The pro-apoptotic effect of chronic contractile activity-induced extracellular vesicles on Lewis lung carcinoma cells

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Introduction

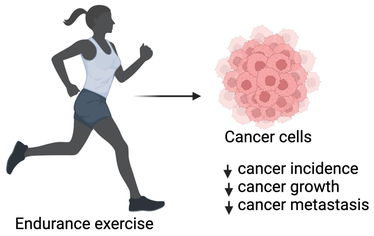

Figure 1. **Benefits of endurance exercise.** Endurance exercise evokes positive systemic benefits such as reduction in cancer incidence, growth and metastasis, but the exact mechanisms have yet to be fully elucidated.

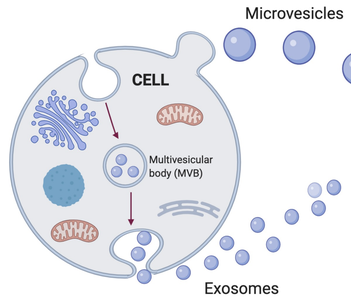

Figure 2. **EV biogenesis and release from cells**. EVs can be differentiated from each other based on their pathway of biogenesis and size, among other criteria. Exosomes are 30-150 nm in size and are released from the fusion of a multivesicular body (MVB) with the plasma membrane. Microvesicles are usually 100-1000 nm in size and are released by pinching outwards from the cell. The new guidelines by MISEV recommend classification of EVs into small EVs (sEVs) or medium/large EVs (m/lEVs), where sEVs are <200 nm and m/lEVs are >200 nm in size.

- **Extracellular vesicles (EVs)** are small lipid membrane-bound structures that enclose biological cargo, and constitute an essential method of cellular communication.
- We have previously shown that chronic contractile activity (CCA) increases the concentration of skeletal muscle-derived EVs, and these in turn increased mitochondrial biogenesis in C2C12 myoblasts.

OBJECTIVE:

To investigate if skeletal muscle-derived EVs post-CCA can mediate the anti-tumorigenic effects associated with chronic exercise.

Methods

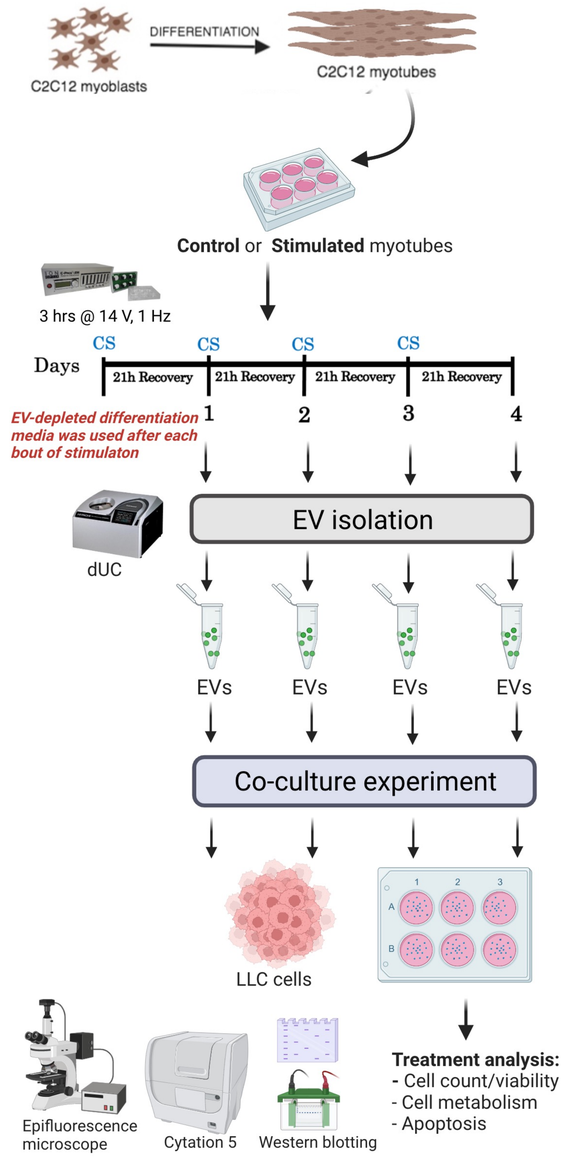

Figure 3. Schematic illustrating the study design. C2C12 myoblasts were differentiated into myotubes. Fully differentiated myotubes were divided into control (CON) and chronic stimulated (CS) groups. CS myotubes were stimulated at 14V for 3 hrs X 4 days to mimic chronic exercise. Media was changed to EV-depleted differentiation media after each bout of stimulation. EVs were isolated from CON and CS myotubes using differential ultracentrifugation (dUC) and stimulation co-cultured with Lewis lung carcinoma (LLC) cells after each bout of stimulation. Treated LLC cells were measured for changes in cell growth, metabolism and death.

