Diabetic Complications Consortium

Application Title: Development and Optimization of Near Single Cell Proteome of Diabetic Nephropathy

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1. Project Accomplishments:

- The study's objective was to perform proteomic analysis of laser captured glomeruli from kidney tissue collected from different stages of DKD. Working on the proposed plan of carrying out near-single-cell proteomics on the laser-captured microdissected glomeruli from kidney tissues we encountered time delay at PNNL in carrying out the assay in time as proposed. It was attributed to COVID -related issues and the issues associated with the availability of the machine that was supposed to carry out the proposed analysis. While waiting for the nscProteomics system to be available, we carried out a newly available "omic" assay platform to profile whole transcriptome and proteins in the same kidney tissues available for the proposed study. We have completed analysis of transcriptome analysis and multiplex protein analysis of 12 kidneys that included 4 kidneys each from a healthy section of nephrectemized kidney, mild DKD, and Severe DKD (Table 1). We have completed data generation and preliminary data analysis. The data is presented in the form of a manuscript. Diacomp grant will be acknowledged in the manuscript.

Table 1. List and information on the phenotypes of kidney tissues and their mounting on slides for transcriptome and proteomics analysis.

Slide #	**Section**	**Sample Group**	**New Sample ID (GeoMx)**
Slide 1	A	Healthy Ctrl	Healthy
	B	Mild DKD (DKDII)	DKD2
	C	Severe DKD (DKD IV)	DKD3
Slide 2	A	Healthy Ctrl	DKD4
	B	Mild DKD (DKD II)	DKD5
	C	Severe DKD (DKD IV)	DKD6
Slide 3	A	Healthy Ctrl	DKD7
	B	Mild DKD (DKD II)	DKD8
	C	Severe DKD (DKD IV)	DKD9
Slide 4	A	Healthy Ctrl	DKD10
	B	Mild DKD (DKD II)	DKD11
	C	Severe DKD DKD (IV)	DKD12

Specific Aims:

Sp. Aim 1. Optimization of nscProteomics for isolated glomeruli.

- This had to be adapted for a new technology because of the reason described in the Project Accomplishments, we successfully developed and optimized analysis of kidney tissue for transcriptome and proteome analysis using GeoMx DSP methods.

Sp. Aim 2. We will perform nscProteomics of kidney glomeruli captured from different stages of CKD due to diabetic nephropathy.

- We have completed analysis of 12 kidneys for transcriptome and proteome analysis using GeoMx DSP. For this we have performed analysis on not only glomerulus but also on proximal tubular component and infiltrating immune cells.

2. Publications:

- A manuscript is in preparation where Diacomp Funds will be acknowledged for the support