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Original Article

Claudin-1 down-regulation in the prostate is associated with aging and increased infiltration of inflammatory cells in BPH

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Abstract: Introduction and Objective: Benign prostatic hyperplasia (BPH) is an age-related disease that is frequently associated with chronic prostatic inflammation. In previous studies, we detected the presence of PSA protein in the stroma of BPH nodules and down-regulation of junction proteins E-cadherin and claudin-1. Transmission electron microscopy (TEM) imaging showed a decrease in tight junctions suggesting the luminal epithelial barrier in BPH tissues may be compromised. Recent *in vitro* studies showed that stimulation of benign prostate epithelial cell lines with TGF-β1 induced a decrease in claudin-1 expression suggesting that inflammation might be associated with alterations in the prostate epithelial barrier. This study explored the potential associations between aging and loss of junction proteins and the presence of inflammatory cells in prostate tissue specimens from young healthy donors and aged BPH patients. Methods: Immunostaining of serial prostate sections from 13 BPH patients and five healthy young donors was performed for claudin-1, CD4, CD8, CD20 and CD68. H-Scores and the number of inflammatory cells were calculated for the same area in donor, normal adjacent prostate (NAP) to and BPH specimens. Quantification and statistical correlation analyses were performed. Results: Claudin-1 immunostaining was inversely associated with increasing age, and inflammation in prostate specimens. B-cell infiltration increased with age and BPH was associated with an increased infiltration of T-cells and macrophages compared to NAP. Conclusions: These findings suggest that aging is associated with down-regulation of claudin-1 and claudin-1 is further decreased in BPH. Claudin-1 down-regulation was associated with increased infiltration of inflammatory cells in both NAP and BPH tissues. Claudin-1 down-regulation in the aging prostate could contribute to increased prostatic inflammation, subsequently contributing to BPH pathogenesis.

Keywords: Claudin-1, prostate, inflammation, BPH, aging

Introduction

Benign prostatic hyperplasia (BPH) is a common age-related prostatic disease and is the most common cause of lower urinary tract symptoms (LUTS) in men due in part to bladder outlet obstruction [1, 2]. There can be a spectrum of clinical manifestations, ranging from urinary hesitancy, urgency, and frequency to acute urinary retention. Although generally not lethal, BPH is associated with significant morbidity, typically urinary bleeding, infections, bladder stones, and renal failure. Aging and androgens are two well-established risk factors for BPH/LUTS [3-7]. With increasing life expectancy, BPH incidence and costs for treatment and management of symptomatic BPH have risen [8]. Morphologic changes indicative of BPH have been consistently documented, as early as 50 years and in approximately 50% of men at the age of 50 [9]. Subsequently its prevalence increases about 10% each subsequent

decade [10]. In parallel, LUTS is present in about 10%-20% of men at ages 50-59; and is present in about one-third of men by age 75 [10]. Prostate volume also increases with age, with the greatest increases appearing in the sixth and seventh decades of life [11]. The changes of BPH in the prostate manifest as a nodular proliferation, typically present in the transition zone of the prostate. Histologically BPH is most commonly an admixture of epithelial and stromal cells. However, in some cases glandular epithelial elements can predominate, while in others fibromuscular-rich stroma comprises the dominant constituent of the BPH nodule [12]. Understanding the mechanisms of BPH development and progression is important because it may eventually lead to novel preventive and/or treatment approaches.

Other factors, including inflammation could also impact BPH/LUTS pathogenesis and progression [13-18]. Inflammation detected in patients enrolled in the Medical Therapies of Prostate Symptoms (MTOPS) study predicted BPH progression events such as symptom worsening, acute urinary retention, and need for surgery [16]. In 8,224 men, statistically significant correlations were found between average and maximum chronic inflammation and IPSS variables, and more severe inflammation was associated with higher IPSS scores [15]. However, the causes and consequences of prostatic inflammation remain poorly understood. Defining the role of prostatic inflammation in BPH pathogenesis and associated LUTS may lead to new approaches to prevent and/or treat BPH/LUTS.

