Diabetic Complications Consortium

Application Title: Mechanisms of Glomerular Hypercellularity in Diabetic Nephropathy

Principal Investigator: K.J. KELLY

1. Project Accomplishments:

Spatial transcriptomics was performed on 10 diabetic kidney specimens and 4 reference samples. The diabetic kidneys were heterogeneous with respect to cellularity and the presence of fibrosis and immune cells. Pathways enriched in areas with degenerating podocytes and endothelial cells included response to hypoxia, morphology and development and angiogenesis.

2. Specific Aims:

The original aims were revised based on new technology (spatial transcriptomics) which we believe will address our goals in a more comprehensive manner (discussed with Dr. Ketchum).

Aim 1. Define the transcriptome of human diabetic kidneys using spatial transcriptomics to determine the changes that may account for the alterations in function and structure. Our working hypothesis is that proximal tubules communicate with glomerular cells and influence the cellularity of the glomerulus and thus glomerular pathology.

Results: we performed an unbiased transcriptomic comparison of diabetic biopsies and reference kidneys. Due to the policies surrounding the COVID19 pandemic, there was a delay in running the transcriptomic data. The analysis of the data is continuing. Examples of the data obtained from reference (control) kidneys and diabetic kidney biopsies are shown in figures 1 and 2.

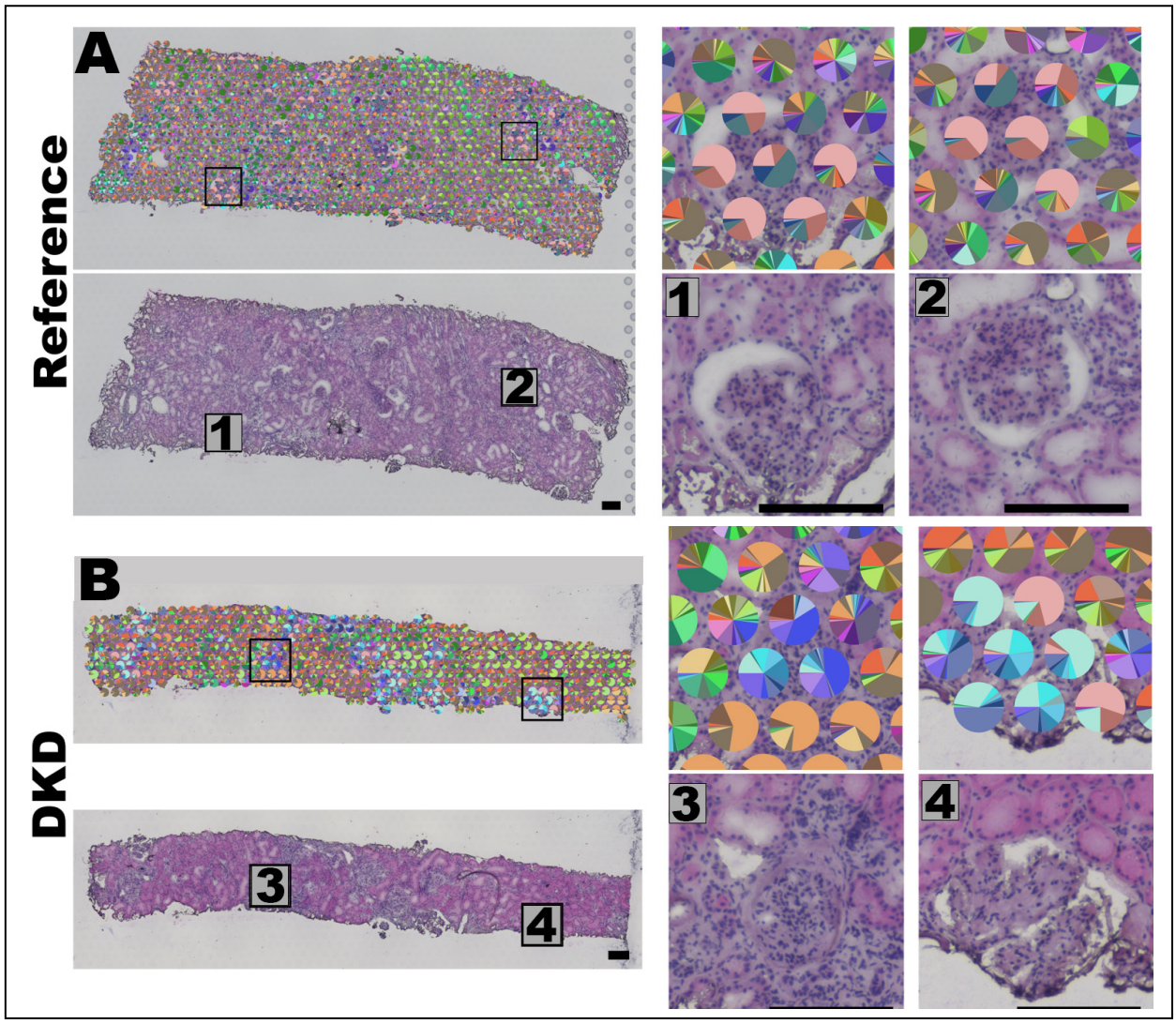

Figure 1. Cell type deconvolution in examples of reference (A) and diabetic kidney disease (B) are presented. The upper panels for each sample show "spots" deconvoluted and the lower panels show hematoxylin and eosin (H+E) staining for the same sections. Glomeruli at higher magnification are shown in the right panels. In the reference tissue, there is a large podocyte signature in the two glomeruli. In the diabetic sample, the glomeruli represent neighborhoods (figure 3) with different cellular signatures. The scale bars represent 200 microns.

