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

Wei Chen, Laura E. Pascal, # and Rajiv Dhir #, Goundappa K. Balasubramani, Chandler Hudson, Pooja Srivastava, Anthony Green, Donald B. DeFranco, Naoki Yoshimura and Zhou Wang

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 TGFβ1 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 BPH patients and 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: Down-regulation of junction protein claudin-1 was associated with increasing age, and inflammation in NAP and BPH compared to young healthy donor prostate. 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.

Funding

We are grateful to Elaine V. Byrnes and Paul Knizner for technical support. This work was funded in part by NIH grants U54 from NIDDK, DK112079 (ZW), R56 DK107492 (ZW), and 1R50 CA211242 (LEP), American Urology Association Award (WC). This project used the UPCI Tissue and Research Pathology Services (TARPS) and the Pitt Biospecimen Core and was supported in part by award P30CA047904 with additional support from the University

Age-related decrease in Claudin-1

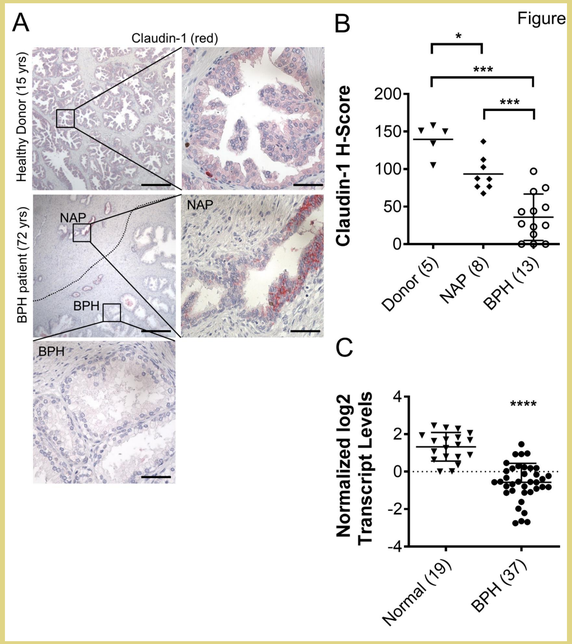

Figure 1. Expression of claudin-1 in the prostate. Immunostaining of claudin-1 expression in young healthy donor, normal adjacent prostate (NAP) and BPH specimens. (A) Representative images showing the expression of claudin-1 (red). Original magnification 5×, inset 40×, scale bars indicate 400 μm in 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 [17]. Number of patients in parentheses. Data represent mean ± S.D.; *, p<0.05; ***, p<0.001; ****, P<0.0001.

Localization of Claudin-1 and Inflammation

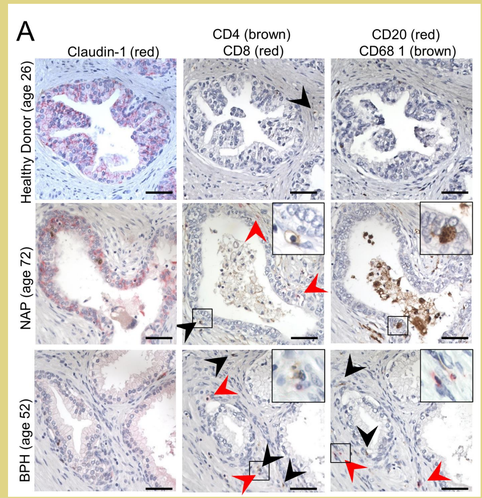

Figure 2. Immunostaining of claudin-1, CD4 (brown), CD8 (red), CD20 (red) and CD68 (brown) in serial sections of young healthy donor, normal adjacent prostate (NAP) and BPH specimens. Age of patient in parentheses. Original magnification 40×, scale bars indicate 50 μm in 40×.

Prostatic Inflammation

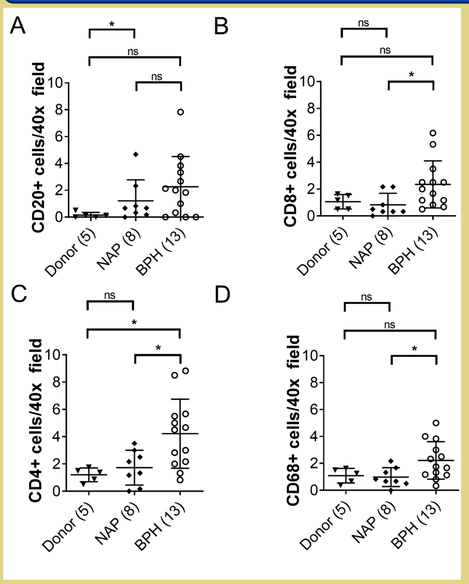

Figure 3. Inflammation in the prostate. Quantification of the number of inflammatory cells in prostate tissues from young healthy donors, normal adjacent prostate (NAP) and BPH. (A) Quantification of CD4 positive T-cells. (B) Quantification of CD8 positive T-cells. (C) Quantification of CD20 positive 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.

Claudin-1 correlation with age and inflammation

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

Table 2. Comparing 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 Average	150 (17)	87.1 (30.8)	**0.0156**	27 (36)	87.1 (30.8)	**0.0002**
Number of CD8 positive cells per filed	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**
Weight of Prostate in grams	36 (9)	49.1 (47.3)	0.3142	55.4 (43.1)	49.1 (47.3)	0.4810
Volume of prostate	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 between groups.

Table 3. Correlation: Claudin-1 down-regulation with Age in the combined samples from groups Donor vs. NAP

	Correlation	P value
Claudin-1 H-Score Average	- 0.64	**0.0182**