TITLE: Mechanisms Contributing to Aberrant Vascular Development in the Brain Following Prenatal Alcohol Exposure through Elevation of Mir-150-5p

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ABSTRACT:
Fetal Alcohol Spectrum Disorders (FASD) are conditions impacting development in children exposed to alcohol *in utero*. Among other associated anatomical defects, the organization of the brain microvasculature is altered in severe forms of FASD. Other vascular anomalies such as defective angiogenesis, permeability, vessel reactivity, and blood flow are found in animal models of prenatal alcohol exposure (PAE). The underlying mechanisms of these vascular defects are not fully understood. We previously found miR-150-5p to be upregulated in brain microvascular endothelial cells (BMVECs) isolated from embryonic mouse cortices following moderate PAE. We identified targets of miR-150-5p in BMVECs and demonstrated that miR-150-5p inhibition could counter alcohol-mediated effects *in vitro* on BMVECS and *in vivo* on the cortical microvasculature. Here, we investigate mechanisms that contribute to miR-150-5p elevation in BMVECs following PAE. We hypothesized that multiple mechanisms contribute to increased miR-150-5p abundance in BMVECs following ethanol (EtOH) exposure. We previously determined that EtOH exposure reduces exosomal secretion of miR-150-5p from BMVECs. We investigated the vacuolar protein sorting-associated protein 4 A (VPS4A), which controls the loading of some miRNA cargo, including miR-150-5p, into exosomes. VPS4A was reduced in primary BMVECs following PAE and *in vitro* following EtOH treatment. Overexpression of wild-type VPS4A reduced intracellular levels and increased exosomal levels of miR-150-5p, while overexpression of a dominant-negative form of VPS4A did the opposite. These results indicate that VPS4A mediates intracellular and exosomal miR-150-5p distribution in BMVECs. Additionally, we investigated the RNA-binding protein KSRP, which binds the stem-loop of select pre-miRNAs, including pre-miR-150, to promote their maturation to miRNAs. KSRP is increased in primary BMVECs following PAE and following *in vitro* EtOH treatment. Ongoing studies will determine whether PAE affects KSRP binding of pre-miR-150 and miR-150-5p levels. In summary, our research implicates multiple mechanisms of miR-150-5p regulation that may contribute to brain microvascular defects following PAE.