STRONG CHILDREN'S RESEARCH CENTER

Summer 2013 Research Scholar

Name: **Faraz Khan**

School: **Rochester Institute of Technology**

Mentor: **George J. Schwartz, MD**

ABSTRACT

Title: Isolation of cortical collecting ducts from rabbit kidney by laser capture microdissection

Background: Heterogeneous tissue, such as the kidney, has been an obstacle in analyzing specific cells of interest. Small pieces of complex tissue contain different cellular components which can skew the interpretation of molecular analysis results. Laser capture microdissection (LCM) is a new technique that allows for the procurement of tissue samples that are localized in specific tissue structures. Once these collected samples have undergone a nucleic acid retrieval assay, it is possible for a molecular analysis to be conducted on a specific cell population found in a complex tissue environment.

Objective: The purpose of this study was to verify previous studies for ion transporter expression levels in rabbit cortical collecting ducts (CCDs) using LCM technique. Once a sufficient amount of tissue was procured, an RNA extraction assay was conducted, followed by a real time reverse transcriptase polymerase chain reaction (qRT-PCR) in order to measure specific gene expression in isolated tubule segments of interest.

Results: We report the enrichment of different ion transporter transcripts in rabbit CCDs through fold changes in the threshold cycles. Our measurements were based on the fold changes in mRNA expression for the B1 subunit of vacuolar proton ATPase (V-ATPase) and the apical bicarbonate/chloride anion exchanger pendrin of intercalated cells and the beta subunit of epithelial sodium channel (ENAC) of the principal cells. All of these ion transporters were normalized to GAPDH, a housekeeping gene.

We compared LCM CCDs to a scrape control (unselected area) as well as LCM CCDs to LCM PT collection. For pendrin, when comparing CCD to scrape, we saw up to a 9 fold increase in expression levels and an 8 fold increase from CCD to PT. Similarly, ENaC-β had up to a 9 fold increase from CCD to scrape and a range of 5-11 fold increase from CCD to PT. B1 V-ATPase levels had up to a 6 fold increase for the CCD to scrape and a range of 9-11 fold increase for CCD to PT.

Conclusion: LCM is a useful tool in isolating specific cell structures in an organ with diverse cell populations, such as the kidney. This application allows for specific molecular analysis from organs of interest and can be used for downstream work such as RNA sequencing.