Diabetic Complications Consortium

Application Title: Epigenomic modification as a mechanism of hyperglycemic memory in the bladder.

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1. Project Accomplishments:

The goal of our proposal is to increase our understanding of the mechanisms that result in hyperglycemic memory in the bladder and potentially identify novel targets to treat DBD that persists in patients that are under glycemic control. A major factor identified as contributing to hyperglycemic memory in other diabetic diseases are epigenomic changes in the methylation patterns of genomic DNA. To determine if similar mechanisms of hyperglycemic memory contribute to the continuation of DBD in patients brought under glycemic control we proposed to perform epigenomic analysis of gDNA isolated from a rat model of diabetes, with or without insulin treatment.

We generated the rat models of type-1-diabetes that were proposed to be used in our epigenetic studies; namely rats with one or three months of streptozocin induced diabetes, with or without an additional further one month of treatment with insulin to bring the animals under glycemic control. Bladder was harvested and separated into mucosal and detrusor tissue, and RNA-free genomicDNA (gDNA) isolated. These samples were submitted to Diagenode Inc. (https://www.diagenode.com/) for quality control of our gDNA preps and performance of reduced-representation bisulfite *sequencing* (*RRBS*-Seq) on July 20, 2019. This first round of samples had levels of low MWt polynucleic acid contamination (possibly degraded DNA or RNA contamination) that might interfere with the RRBS-Seq. We subsequently improved our methods of gDNA purification and samples were submitted for RRBS-Seq on Oct. 3, 2019. Unfortunately, because of the delay in performing epigenomic analysis, we are still waiting for these results, which we anticipate being available in the next 4-6 weeks. We remain confident that this data will provide novel, publishable, insights into the genes most likely to exhibit hyperglycemic memory in the bladder and intend to use this data as preliminary results to support an R01 type grant application.

2. Specific Aims:

Specific Aim 1: Determine if diabetes causes epigenetic modifications in the bladder genome and investigate if glycemic control can reverse identified epigenetic modifications.

Results: As detailed above, because of the delay in initiating epigenomic analysis, we are still waiting for our main data and results. Once we have the analysis we are confident that our findings will be novel and publishable and intend to use this data as preliminary results for an R01 type study. All epigenomic data generated and used in support of future publications or grant proposal will be made available through publicly accessible websites.

Specific Aim 2: Confirm epigenetically modified loci correlates with levels of gene expression in these loci.

Results: As detailed above because of the delay in initiating epigenomic analysis, we have not yet been able to determine precisely which genes to investigate to determine if epigenomic modification of specific loci correlates with expression of genes in that loci. However, we have performed metabolomic analysis in several tissues related to this research and so will be able to determine if epigenomic modification of specific loci correlates with changes in metabolic pathways which involve enzymatic activity of genes located in these loci.

3. Publications:

We have high confidence that upon availability of our epigenomic data the findings will be novel and rapidly published. We also intend to use this data as preliminary results for an R01 type study.