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mir-206 expression in plasma-derived extracellular vesicles in children with cerebral palsy

BACKGROUND: Cerebral palsy (CP) is a non-degenerative neurological disorder that leads to muscular dysfunction over time, causing decreased muscle size and function such as a decrease in contractility, increase in stiffness and fibrosis, and increase in muscle dysfunction. Skeletal muscle differentiation (myogenesis) occurs when satellite cells become activated to proliferate myoblast stem cells that differentiate into myotubes and later fuse forming muscle fibers. Micro-RNA (miRNA) is a small non-coding RNA molecule that regulates gene expression by primarily binding to 3' UTR (untranslated region). Muscle-enriched miRNAs, such as miR-206, act as a "post-transcriptional switch" that can push skeletal muscle cells from a proliferative to a differentiated state, causing an increase in myotube size and fusion by targeting anti-differentiation genes. Delivering exogenous mir-206 using plasma from EVs (extracellular vesicles) can improve differentiation, thus improving muscle health in CP. An initial step is to optimize ddPCR to determine the endogenous miR-206 levels in plasma-derived EVs.

OBJECTIVE: The purpose of this study was to assess miR-206 expression in plasma derived EVs in children with CP and identify predicted targets of miR-206.

METHODS: Blood was collected from 7 participants with CP between ages 6-25 years. EVs were isolated from plasma (500, 250, 175, and 100 µl) using the ultracentrifugation method. RNA was extracted from both isolated EVs and the EV depleted plasma using the miRNeasy Micro Kit, and 10ng of RNA was then reverse transcribed into cDNA. The expression of miR-206 was determined in the plasma EVs using ddPCR (Digital Droplet PCR). Specific target genes of miR-206 were identified using databases TargetScanHuman, miRTARGET, and miRDB. Cut off score for miRTARGET and TargetScanHuman data was <50, and mirDB was n=50. DAVID Bioinformatics was used to identify the top 10 targets, and common target genes from the three databases. Subcategories used were "KEGG PATHWAY", "UP KW CELLULAR COMPONENT", "HPA NORMAL TISSUE", "HPA NORMAL TISSUE CELLTYPE", "GAD DISEASE", and "OMIM DISEASE" to determine common target genes among the 3 databases and the functions of those genes.

RESULTS: From DAVID Bioinformatics in the top 10 most significant genes from the 3 databases, TAGLN2 (transgelin 2) was one of the common genes, and its function was aligned with the cerebral cortex. KCNIP3 (potassium voltage-gated channel interacting protein 3, calsenilin) was an additional common gene which aligned with seminal vesicles. PAX7 (paired box 7) functions in maintaining skeletal cells in proliferative states, preventing myogenic differentiation.

SUMMARY: We have identified target genes of miR-206, and future studies will determine the functional relevance of these genes in the skeletal muscle differentiation of children with CP.