Diabetic Complications Consortium

Application Title: Molecular and pathological signature of the human kidney tubule in progression of diabetic nephropathy

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1. Project Accomplishments:

Please note that we are in the midst of a 1 year no cost extension, granted in August 2017. Despite the relatively short duration of the award, we are very excited with our progress, which I will discuss below (see specific aims section 2). The project has already yielded 2 publications with another under review (see publications section 3).

2. Specific Aims:

1. ***Define the transcriptome expressed by the tubular subsegments from biopsies of patients with diabetic nephropathy and different rates of progression.***

Based on existing literature and preliminary data, the *working hypothesis* for this aim is that the transcriptome of tubular subsegments from initial kidney biopsies of patients with diabetic nephropathy that over time rapidly progressed (decrease in eGFR > 5 mL/min/1.73m^2/year) will exhibit a unique molecular signature that is distinct from that seen in diabetic patients who did not rapidly progress.

Progress on aim1: We have optimized our ability to micro-dissect tubular segments from kidney biopsies. We had to perform several quality control measures, since we came to realize that the yield of RNA varies between biopsies, and such work is crucial to build a solid base for the project. This essential work yielded a manuscript: "*Integrity and expression of low-input, degraded renal mRNA for precision medicine applications*", funded through DiaComp, which is currently being reviewed for publication.

We are pleased to report that we have identified the appropriate set of kidney biopsies. For 8 biosies, we have completed tissue collection, RNA isolation, and have RNA sequencing data for the Glomerulus, S1, S3, TAL, CD, and tubulointerstitium of these biopsies. Our goal during the no cost extension is to complete segmental RNA isolation and perform transcriptomic analysis on the next 10 biopsies.

2. ***Determine the abundance and distribution of immune cell subtypes in the same set of patient biopsies.***

Based on existing literature and preliminary data, the *working hypothesis* for this aim is that the abundance and distribution of immune cell subtypes in initial kidney biopsies obtained from patients with diabetic nephropathy that over time rapidly progressed will exhibit a unique relationship relative to specific tubular segments distinct from that seen in diabetic patients who did not rapidly progress.

Progress on aim2: We are pleased to report that we are able now to reliably perform large-scale 3D imaging of biopsy section with 8 different markers, staining simultaneously for basic tubular and immune cells markers. We have also made important advancement in our ability to analyze multi-parametric data that is generated by our imaging through the software platform that we developed (J Am Soc Nephrol. 2017 Jul;28(7):2108-2118). Optimization of this work was a necessary step before we utilize the precious diabetic nephropathy biopsies. This work and the current state of the art of 3D tissue cytometry yielded another manuscript (Transl Res. 2017 Nov;189:1-12. PMID: 28784428) also funded by DiaComp.

Over the coming 8-10 months we plan to complete the multi-fluorescence 3D imaging on the identified diabetic nephropathy biopsies, and the corresponding tissue cytometry analysis to correlate with the transcriptomics findings.

3. Publications:

Accepted:
Quantitative Three-Dimensional Tissue Cytometry to Study Kidney Tissue and Resident Immune Cells. Winfree S, Khan S, Micanovic R, Eadon MT, Kelly KJ, Sutton TA, Phillips CL, Dunn KW, El-Achkar TM. J Am Soc Nephrol. 2017 Jul;28(7):2108-2118.
PMID: 28154201

Large-scale 3-dimensional quantitative imaging of tissues: state-of-the-art and translational implications. Winfree S, Ferkowicz MJ, Dagher PC, Kelly KJ, Eadon MT, Sutton TA, Markel TA, Yoder MC, Dunn KW, El-Achkar TM. Transl Res. 2017 Nov;189:1-12.
PMID: 28784428

Under review:
Integrity and expression of low-input, degraded renal mRNA for laser micro-dissection. Michael T. Eadon; Tarek M. El-Achkar; Ying Hua Cheng; Kenneth W Dunn; Hongyu Gao; Jospeh Ipe; Katherine J. Kelly; Sarah N. Lipp; Yunlong Liu; Jeanette N. McClintick; Samir V. Parikh; Brad H. Rovin; Todd C. Skaar; Timothy A. Sutton; Seth Winfrees; Xiaoling Xuei; Pierre C. Dagher. Under Review at BMC Nephrology.