Diabetic Complications Consortium

Application Title: The renal microvasculature is highly sensitive to hyperglycemia.

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1. Project Accomplishments:

Maternal hyperglycemia is a significant risk factor for the development of genitourinary defects and renal malformations, and there is evidence from animal models that prenatal exposure to hyperglycemia can lead to reduced nephron endowment. In this project, we hypothesized that maternal hyperglycemia results in impaired nephrogenesis as a result of altered miRNA function and aberrant differentiation of the kidney vasculature. We have begun to characterize the histological and morphological features of aberrant kidney development in mouse and human developing kidneys exposed to hyperglycemia *in utero,* and anticipate that we will generate small RNA and RNA sequencing information on the developing renal microvasculature when exposed to hyperglycemia in mouse and human.

2. Specific Aims:

Aim 1: To characterize the RNA and small RNA transcriptome of human fetal kidneys and isolated fetal endothelial cells exposed to hyperglycemia.

Results:
We obtained de-identified neonatal kidney samples from an infant of gestational age 31 weeks (control) and 32 weeks (infant of diabetic mother) from the International Institute for the Advancement of Medicine (IIAM), along with clinical and demographic information from the neonatal donors, including maternal age, maternal diabetes (type 1 versus type 2), maternal hemoglobin A1c, maternal ethnicity, maternal smoking, maternal obesity and infant gender. Both of these samples have anencephaly, but no other extra-renal congenital malformations. We continue to have an active process in place to collect human fetal kidneys from the University of Pittsburgh Health Sciences Tissue Bank and neonatal infant kidneys from the IIAM for controls and infants of diabetic mothers.

We performed a detailed histological characterization of these samples, which showed that the kidney of the infant of the diabetic mother displayed narrower developing renal vasculature (as detected by PECAM staining), decreased expression of the nephron progenitor marker Six2, and larger Jagged1-positive structures (early developing nephrons) (Figures 1 and 2).

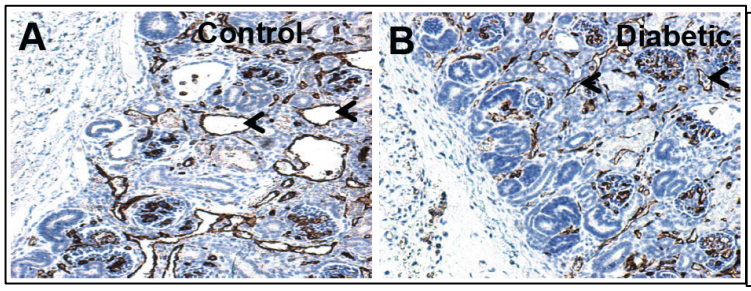

Figure 1. Immunohistochemistry for PECAM in a control kidney at 31 weeks gestational age (A) and a kidney from the infant of a diabetic mother at 32 weeks gestational age (B).

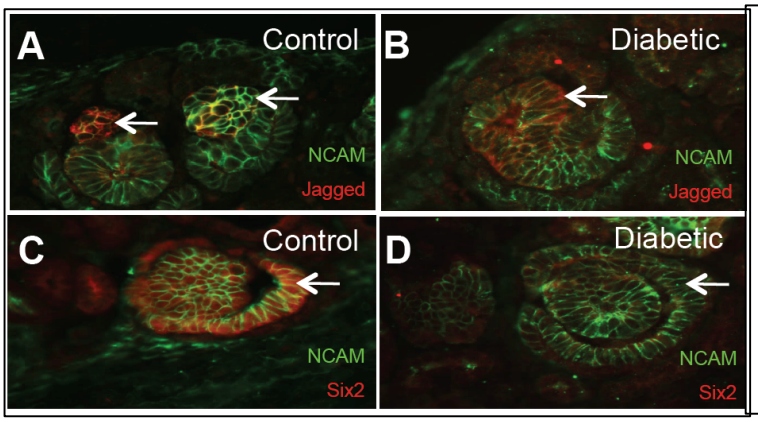

Figure 2. Immunofluorescent staining for markers of nephron progenitors and early developing nephrons (NCAM), early developing nephrons (Jagged1) and nephron progenitors (Six2) in a control kidney at 31 weeks gestational age (A, C) and a kidney from the infant of a diabetic mother at 32 weeks (B, D).

We isolated human fetal endothelial cells from each of the kidneys listed above, and total RNA was isolated from human fetal kidney cortex and human fetal endothelial cells for high-throughput RNA and small RNA sequencing at Children's Hospital of Pittsburgh Center for Genomic Medicine. The RNA sequencing data from these experiments is now being analyzed by Andrew Clugston, a bioinformatics student in the laboratory. His analysis will consist of processing and quality control of sequencing reads, read alignment, gene/transcript abundance quantification, and identification of differentially expressed gene/transcripts and miRNAs. He will then utilize miRNA target prediction algorithms and databases (like TargetScan, MicroT, MAMI and MiRanda) to identify mRNA-miRNA interactions.