Our studies suggest that epithelial barrier proteins are altered in BPH and may contribute to prostatic inflammation. We previously reported the presence of secretory proteins prostate specific antigen (PSA) and kallikrein 2 (KLK2) in the stromal compartment and down-regulation of adherens junction protein E-cadherin and claudin-1 in mixed nodular BPH compared to associated normal adjacent tissues [19-21], suggesting that the epithelial barrier of BPH glands may be altered and leaky. Tight junction 'kiss points' in BPH luminal epithelial cells were decreased compared to normal adjacent prostate glands [22]. The presence of secretory proteins in the prostate stroma might induce stromal inflammation, proliferation or fibrosis, which are also frequently observed in BPH [14, 23]. In rats, formalin-induced prostatic inflammation resulted in activation of the transforming growth factor beta 1 (TGF-β1) signaling pathway and down-regulation of E-cadherin immunostaining [24]. In benign prostate epithelial cell lines BHPrE1 and BPH-1, stimulation with TGF-β1 induced an increase in epithelial barrier permeability and a decrease in tight junction 'kiss points' [20, 21], suggesting that inflammatory mediators could contribute to the down-regulation of E-cadherin and claudin-1. Chronic prostatic inflammation and increased TGF-β1 have been associated with age-related prostatic disease and the onset of LUTS (Reviewed in [13]). Furthermore, BPH patients with inflammation were shown to be at increased risk of clinical progression to acute urinary retention or urinary incontinence [17]. Down-regulation of junction proteins such as E-cadherin and claudin-1 may be associated with a decline in prostate epithelial barrier function and increased prostatic inflammation, thus contributing to the pathogenesis of BPH.

One of the single greatest risk factors for BPH is age [25]. Our previous studies showed profound claudin-1 down-regulation in clinical BPH specimens [21]. However, the potential mechanism for claudin-1 down-regulation in the prostate is unknown. Aging has been associated with changes in cell adhesion complexes and an increase in epithelial barrier permeability (Reviewed in [4]). Aging is also associated with increased prostatic inflammation [26, 27]. Here we explored the potential association of claudin-1 and inflammation with aging in prostate tissue specimens from young healthy donor and BPH patients. Immunostaining of claudin-1, CD4, CD8, CD20 and CD68 was performed on serial sections from young healthy donor, normal adjacent prostate to BPH (NAP) and BPH tissues and scored for comparison. This study provides new insights into the potential association of aging with prostatic epithelial barrier permeability and prostatic inflammation.

Materials and methods

Patient cohort

The cases selected included 14 patients with symptomatic BPH who required surgical intervention via either a transurethral resection of

Table 1. Demographics of human prostate tissue specimens for immunostaining study

Tissue type	Mean age (range)	No. patients	Mean Prostate mass (g)	Mean Prostate volume (cm^3)
BPH	62.5 (50-77)	13	81.4	74.7
NAP	62 (50-77)	8		
Donor	20.2 (15-26)	5	39.1	24.1

BPH: benign prostatic hyperplasia. NAP: normal adjacent prostate.

the prostate or a simple prostatectomy. BPH consisted of mixed hyperplastic nodules comprised of both glandular and stromal proliferation. In addition, prostate specimens were obtained from 6 organ donors. Any case with the presence of moderate to severe prostatitis was excluded. The criteria used for defining moderate prostatitis was the presence of inflammatory cells in the prostate stroma infiltrating into prostate glands with or without presence of crypt abscess formation. Two patients were identified with prostatitis, one donor and one BPH, and were excluded from the analyses. None of these 20 patients had any prior history of chemo-, radio-, or hormone therapy. These "deidentified" specimens were retrieved from the clinical files of UPMC by the University of Pittsburgh Biospecimen Core (PBC) with approval from the University of Pittsburgh Institutional Review Board for this research project. PBC also provided deidentified pathology reports for the patients constituting the study cohort. All participating patients or their next of kin were asked to give consent for the banking protocol. The University of Pittsburgh has designed a generic consent form for tissue and biological specimen collection. This consent form also gives permission for tracking of patient progression, gathering of patient demographics and collection of clinically relevant information. Patient demographics for the analyzed cohort were listed in **Table 1**.

Histopathologic analysis and immunohistochemistry

Samples were fixed in 10% formalin for at least 24 hrs, then embedded in paraffin. Immunohistochemical staining was performed on five-micron sections of paraffin-embedded prostate tissue specimens as described previously [22]. Briefly, sections were deparaffinized and rehydrated through a graded series of ethanol. Heat-induced epitope retrieval was performed using a BioCare Decloaking Chamber (Biocare Medical, Pacheco, CA), followed by 5 minutes rinsing in TBS buffer. Primary antibodies for immunostaining of prostate tissue sections were mouse monoclonal anti-claudin-1 (XX7, sc-81796, Santa Cruz Biotechnology, Dallas, TX), rabbit monoclonal anti-CD4 (EPR6855, ab133616, Abcam, Cambridge, MA, USA), mouse monoclonal anti-CD8 (144B, M7103, Dako), mouse monoclonal anti-CD20 (L26, 14-0202-82, Invitrogen, Carlsbad, CA, USA), and mouse monoclonal anti-CD68 (KP1, CM033, BioCare Medical). Serial sections were cut and stained using the following scheme: slide 1 was double stained with CD4 and CD8; slide 2 with CD20 and CD68, slide 3 with hematoxylin and eosin (H&E), and slide 4 with claudin-1. Slides were then counterstained in hematoxylin and cover-slipped. Stained sections were imaged with a Leica DM LB microscope (Leica Microsystems Inc., Bannockburn, IL, USA) equipped with an Imaging Source NII 770 camera (The Imaging Source Europe GmbH, Bremen, Germany) and NIS-Elements Documentation v 4.6 software (Nikon Instruments, Inc., Mellville, NY). All tissues were examined by a board-certified genitourinary pathologist (R.D.) using light microscopy. Scores for each antibody were determined for prostate glands in the same field for all slides by three independent investigators (L.E.P., W.C., and C.N.H.).

