Diabetic Complications Consortium

Application Title: "Spatial Representation of Single Cell RNA-sequencing Data in Embryonic Kidneys"

Principal Investigator: Thomas Carroll

1. Project Accomplishments:

The purpose of this project was to establish protocols and data to generate a virtual atlas of gene expression in the newborn mouse kidneys using in situ single cell mRNA sequencing. Initially, the project was based with a group out of Sweden who had created a startup company named Cartana. This group was going to provide us with reagents and techniques to complete this project. Timely completion of the aims was complicated by several issues including work stoppage/slowdown at both Cartana and UTSW due to the Covid crisis. Once these issues were overcome, progress was once again halted when Cartana was purchased by 10X Genomics. 10X ordered all existing projects to be put on hold until a to be determined date. In early 2021, we entered into an agreement with 10X to proceed with our proposed research. The in situ sequencing has now been performed and imaged. Although we are still in the process of analyzing the data, our results so far suggest that the project was a great success. We hope to submit a manuscript including this data in the next 6-9 months.

2. Specific Aims:

Specific Aim 1: Map E18.5 whole kidney single cell RNA-Seq to reference kidney

Specific aim1a: Perform in situ single cell sequencing with a set of logically chosen reference genes.

Results-We developed software to select candidate genes from single cell RNA sequencing data that could be visualized using standard molecular and cellular biology techniques. A manuscript on this work was published in the Journal of Molecular Biology earlier this year and the Diacomp grant has been cited. Using genes selected with this tool, we simultaneously visualized the expression of 93 mRNAs in E15.5, E18.5, P1, P3 and P5 kidneys. Mapping gene expression to specific cell types has proven to be somewhat of a challenge as the Cartana technique has extremely high (sub-cellular) resolution for each gene product. Transcripts can be detected in discontinuous cell bodies (anuclear tissue). This data needs to be discarded as we cannot assign the expression to a specific cell with any confidence. However, we believe we have overcome this issue and are currently continuing curation of the data.

Specific aim1b: Generate high definition, single cell expression map E18.5 kidney using Spatial Transcriptional Assignment Tool (STAT).

Results-As discussed above, the curation work is currently underway. We anticipate completion within 2-3 months. Once it is completed, we can quiclky generate the virtual map as the computer code for this tool has already been generated.

3. **Publications:**
4. Chaney CP, Drake KA, Carroll TJ. Integration of Multiple, Diverse Methods to Identify Biologically Significant Marker Genes. J Mol Biol. 2022 Oct 15;434(19):167754. doi: 10.1016/j.jmb.2022.167754.