Online Proceedings

Session 1E

From Viral Pathogenesis to Genetic Diseases to Building a Better Kidney

Session Moderator: Michael Lagunoff, Microbiology
MGH 231
12:30 PM to 2:15 PM
* Note: Titles in order of presentation.

Using CRISPR-Cas9 Gene Editing to Discover Which Transporters Are Responsible for Glucose Absorption in Kidney Organoids
James David Whiteley, Junior, Biology (Molecular, Cellular & Developmental)
Mentor: Benjamin Freedman, Medicine/Nephrology
Mentor: Ramila Gulieva, Nephrology

Kidneys are responsible for regulating blood glucose levels in the body through the reabsorption of sugars in the proximal tubule of the nephron. Issues with glucose absorption can lead to diabetic nephropathies where too much glucose is excreted. To study kidney glucose absorption, the lab made kidney organoids, which are miniature stem cell-derived in vitro organs that mimic the structure and function of kidneys. We used induced pluripotent stem cells (iPSCs), a type of stem cell that can be generated directly from one's own adult stem cells. In a previous experiment, the lab found glucose absorption was present through the Sodium Glucose Co-Transporter 2 (SGLT2). The lab wanted to see if this was the only channel responsible for glucose absorption. Using the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) system, we knocked out SGLT1 and SGLT2 separately and also knocked out Glucose Transporters (GLUT1 and GLUT2) separately in the WTC11 kidney stem cell line. We selected a guide RNA corresponding with each glucose transporter in the cell membrane of kidney organoids. Then RNA-guided Cas9 created the mutation by breaking the sequence for each glucose transporter in a specific site of the genome. We verified our mutations were done correctly by sending our results to a DNA sequencing company. We expect to find mutant lines will have less glucose absorption than our control and hope to learn which transporters are more active. We are further using this method to create double and quadruple knockout lines with multiple glucose transporters knocked out at once. We hope to learn which transporters are most responsible for glucose absorption, and in the future, can assess when certain transporters are used over others, which will open the doors for disease models for diabetes not only in kidneys but also in other organs.

Session 1E

From Viral Pathogenesis to Genetic Diseases to Building a Better Kidney

Session Moderator: Michael Lagunoff, Microbiology
MGH 231
12:30 PM to 2:15 PM
* Note: Titles in order of presentation.

Using Induced Pluripotent Stem Cells to Change Urine into Kidney Tissue
Kezia Caroline Philip, Senior, Bioengineering
Mary Gates Scholar
Mentor: Benjamin Freedman, Medicine/Nephrology
Mentor: Nelly Cruz, Medicine
Mentor: Ramila Gulieva, Nephrology

Approximately 14% of the United States population is currently living with chronic kidney disease (CKD), demonstrating the importance of developing effective treatments. Presently, the mechanisms and driving forces of the many diseases that comprise the spectrum of CKD are not well understood. Current methods for acquiring kidney cells for research purposes require an invasive biopsy taken directly from the kidney. We theorized that patient kidney cells can be acquired from a significantly less invasive urine sample, be expanded, and subsequently differentiated into new kidney tissues. We developed a method for accomplishing this, which enabled the collection of cells from the urine samples of over 40 different patients located across the globe with various subtypes of CKD. Reverse transcription polymerase chain reactions (RT-PCRs) probing for the expression of XIST, a RNA gene exclusively expressed in females, were conducted on urinary cells from patients who have had kidney transplants with donors of the opposite gender. Using this assay, we show that these urinary cells originated in the

donor kidney. By introducing a set of transcription factors expressed in embryonic stem cells, urinary cells were further reprogrammed to induced pluripotent stem cells (iPSCs), an undifferentiated, stem cell-like state. Subsequently, the iPSCs were differentiated into patient-specific kidney organoids, marking the first time that new kidney-like structures have been generated from a urine sample. Hollow tubules, which better mimic the architecture of the kidney, were created by incorporating these urinary cells or iPSC-derived kidney organoid cells into three-dimensional microfluidic devices wherein normal cell viability and morphology was maintained up to twenty days. Immunohistological stainings indicate similar protein expression between the cell types, which will be further investigated in the upcoming months. These studies improve our ability to regenerate kidney tissue and disease processes from patients' own bodies, starting with a simple urine sample.