Diabetic Complications Consortium

Application Title: Simultaneous Spatially-Preserved Protein and mRNA expression Profiling of the Human Kidney

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1. Project Accomplishments:

During the year of funding from DiaComp, we were able to develop a protocol to successfully detect mRNA and proteins in the same formalin-fixed, paraffin embedded human kidney section using either immunofluorescence or imaging mass cytometry. We have also validated a library of 26 antibodies for human kidney to simultaneously identify and segment podocytes, mesangial cells, endothelial cells, proximal tubule, thick ascending limb, macula densa, distal convoluted tubule, collecting duct, fibroblasts, macrophage, T cells and PMNs. We have now banked and imaged 6 normal donor kidney biopsies and 11 normal regions of kidney tumor resection specimens.

2. Specific Aims:

Specific Aim 1. Protocol development for the use of ProGene IMC analysis of human tissue sections

Results: We were unsuccessful with attempts to detect mRNA *in situ* using a variant of rolling circle mRNA amplification termed PLAYR. In addition, through work with a collaborating group at Yale, we observed that that the background level was unacceptably high with this approach, limiting signal specificity. We therefore investigated alternative approaches for simultaneous RNA and protein detection, and have succeeded in using a different method of RNA detection, RNA Scope (ACD Biotechnology), to faithfully detect mRNA of interest in formalin-fixed paraffin-embedded human kidney tissue. Additionally, we have developed a protocol to perform simultaneous detection of protein and transcript for the same gene, using both immunofluorescence and, more recently, IMC (Figure 1). Our current efforts are focused on increasing and optimizing signal strength for the detection of a range of mRNAs with IMC.

Specific Aim 2. Use of ProGene IMC to develop a spatial atlas of the protein-mRNA expression profiles of multiple cell types in the normal and diabetic human kidney.

Results: We have made much progress towards our goal of developing combined Protein-Gene Imaging Mass Cytometry ("ProGene IMC") to investigate the resident cell types, immune cell types, and injury pathways active in the diabetic versus reference kidney. Our current panel of validated, metal-conjugated antibodies stands at 26 markers of resident renal cells and immune cells which can be detected in parallel, with six more antibodies currently in the development pipeline. We have developed protocols using the machine learning program Ilastic and Cell Profiler to perform single cell segmentation on our images, and ascribe a cellular phenotype to each cell detected in a complex multiplexed data set (Figure 1).

In light of our very successful use of RNA Scope, we can now expand our panel of RNA detection probes, as originally proposed, from 1 to 6-8 - to complement our panel of 22-31 metal-conjugated antibodies - prior to performing analysis on human tissue. Once we have validated the requisite number of probes, we will perform simultaneous analysis (to minimize

variability) on 10 reference kidneys and ten class II diabetic samples. Reference human kidney has been obtained by biopsy of living donors at the time of collection (we currently have 6 such samples and expect to accrue 2-3 additional in each of the next two months). We have obtained Yale Human Investigations Committee approval to perform analysis on class II diabetic nephropathy samples that have been banked by Yale since 2010. We have requested a no-cost extension to carry over remaining DiaComp funds in order to image these Diabetic Kidney samples.

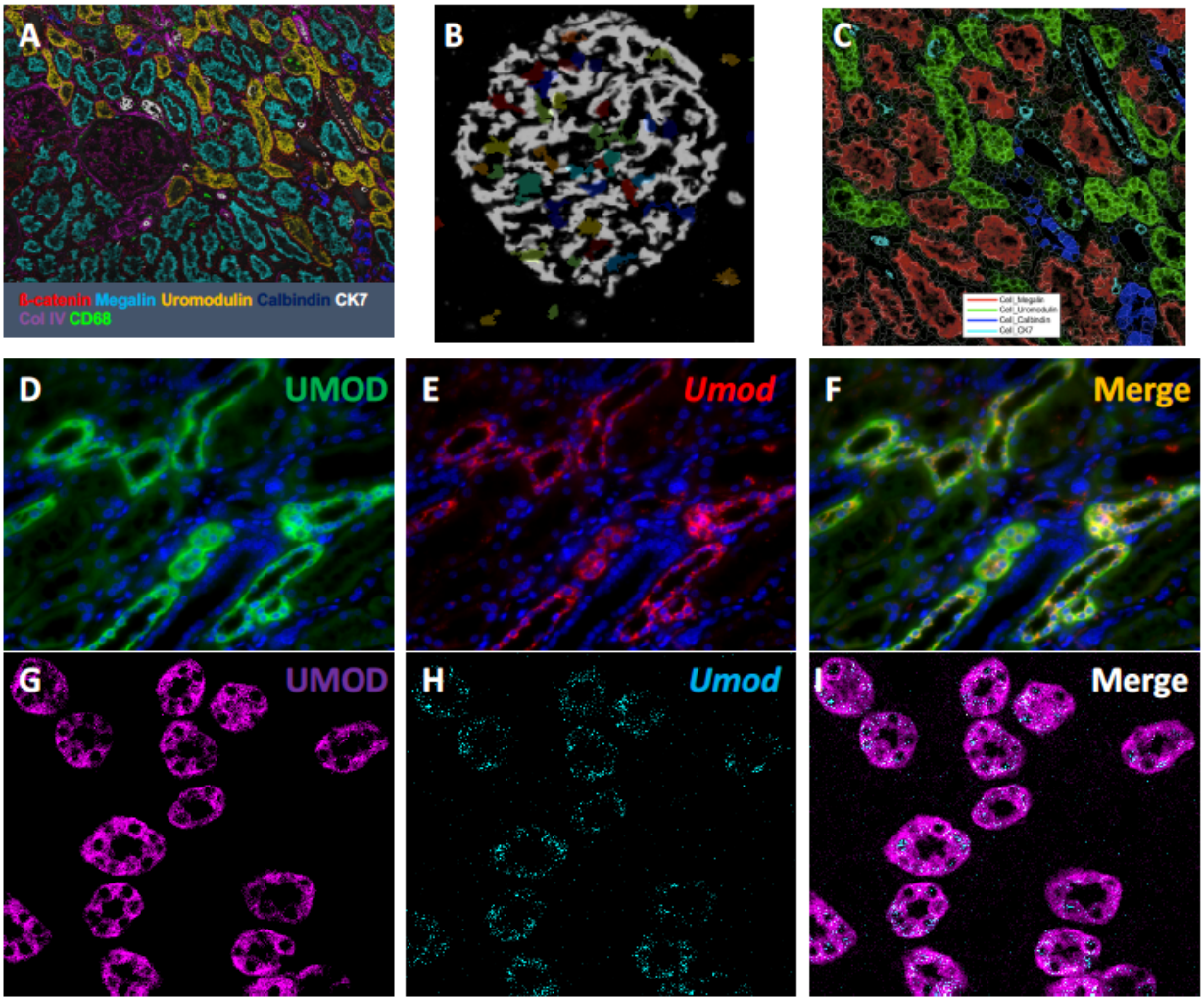

Figure 1. Successful use of Imaging Mass Cytometry to detect and segment differentiated cell types. **A.** Multiplexed image showing simultaneous detection of 7 protein targets in human formalin-fixed paraffin-embedded kidney. **B.** Mask generated showing a glomerulus in which a podocyte mask is overlaid upon endothelial cells (multicolored). **C.** Mask generated from tubulointerstitum, segmenting individual cells by marker expression. **D-I.** Parallel detection of Uromodulin protein and *Umod* transcript with immunofluorescence (D-F) and Imaging Mass Cytometry (G-I).

3. Publications: None