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

Lower urinary tract dysfunction (LUTD) in men and associated symptoms (LUTS) including weak stream, nocturia, incomplete emptying and intermittence or hesitancy, can adversely affect the patient’s quality of life. The progression of LUTS has 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 element 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 carcinogen-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 Repository. 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 prostatic 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 (AbDABO, Abcam). Images (6/specimen) were captured with a NanoView multispectral system and H-score was calculated using after exclusion of luminal area and cell segmentation using a 4× 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 immunoblot (BPH-S1 and epithelial BPH-1, NHPR-E1) cell lines and was compared to prostate cancer cells (LNCaP, CA-22, 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 IL-6 were analyzed on the expression of COL1A1, COL1A2, SPARC, alleles, TGFB1, IL6, CXCL1, CXCL2, CXCL3, MMP1, MMP2, MMP9, TIMP1 and TIMP2 were analyzed by qPCR.

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 BPH (1-BPH and S-BPH). (B) OPN levels were significantly increased in S-BPH compared to 1-BPH and (C) were most increased in patients who showed combination treatment of a-bladder (a-B and S-B-reduction inhibitors (SARI)). Sari was scored with immunohistochemistry and followed by tissue- and cell segmentation and automatic determination of H-score by an algorithm developed with inform software. Western blot analysis confirmed the upregulation of OPN and its cleaved form at approximately 32 kD.

Conclusion

• 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 α and SARI indicating a role for OPN in the pathogenesis of prostatic fibrosis.
• Resident cells in the prostate are capable of secreting OPN in response to inflammatory signals.

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

BPH tissue was provided by the NCI funded Cooperative Human Tissue Network. Use of human tissue is approved by the SCMC IRB2010-056 and the CM (K12DK007915-05) RRs. We thank the patients who have generously donated tissue for this study.

References


Figure 3: IL-1β and TGF-β1 stimulates OPN secretion in prostate cell lines. (A) OPN is expressed in benign prostatic stromal (BPH-S1) and epithelial (BPH-E1, NHPR-E1) grown in monolayer or 3D Matrigel cultures. OPN levels in BPH-S1 were comparable to what was observed prostatic carcinoma cell line (LNCaP) CA-22, 22Rv1. (B) IL-1β and TGF-β1 stimulated OPN secretion in BPH-S1 benign stromal cells whereas only TGF-β1 showed stimulated in NHPR-E1 epithelial cells. *p < 0.05; **p < 0.01; ***p < 0.001

Figure 4: Osteopontin possesses a robust, immediate stimulatory action on inflammatory genes. (A) Human recombinant OPN stimulated the expression of IL-6, TNF-α (protein and mRNA) induced inflammatory genes and, decreased expression of IL-6, TNF-α. (B) Expression of genes related to fibrosis and tissue remodeling was significantly altered except MMP9 after 2-12 hours of stimulation with 0.5 μM/mL OPN. *p < 0.01; **p < 0.001.

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.

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References


Figure 6: Osteopontin possesses a robust, immediate stimulatory action on inflammatory genes. (A) Human recombinant OPN stimulated the expression of IL-6, TNF-α (protein and mRNA) induced inflammatory genes and, decreased expression of IL-6, TNF-α. (B) Expression of genes related to fibrosis and tissue remodeling was significantly altered except MMP9 after 2-12 hours of stimulation with 0.5 μM/mL OPN. *p < 0.01; **p < 0.001.

Figure 7: Osteopontin possesses a robust, immediate stimulatory action on inflammatory genes. (A) Human recombinant OPN stimulated the expression of IL-6, TNF-α (protein and mRNA) induced inflammatory genes and, decreased expression of IL-6, TNF-α. (B) Expression of genes related to fibrosis and tissue remodeling was significantly altered except MMP9 after 2-12 hours of stimulation with 0.5 μM/mL OPN. *p < 0.01; **p < 0.001.

Figure 8: Osteopontin possesses a robust, immediate stimulatory action on inflammatory genes. (A) Human recombinant OPN stimulated the expression of IL-6, TNF-α (protein and mRNA) induced inflammatory genes and, decreased expression of IL-6, TNF-α. (B) Expression of genes related to fibrosis and tissue remodeling was significantly altered except MMP9 after 2-12 hours of stimulation with 0.5 μM/mL OPN. *p < 0.01; **p < 0.001.

Figure 9: Osteopontin possesses a robust, immediate stimulatory action on inflammatory genes. (A) Human recombinant OPN stimulated the expression of IL-6, TNF-α (protein and mRNA) induced inflammatory genes and, decreased expression of IL-6, TNF-α. (B) Expression of genes related to fibrosis and tissue remodeling was significantly altered except MMP9 after 2-12 hours of stimulation with 0.5 μM/mL OPN. *p < 0.01; **p < 0.001.