CHRD 2022: Abstract & Poster Submission Form

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Presenter Status
- ○ Undergraduate Students
- ○ Masters Student
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Research Category
- ◉ Basic Science
- ○ Clinical
- ○ Community Health / Policy

Role in the project
- ☑ Design
- ☑ Perform Experiments
- ☑ Analyze Data
- ☑ Write Abstract
- ☐

Title
Potential of extracellular vesicles as biomarkers of mitochondrial dysfunction

Background

Mitochondrial disorders (MD) are caused by dysfunction within the respiratory chain and can be classified as primary or secondary MD. Extracellular vesicles (EVs) are important mediators of intercellular communication and show promise as disease biomarkers, but remain unexplored in MD. We hypothesize that EVs can serve as biomarkers of MD.

Objective

To test our hypothesis, we characterized plasma-EVs from a known PMD (MELAS, N=1), 2 patients with hypophosphatasia (HPP) a disorder of skeletal mineralization recently associated with mitochondrial dysfunction and matched controls and compared results to EVs from a hydrogen peroxide (H2O2)-induced model of mitochondrial dysfunction.

Methods

EVs were isolated from MD patients and controls using size exclusion chromatography and ultrafiltration, and differential ultracentrifugation from conditioned media. EVs were analyzed biophysically, and expression of proteins associated with small-EVs (TSG101, Flotillin-1), medium/large-EVs (Cytochrome-C) and non-specific lipoprotein marker (ApoA1) by Western blotting. EVs were similarly studied in C2C12 myoblasts treated with H2O2 (0.5mM, 6hrs, N=5-6), optimized to induce mild mitochondrial dysfunction (10% decrease in MitoTracker Red).

Results

EV concentration (particles/ml) was 1.96, 1.48, and 2.96-fold higher in MELAS, HPP1, HPP2 vs. controls, and 1.58-fold higher in H2O2-EVs vs. control-EVs, though not significant. EV size and stability remained unchanged across conditions. EV protein yield was 1.70, 1.52 and 2.29-fold higher in MELAS, HPP1 and HPP2, and 1.90-fold in H2O2-EVs ($p=0.02$). Flotillin-1 expression was reduced by 69.54% in patient-EVs, and by 35.4% in H2O2-EVs ($p=0.03$). TSG101 was reduced by 39.1% in MELAS-EVs, 30.48% in HPP-EVs, and 51.81% in H2O2-EVs ($p=0.08$). Cyt-C was 2.9-fold higher in HPP-EVs and undetectable in H2O2-EVs. ApoA1 expression was lower in patient-EVs vs. controls.

Conclusion

Mitochondrial dysfunction increased EV concentration and protein yield, and decreased Flotillin-1 expression in both patient-EVs and H2O2-EVs. EV subtype-related proteins were expressed differentially. EVs as biomarkers for MD require further investigation but hold viable potential.

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