

**Epigenetic regulation in neonatal ECFCs following intrauterine exposure to gestational diabetes**Emily K. Blue<sup>1,2</sup>, BreAnn M. Sheehan<sup>1,2</sup>, Zia V. Nuss<sup>1,2</sup>, Cassandra R. Gohn<sup>3</sup>, Kaela M. Varberg<sup>3</sup>,  
Jeanette N. McClintick<sup>4</sup>, and Laura S. Haneline<sup>1,2,3,5,6</sup><sup>1</sup>Department of Pediatrics, the <sup>2</sup>Herman B Wells Center for Pediatric Research, <sup>3</sup>Department of Cellular & Integrative Physiology, <sup>4</sup>Department of Biochemistry and Molecular Biology, <sup>5</sup>Department of Microbiology & Immunology, and the <sup>6</sup>Indiana University Simon Cancer Center  
Indiana University School of Medicine  
Indiana University-Purdue University Indianapolis

Gestational diabetes (GDM) complicates up to 10% of pregnancies. In addition to acute risks, the children of diabetic mothers have an increased risk of obesity, diabetes, and hypertension, starting in childhood. While the causes of this increased risk are unknown, previous studies in our lab have identified functional deficits in endothelial colony forming cells (ECFCs) isolated from the cord blood of GDM pregnancies. This study focused on identifying genes that have altered epigenetic modifications that result in abnormal mRNA and protein expression in ECFCs from the cord blood GDM pregnancies. The objective of this study was to identify mRNA expression and DNA methylation alterations in ECFCs that may help identify the causes of ECFC dysfunction following intrauterine exposure to GDM. ECFCs were obtained from control and GDM pregnancies. DNA, RNA, and protein samples were isolated in parallel from ECFCs. RNA microarray analysis using the Affymetrix Human 1.0 Gene Array was used to identify gene expression alterations in GDM ECFCs compared to control ECFCs. Genome-wide DNA methylation was assessed using an Infinium 450K Methylation Array for DNA samples at >450,000 CpG sites. Correlation analysis was performed to identify possible sites that have altered CpG methylation and RNA expression. RNA expression results were validated using qRT-PCR and western blotting. Bisulfite sequencing of genomic DNA from the ECFCs was performed to identify additional sites with altered methylation for regions not included in the DNA methylation array. Of the 28,000 genetic loci tested, 596 mRNAs were altered between control and GDM ECFCs ( $p < 0.01$ ). More stringent criteria identified 38 genes for further investigation by limiting analysis to genes that exhibited increased or decreased expression by at least 50%, with a  $p < 0.01$ . PLAC8 was identified as being increased 5-fold by microarray analysis, a result which was confirmed in two cohorts by qRT-PCR and western blotting. Analysis of the methylation array and bisulfite sequencing results revealed 3 regions surrounding the transcriptional start site of PLAC8 gene whose CpG methylation negatively correlate with RNA expression in samples from control and GDM ECFCs. In contrast, a CpG island is fully unmethylated in both control and GDM ECFCs. The discovery of CpG sites whose methylation correlates with PLAC8 mRNA expression in ECFCs is consistent with the hypothesis that intrauterine exposure to GDM results in epigenetic changes. Analysis of methylation at this site could be used as a biomarker for children of mothers with GDM who may be at risk for disease later in life. Using bisulfite pyrosequencing, we are currently developing assays to quickly determine if methylation of the PLAC8 putative promoter region is altered in cord blood mononuclear cells obtained from GDM or healthy control pregnancies. We are also investigating the role of methylation in regulating PLAC8 RNA expression, determining if there is altered histone modifications and transcription factor binding in these regions, and examining other genes that may comprise a molecular signature of ECFC dysfunction.