In silico analysis of claudin-1 gene expression in BPH and normal adjacent epithelium

Publicly available RNA-Seq data from prostate cancer specimens with concurrent BPH was queried to determine the differential expression of claudin-1 [28]. Expression level data for claudin-1 (CLDN1) was available for 19 specimens of normal peripheral zone prostate tissue and 37 BPH specimens as normalized $\log_2$ transcript levels.

Statistical methods

Summary statistics were provided for the three tissue groups: donor, NAP and BPH. Due to the smaller sample size in all three groups, the group comparison between donor vs. NAP and

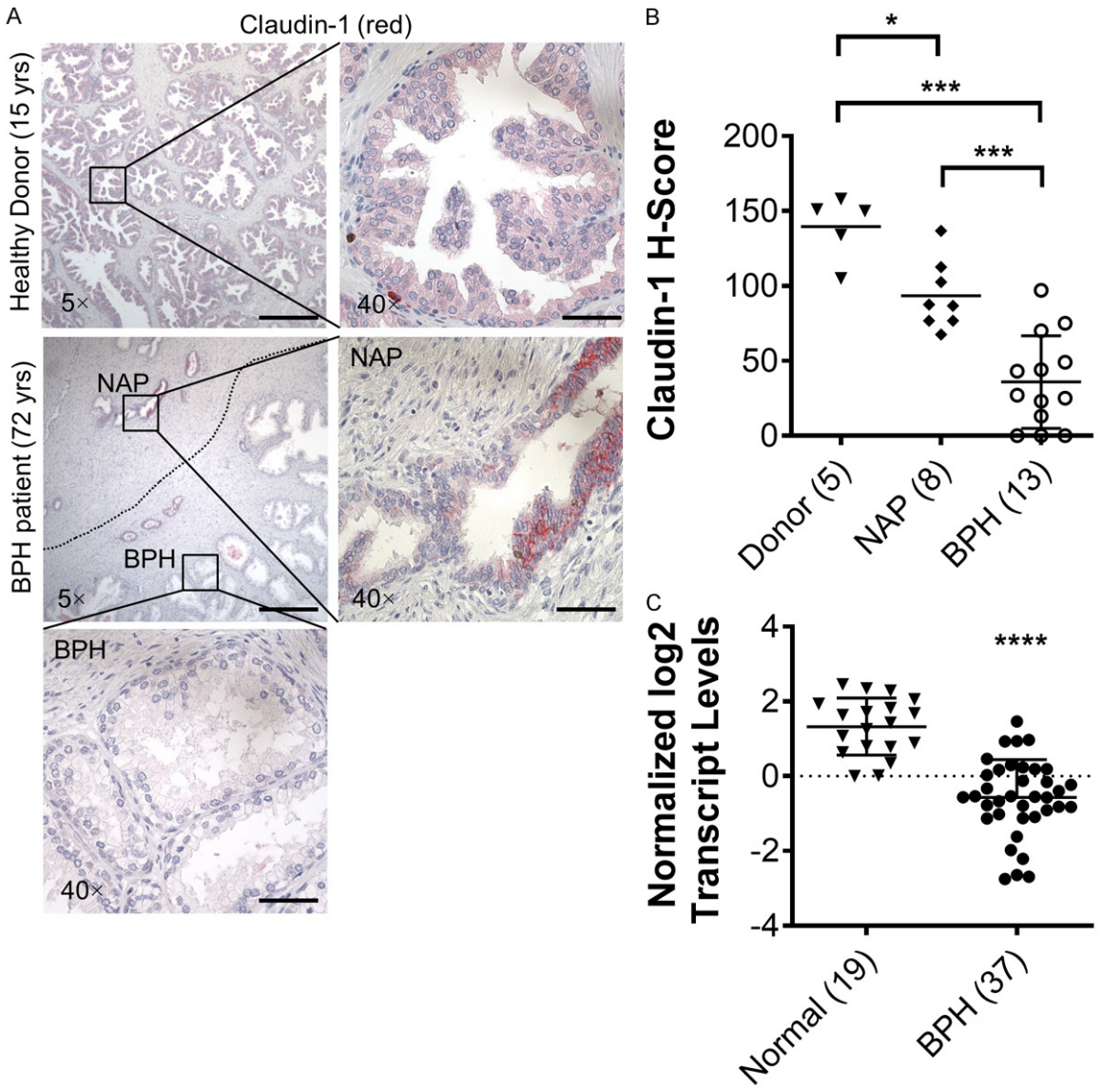

Figure 1. Expression of claudin-1 in the prostate. Immunostaining of claudin-1 expression in young healthy donor, normal adjacent prostate (NAP) and BPH specimens. A. Representative images showing the expression of claudin-1 (red). Scale bars indicate 400 μm in 5×, 50 μm in 40×. Age of patient in parentheses. B. Quantification of mean claudin-1 staining intensity H-score. C. Quantification of claudin-1 mRNA expression from in silico analysis of RNA-Seq data [28]. Number of patients in parentheses. Data represent mean ± S.D.; *, P<0.05; ***, P<0.001; ****, P<0.0001.

BPH vs. NAP for all the outcomes was presented with median and inter-quartile range values, and the significance was tested using Wilcoxon signed-rank test. The Pearson correlation coefficient was used to determine the correlation between claudin-1 and age in the combined samples from groups donor vs. NAP. To study the linear relationship, a simple linear regression analysis was used between the dependent variables (outcomes) and the group of the independent variable. A multiple regression method was used to include the predictor variable 'Age' along with the group (donor, NAP and BPH). Several regression models were built to see the significance of the primary predictor group and 'Age'. A type I error or a *p*-value <0.05 was used as a threshold to identify statistical significance. Data were analyzed using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA). GraphPad Prism version 9 was used for graphics (GraphPad Software, San Diego, CA, USA). Values are expressed as means ± S.D.

