They are grateful to the Department of Cellular Pathology at the Royal United Hospital, Bath, for specimen handling and curation. The authors would like to thank Professor Robert Kelsh at the University of Bath for providing use of imaging facilities.

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