Mitochondrial dysfunction due to aging contributes to prostatic fibrosis

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Introduction

Aging is the single largest risk factor for many common diseases that burden public health. For example, as men age, the prostate undergoes a prototypical aging change, fibrosis. The aged fibrotic prostate contributes to urinary symptoms, which will afflict nearly every man if they live long enough. However, the molecular mechanisms responsible for the aging-dependent promotion of fibrosis in prostate are largely unknown and understudied. We sought to reveal the contribution of mitochondrial dysfunction to fibrosis in the aging prostate, ultimately leading to urinary symptoms, and overall poor health.

Hypothesis

We hypothesize that fibrosis-mediated lower urinary tract symptoms (LUTS) in benign prostatic hyperplasia (BPH) patients is a result of an increase in mitochondrial dysfunction and cellular senescence.

Materials and Methods

- *Young (2 months) and old (24 months) C57Bl6 mice were obtained from Jackson Laboratory.*
- *Compressed hormone pellets (2.5mg 17ß-estradiol (E2) and 25mg T) were implanted subcutaneously in treated mice; sham surgeries were also performed on control mice.*
- *Aged mice were fed Pirfenidone (anti-fibrotic/senolytics) in peanut butter to reverse age-mediated LUTD.*
- *Rotenone (2.8mg/kg/day) treated Lewis rats were treated for 5 days to disrupt complex I.*
- *Urinary function in rodents were assessed using void spot assays and quantified using VoidWizzard plugin in FIJI.*
- *Human prostate samples were obtained through the UW-Madison tissue biobank.*
- *Collagen was assessed using picrosirius red (PSR) staining and birefringence imaging*
- *Immunohistochemistry (IHC) staining was performed on FFPE human and rodent prostate tissues.*
  - *p16/INK4A (1:4000) was used for cellular senescence.*
  - *Ndufs3 (1:1000) is a critical component of the electron transport chain complex I, and mitochondrial dysfunction, particularly reduced complex I activity, are associated with reduced NDUFS3 expression*
  - *PINK1 (1:1000) is essential to maintain mitochondrial homeostasis by promoting mitophagy of damaged mitochondria.*
- *IHC staining was imaged using the Mantra multispectral imaging platform and quantified with InForm.*
- *Cultured human prostate stromal cells (BHPrS1) were treated with rotenone to selectively inhibit mitochondrial complex I.*
- *qRT-PCR was performed to examine cellular scenescence, mitochondrial dysfunction, and collagen gene expression.*

Results

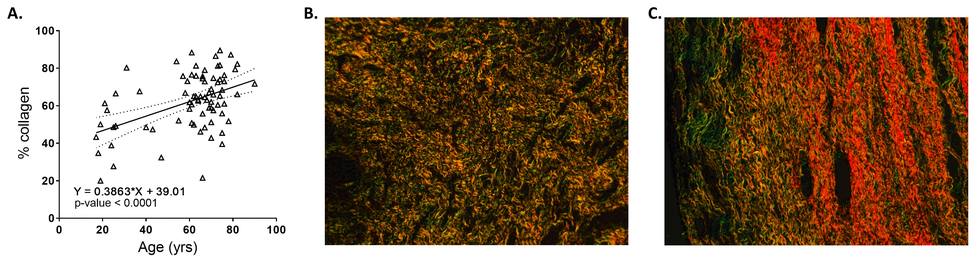

Figure 1. Prostatic fibrosis increases with age. A. The percent collagen within human prostate tissue is positively correlated with age. **B.** Representative PSR image of young human prostate. **C.** Representative PSR image of old human prostate.

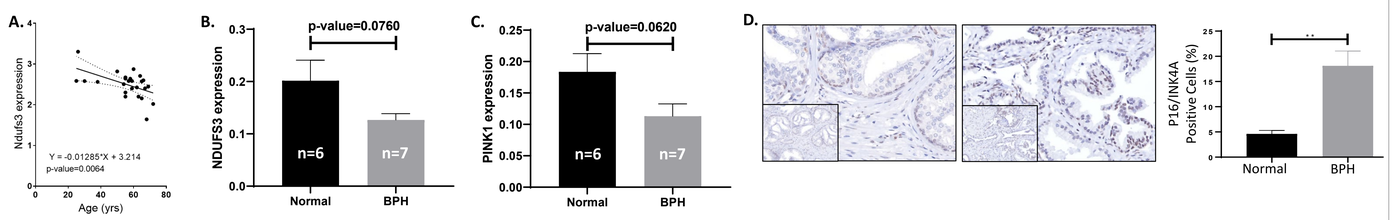

Figure 2. Mitochondrial dysfunction and cellular senescence is increased in aged prostate and BPH. A. Meta-analysis of NDUFS3 gene expression is negatively correlated with age. **B.** RNA-seq analysis of age-matched organ donor and BPH patients show a decreased NDUFS3 expression. **C.** RNA-seq analysis of age-matched organ donor and BPH patients show a decreased PINK1 expression. **D.** IHC staining of normal and BPH human prostate show a significant increase in p16/INK4A.