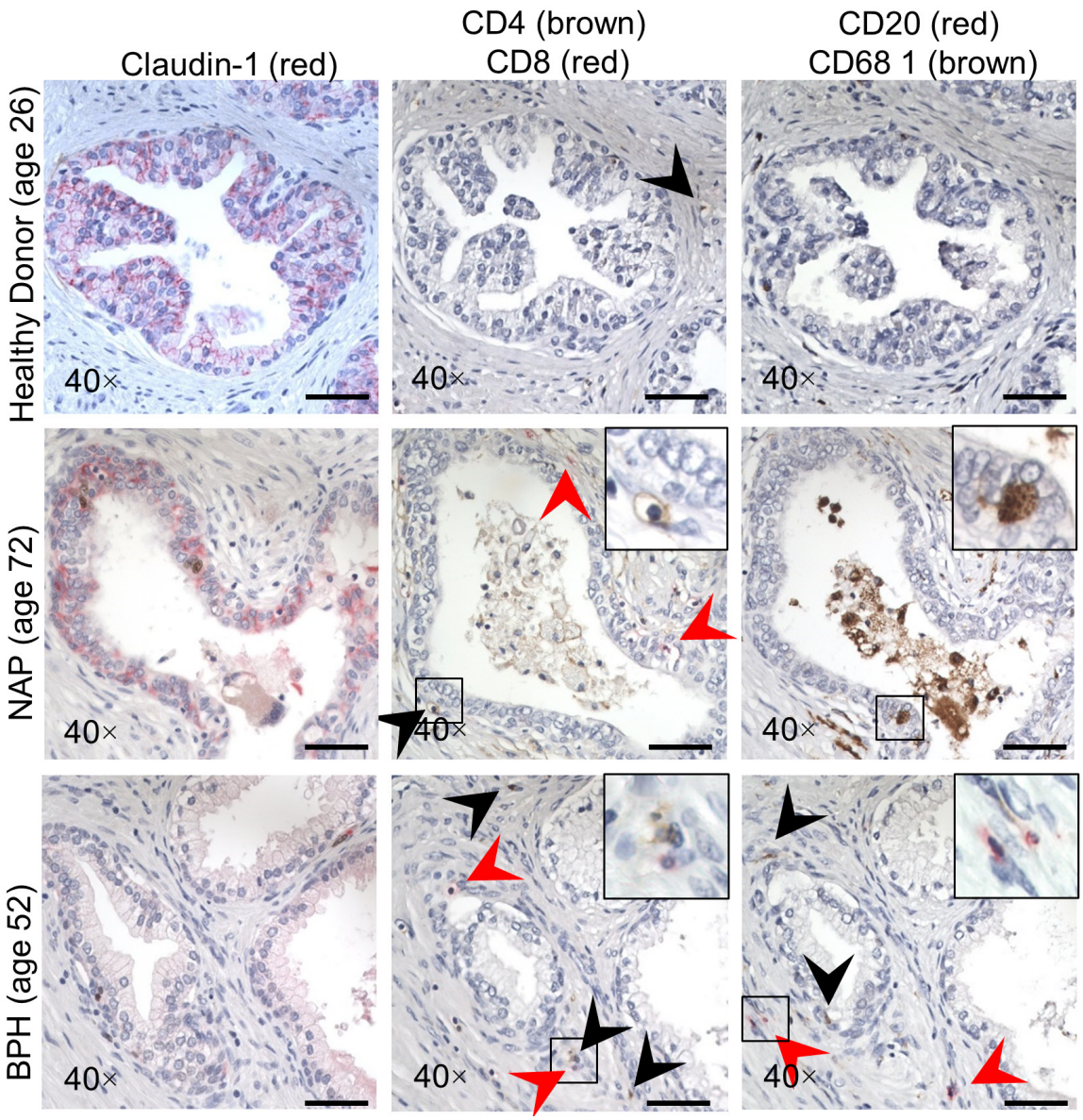

Figure 2. Immunostaining of claudin-1, CD4 (brown), CD8 (red), CD20 (red) and CD68 (brown) in serial sections of young healthy donor, normal adjacent prostate (NAP) and BPH specimens. Age of patient in parentheses. Scale bars indicate 50 μm in 40×.

Results

Expression of tight junction protein claudin-1 in the aging prostate and in BPH

Claudin-1 expression was previously reported to localize in the basal cell layer of BPH but was absent or very rare in prostate cancer tissue [29]. In a more recent study, claudin-1 expression was increased in a fraction of prostate cancers and associated with a favorable prognosis in ERG-positive cancer [30]. We previously reported a reduction in claudin-1 expression in BPH tissue compared to NAP [21]. However, no previous reports of claudin-1 expression have compared expression in young healthy donor prostate to older BPH patient and normal prostate adjacent to BPH tissues. In our analysis, cytoplasmic claudin-1 immunostaining was observed in the luminal and basal epithelial cells of healthy young donor prostate glands (**Figure 1A** top panels). NAP glands in aged patients displayed a similar staining pattern (**Figure 1A** center panels), while claudin-1 im-