As an alternative approach to evaluating the effect of hyperglycemia on the developing renal vasculature, given the difficulties with obtaining the kidneys of infants of diabetic mothers, we grew primary human fetal renal endothelial cells, and exposed them to 5 mM, 10 mM and 20 mM glucose concentrations in their media. We have preliminary data that the expression of KDR, a receptor that is critical in endothelial proliferation and survival, and PECAM, which is required for leukocyte migration during inflammation, are altered in human fetal renal endothelial cells exposed to varying concentrations of glucose (Figure 3). We have isolated total RNA from these samples, and performed RNA and small RNA sequencing on these samples. This RNA sequencing data is also currently being analyzed as noted above.

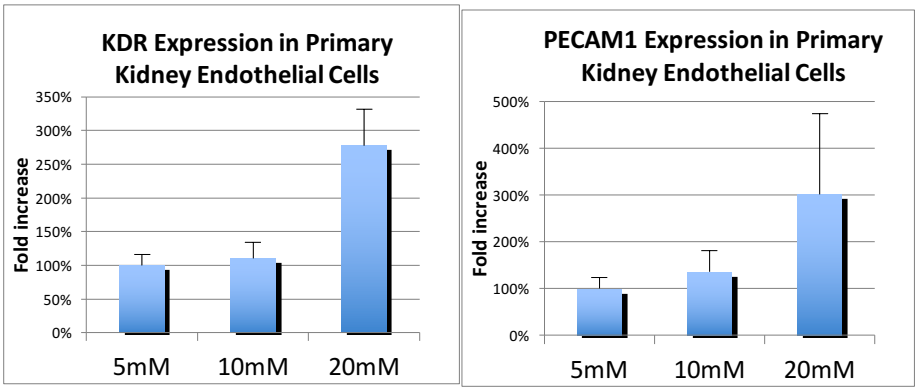

Figure 3. Quantitative PCR for KDR (left) and PECAM1(right) for primary human kidney endothelial cells, cultured in varying glucose concentrations. n=3.

Aim 2: To validate the mis-regulation of mRNA and miRNA expression in response to hyperglycemia *in vitro* and in the streptozotocin-induced mouse model of diabetes.

Results:
We performed a detailed histological analysis of embryonic mouse kidneys from control pregnant CD1 dams and pregnant dams in which diabetes was induced with streptozotocin at embryonic day 5. Interestingly, embryonic mouse kidneys exposed to a hyperglycemic environment appear to have more enlarged developing nephron structures and changes in the appearance of the developing renal vasculature (Figures 4-5). The developing renal endothelial cells from control embryos and embryos of diabetic dams was also isolated for RNA and small RNA sequencing as noted above. The analysis is pending.

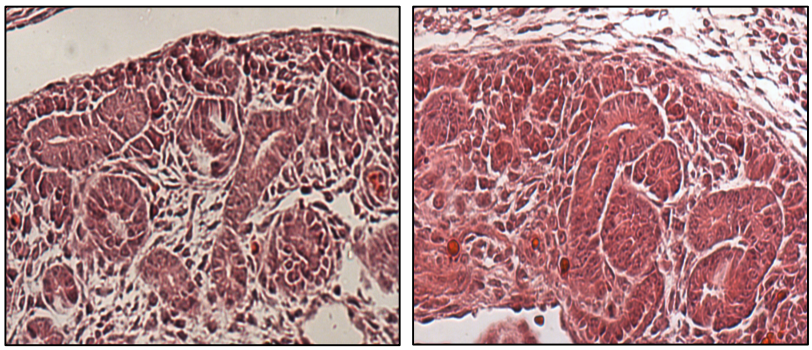

Figure 4. H&E staining of control embryonic mouse kidneys (left) and embryonic mouse kidneys exposed to hyperglycemia (right) at embryonic day 14. The developing nephron structures appear larger on the right.

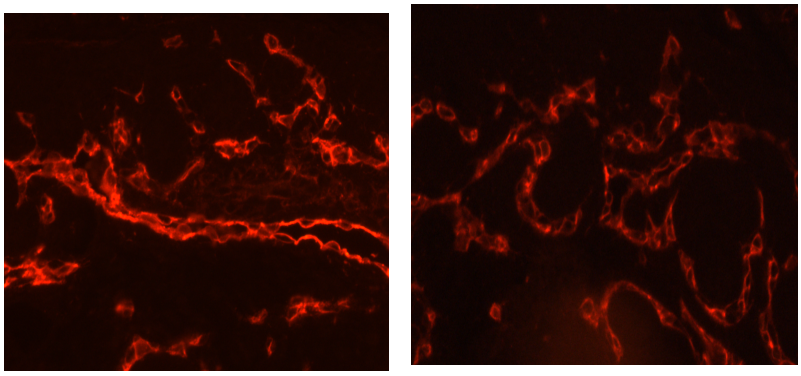

Figure 5. Immunofluorescent staining for endomucin in control embryonic mouse kidneys (left) and embryonic mouse kidneys exposed to hyperglycemia (right) at embryonic day 14. There is less robust endomucin staining on the right.

3. Publications:

Pending.