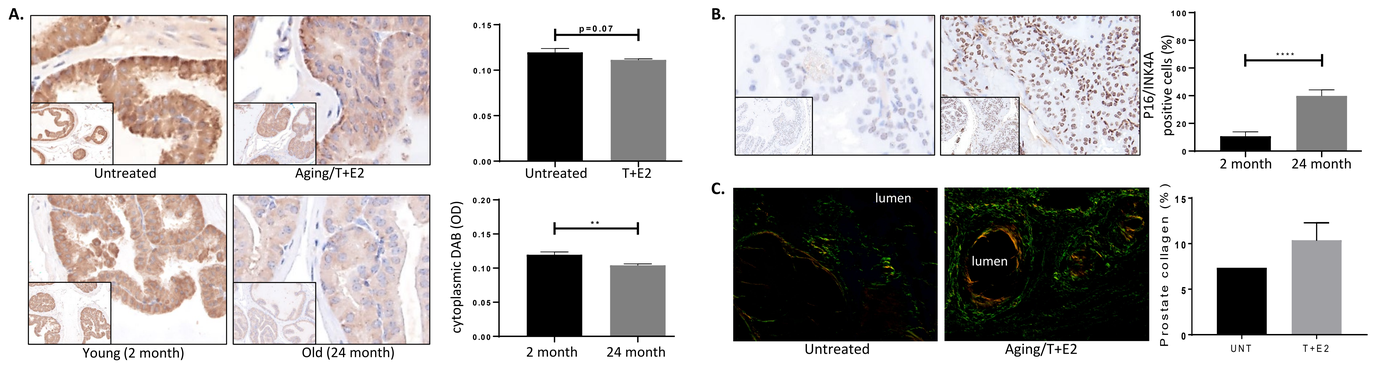

Figure 3. Mitochondrial dysfunction, cellular senescence, and fibrosis is increased in animal models of lower urinary tract dysfunction (LUTD). A. IHC of the anterior prostate (AP) shows a decrease in PINK1 in both the T+E2 steroid hormone and aging model for LUTD. **B.** IHC of the AP shows a significant increase in p16/INK4a in the aging model of LUTD. **C.** PSR staining of the AP shows an increase in collagen deposition. **, p-value < 0.01; ****, p-value < 0.0001.

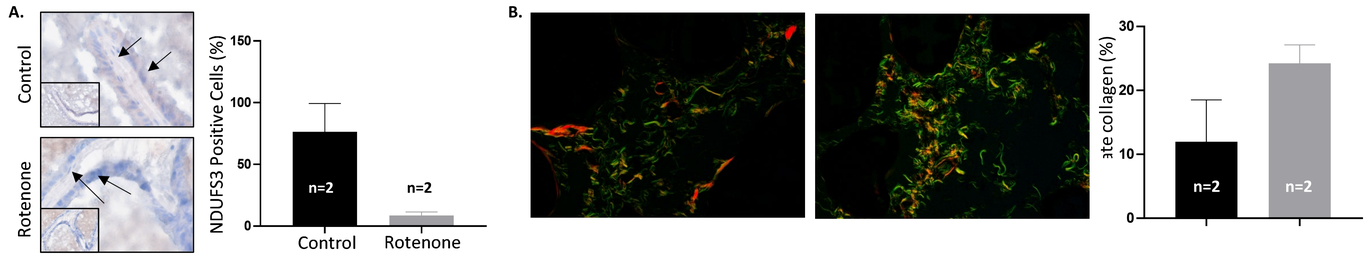

Figure 4. Disruption of complex I leads to an increase in mitochondrial dysfunction and collagen deposition in rat prostate. A. IHC of rat DLP shows a decrease in Ndufs3 positivity in response to rotenone. **B.** PSR staining of rat DLP shows an increase in collagen deposition in response to rotenone.

Results (cont.)

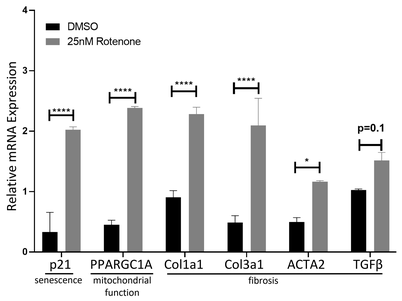

Figure 5. Disruption of complex I increases expression of collagen genes in human prostate stromal cells. qPCR analysis showed significant increases in genes associated with cellular senescence, mitochondrial function, and fibrosis.
*, p = 0.05; ****, p < 0.0001

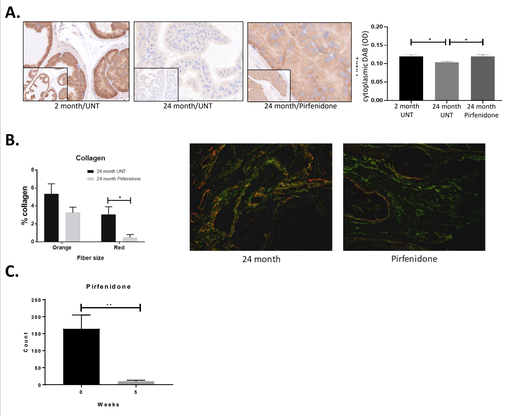

Figure 6. Pirfenidone reverses mitochondrial dysfunction, fibrosis, and LUTD in aging mice. A. IHC of AP show a decrease in PINK1 with age that can be reversed with pirfenidone. **B.** PSR staining of mouse AP show a significant decrease in large collagen bundles. **C.** LUTD assessed by void spot assays show a significant decrease in void counts suggesting a resolution of urinary dysfunction upon Pirfenidone treatment. *, p-value < 0.05;
**, p-value < 0.01

Conclusions

- Age significantly increases prostatic fibrosis, mitochondrial dysfunction, and cellular senescence.
- BPH is associated with mitochondrial dysfunction, cellular senescence, and fibrosis.
- Rodent models of LUTD show an increase in fibrosis, mitochondrial dysfunction, and cellular senescence.
- Specific disruption of complex I by rotenone increases mitochondrial dysfunction and fibrosis in a rat model and human prostate stromal cell lines.
- Treatment of aging mice with Pirfenidone reverses age-associated mitochondrial dysfunction and LUTD.

Acknowledgements

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