Table 2. Comparing claudin-1 H-Score and number of inflammatory cells per field in young healthy donor (Donor), normal adjacent prostate to BPH (NAP) and BPH tissues. Donor vs. NAP and BPH vs. NAP

Outcome	Donor (n=5) vs. NAP (n=8)		*P* value[1]	BPH (n=13) vs. NAP (n=8)		*P* value[1]
	Median (IQR)	Median (IQR)		Median (IQR)	Median (IQR)	
Claudin-1 H-Score	150 (17)	87.1 (30.8)	**0.0156**	27 (36)	87.1 (30.8)	**0.0002**
Number of CD8 positive cells per field	1.2 (1)	0.4 (1.2)	0.3353	2 (1.3)	0.4 (1.2)	**0.0135**
Number of CD4 positive cells per field	1.4 (0.6)	1.8 (2.1)	0.6084	4.5 (3.3)	1.8 (2.1)	**0.0271**
Number of CD20 positive cells per field	0.1 (0.1)	0.7 (1.3)	**0.0463**	2 (3)	0.7 (1.3)	0.4240
Number of CD68 positive cells per field	1.3 (0.8)	0.8 (0.9)	0.8260	1.8 (1.8)	0.8 (0.9)	**0.0326**
Prostate mass (g)	36 (9)	49.1 (47.3)	0.3142	55.4 (43.1)	49.1 (47.3)	0.4810
Prostate volume (cm^3)	19.1 (2.6)	33.5 (30.2)	**0.0135**	45.4 (40.7)	33.5 (30.2)	0.6053

IQR: Inter-Quartile range (difference between third quartile-First Quartile). [1]*P* value based non-parametric test using Wilcoxon Signed Rank Test due to smaller sample size. Bold indicates statistically significant difference between groups. BPH: benign prostatic hyperplasia. NAP: normal adjacent prostate.

