Expression of PPP1r Protein Regulators of Smooth Muscle Relaxation is Altered in Prostates of Aged Mouse Models of LUTS/BPH and in Human Cells Cultured from BPH Diseased Prostate

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Benign prostate hyperplasia (BPH) incidence increases with age and is common in older men. Quality of life is severely impacted by BPH, which leads to bladder outlet obstruction (BOO) and lower urinary tract symptoms (LUTS). Sustained contraction of prostrate smooth muscle around the bladder and urethra is a major contributor to lower urinary tract symptoms (LUTS) associated with benign prostate hyperplasia (BPH). Smooth muscle contraction requires phosphorylation of myosin light chain (MLC) by myosin light chain kinase (MLCK), whereas dephosphorylation of MLC and muscle relaxation in the prostate is promoted by protein phosphatase 1c (PP1c). PP1c forms a complex with the poorly characterized M20 protein and a member of the family of protein phosphatase 1 regulators (PPP1Rs) including PPP1R12a/b/c, PPP1R14a/b/c and PPP1R16a/b that increase or decrease PP1c activity. The relative expression and activity of PPP1r proteins in normal and BPH prostate tissue is not well characterized. Our objective was to understand how smooth muscle relaxation is regulated in normal and BPH/LUTS prostate by assaying PPP1R protein expression in mouse and human prostate models. Direct comparisons of Ppp1r mRNA quantities by RT-qPCR in mouse prostate extracts revealed that Ppp1r12c (a PP1c activator) and Ppp1r16b (a PP1c inhibitor) were the highest expressed Ppp1r mRNAs in mature mice (6 months-old) and that Ppp1r12c and Ppp1r16b mRNA levels were induced a further 2.0- and 3.6-fold (n=5), respectively in prostates from aged (21-month-old) mice that have LUTS characteristics. These results are the first characterization of relative PPP1r mRNA expression in prostate tissue and identify Ppp1r12c and Ppp1r16b as the major regulators of PP1c in normal and aged mouse prostate. In human stromal cells cultured from normal or BPH diseased regions of the same prostate we found that most cells were positive for smooth muscle cell markers. We also found phosphorylated MLC immunostaining intensity (marker for increased cell contraction) was greater in BPH than control prostate cultures. This finding supports the hypothesis that the SM relaxation pathway is less active in BPH cultures. Studies of Ppp1r mRNAs in human prostate cell cultures determined that expression of mRNA encoding PPP1R14A, an inhibitor of PP1c, was 28.7-fold higher in BPH than normal prostate cells (n=3). This observation suggests that PPP1R14a-mediated inhibition of PP1c may contribute to initiation and/or maintenance of LUTs in humans. Mining of published RNA-seq studies revealed PPP1r14c as well as PPP1R16b inhibitors of PP1c are induced 3.7- and 3.4-fold, respectively in human BPH tissue. Future studies will focus determining whether decreasing androgen:estrogen ratios that occur in aging men and in association with BPH causes altered expression of PPP1R proteins and their interactions with PP1c to decrease smooth muscle relaxation. In conclusion, our data indicate that in 1) aged mouse models of BPH/LUTS, 2) cultured cells from human BPH and 3) human BPH tissue, expression of PPP1r regulators of PP1c and SM relaxation is altered to promote sustained SM contraction, a cause of LUTS. Our studies have identified the most likely PPP1R proteins responsible for regulation of PP1c and SM relaxation in prostate. Our results also provide a mechanistic explanation for sustained SM contraction around the prostatic urethra, restriction of urine flow and LUTS including voiding dysfunction that occurs with aging-associated altered hormone levels. Our findings may provide new targets for LUTS therapies to reverse aging associated LUTS. Supported by 1R21AG077740-01A1.