MP06-06 INCREASED EXPRESSION OF OSTEOPONTIN IN THE PROSTATE IS ASSOCIATED WITH THE CLINICAL PROGRESSION OF BPH

Petra Popovics[1,2,3], Wisam N. Awadallah[3], Sarah E. Kohrt[4], Thomas C. Case[5], Nicole L. Miller[4], Emily Ricke[1,2], Marisol Ramirez-Solano[6], Qi Liu[6], Robert J. Matusik[5], William A. Ricke[1,2] and Magdalena M. Grabowska[3,4]

[1]Department of Urology, School of Medicine and Public Health, University of Wisconsin-Madison, Madison, WI, [2]George M. O'Brien Center of Research Excellence, University of Wisconsin School of Medicine and Public Health, Madison, WI.[3]Department of Urology, Case Western Reserve University, Cleveland, OH, [4]Department of Pharmacology, Case Western Reserve University, Cleveland, OH [5]Department of Urology, Vanderbilt University Medical Center, Nashville, TN [6]Center for Quantitative Sciences, Vanderbilt University Medical Center, Nashville, TN

Wisconsin Multidisciplinary K12 Urologic Research Career Development Program

George M. O'Brien Urology Research Center UNIVERSITY OF WISCONSIN-MADISON

Introduction

Lower urinary tract dysfunction (LUTD) in men and associated symptoms (LUTS) including weak stream, nocturia, incomplete emptying and intermittent or hesitant urination, can adversely affect the patient`s quality of life. The progression of LUTS have been linked to age-related changes including alterations in steroid levels, but there is a growing body of evidence demonstrating that chronic inflammation has a central role in its pathogenesis, in part, by stimulating prostatic fibrosis. Fibrotic pathology is associated with the accumulation of collagen fibers in the stroma and the rearrangement of the periurethral prostatic architecture often resulting in obstructive urinary symptoms. Current therapies do not target fibrosis, however, it is a key event in driving medical resistance to LUTS. Consequently, the development of novel medical strategies requires a better understanding of the molecular aspects of this prostatic disease. Our previous study identified osteopontin (OPN) as a novel gene responding to inflammation with an exceptional increase in its expression in a carrageenan-induced prostatic inflammation model. OPN is implicated in various fibrotic diseases, including delaying the resolution of thioacetamide-induced liver fibrosis in mice partly due to its stimulatory action on collagen-I deposition. Accordingly, OPN may serve as a valuable molecular target to develop novel pharmacological therapies to prostatic fibrosis and LUTS. Our study focused on determining whether OPN levels are increased in clinically progressed BPH patients and to identify key aspects of its production in prostate cells.

Methods

Analysis of human prostate samples: Following Institutional Review Board approval, prostate tissues were acquired from the Vanderbilt University Medical Center (VUMC) BPH Tissue and Data Biorepository. Surgical BPH specimens were obtained from patients who had failed medical therapy and undergone one of several types of operations for benign prostatic hyperplasia (holmium laser enucleation of the prostate, or open/simple prostatectomy) to relieve LUTS. Incidental BPH was isolated from the transitional zone of men undergoing radical prostatectomy for low volume, low grade prostate cancer confined to the peripheral zone (PZ). Samples were selected in which the malignancy is low risk (Gleason Score 7 or less) small volume (≤1 cc), and localized in the PZ of the prostate, to minimize any field effects. Patients who received α-blockers were also excluded from this group. Tissues were paraffin-embedded for immunohistological detection of osteopontin (ab8448, Abcam). Images (6/specimen) were captured with a Nuance multispectral system and H-score was calculated using after exclusion of luminal area and cell segmentation using a 4-tier scoring system with an algorithm developed with inForm software specifically for this dataset (Figure 1.). Protein levels of OPN and GAPDH from tissue lysates were determined with Western blot.

