Diabetic Complications Consortium

Application Title: Innervation of adult kidney (human and mouse)
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1. Project Accomplishments:

This project's overall goal was to implement innovative technologies that can advance the single cell and 3D relationships between peripheral nerves and the kidney for application in diabetic kidney disease. We have two main accomplishments for this proof of concept study. First, we established a method to label sensory neurons that specifically project to the kidney, isolated them and successfully generated gene expression profile using FACS-seq. Second, we established a method to perform whole mount 3D lighsheet microscopy of renal nerves and their projection to major kidney structures using full thickness kidney lobe (~2-4mm wide) encompassing cortex and medulla in control and diabetic kidney tissue.

2. Specific Aims:

Specific Aim 1. **Single cell transcriptome of kidney innervating sensory neurons.**
Results:
1) We injected the retrograde tracer WGA into different parts of the mouse kidney (cortex and medulla) at different sites and examined spinal levels of DRG for retrograde labeling. We determined that T10-L1 DRG showed the most labeling for kidney specific sensory neurons. Mice with no WGA injection did not label DRGs.

2) We isolated T10-L1 sensory neurons after dissociation subjected them to FACS. We were able to successfully obtain >80% viable WGA-labeled cells. We collected these as pools of 100 cells or single cells. We subjected these to cDNA library preps and successfully obtained high quality libraries and RNA sequencing data. As a control, we also in parallel did FACS-seq on DRG from NaV1.8Cre lineage sensory neurons from these spinal levels without WGA injection. Principal component analysis clearly showed separation of kidney specific sensory neurons from general nociceptors. Initial analysis using WGCNA shows enrichment of module with genes associated with neuronal projection, cell migration, blood vessel development, neuron differentiation and neuron development thus indicating successful establishment of a process to isolate kidney specific sensory neurons and associated genes.

Specific Aim 2. **Determine 3D innervation of sensory and sympathetic neurons that innervate distinct structures within the mature male and female human kidney.**
Results:
To enable 3-D visualization human kidney innervation and its specific targets we developed different methods. We first validated immunoreactivity of a number of antibodies targeting peripheral nerves (pan-neuronal, sympathetic, sensory, nerve endings) using 3D confocal microscopy. We confirmed multiplexed immunostaining of these antibodies with presence of receptor tyrosine kinase RET and TOH positive nerves, nonTOH labeled sensory nerves in the papilla, dense TOH staining in the blood vessels of the juxtaglomerular apparatus and strong puncta of synapsin staining on several tubules including the collecting duct. We also established a method for successfully performing lightsheet microscopy using two tissue-clearing methods, PACT and CUBIC, on human kidney samples that span the entire kidney depth (Cortex-medulla) ranging from 2-4mm in thickness and about 1.5-2cm long. We multiplexed four different antibodies to target podocytes, endothelial cells, tubular epithelium and nerves. Both methods were successful. The data dhow peripheral nerves encapsulating the entire renal corpuscle with nerve endings in contact with the outer layer of the Bowman's capsule, heavily innervating the juxtaglomerular apparatus and to some of the tubular cells. Segmentation analysis is ongoing.

3. Publications:

We are currently preparing two methods papers with data described above.