54 Distinct neuronal subtypes in fetal CSF characterized by single-cell transcriptomics

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OBJECTIVE: Cells within the fetal cerebrospinal fluid (CSF) at 22-26 weeks' gestation are only now being characterized. During fetoscopic myelomeningocele repair, fetal CSF is drawn from the lesion as part-and-parcel of the repair. We hypothesized most of these cells would be neuronal and neuroepithelial cell-types.

STUDY DESIGN: We have generated a repository of paired amniotic fluid (AF) and fetal CSF specimens from >50 fetoscopic surgeries consenting for use. We used orthogonal methods to catalog cell populations in the fetal CSF during gestation. We performed flow cytometry to quantify $CD34^+$ hematopoietic stem cells, $CD56^+$/$CD45^+$ NK cells, and $CD133^+$/$CD45^+$ hematopoietic progenitor cells (N=4) and established a neurosphere culture system that could be maintained for 4 weeks (N=3). Lastly, we performed single-cell RNA-sequencing (scRNA-seq) as an unbiased approach to characterize paired fetal CSF and AF cell populations (N=9).

RESULTS: By flow, neural precursor cells represented approximately 39% of the CSF cells, leaving many cell-types uncharacterized. Therefore, we utilized scRNA-seq resulting in 22,058 total cells clustered into 18 distinct cell-types (Fig 1a). We then focused on 3,742 transcriptomes of neuronal origin (Fig 1b), revealing fetal CSF neuronal cells were mainly composed of microglia, neuroblasts, and immature neurons (Fig 1c). A cluster of putative ARC capsids, which are endogenous 40nm virus-like capsids carrying neurotransmitter RNA, demonstrated that *ARC* was expressed in 261 CSF cells from 87% of the samples (Fig 2b). 73 transcriptomes putatively annotated as ARC capsids revealed RNA content payloads associated with cadmium ion response, cell cycle, and transcription regulation (GO pathways, $f< 0.05$ controlling for multiple comparisons).

CONCLUSION: Neuronal cell-types in the mid-gestation fetal CSF of ongoing pregnancies provide an unparalleled picture of neuronal development. Further analyses of the recently discovered ARC capsid neuronal signaling system will unveil the sophisticated differentiation trajectories of neurogenesis *in utero* and how neurons may be engineered or repaired.

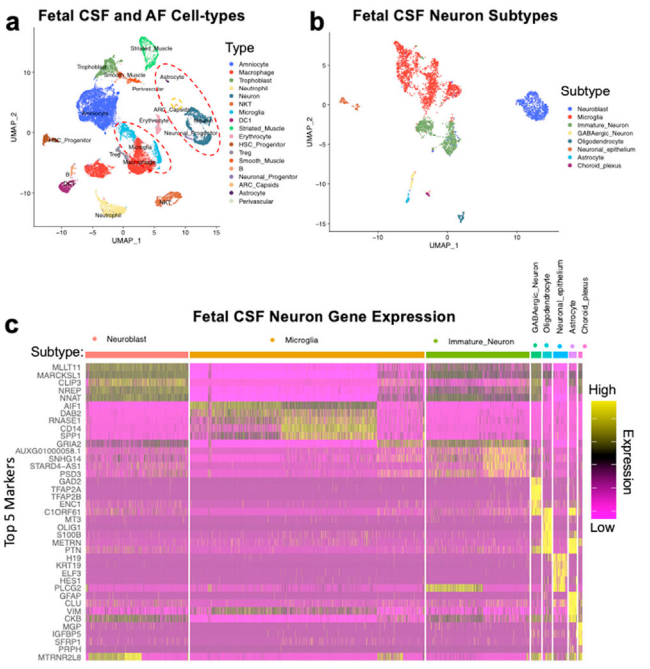

Figure 1. Single-cell Characterization of Neuronal Cell-types in Fetal CSF Obtained During Neural Tube Defect Repair Surgeries. a CSF and amniotic fluid (AF) cells from 22-26 weeks' gestation from ongoing pregnancies were subjected to 10x Genomics 3' scRNA-seq resulting in 22,058 cells. **b** 3,742 neuronal cell subtypes were subset and re-analyzed, identifying 8 distinct subtypes. **c** The top 5 marker transcripts for each neuronal subtype.

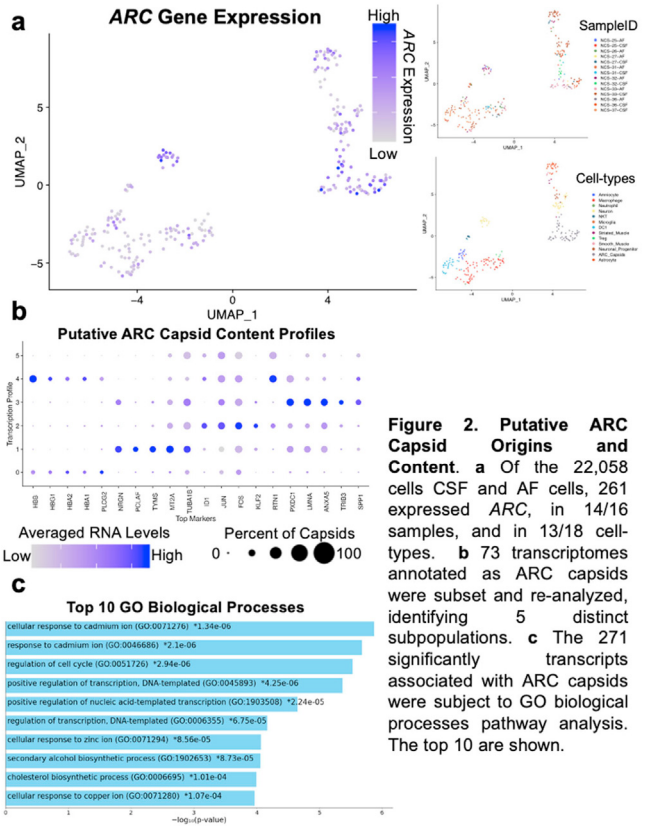

Figure 2. Putative ARC Capsid Origins and Content. a Of the 22,058 cells CSF and AF cells, 261 expressed *ARC*, in 14/16 samples, and in 13/18 cell-types. **b** 73 transcriptomes annotated as ARC capsids were subset and re-analyzed, identifying 5 distinct subpopulations. **c** The 271 significantly transcripts associated with ARC capsids were subject to GO biological processes pathway analysis. The top 10 are shown.