May 11th, 11:30 AM - 1:30 PM

Activation of Aryl Hydrocarbon Receptor Signaling in Human Trophoblasts Alters Markers of Growth and Differentiation

Asmaa Alsousi

Activation of Aryl Hydrocarbon Receptor Signaling in Human Trophoblasts Alters Markers of Growth and Differentiation

Submitting/Presenting Author (must be a trainee): Asmaa Alsousi
Primary Email Address: aalsousi@cmh.edu

☐ **Medical Student**
☐ **Resident/Psychology Intern (≤ 1 month of dedicated research time)**
☐ **Resident/Ph.D/post graduate (> 1 month of dedicated research time)**
☐ **Fellow**

Primary Mentor (one name only): Carrie Vyhlidal
Other authors/contributors involved in project: Rebecca Biswell, Boryana Koseva, Jeffrey Johnston, Elin Grundberg, Michael J. Soares

Describe role of Submitting/Presenting Trainee in this project (limit 150 words):
The Trainee cultured, treated the human trophoblast stem cells (hTS), Extracted RNA and DNA, performed experiments of WGBs and ATAC-seq to a stopping point then submitted to Genome center. Analyzed RNAseq, WGBS, and ATAC-seq data with Ingenuity Pathway Analysis software (IPA), R studio and others. The trainee ran QPCR, and analyzed data to confirm the role of AHR signaling in hTS

Background, Objectives/Goal, Methods/Design, Results, Conclusions limited to 500 words

Background: It is estimated that 1.7% of pregnant women smoke during their pregnancy globally, with the highest levels observed in Europe at 8.1%, and lowest in Africa at 0.8. The association of maternal cigarette smoking with increased risk of poor birth outcomes such as preterm birth, congenital anomalies, and neonatal mortality is well-established. In addition, evidence suggests that intrauterine exposure to maternal smoking impacts the risk of developing diseases later in life; however, we still do not understand the exact mechanism(s) leading to these outcomes. Once components of cigarette smoke (CS) cross the placenta and enter the fetal compartment, several metabolic pathways are involved and may affect development, including via the aryl hydrocarbon receptor (AHR) pathway.

Objectives/Goal: The role of AHR pathway in trophoblasts remain the subject of active investigation.

Methods/Design:

- CT27 human trophoblast stem cells (hTS) were used for investigating the effects of CS.
- Total RNA was extracted and used for library construction employing the TruSeq Stranded RNA Protocol. The libraries were sequenced on the Illumina NovaSeq with 2 x151 paired end reads. Sequencing reads were pseudo-aligned to the GRCh38 GENCODE v19 transcript annotations using kallisto with default parameters. The resulting estimated counts per transcript were combined into gene-level counts, followed by differential expression analysis (padj < 0.05) using the standard workflow described in the DESeq2 vignette.

- Genomic DNA was purified and used for library preparation according to ACCEL-NGS Methyl-Seq DNA Library protocol to perform whole-genome bisulfite sequencing (WGBS). Samples were analyzed using the Illumina DRAGEN Methylation pipeline. Methylated and unmethylated read counts were extracted using Bismark.
- Omni-ATAC-sequencing protocol was used to determine chromatin accessibility. The sequencing data were aligned to (GRCh37/hg19). The number of aligned reads across the genome was counted in 100-bp bins for each sample. Bins were ordered in descending order of reads, and the top 30,000 bins ("t30K") were selected for downstream analyses. All windows were submitted to the Genomic Regions Enrichment of Annotations Tool (GREAT) in order to find nearby genes whose regulatory domains overlap windows.
- QPCR was used to confirm gene expression with AHR activators 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), activated Kynurenine (Kyn), and the AHR Inhibitor (CH223191).
- Data interpretation and pathway networks were analyzed with IPA, R Studio Analysis, and GraphPad Prism 8.

Results:

- Results from RNA-seq, WGBS, and ATAC-seq indicate that components of CS affect hTS by activating AHR signaling and altering the expression of genes involved in processes underlying trophoblast cell fate, such as differentiation.
- EVT treatment with CH223191 on day 5 and ST on day 1 of differentiation was selected for experiments with AHR activators. In the hTS cell state, treatment with TCDD increased *CGB3*, and TIPARP gene expression but not TP63, while Kyn upregulated *CGB3*, *TIPARP*, and TP63 expression. During EVT differentiation, both activators downregulated *CGB3* expression and upregulated *TP63* during differentiation, resulting in the maintenance of the undifferentiated state of cells through AHR signaling.

Conclusions: CS alterations of gene expression in hTS cells including via the AHR pathway, and genes encoding proteins implicated in the regulation of trophoblast cell differentiation may impair proper development and function of the placenta and pregnancy outcomes. Ongoing RNA-seq study is assessing the role of Kyn and TCDD through AHR signaling.

Supported by NIH R01ES029280