Mitochondrial complex 1 regulation of epithelial barrier integrity via NDUFS3 in prostate epithelial cell line RWPE-1

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Abstract

INTRODUCTION AND OBJECTIVE: The NDUFS3 gene encodes a protein subunit that belongs to a part of mitochondrial respiratory chain complex I. Knockdown of NDUFS3 has been reported previously to impair complex I activity in different cell types including the breast cancer cell line, MCF-7, and HeLa cells. NDUFS3 expression is decreased with age in the human prostate and is further decreased in BPH tissues, however the function of NDUFS3 in the prostate is unknown.

METHODS: In vitro cell line assays with benign prostatic epithelial cell line RWPE-1 were utilized to determine the impact of complex 1 inhibition via rotenone and NDUFS3 knockdown on the prostate epithelial barrier. Western blotting and qPCR were used to determine alterations in protein and/or mRNA expression of E-cadherin, occludin and tight junction protein 1 following rotenone stimulation or siRNA knockdown of NDUFS3. FITC diffusion and TEER assays were used to assess epithelial barrier function following knockdown or rotenone stimulation.

RESULTS: Expression levels of epithelial barrier genes was decreased and FITC diffusion was increased following rotenone-induced mitochondrial complex 1 inhibition. Rotenone induced a dramatic decrease in p62 and increased LC3B-11 expression and increased expression of NDUFS3 protein. NDUFS3 knockdown increased p62 and decreased PRDX3.

CONCLUSIONS: Inhibition of mitochondrial complex 1 in prostate epithelial cells dramatically altered autophagy and mitochondrial activity and reduced epithelial barrier integrity. Mitochondrial complex 1 dysfunction could play a role in BPH pathogenesis via reduced epithelial barrier function and subsequent increased prostatic inflammation.

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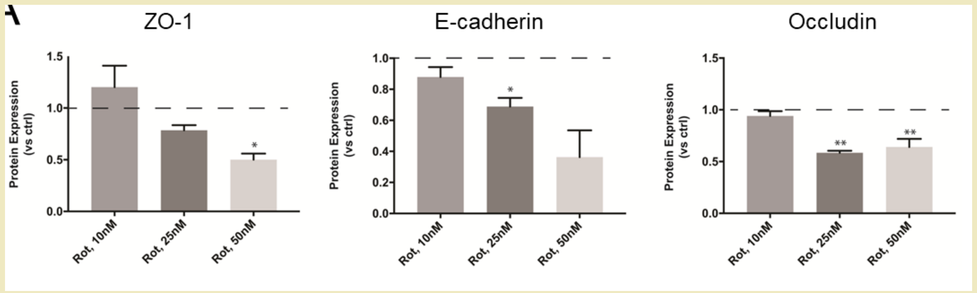
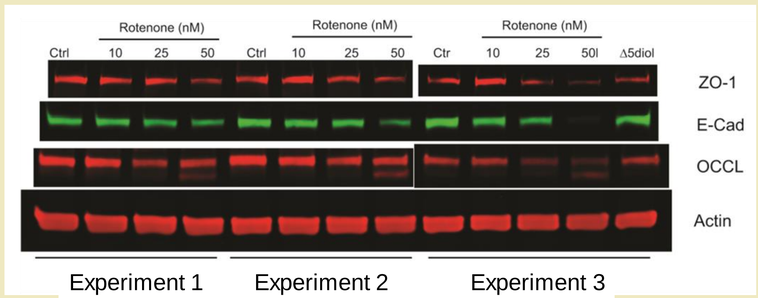
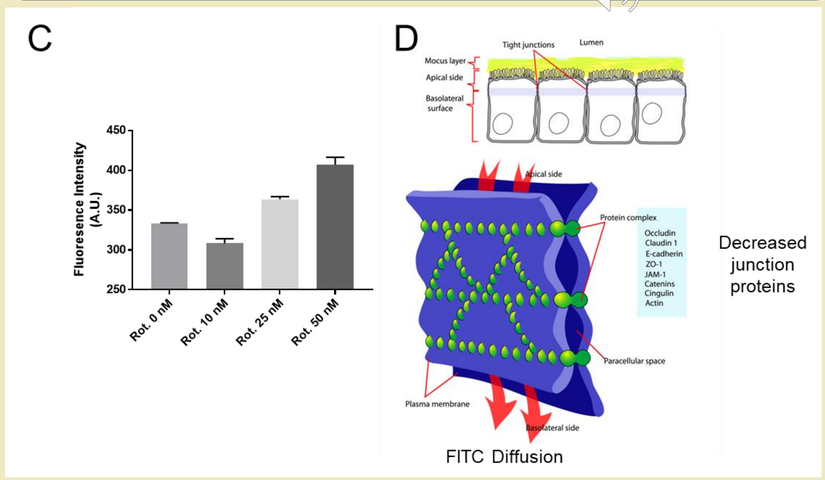
Mitochondrial complex 1 function is important for epithelial barrier protein expression in RWPE-1 cells

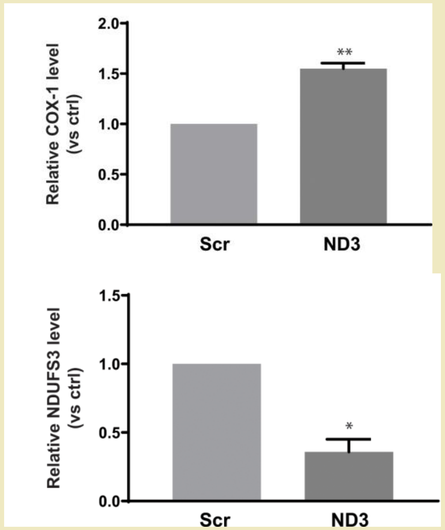


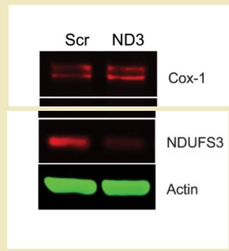


Mitochondrial complex 1 inhibition increases FITC Diffusion through the monolayer is increased



NDUFS3 is increased by complex I inhibition and NDUFS3 knockdown increases inflammatory mediators





Conclusions: Mitochondrial dysfunction and epithelial barrier function have been postulated to play a role in BPH pathogenesis. Our findings suggest that mitochondrial complex 1 activity may be important for the maintenance of the prostate epithelial barrier