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Phonetic spelling of your (full) name

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Presenting Author Category

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Research Category

Basic Science

Abstract Title

143B Osteosarcoma cybrids as an in vitro model to investigate extracellular vesicles in MELAS syndrome

Authors

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Background

Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS) syndrome is most often caused by an A3243G mtDNA mutation. It typically presents in childhood with metabolic insufficiency. Diagnosis is challenging due to multisystemic manifestations and variations in mutant mtDNA levels (heteroplasmy). No robust animal models exist. Cybrids (cytoplasmic hybrid cells with MELAS mitochondria on a uniform nuclear background) offer a unique in vitro system to investigate MELAS. We recently showed that plasma-derived extracellular vesicles (EVs) are differentially expressed in MELAS patients and may serve as biomarkers.

Objective

We hypothesized that MELAS-cybrid EVs carry a signature similar to patients, and that these cells recapitulate key features of MELAS metabolic insufficiency.

Methods

Human 143B osteosarcoma cybrids harbouring wild-type or A3243G mutant mtDNA (MELAS, 100% heteroplasmy) were cultured in regular or EV-depleted growth medium. Cell growth was assessed by sulphorhodamine B (SRB) assay (24-72 h), and expressed relative to wild-type controls. Metabolic phenotype (oxygen consumption rates (OCR), ATP production) was determined using an XFe24 analyzer. Cybrid-EVs were quantified by single-particle analysis. Data were analyzed by repeated-measures two-

way ANOVA with Dunnett's post-hoc test, or paired t-tests.

Results

Relative to wild-type controls, MELAS-cybrid growth was significantly reduced (main effect of group, $p=0.0017$; $N=4$). MELAS-cybrids showed an approximate 33% growth deficit at 24 h in regular medium ($p=0.0011$; $N=4$) and 49–51% lower growth at 24–48 h in EV-depleted medium ($p=0.010$ – 0.016 ; $N=4$). MELAS-cybrids also exhibited reduced basal OCR (90%), maximal OCR (87%), and total ATP production (11%; $N=2$). EV secretion per cell was 1.78-fold higher in MELAS-cybrids ($p=0.0358$, $N=4$), while EV size was unchanged.

Conclusion

MELAS-cybrids exhibit slower growth, profound metabolic impairment, and elevated EV secretion that together mimic key features of the patient phenotype. These cells constitute an effective in vitro model for studying EV biology and the mechanisms of increased EV release in MELAS patients.