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Research Category

Basic Science

Abstract Title

Chronic contractile activity derived extracellular vesicle uptake in recipient myoblasts

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Background

Extracellular vesicles (EVs) are nanoparticles released by all cells that deliver biological cargo to recipient cells. We previously demonstrated that skeletal muscle-derived EVs (Skm-EVs) isolated from fully differentiated myotubes after chronic contractile activity (CCA), increase mitochondrial biogenesis in recipient murine myoblasts, an effect dependent on membrane proteins, which are necessary for EV docking and internalization by recipient cells.

Objective

It is unknown whether CCA alters EV uptake by recipient cells. We hypothesized that CCA-EVs are internalized by recipient cells more readily than control-EVs.

Methods

C2C12 myoblasts were differentiated into myotubes, and subjected to CCA (14 V, 3 h/day, 4 days). COX

activity was used to confirm increased mitochondrial content with CCA in contracted myotubes. Skm-EVs were isolated from conditioned media from control and CCA myotubes on day1 and day 4, and characterized using single-particle analysis. EVs were labelled with PKH67 and incubated with recipient myoblasts for 24 h. Fluorescence was measured at multiple cell densities, with dye-only and other controls to rule out artefacts. Data were analyzed by paired t-tests, one-way or two-way ANOVAs.

Results

CCA produced a 1.48-fold increase in COX activity ($p=0.12$; $N=4$), in line with an expected rise in mitochondrial content albeit not significant. EV concentration, size, and protein yield remained unchanged between groups and with time (all $p>0.05$; $N=4$), consistent with EV size data from prior work, but without the previously reported increase in EV secretion and yield. PKH67+EV fluorescence in treated cells increased with cell density, with the highest observed at 18,000 cells/well, and exceeded dye-only controls. Preliminary uptake data ($N=2$) showed a 16% higher fluorescence signal in recipient myoblasts with CCA-EVs versus control-EVs.

Conclusion

These preliminary findings suggest that CCA-EVs may be taken up more readily by recipient myoblasts. Larger sample sizes are required to confirm differences in EV uptake and to identify the underlying endocytic pathways.