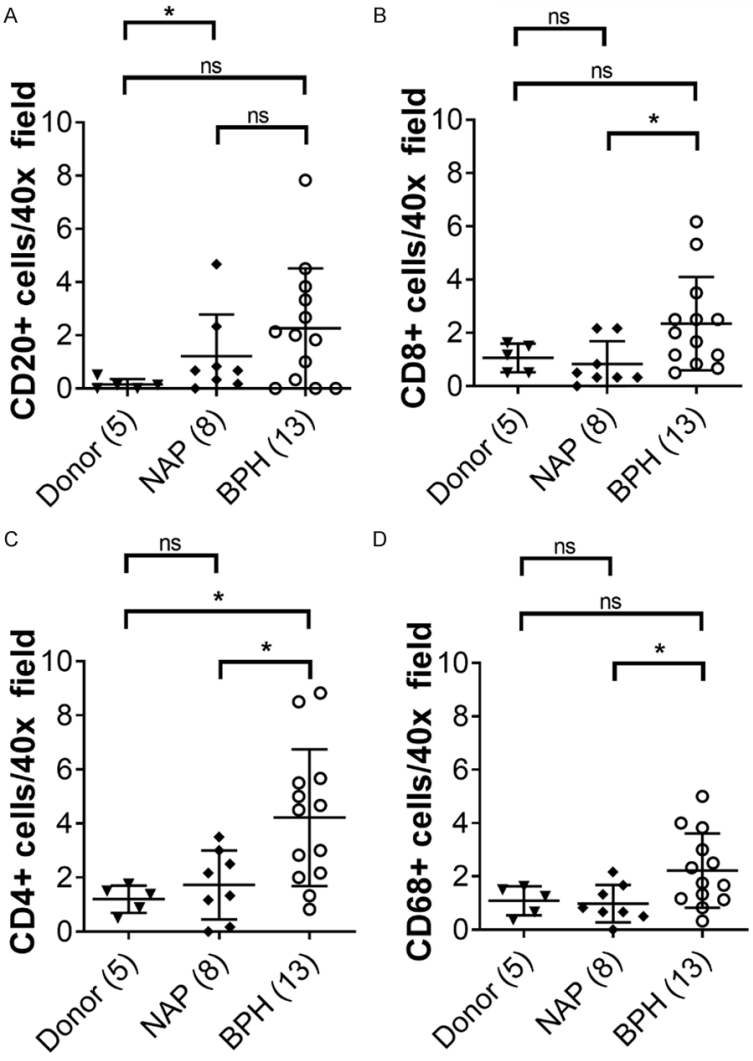

Figure 3. Inflammation in the prostate. Quantification of the number of inflammatory cells in prostate tissues from young healthy donors, normal adjacent prostate (NAP) and BPH. A. Quantification of CD4 positive T-cells. B. Quantification of CD8 positive T-cells. C. Quantification of CD20 positive B-cells. D. Quantification of CD68 positive macrophages. Number of patients in parentheses. Data represent mean ± S.D.; *, P<0.05; **, P<0.01. ns, not significant.

munostaining in BPH glands was observed in the basal epithelial cell layer as previously reported [21, 29], or was completely absent (**Figure 1A** bottom panels). Quantification of the H-Score for each tissue type showed that claudin-1 expression was decreased with age and was further decreased in BPH (**Figure 1B**). *In silico* analysis of recently published RNA-Seq data for BPH and matched normal prostate from radical prostatectomy specimens (performed for prostate cancer) with concurrent BPH [28] showed that claudin-1 was down-regulated at the mRNA level as well (**Figure 1C**), in agreement with our previous findings that claudin-1 down-regulation was also evident at the mRNA level in the epithelium of BPH tissues [20].

Inflammation in the aging prostate and BPH

Serial sections of the same prostate specimens used to examine claudin-1 were also stained for inflammatory cell markers. The number of infiltrating inflammatory cells was counted per 40× field in each corresponding area of healthy young donor tissue and older patients with BPH and corresponding NAP (**Figure 2**). Com-

Table 3. Generalized Linear Model (Donor vs. NAP). The unadjusted and covariate adjusted model with intercept, estimate, SE of both and its significance

Outcome Model	Simple Regression Model			Adjusted with Covariate		
	Estimate (SE)	*P* value	R-square	Estimate (SE)	*P* value	R-square
Claudin-1 H-Score Average			0.41			0.56
Intercept	151.96 (16.33)	–		130.47 (18.73)	—	
Age	-0.89 (0.32)	0.0182		0.45 (0.78)	0.5735	
Group (ref.=Donor)	—	—		-65.15 (34.98)	0.0921	
Number of CD8 positive cells per field			0.01			0.05
Intercept	1.042 (0.49)	–		0.77 (0.64)	—	
Age	-0.003 (0.01)	0.7896		0.02 (0.030	0.5939	
Group (ref.=Donor)	—	—		-0.83 (1.19)	0.4991	
Number of CD4 positive cells per field			0.07			0.07
Intercept	0.96 (0.68)	—		0.99 (0.91)	—	
Age	0.01 (0.01)	0.3796		0.01 (0.04)	0.7911	
Group (ref.=Donor)	—	—		0.10 (1.70)	0.9546	
Number of CD20 positive cells per field			0.11			0.18
Intercept	-0.08 (0.83)	—		0.51 (1.07)	—	
Age	0.02 (0.02)	0.2655		-0.02 (0.04)	0.6981	
Group (ref.=Donor)	—	—		1.80 (1.99)	0.3898	
Number of CD68 positive cells per field			0.06			0.22
Intercept	1.34 (0.40)	—		1.76 (0.49)	–	
Age	-0.07 (0.01)	0.4032		-0.03 (0.02)	0.1289	
Group (ref.=Donor)	—	—		1.30 (0.91)	0.1865	
Prostate mass (g)			0.37			0.51
Intercept	23.7 (12.86)	—		6.01 (17.04)	—	
Age	0.64 (0.28)	0.0468		1.67 (0.74)	0.0551	
Group (ref.=Donor)	—	—		-45.17 (30.65)	0.1787	
Prostate volume (cm^3)			0.61			0.84
Intercept	-2.85 (11.45)	—		-29.07 (10.94)	—	
Age	0.94 (0.25)	0.0045		2.46 (0.48)	0.0009	
Group (ref.=Donor)	—	—		-66.94 (19.67)	0.0093	

