Mitochondrial dysfunction contributes to fibrosis in aging-associated benign prostatic hyperplasia (BPH)

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INTRODUCTION

Benign prostatic hyperplasia (BPH) is characterized by proliferation, smooth muscle changes, and fibrosis of the prostate. The single greatest risk factor for BPH is age, with 90% of men in their eighties affected. Many men with BPH develop lower urinary tract symptoms (LUTS), which significantly reduce their quality of life as disease severity progresses. Given the multifactorial nature of the disease, current treatment options fail to provide long-term relief for the majority of BPH/LUTS patients. While the physiological link between BPH and aging is clear, the molecular mechanisms have yet to be fully elucidated. Recently, it has been demonstrated that an aged mouse model recapitulated the lower urinary tract dysfunction of BPH/LUTS. Additionally, the aging process is characterized by multiple molecular changes including cellular senescence, mitochondrial dysfunction, deregulated nutrient-sensing, and loss of proteostasis. This study aims to identify the role of mitochondrial dysfunction in the prostate of aged mice and determine how it contributes to fibrosis and disease progression.

HYPOTHESIS

We hypothesize that mitochondrial dysfunction is occurring due to a poorly functioning complex I of the electron transport chain, and that this dysfunction is contributing to fibrosis in BPH/LUTS.

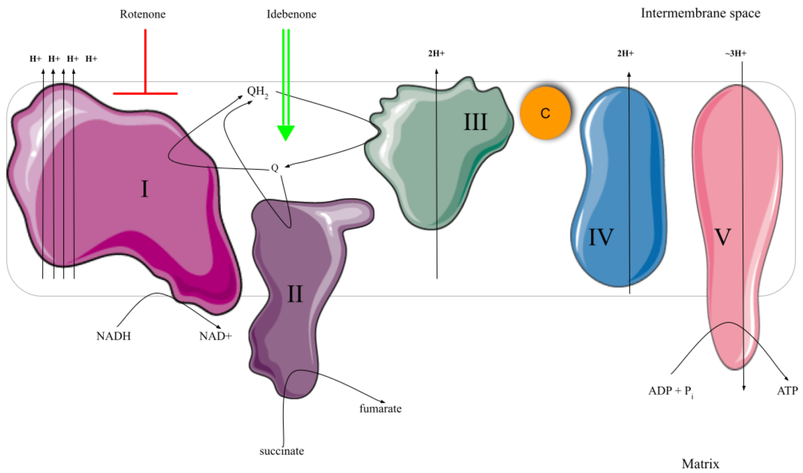


Figure 1. Electron Transport Chain. Cartoon diagram visualizing the complexes of the electron transport chain inside the mitochondria. Rotenone targets and inhibits complex I, while idebenone "bypasses" complex I dysfunction by acting as a coenzyme Q (Q; CoQ_{10}) analog.

METHODS

- C57Bl/6J mice were obtained from Jackson Laboratory (2 months) and the NIA (24 months)
- Mice were euthanized and the prostate harvested; tissues were formalin-fixed and paraffin-embedded
- Tissue sections were stained using multispectral quantitative immunofluorescence via the OPAL kit for NDUFS3 and PINK1. Using InForm® software, we spectrally unmixed each fluorophore and quantified optical density
- BHPrS1 cells were cultured and 24 hours post-plating, cells were treated with DMSO vehicle, 25 nM rotenone, or 25 nM rotenone in combination with 1 μM idebenone

RESULTS

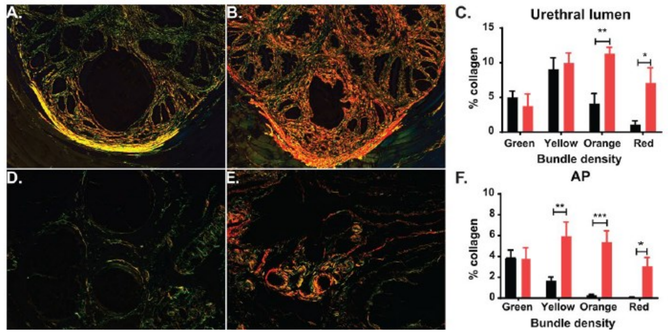


Figure 2. Aging leads to changes in collagen deposition in mice. Representative images visually demonstrate the change in amount and thickness of collagen deposition between young (**A, D**) and old (**B, E**) mice in the anterior prostate (AP) and urethral lumen. Quantification (**C, F**) of the picrosirius red (PSR) staining demonstrates significant differences between bundle density sub-groups for both the urethral lumen and anterior prostate. *, p< 0.05; **, p< 0.01; ***, p <0.0001

