Diabetic Complications Consortium

Application Title: Redefining DM by Agnostic Kidney Interrogation: A Pilot Grant

Principal Investigator: Barasch, J.

1. Project Accomplishments:

A. Isolation of nuclei from frozen human kidneys by optimizing buffer components
B. Optimization of cell and nuclear capture
C. Identification of gene clusters; identification of glomerular genes.
D. Authentication of genes

E. Specific Aims:

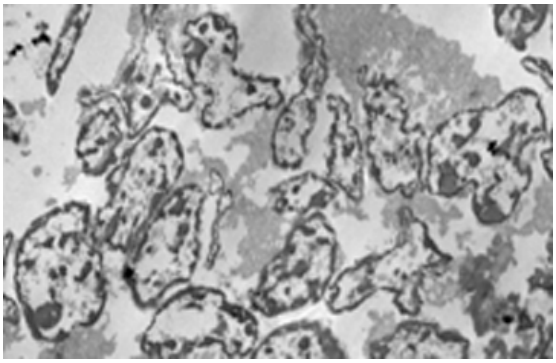
Aim 1, 2: Isolation of Nuclei from frozen kidneys and RNAseq: We have performed both single cell isolation from fresh tissue and nuclei from frozen tissues. We recognized that the two sources of gene expression have merit and also limitations, listed in the Table below. Because while nephrectomy samples are abundant, biopsy tissues are intensely limiting. As a result, we have spent the better part of the year redesigning the flow-capture cells so that we have a high rate of both nuclear capture. The flow cell is made from polydimethylsiloxane in Dr Sims lab. The polydimethylsiloxane has embedded microwells 50um in diameter. The reengineered wells are arranged in parallel arrays so that each cell or nucleus has equal exposure to lysis reagents and RNA capture reagents (each lane has 30,000 wells; see Figure Below). The RNA capture reagents are doubly bar-coded, once during RNA capture and second upon conversion to cDNA. The device is automated and scalable and solid state meaning that the biochemistry of lysis and capture are driven automatically by programmable pumps (Sims Scientific Reports, 2016).

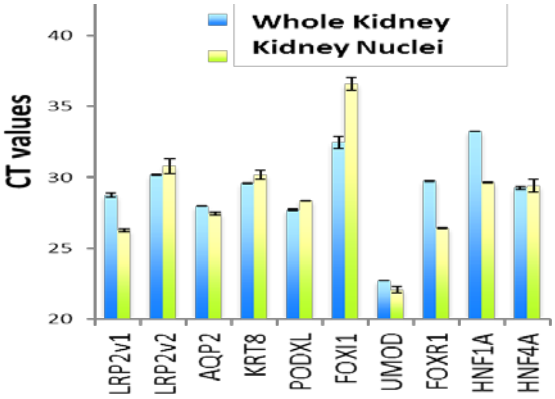
	Single Cell	Single Nucleus
Pros	Fresh tissue: less manipulation	Cell level analyses on challenging tissue sources (frozen biopsies) – human postmortem tissue repositories
	Sequence mature cytoplasmic mRNA	Stabilized RNA – not reliant on live/dead state
	Many established protocols for the kidney	Scalable – cryo preservation of sorted nuclei for deeper sampling
		Mixture of intronic and exonic data (nascent transcripts)
Cons	Difficult to obtain fresh human tissue - time restraints with time lag	Nuclear RNA, not cytoplasmic
	Cells need to be viable and intact	Gene length bias: Long genes better than shorter genes
		Mitochondria content contamination

	Cells	Nuclei
Starting concentration	**$2.7x10^6$** ($1.35x10^6$/mL in 2 mL)	**$9.14x10^5$** ($4.57x10^5$/mL in 2mL)
Live/Dead Stain	96% live 4% dead	1% live 99% dead
Final loading concentration	**~$1x10^5$** ($5x10^5$ cells/mL in 200µL)	**~$1x10^5$** ($5x10^5$ cells/mL in 200µL)

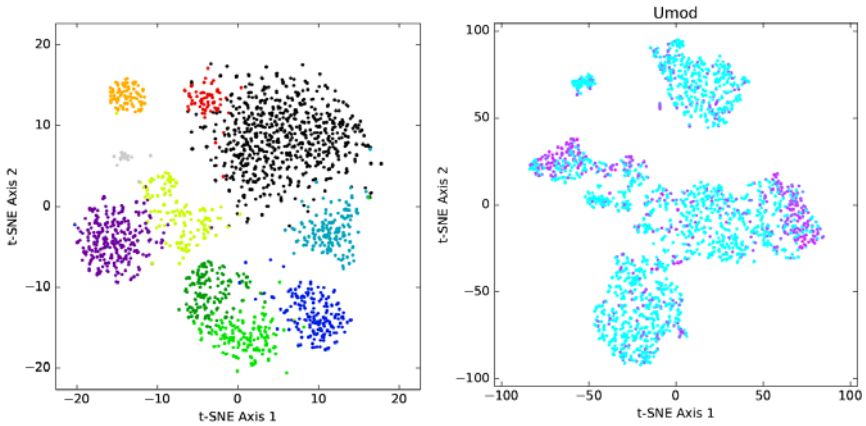
We have used human kidney as a source of both cells and nuclei for comparisons of bulk RNA, single cell and single nuclear RNA, with detection of

7.28ng of cellular RNA and 22ng of nuclear RNA. Highly purified nuclei (and cells) were confirmed with EM (Left, below) and by PCR (Right, below) for segment specific genes

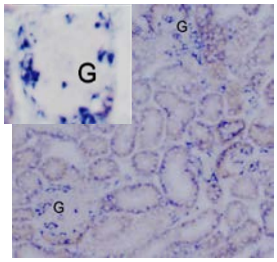




Representative cellular (Left) and nuclear (Right) t-SNE plots are in the Figures Below



One of the nuclear clusters was marked by anchor genes including synpo, synpo2, wt1 indicating glomerular nuclei. Focus on this cluster revealed a host of channels (e.g. Kcnma1), secreted proteins (e.g. Igfbp7), receptors e.g Flt). Some of the genes are already known to be associated with glomerulopathy (such as Pdss2, Plpp1, Timp3). We chose in situ to authenticate gene expression. IGFBP7 is shown in Figure Left-it is known as an AKI biomarker (Nephrocheck), but we found it at baseline in tubules and most intensely in glomeruli (G). These data provide our pipeline—biopsies from an array of glomerular diseases—completed identification of isolation buffers, miniaturization of nuclear capture, identification of lysis conditions- gene discovery in comparison to anchor genes, authentication via in situ (most recently RNA-Scope), Protein Atlas, Tabula Muris etc. In short, technical advances have been made to maximize the amount and quality of information that can be obtained from biopsies. We will upload our geneset once we complete our authentication.



F.Publications: Park J, Shrestha R, Qiu C, Kondo A, Huang S, Werth M, Li M, Barasch J, Suszták K. Single-cell transcriptomics of the mouse kidney reveals potential cellular targets of kidney disease. *Science*. 2018 May 18;360(6390):758-763. doi: 10.1126/science.aar2131. Epub 2018 Apr 5. PubMed PMID: 29622724; PubMed Central PMCID: PMC6188645.

I was fortunate to work with the Susztak lab involving a novel kidney cell that we both identified in a single cell mapping project by Dr Susztak.