R-Square: Square of correlation. NAP: normal adjacent prostate.

pared to young donor tissues, older NAP tissues displayed a decrease in claudin-1 immunostaining, an increase in the infiltration of CD20 positive B-cells and an increase in prostate volume (**Table 2**). BPH tissues displayed a further decrease in claudin-1 immunostaining as well as an increased infiltration of CD4 and CD8 positive T-cells and CD68 positive macrophages (**Table 2**; **Figure 3**). The average number of CD20 positive B-cells was increased with age (**Figure 3A**), while BPH displayed a significant increase in the average number of both CD4 and CD8 positive killer T-cells compared to NAP (**Figure 3B**). The overall average number of CD20 positive B-cells and CD68 positive macrophage cells was not differentially expressed in donor compared to older NAP tissues, but BPH displayed a significant increase in CD68 positive macrophages compared to NAP (**Figure 3C**, **3D**).

Correlation of claudin-1 and inflammation with age and BPH

Correlation analyses identified a significant negative correlation between claudin-1 and age in the prostate (-0.64, P = 0.0182). A simple linear regression model comparing donor versus NAP revealed a significant association between claudin-1 down-regulation and increasing age (**Table 3**). Aging was also associated with an increase in prostate mass and volume (**Table 3**). When comparing BPH to NAP, there was a significant association between claudin-1

Table 4. The unadjusted and covariate adjusted model with intercept, estimate, SE of both and its significance (NAP vs. BPH)

Outcome Model	Simple Regression Model			Adjusted with Covariate		
	Estimate (SE)	*P* value	R-square	Estimate (SE)	*P* value	R-square
Claudin-1 H-Score Average			0.52			0.52
Intercept	93.33 (9.97)	--		80.01 (43.05)	---	
Group (ref.=NAP)	-57.49 (12.67)	0.0002		-57.60 (12.98)	0.0003	
Age	---	---		0.22 (0.67)	0.7537	
Number of CD8 positive cells per filed			0.21			0.23
Intercept	0.83 (0.52)	---		-0.69 (2.24)	--	
Group (ref.=NAP)	1.51 (0.67)	0.0352		1.50 (0.68)	0.0398	
Age	---	---		0.02 (0.04)	0.4924	
Number of CD4 positive cells per field			0.26			0.30
Intercept	1.73 (0.76)	---		4.92 (3.20)	---	
Group (ref.=NAP)	2.49 (0.97)	0.0186		2.52 (0.97)	0.0179	
Age	---	---		-0.05 (0.05)	0.3180	
Number of CD20 positive cells per field			0.07			0.14
Intercept	1.21 (0.72)	---		4.88 (2.97)	---	
Group (ref.=NAP)	1.06 (0.91)	0.2600		1.09 (0.90)	0.2401	
Age	---	---		-0.06 (0.05)	0.2193	
Number of CD68 positive cells per field			0.22			0.25
Intercept	0.98 (0.42)	---		2.43 (1.78)	---	
Group (ref.=NAP)	1.24 (0.53)	0.0310		1.25 (0.54)	0.0314	
Age	---	---		-0.02 (0.03)	0.4133	
Prostate mass (g)			0.04			0.38
Intercept	58.94 (23.18)	---		-158.28 (77.91)	---	
Group (ref.=NAP)	22.43 (28.39)	0.4410		12.25 (23.80)	0.6142	
Age	---	---		3.70 (1.29)	0.0115	
Prostate volume (cm^3)			0.03			0.37
Intercept	48.29 (31.17)	---		-241.37 (105.23)	---	
Group (ref.=NAP)	26.36 (38.18)	0.4998		12.78 (32.15)	0.6965	
Age	---	---		4.94 (1.74)	0.0124	

R-Square: Square of correlation. BPH: benign prostatic hyperplasia. NAP: normal adjacent prostate.

down-regulation and increased CD4 and CD8 positive T-cells and CD68 positive macrophages (**Table 4**). BPH was also associated with an increase in prostate mass and volume (**Table 4**). Pearson correlation analysis comparing claudin-1 to age, inflammatory cells, prostate mass and volume in all prostate tissues analyzed showed that claudin-1 expression was negatively correlated with age, CD4, CD20 and CD68 (**Table 5**).