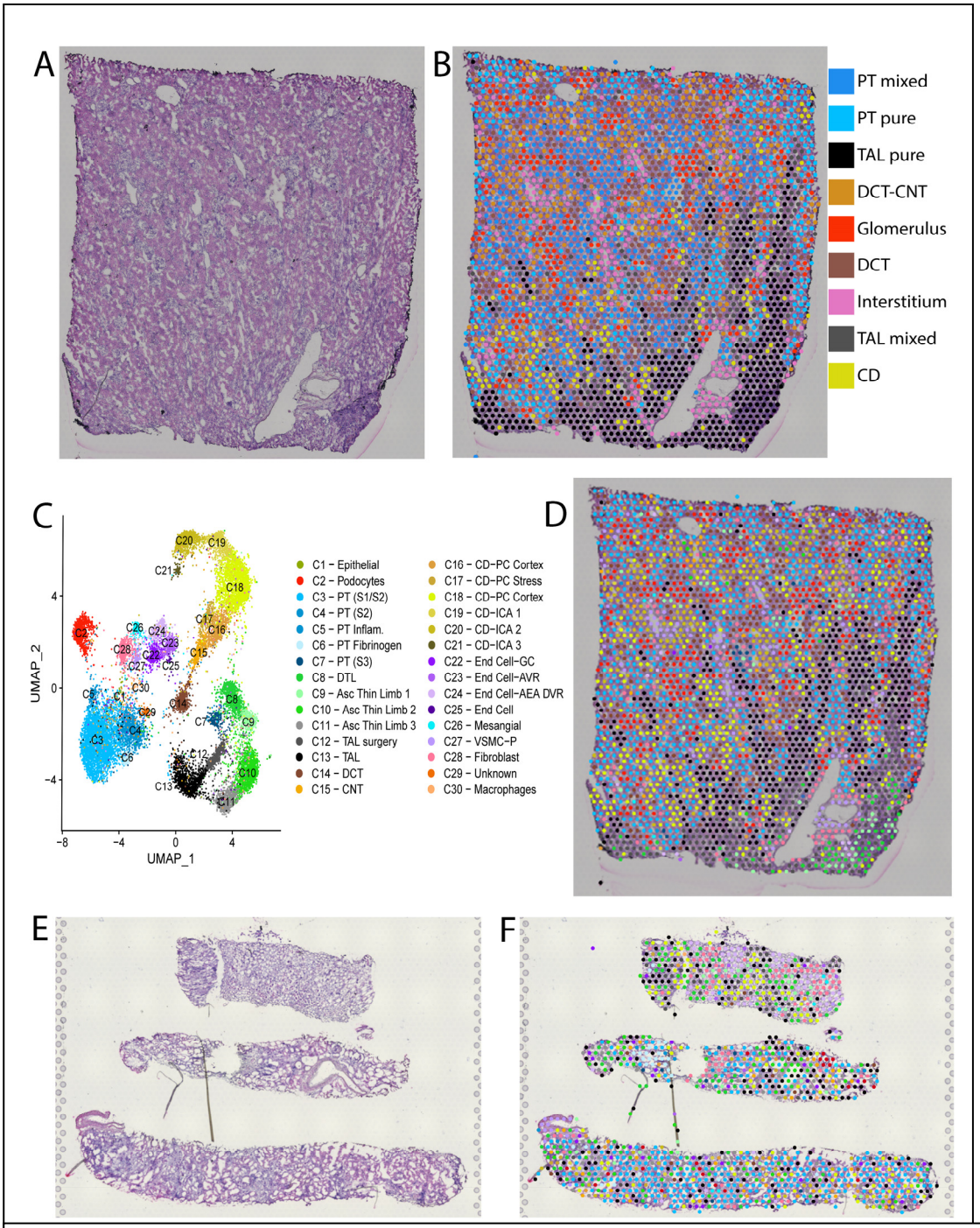

Figure 2. In A is shown H+E staining of a slice of a reference (healthy) human kidney, where is possible to identify several histological features, such as glomeruli, tubules, vascular structures and a portion of medullary rays. In B, the tissue is overlain. These clusters were classified using histological properties of the tissue along with differentially expressed genes. In C is shown the UMAP of the human kidney snRNAseq. Figure 1D presents those clusters transferred over the spatial