A Cellular Resource for Studying Male Infertility in Cystinosis

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Lay-oriented description: To this date, the exact pathophysiology of infertility observed in males with cystinosis is not fully understood, which is crucial, as when treated with cysteamine, many are reaching young adulthood. In-lab model to study male infertility in cystinosis is an unmet need. We have successfully created and characterized an in-lab model that can mimic the disease and can be used to study cystinosis-mediated infertility. We have also identified possible key players in this type of infertility by comparing data obtained from our in vitro model and testicular biopsy samples from males living with cystinosis. Hence, our in-lab model and data from this study can be used not only to enhance our knowledge in this area but also can be subsequently used for screening of therapeutic agents that could be protective to male fertility.

Scientific abstract:

Background: Absence of a suitable in-vitro model to study the pathophysiology associated with male infertility in cystinosis is a critical unmet need to the growing population of individulas with cystinosis, who were treated with cysteamine is reaching young adulthood.

Method: We used CRISPR-Cas9 to generate human immortalized *CTNS*$^{-/-}$ testicular cells, and performed HPLC-MS to confirm its phenotype. We isolated RNA and performed bulk RNA-sequencing. The transcriptomic data was analyzed by using machine learning algorithms to differentiate transcriptomic states between healthy and cystinosis phenotypes.

Results: We have successfully generated *CTNS*$^{-/-}$ testicular cells with 86% efficiency, phenotypically confirmed by quantifying the inter-cellular cystine accumulation, which was similar to the range observed in individuals with cystinosis. Our transcriptomic data distinctly separates the control and *CTNS*$^{-/-}$ form into two distinct clusters. We have further identified significantly downregulated testicular cells-specific VATPases, which are known to facilitate autophagosome-lysosome fusion, normal spermatogenesis and motility, intra-cellular organell acidification, energy metabolism/catabolism, and also restricts inflammasome activation in *CTNS*$^{-/-}$ cells. All these processes are required to maintain normal testicular physiology. Also, we have identified NOTCH Signaling pathway as the most significantly affected pathway.

Conclusion: In conclusion, we performed bulk RNA-sequencing on our in-lab generated cystinosis cell-model, and compared it with our transcriptomic data obtained from cystinosis-testicular biopsy tissue, to understand the etiology of azoospermia associated with cystinosis. We identified common V-ATPases and signaling pathways that are disrupted in testicular cells and tissues in men with cystinosis. Data obtained from this small project can be used to enhance our knowledge about the cystinosis-mediated infertility and hence find a drug target for cure or to protect male fertility.