Inhibition of mitochondrial complex I influences the expression of inflammatory mediators in a human prostatic stromal cell line

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Project Overview

Introduction and Objective

Benign prostatic hyperplasia (BPH) and associated lower urinary tract symptoms (LUTS) are extremely common in aging men. BPH is characterized by fibrosis and inflammation, however their role in BPH etiology is not well understood. Mitochondrial dysfunction is frequently associated with age-related diseases and stromal inflammation. We sought here to determine if disruption of mitochondrial complex 1 in prostate stromal cells could influence the expression of genes associated with fibrosis and inflammation. Our further objective was to see if this process could be reversed by administration of Nicotinamide mononucleotide (NMN), which restores NAD+ balance.

Methods The prostate stromal cell line BHPrS1 was treated with rotenone to induce mitochondrial damage and then treated with NMN, to reverse the mitochondrial damage by increasing NAD+ biosynthesis. qRTPCR was utilized to determine the mRNA expression levels of markers associated with inflammation (COX-1, IL-6, IL-8) and fibrosis (collagen 1A1) in cells treated with rotenone and/or NMN. Immunofluorescence staining for smooth muscle marker calponin was performed to visualize the influence of rotenone on stromal cell differentiation.

Results: COX-1 and IL-6 mRNA levels were increased, while IL-8 was decreased in response to rotenone treatment in BHPrS1 cells. While collagen 1A1 levels were altered, the results were not consistent. Calponin expression was decreased.

Conclusions: Disruption of mitochondrial complex 1 induced an increase in inflammatory mediators COX-1 and IL-6 in BHPrS1 cells, suggesting that mitochondrial damage could induce an inflammatory response in prostate stromal cells. Furthermore, smooth muscle marker calponin was decreased by rotenone, suggesting that mitochondrial damage could contribute to stromal fibrosis in the prostate. The variable influence of rotenone stimulation on collagen levels and on the cytokines is something that must be further explored. It may be that collagen deposition is not part of the acute response to mitochondrial disruption. Taken together, these results suggest that mitochondrial disruption may play a role in BPH pathogenesis and that restoring NAD+ balance could have therapeutic potential for BPH treatment.

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Mitochondrial Dysfunction is increased in aged prostate and BPH

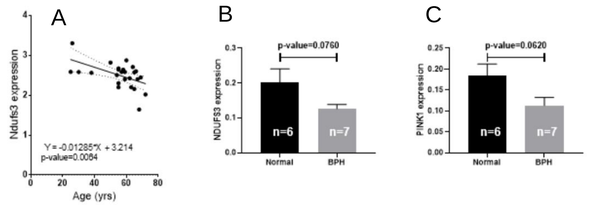

Figure 1. Expression of mitochondria-associated genes NDUFS3 and PINK1 in prostate and BPH. (A) Impact of age on NDUFS3 mRNA levels in prostate. (B) NDUFS3 and (C) mRNA expression in BPH compared to normal prostate tissue. (D) NDUFS3 (green) and PINK1 (red) immunostaining in normal prostate and BPH tissue. (E) Quantification of NDUFS3 and PINK1 positive cells in BPH tissue compared to normal prostate. *, $p < 0.05$.

Mitochondrial complex 1 disruption induced loss of smooth muscle marker calponin

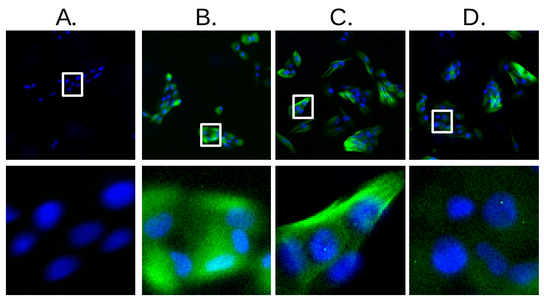

Figure 2. Expression of Calponin in BHPrS1 stromal cells following mitochondrial complex 1 inhibition. (A) No Antibody control. (B) No treatment. (C) Vehicle. (D) 25nM rotenone. BPHrS1 cells displayed a reduction in calponin (green) expression. Nuclei stained with DAPI (blue).