transcriptomics spots. The H+E staining of a diabetic biopsy is presented in E, and the single nucleus data is transferred and presented in F.

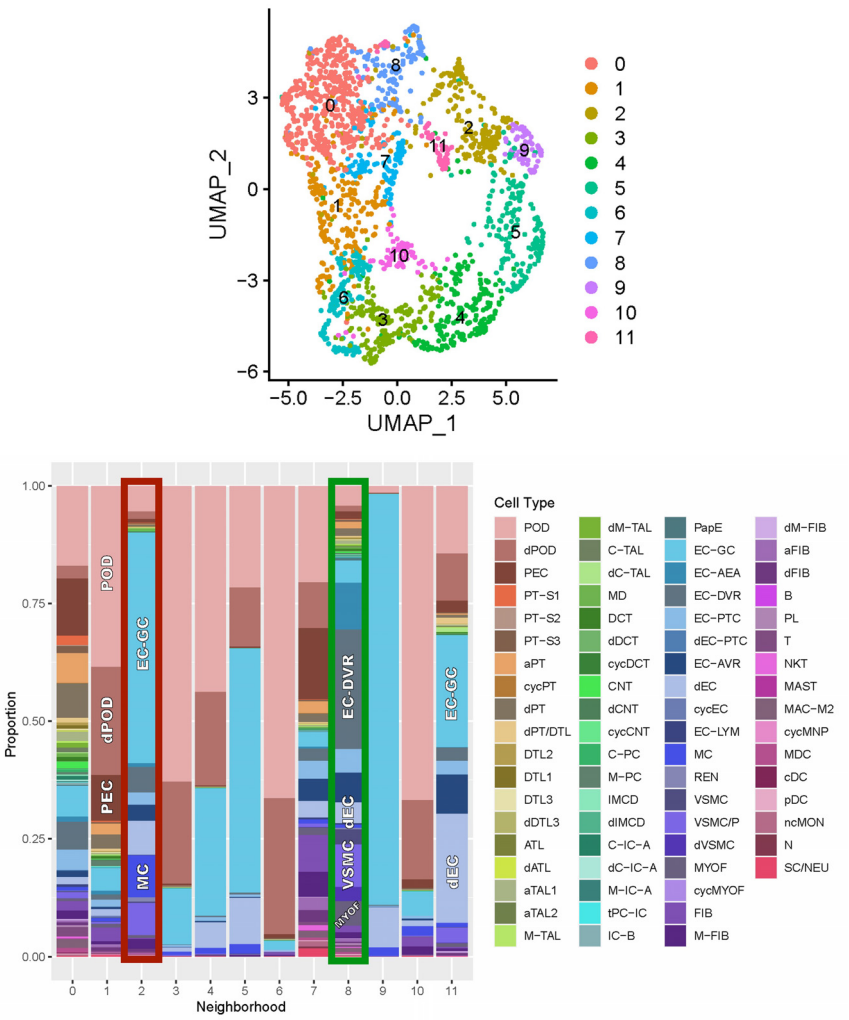

Figure 3. In the upper panel is shown a UMAP with clustering of glomerular "spots" by cell number (aim 2) and type, thus defining "neighborhoods". In the lower panel is shown the mean cell proportion of each neighborhood. In some neighborhoods in diabetic kidneys, for example 2 and 8, there is a reduced podocyte signature with maladaptive endothelial cells.

