

Amniotic fluid transcriptomic changes in fetuses with myelomeningocele

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Objective

To identify fetal molecular pathophysiological changes and novel disease mechanisms specific to fetuses with myelomeningocele (MMC) by analyzing amniotic fluid supernatant (AFS) cell-free (cf) RNA transcriptome.

Background

cf RNA in (AFS) reflects developmental changes in gene expression in the living fetus, including genes specific to the central nervous system (CNS). Although CNS-specific transcripts have been identified in AFS, it is not known whether changes in the AFS transcriptome reflect the specific pathophysiology of fetal CNS disorders.

Methods

AFS was collected from 10 pregnant women at the time of the open MMC repair. Archived AFS from sex and gestational age-matched euploid fetuses (n=10) without MMC were used as controls. AFS cf RNA was isolated, processed and hybridized to Affymetrix GeneChip® Human Genome U133 Plus 2.0 arrays. Differentially-regulated genes were identified using paired t-tests ($p < 0.05$). Significantly altered expression in genes, pathways and networks was identified using Ingenuity Pathway Analysis® (right-tailed Fisher Exact Test, $p < 0.05$). Multiple testing was adjusted with the Benjamini-Hochberg corrected p-values (< 0.05). Tissue specific gene expressions were assessed by utilizing BioGPS® database.

Results

MMC samples were from fetuses at slightly older gestational ages (24.5±1.0 wks) than controls (20.9±0.9 wks). Fetuses with MMC had 284 differentially-regulated genes (176 up- & 108 down-regulated) in AFS. Pathway analysis demonstrated a significant contribution of inflammation (Z-score 2.45) to pathology and a broad influence of Wnt signaling pathways (*Wnt1*, *Wnt5A*, *ITPR1*, Z-score -1.3). Known genes associated with MMC (*PRICKLE2*, *GLI3*, *RAB23*, etc) were differentially regulated. In addition, novel dysregulated genes were identified in association with neurodevelopment and neuronal regeneration (*GAP43* and *ZEB1*) or axonal growth and guidance (*ACAP1*). Of 28 genes (10%) that were highly specific in tissue origin, 12 were identified as CNS highly specific genes.

Conclusions

Transcriptomic analyses of living fetuses with MMC using AFS cfRNA demonstrated differential regulation of specific genes and molecular pathways relevant to this CNS disorder, resulting in a new understanding of pathophysiological changes. The data also suggested the importance of pathways involving secondary pathology, such as inflammation and neuronal regeneration, in MMC. These newly identified pathways may lead to hypotheses that can test novel therapeutic targets as adjuncts to fetal surgical repair.

Manuscript from this study:

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