***In vitro* studies:** OPN expression was analyzed in immortalized benign stromal (BHPrS-1) and epithelial (BHPrE-1, NHPrE-1) cell lines and was compared to prostate cancer cells (LNCaP, C4-2B, 22RV1). To determine whether inflammatory signals can mediate OPN secretion, we tested the effects of lipopolysaccharide (LPS) and recombinant human (rh) interleukin-1β (IL-1β) and transforming growth factor-β1 (TGF-β1). The effects of 500nM rhOPN on the expression of *COL1A1, COL1A2, SPP1, ACTA2, TGFB1, IL6, CXCL8, CXCL1, CXCL2, CXCL12, MMP1, MMP2, MMP9, MMP14, TIMP1* and *TIMP2* were analyzed by qPCR.

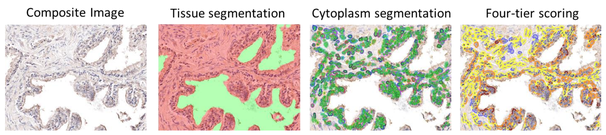


Figure 1: Tissue- and cell segmentation. Tissue was first segmented from lumen and tissue-free areas which was followed by the identification of nuclei were based on hematoxylin staining. Cytoplasm was then determined as a 20-pixel wide area immediately surrounding the nucleus. Four intensity bins were set manually based on the variability observed in OPN staining across the training set.

Results

OPN expression is associated with the clinical progression of LUTD/LUTS

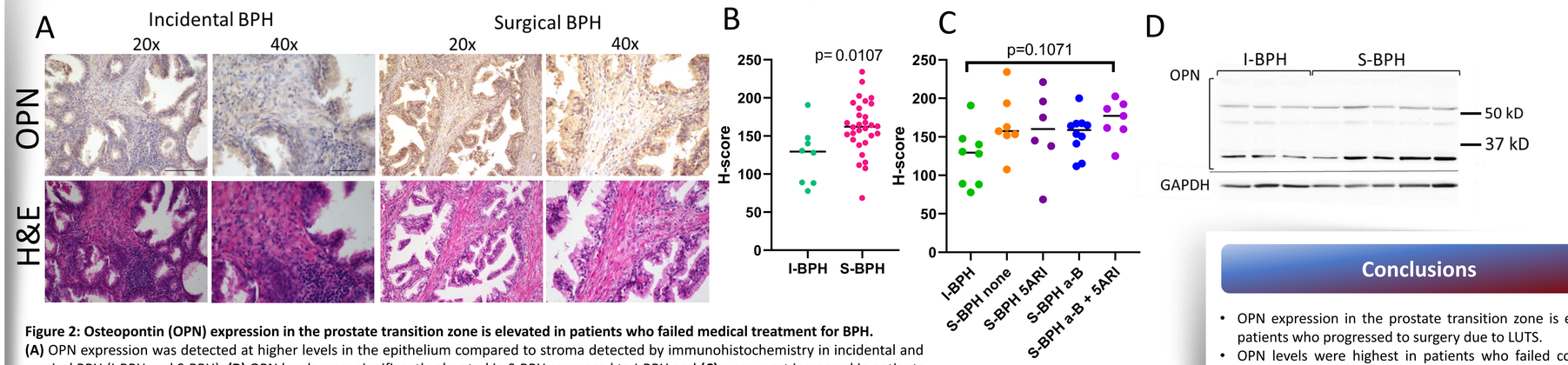


Figure 2: Osteopontin (OPN) expression in the prostate transition zone is elevated in patients who failed medical treatment for BPH. **(A)** OPN expression was detected at higher levels in the epithelium compared to stroma detected by immunohistochemistry in incidental and surgical BPH (I-BPH and S-BPH). **(B)** OPN levels were significantly elevated in S-BPH compared to I-BPH and **(C)** were most increased in patients who received combination treatment of α-blockers (a-B) and 5-α-reductase inhibitors (5ARI). Staining was scored with multispectral imaging followed by tissue- and cell segmentation and automatic determination of H-score by an algorithm developed with inForm software. **(D)** Western blot analysis confirmed the upregulation of OPN and its cleaved form at approximately 32 kD.

