Extracellular vesicles from cardiosphere-derived cells show immunomodulatory properties in vitro and improve muscle activity in a mouse model of Duchenne Muscular Dystrophy

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Introduction

- Early immune infiltration can contribute substantially to the initiation and progression of muscle pathology. Activated T cells and macrophages play a critical role in muscle wasting.
- CDCs are cleared shortly after administration and paracrine signals play an essential role in the activation of the regenerative pathways.
- EVs from CDCs are able to recapitulate most of the effects mediated by the cells.

Methods

Isolation of MSCs and CDCs

- MSCs were obtained from Lonza
- CDCs: Donor heart from organ procurement organization
- Cardiophere formation
- Cardiophere-Derived Cells
- CDC potency is determined by functional improvement of ejection fraction in an in vivo myocardial infarction mouse model

Isolation and characterization of EVs

- EV isolation:
  - EV isolation:
  - EV isolation:
  - EV isolation:
  - EV isolation:
  - EV isolation:

In vitro T cell assay

- Human T lymphocytes activated by PHA were treated with CDC-EVs. T cells were labeled with CFSE and proliferation analyzed by flow cytometry.

In vitro mouse macrophage assay

- Macrophages were isolated from peritoneal exudates from mice primed with 3% Brewer’s thioglycolate and phenotype tested by PCR.
- EV dosing: 2500 particles per cell

In vivo mouse macrophage assay

- Zymosan injection (i.p.)
- D1
- D2
- D3
- Sacrifice

DMD mouse model

- 10-12 months old female mdx mice
- Weekly IV injection: PBS or 2x10⁶ EVs
- Exercise capability (treadmill):
  - Starting at 10 m/min
  - Increase of 1 m/min every minute
  - Distance traveled in meters was recorded and difference between exercise capability at week 3 and week 0 were calculated as percent change from baseline.

Summary and Conclusions

- EVs from CDCs and MSC have been characterized to identify surface markers and cargo nucleic acids able to discriminate between them.
- CDC-EVs have immunomodulatory capabilities on T cells and macrophages. Therapeutic capabilities of CDC-EVs have been tested in vivo in a sepsis mouse model and in a Duchenne Muscular Dystrophy model.

- EVs from CDC and MSC showed a different expression of surface markers and a different cargo content.
- CDC-EVs have strong immunomodulatory capabilities on human activated T-lymphocytes.
- EVs from potent CDCs showed a stronger dose-dependent upregulation of anti-inflammatory genes in activated mouse macrophages compared to EVs obtained from non-potent CDCs or from MSCs.
- EVs from potent CDC cell lines reduce the increased accumulation of activated macrophages in an in vivo peritonitis mouse model.
- Repeat dosing of EVs from primary or immortal CDCs are effective improving exercise capabilities in mdx mice.

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