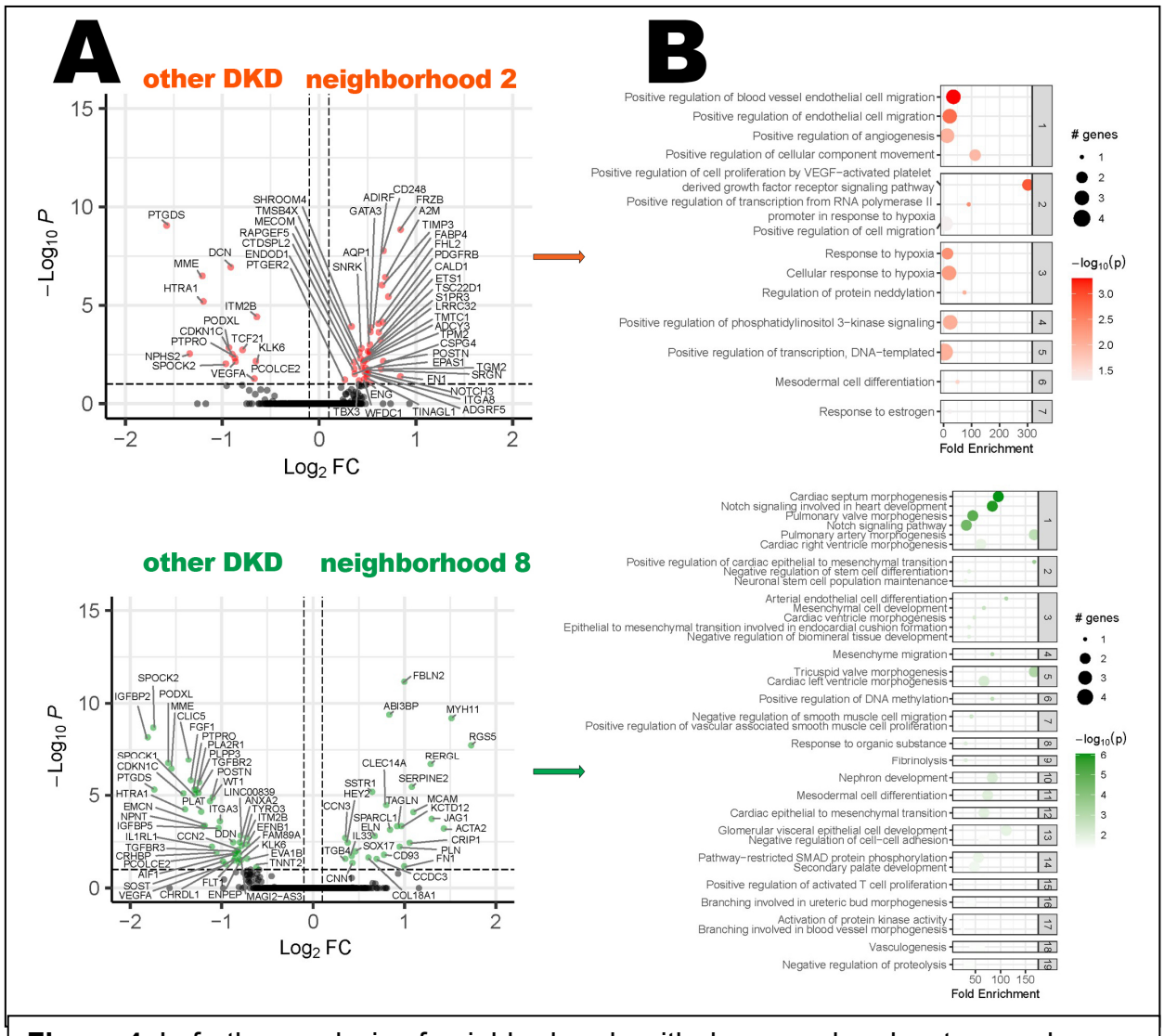

Figure 4. In further analysis of neighborhoods with decreased podocytes, each neighborhood was compared to all other spots from diabetic kidney samples. Differential expression was tested with Wilcoxon rank-sum test (A). Gene ontology biological processes pathways is shown in B. Some of the pathways include response to hypoxia and epithelial to mesenchymal transition.

Aim 2. Characterize the cellularity in glomeruli from human diabetic nephropathy biopsies using 3D cytometry. The working hypothesis for this aim is that the cellularity of DN glomeruli consists of increases in both mesangial and endothelial cells as compared to reference human kidney tissue.

Results: we have found varying cellularity in the glomeruli of biopsies from diabetic (n=19 glomeruli) patients. Within each specimen, cellularity was consistent among glomeruli. The differences in cell types within glomeruli are shown above.

reference	diabetic
259556 ± 8107	592715 ±116469
	p<0.05

We are analyzing this data further. These studies suggest the existence of a temporal and spatial trajectory for mesangial cells in the progression of diabetic kidney disease and we plan to further define the trajectory and describe the unique milieu of diabetic kidneys.

3. Publications:

Publications are pending.