Discussion

The tight junction protein claudin-1 is critical for the maintenance of the epithelial barrier [31] and is expressed by tight epithelia [32]. Deletion of claudin-1 in a mouse model resulted in severe dehydration and death within 1 day of birth due to the loss of a tight junction barrier and subsequent excessive transepidermal water loss across the skin [33]. Previous studies of claudin-1 expression in prostate tissues have mainly focused on its differential expression in prostate cancer. Loss of claudin-1 was associated with prostate cancer invasion, progression and metastatic transformation [29, 34], while increased claudin-1 expression was correlated with better prognosis [30]. We recently reported that claudin-1 expression was down-regulated in BPH [20, 21], which is another prevalent prostate disease associated with aging in men [35, 36]. Our immunostaining results reveal that immunostaining of claudin-1 was significantly decreased with age in the prostate, while the average number of B-cells was increased with age. Claudin-1 immunos-

Table 5. Pearson correlation of claudin-1 with inflammatory cells

Claudin-1 vs.	Age	CD8	CD4	CD20	CD68	Mass	Volume
Pearson r							
r	-0.5838	-0.2751	-0.6099	-0.5560	-0.4246	-0.2660	-0.2745
95% CI	-0.7921 to -0.2539	-0.5986 to 0.1257	-0.8067 to -0.2915	-0.7762 to -0.2149	-0.6973 to -0.04460	-0.6112 to 0.1642	-0.6169 to 0.1553
R squared	0.3409	0.07566	0.3720	0.3091	0.1803	0.07075	0.07534
P value							
P (two-tailed)	**0.0017**	0.1738	**0.0009**	**0.0032**	**0.0306**	0.2199	0.2050

CI: confidence interval. Bold indicates statistically significant between groups.

taining was further decreased in mixed hyperplastic epithelial and stromal nodules of BPH tissues compared to NAP, and this decrease was associated with increased infiltration of T-cells and macrophages. A reduction in claudin-1 within tight junctions of BPH glands could help explain our previous findings of PSA in the prostatic stroma of BPH tissues [19]. This could be a consequence of claudin-1 down-regulation resulting in a loss of tight junctions and a "leaky" prostate luminal epithelial barrier in BPH glands. Increased leakiness and decreased claudin protein expression with age has been reported in other tissues [37] and could contribute to the development of age-related diseases. Taken together, these findings and ours suggest that claudin-1 plays a critical role in the maintenance of the prostate epithelial barrier and altered claudin-1 expression in the aging prostate could contribute to the development of age-related prostatic disease.

The presence of increased inflammatory cell infiltration in the current study is consistent with the frequent histologic finding of inflammation associated with BPH [15, 38]. Chronic prostatic inflammation has been associated with prostatic enlargement and LUTS; and has been implicated as a cause of prostatic fibrosis and subsequent bladder outlet obstruction (Reviewed in [39]). Chronic prostatic inflammation has also been associated with the severity of bladder voiding dysfunction in patients [38]. Several rodent studies have shown that chemical- or bacterial-induced prostatic inflammation can induce bladder overactivity [24, 40, 41], further implicating prostatic inflammation as a potential contributing factor to BPH pathogenesis. We have also previously shown that inflammatory cytokine, TGF-β1, could down-regulate claudin-1 mRNA and increase transepithelial electrical resistance and FITC-dextran diffusion in benign prostatic epithelial cell lines grown in a monolayer [20, 21], suggesting that inflammation could inhibit claudin-1 expression and contribute to increased epithelial barrier permeability. Here, we showed that claudin-1 was inversely correlated with age and the infiltration of inflammatory cells in the prostate, suggesting that alterations to the epithelial barrier were associated with increased prostatic inflammation. Thus, age-related claudin-1 down-regulation could result in the loss of tight junctions and a leaky prostate epithelial barrier. Defects in the prostate epithelial barrier could subsequently trigger the infiltration of inflammatory cells and secretion of inflammatory cytokines, the further inhibition of claudin-1 expression, and a chronic inflammatory response.

In summary, our results suggest that a compromise of tight junction structure and function as manifested by reductions in claudin-1, could be a consequence of normal aging in the prostate and ultimately contribute to inflammatory cell infiltration in the prostate and the development of BPH.

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Disclosure of conflict of interest

None.

Abbreviations

BPH, benign prostatic hyperplasia; LUTS, lower urinary tract symptoms; NAP, normal adjacent prostate.

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