Mitochondrial dysfunction induced differential expression of inflammatory cytokines and collagen genes

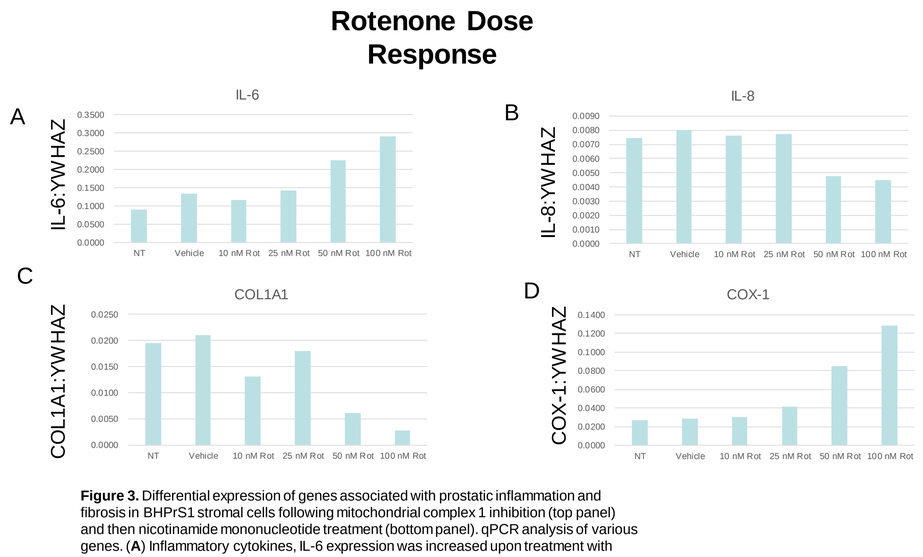

Figure 3. Differential expression of genes associated with prostatic inflammation and fibrosis in BHPrS1 stromal cells following mitochondrial complex 1 inhibition (top panel) and then nicotinamide mononucleotide treatment (bottom panel). qPCR analysis of various genes. (**A**) Inflammatory cytokines, IL-6 expression was increased upon treatment with rotenone (**B**) IL-8 expression was decreased upon rotenone treatment. (**C**) Collagen 1A1 expression was altered, however results were not consistent. (**D**) Inflammatory mediator COX-1 expression showed a positive correlation with increasing concentration of rotenone treatment

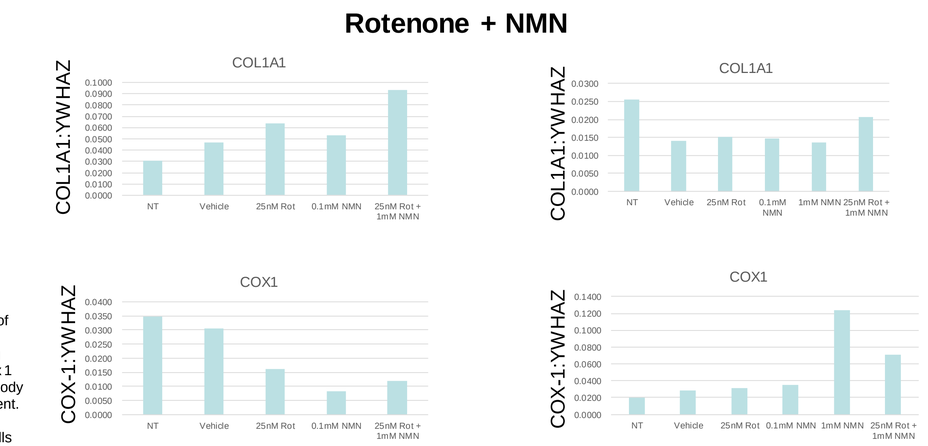

Figure 4. Differential expression of genes associated with prostatic inflammation and fibrosis in BHPrS1 stromal cells following mitochondrial complex 1 inhibition and then nicotinamide mononucleotide treatment . qPCR analysis showed results that were inconsistent for both COL1A1 And COX-1 genes.