Results

1. Effect on cell count and viability

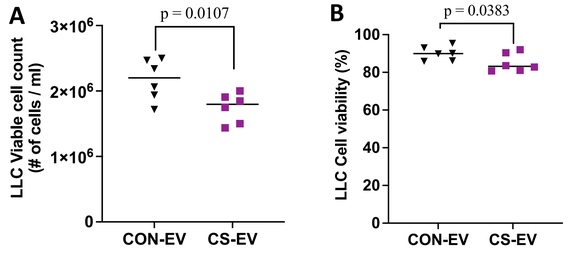

Figure 4. Effect of CS-derived skeletal muscle-EV on cell count and viability in LLC cells. (A) Cell count decreased by ~6% (p=0.0107), and **(B)** cell viability decreased by ~20% (p=0.0383) in LLC cells with CS-EV treatment *vs*. CON-EVs. Data were analyzed using an unpaired Student's t-test, are expressed as scatter plots with mean (N=6). Exact p values for significant results is shown.

2. Effect on cell metabolism

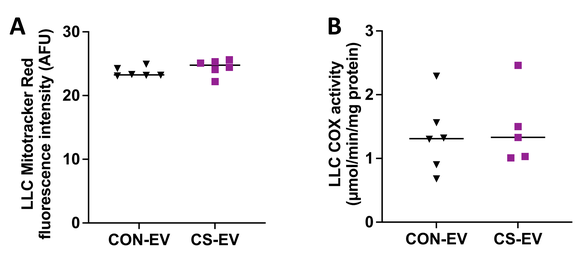

Figure 5. Effect of CS-derived skeletal muscle-EV on markers of mitochondrial biogenesis in LLC cells. (A) MitoTracker Red CMXRos, and **(B)** COX activity did not change in LLC cells with CS-EV treatment *vs*. CON-EVs. Data were analyzed using an unpaired Student's t-test, are expressed as scatter plots with mean (N=5-6). Exact p values for significant results is shown.

3. Effect on apoptosis

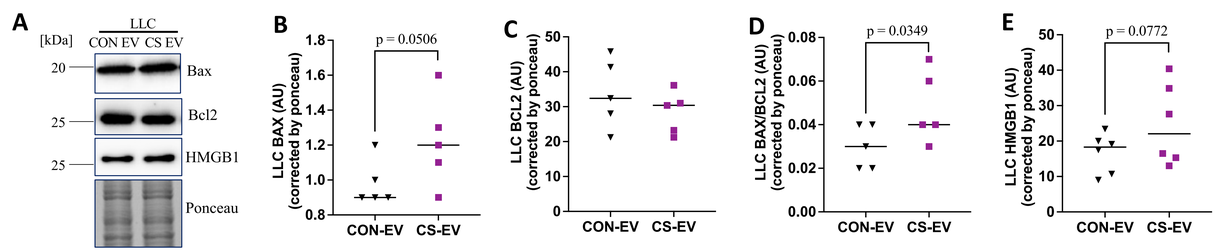

Figure 6. Effect of CS-derived skeletal muscle-EV treatment on markers of apoptosis in LLC cells. (A) Representative immunoblots for Bax, Bcl2 and HMGB1 expression are shown. Ponceau staining was used as loading control. **(B)** Quantification of immunoblot analysis showed that expression of Bax (20 kDa) was significantly increased by 24% in LLC cells treated with CS-EVs compared with CON-EVs (p=0.0506). **(C)** Quantification of immunoblot analysis showed no difference in the expression of Bcl2 (26 kDa) between groups. **(D)** The ratio of Bax/Bcl2 was higher by 60% in LLC cells treated with CS-EVs compared with CON-EVs (p=0.0349). **(E)** Quantification of immunoblot analysis showed that expression of HMGB1 (29 kDa) increased by 48% in LLC cells treated with CS-EVs compared with CON-EVs (p=0.0772). Data were analyzed using an unpaired Student's t-test, are expressed as scatter plots with mean (N=5-6). Exact p values for significant results is shown.

Summary and Conclusion

- CCA-induced skeletal muscle-EVs reduced cell count and viability but did not change cell metabolism.
- CCA-induced skeletal muscle-EVs increased the expression of some pro-apoptotic markers in LLC cells.

Altogether, our novel findings illustrate the potential of CCA-derived skeletal muscle-EVs in mediating the anti-tumorigenic effects of chronic exercise.

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