**Materials and Methods**

- **Young (2 months) and old (24 months)**: C57Bl6 mice were obtained from Jackson Laboratory.
- **Compressed hormone pellets (2.5mg 17βestradiol (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.**
- **Human prostate samples were obtained through the UW-Madison tissue biobank.**
- **Mitochondria was isolated by homogenizing in sucrose media.**
- **Immunoblotting was performed to detect protein expression.**

**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.

**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 NDUF53 gene expression is negatively correlated with age. B. RNA-seq analysis of age-matched organ donor and BPH patients show a decreased NDUF53 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 Ndufs3 positivity in young rats compared to aged rats. B. Immunohistochemistry (IHC) staining of normal and BPH human prostate show a significant increase in p16/INK4A. C. Representative PSR image of old human prostate.

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.

Figure 5. Disruption of complex I increases expression of collagen genes in human prostate stromal cells. qRT-PCR analysis showed significant increases in genes associated with cellular senescence, mitochondrial function, and fibrosis.

**Conclusions**

- **Age significantly increases prostatic fibrosis, mitochondrial dysfunction, and cellular senescence.**
- **BPH is associated with mitochondrial dysfunction, cellular senescence, and fibrosis.**
- **Robust 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**

Special thanks to Drs. Timothy Gervasio and Roberta DiMaro at the University of Pittsburgh for the incentive treated rat prostates. This research was supported by U54DK104282 (TTL), U10AG038899 (TTL), U54DK104302 (JDL), and U54DK104303 (WAM).