Silencing *COQ8B* in aortic smooth muscle cells reveals cellular dysfunction related to changes in cell proliferation

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Background/Objective:

Thoracic aortic aneurysm (TAA) is a prevalent disorder that predisposes to aortic dissection. Prior work identified the ubiquinone biosynthesis gene *COQ8B* as a genetic modifier of TAA progression. We sought to determine the impact of decreased *COQ8B* on global transcription in aortic smooth muscle cells (SMCs).

Methods:

Primary human aortic SMCs from a healthy donor were seeded in 12-well plates. Six experimental conditions were created, each with 3 replicates: 1) siRNA targeting *COQ8B* (siCOQ8B); 2) siRNA targeting the dominant TAA gene *SMAD3* (siSMAD3); 3) negative control siRNA (siNeg); 4) siCOQ8B and siSMAD3; 5) siCOQ8B with Angiotensin II (AngII) stimulation (siCOQ8B+AngII); 6) siNeg+AngII. RNA was extracted approximately 48 hours post-siRNA transfection and, for AngII conditions, after 1 hour of incubation with AngII (100 nM). mRNA-sequencing was performed and downstream analysis utilized R packages EdgeR and topGO.

Results:

Multidimensional scaling identified distinct clustering of samples by experimental condition. Downregulated genes in siCOQ8B were enriched for Gene Ontology pathways related to cell proliferation including cell cycle regulators, DNA replication, and mitosis. *MYOCD*, a master regulator of SMC homeostasis, was downregulated. Similar proliferation-related pathways were enriched in siCOQ8B+siSMAD3 and siCOQ8B+AngII compared to siNeg. Pathways related to cell proliferation in siCOQ8B+AngII cells were downregulated when compared to siCOQ8B which indicates that AngII infusion in the context of COQ8B silencing may further dysregulate cell proliferation pathways.

Conclusion:

The results indicate that *COQ8B* has an important role in cell cycle processes in aortic SMCs, including when SMCs are exposed to stressors associated with TAA development. Stimulation of angiotensin receptors may exacerbate the effects of decreased *COQ8B* in these processes. To investigate these experimental results in human pathology, bulk RNA samples and intact nuclei have been isolated from frozen human aortic specimens and prepared for transcriptomic analysis.