OPN is produced in both prostate epithelial and stromal cells

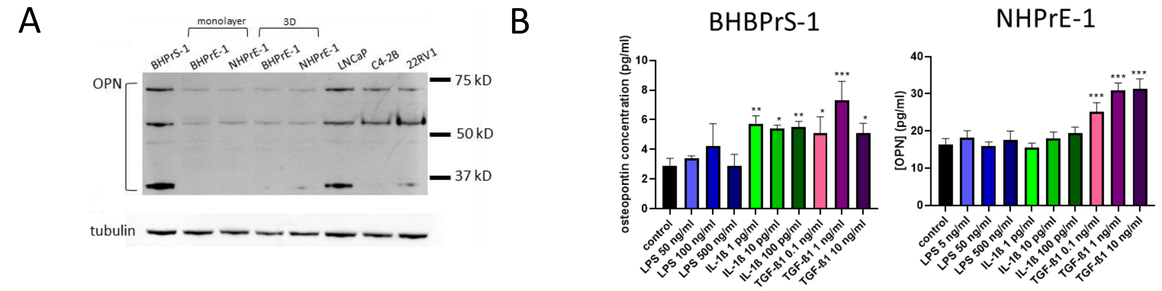
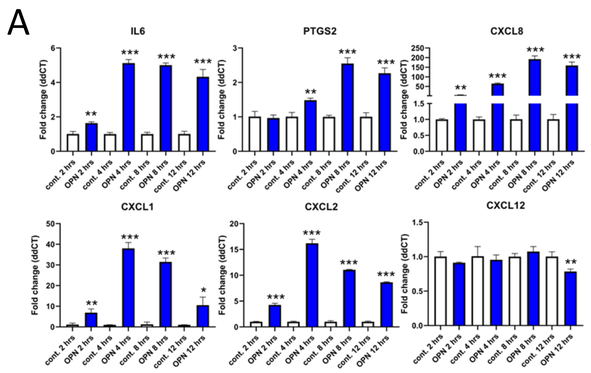


Figure 3: IL-1β and TGF-β1 stimulates OPN secretion in prostate cell lines. (A) OPN is expressed in benign prostate stromal (BHPrS-1) and epithelial (BHPrE-1, NHPrE-1 grown in monolayer or 3D Matrigel cultures) cells. OPN levels in BHPrS-1 were comparable to what was observed in prostate cancer cell lines (LNCaP, C4-2B, 22RV1). Two OPN isoforms between 50 and 75 kD were detected in all cell lines whereas the cleaved product at around 32 kD was dominant in BHPrS-1 cells. **(B)** IL-1β and TGF-β1 stimulated OPN secretion in BHPrS-1 benign stromal cells whereas only TGF-β1 showed stimulation in NHPrE-1 epithelial cells. *p < 0.05; ** p < 0.01; *** p < 0.001

OPN triggers the expression of multiple cytokines



B

fold elevation (ddCT)