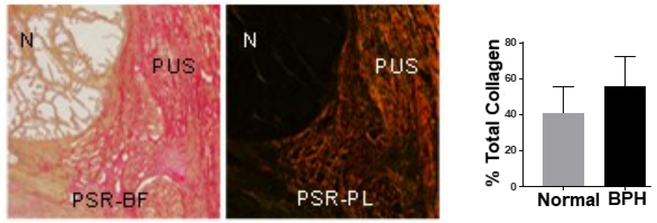


Figure 3. Localization of collagen from TURP specimens. Representative images visually demonstrate the accumulation of collagen in TURP specimens. Collagen bundles (red) predominately accumulate outside the nodule. Graphical quantification (right) shows the increased percent collagen from the total tissue area from BPH in comparison to normal controls. N-Nodule, PUS-Peri-urethral stroma

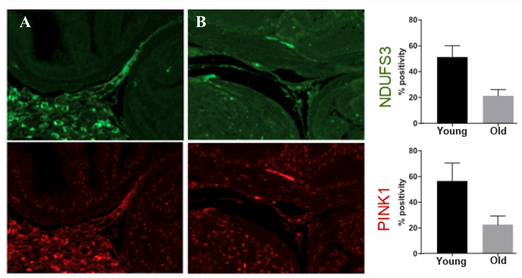


Figure 4. Decreased NDUFS3 and PINK1 expression in the anterior prostate of aged mice. Representative images for young (**A**) and old (**B**) mouse prostate stained for NDUFS3 (green) and PINK1 (red). Quantification of the percent of positive cells for both NDUFS3 and PINK1 are shown (right).

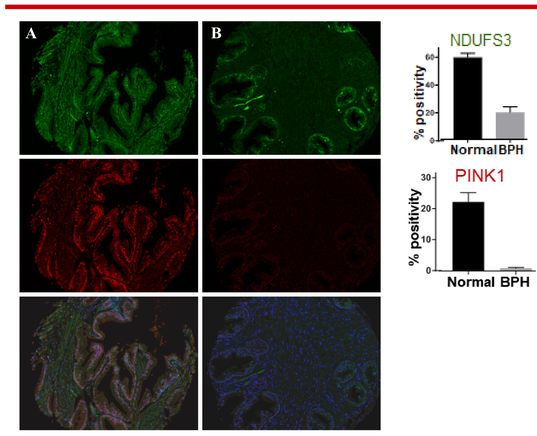


Figure 5. Human BPH and normal prostate samples show a decrease in NDUFS3 and PINK1 in BPH tissue. Tissue samples from BPH patients (**B**) have decreased expression of both NDUFS3 (green) and PINK1 (red) in comparison to normal (**A**). Merged images for both BPH and normal are also shown. Quantification based on the percent positive of total DAPI stained cells is represented on the right for both NDUFS3 (top) and PINK1 (bottom).

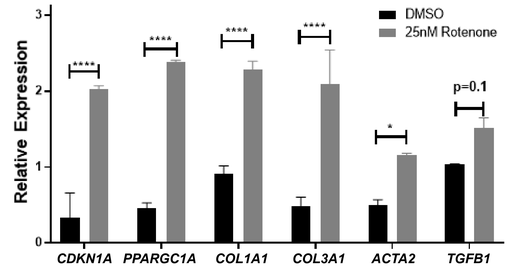
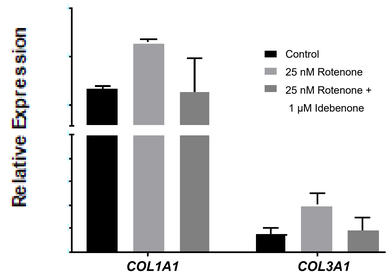


Figure 6. Rotenone induces gene expression changes in a human prostate stromal cell line. The complex I inhibitor, rotenone, induces expression increases in genes associated with senescence, mitochondrial dysfunction, fibrosis, and smooth muscle in BHPrS1 cells. *, p<0.05; ****, p<0.0001

Figure 7. Idebenone, a CoQ_{10} analog, can be used to bypass collagen gene induction in rotenone treated human prostate stromal cells. Human prostate stromal cells (BHPrS1) treated with 25 nM rotenone show an increase in both *COL1A1* and *COL3A1* gene expression. Concurrent treatment with 1 μM idebenone rescues the collagen gene expression, nearly to the level of the control.



CONCLUSIONS

- NDUFS3 is decreased in old mice, suggesting mitochondrial function associated with complex I of the electron transport chain is diminished in the aged prostate
- PINK1 is decreased in old mice, suggesting Parkin-dependent mitophagy of dysfunctional mitochondria is decreased in prostate
- Rotenone treatment of BHPrS1 cells results in an increase in markers associated with mitochondrial dysfunction and senescence, in addition to collagen genes, suggesting a connection between mitochondrial dysfunction and collagen production
- Collagen gene expression induction can be bypassed with a synthetic CoQ_{10} analog, idebenone, suggesting restoration of the electron transport chain reduces collagen gene expression

FUTURE DIRECTIONS

- Treat an aging mouse model of BPH with idebenone or other bypass agents to determine if mitochondrial dysfunction and fibrosis can be ameliorated *in vivo*
- Characterize mitochondrial bioenergetics and overall dysfunction

ACKNOWLEDGEMENTS

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