gene	cont.	OPN 2 hrs	cont.	OPN 4 hrs	cont.	OPN 8 hrs	cont.	OPN 12 hrs
IL6	1.01±0.15	**1.64±0.07****	1.00±0.09	**5.12±0.21*****	1.00±0.10	**5.00±0.13*****	1.01±0.15	**4.33±0.43*****
PTGS2	1.01±0.15	0.97±0.09	1.01±0.12	**1.48±0.06****	1.00±0.05	**2.55±0.17*****	1.00±0.12	**2.27±0.16*****
CXCL8	1.00±0.02	**5.26±1.13****	1.00±0.08	**64.94±3.06*****	1.01±0.13	**192.08±17.01*****	1.01±0.15	**158.8±17.87*****
CXCL1	1.13±0.70	**6.91±1.79****	1.00±0.07	**38.04±2.83*****	1.28±1.07	**31.49±1.87*****	1.00±0.05	**10.54±3.91***
CXCL2	1.00±0.07	**4.25±0.33*****	1.00±0.11	**16.20±0.78*****	1.01±0.19	**11.06±0.08*****	1.01±0.13	**8.64±0.10*****
CXCL12	1.00±0.07	0.91±0.01	1.01±0.14	0.95±0.07	1.00±0.05	1.07±0.07	1.00±0.07	**0.78±0.04****
ACTA2	1.00±0.07	1.1±0.06	1.00±0.07	1.10±0.07	1.00±0.12	1.05±0.06	1.00±0.09	0.95±0.02
SPP1	1.00±0.05	0.80±0.15	1.01±0.21	1.38±0.23	1.01±0.18	1.08±0.23	1.01±0.14	0.89±0.12
TGFB1	1.00±0.04	1.07±0.02	1.00±0.07	0.99±0.05	1.00±0.08	1.10±0.06	1.00±0.07	0.89±0.19
MMP1	1.01±0.17	0.97±0.12	1.00±0.08	1.04±0.02	1.00±0.05	1.14±0.20	1.00±0.11	1.07±0.18
MMP2	1.00±0.03	1.02±0.05	1.00±0.12	1.01±0.05	1.00±0.08	1.15±0.10	1.00±0.09	0.84±0.10
MMP9	1.00±0.04	**1.16±0.01****	1.01±0.14	1.19±0.10	1.00±0.07	1.36±0.21	1.01±0.16	1.26±0.08
TIMP1	1.00±0.04	1.07±0.02	1.00±0.07	0.99±0.05	1.00±0.08	1.10±0.06	1.00±0.07	0.89±0.19
COL1A1	1.01±0.14	0.75±0.05	1.02±0.22	0.84±0.05	1.00±0.06	1.17±0.10	1.00±0.04	0.96±0.11
COL1A2	1.00±0.08	1.05±0.04	1.00±0.11	1.00±0.06	1.00±0.05	1.10±0.18	1.00±0.05	0.89±0.07

Figure 4: Osteopontin possesses a robust, immediate stimulatory action on inflammatory genes. (A) Human recombinant OPN stimulated the expression of IL-6, PTGS2 (protein: COX2), CXCL8 (protein: IL-8), CXCL1 and CXCL2, but decreased that of CXCL12. **(B)** Expression of genes related to fibrosis and tissue remodeling was not significantly altered except MMP9 after 2-12 hours of stimulation with 0.5 µg/ml OPN. ** p < 0.01; *** p < 0.001.

Conclusions

- OPN expression in the prostate transition zone is elevated in patients who progressed to surgery due to LUTS.
- OPN levels were highest in patients who failed combination therapy of a-B and 5ARI indicating a role for OPN in the development of treatment resistance
- Resident cells in the prostate are capable of secreting OPN in response to inflammatory signals

Figure 5. OPN exacerbates inflammation and fibrosis in the prostate. OPN secretion is stimulated by inflammatory cytokines primarily from epithelial cells and promotes inflammation by triggering cytokine expression in stromal cells leading to further OPN release, chronic inflammation and fibrosis.

Future Directions

- Determine whether OPN stimulates fibrosis in stromal-epithelial co-culture
- Define the primary cellular source of OPN in BPH
- Evaluate the therapeutic efficacy of pharmacological inhibition of OPN in BPH

Acknowledgements

Acknowledgements

BPH tissue was provided by the NCI funded Cooperative Human Tissue Network. Use of human tissue is approved by the VUMC (#120944) and the CWR (#STUDY20190025) IRBs. We thank the patients who have generously donated tissue for this study.

Funding

Start-up fund provided by the Department of Urology, Case Western Reserve University (to MMG), NIH NIDDK K12 DK100022 (to PP), 5R01 DK111554-03 (to RJM), T32GM008056-37 (to SEK) and U54 DK104310 (to WAR)

References

1. Popovics P, Cai R, Sha W, Rick FG and Schally AV. Growth hormone-releasing hormone antagonists reduce prostatic enlargement and inflammation in carrageenan-induced chronic prostatitis. Prostate. 2018; 78: 970-980.
2. Popovics P, Schally AV, Salgueiro L, Kovacs K and Rick FG. Antagonists of growth hormone-releasing hormone (GHRH) inhibit proliferation induced by inflammation in prostatic epithelial cells. Proceedings of the National Academy of Sciences of the United States of America. 2017; 114(6):1359-1364.

popovics@wisc.edu @PopovicsPetra