Extracellular Vesicles from Cardiosphere-Derived Cell and from Mesenchymal Stem Cells Show Different Immunomodulatory Capabilities and Distinct RNA Cargo

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Introduction

- Cardiosphere-derived cells (CDCs) possess cardioprotective, regenerative, and immunomodulatory characteristics when delivered to the heart post-myocardial infarction (MI) which appear to be unique traits to CDCs
- Human CDC-derived extracellular vesicles (CDC-EVs) recapitulate the effects of CDCs in acute and chronic in vivo models of MI suggesting most of the therapeutic effects of CDCs are mediated by CDC-EVs
- Here we tested the hypothesis that a distinct cargo profile will define the functional efficacy between CDC-EVs and mesenchymal stem cell-derived EVs (MSC-EVs)
- CDCs derived from different donors possess variable regenerative potency in an in vivo MI animal model thus we compared EVs derived from potent and non-potent CDCs (Cheng et al., JACC Heart Failure, 2014)
- To assess the immunomodulatory role of CDCs and CDC-EVs, we investigated the immunomodulatory effect of EVs in a macrophages and a T-cell based assay (de Couto et al., JCI, 2015; de Couto et al., Circ, 2017).

Methods

Isolation of MSCs and CDCs

- MSCs were obtained from Lonza
- CDCs:
  - Donor heart from organ procurement organization
  - Cardiosphere formation
  - Cardiosphere-Derived Cells

Isolation and characterization of EVs

- EV isolation:
  - 4x washes + culture with serum-free medium
  - 5-45 μm PES
  - 10kDa MWCO

- EV characterization:
  - Particle size and concentration (Nanoparticle tracking technology)
  - Protein concentration (DC protein assay)
  - CDC-EV samples: n=15 (15 days); MSC-EV samples: n=4 (15 days), n=2 (48h)

Exosomal small RNA sequencing

- miRNeasy Serum/Plasma kit to isolate total RNA
- NextGen 500 Sequencing (Illumina); 30 ng/sample RNA input
- CDC-EV samples: n=10 (15 days); MSC-EV samples: n=4 (15 days), n=2 (48h)

In vitro macrophage assay (mouse and rat)

- EV dosing: 500 or 2500 particles per cell

In vivo macrophage assay (mouse)

- Zymosan injection (i.p.)

In vitro T-cell assay

- Negative Selection

Summary and Conclusions

- The size and particle and protein concentration of CDC-EVs are significantly greater and higher than MSC-EVs irrespective from potency
- CDC-EVs and MSC-EVs show a differential cargo composition with a higher Y-RNA and miRNA content in CDC-EVs compared to 15 days and 48 hour MSC-EVs
- miRNA clustering analysis showed that CDC-EVs and MSC-EVs cluster separately with a sub-cluster present in MSC-EVs (MSC-EVs 48h)
- CDC-EVs demonstrated differential clustering between in vivo potent and non-potent CDCs
- Potent CDC-EVs showed a significant upregulation of miR#1 and miR#2 and downregulation of miR#3 compared to non-potent CDC-EVs
- EVs from potent CDCs showed a stronger dose-dependent upregulation of anti-inflammatory genes in activated rat and 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
- CDC-EVs have strong immunomodulatory capabilities on human activated T-lymphocytes

Acknowledgements: DoD Award # (PR150618)
Efficacy and in vitro Uptake of EVs from Cardiosphere-Derived Cells

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ABSTRACT

Capricor Therapeutics has an ongoing clinical trial for the treatment of Duchenne muscular dystrophy (DMD) using an allogeneic cell therapy product, cardiosphere-derived cells (CDCs). It is widely accepted that most of the therapeutic effects observed in cell therapies using non-engrafting cells are caused by paracrine factors secreted by the delivered cells early after administration. In early studies in a mouse model of myocardial infarction, the therapeutic benefits of CDCs were recapitated using the extracellular vesicles (EVs) they secrete. In more recent studies, we have shown that CDC-EVs have a strong immunomodulatory capacity on macrophages and T cells, making them very attractive for the treatment of inflammatory disorders. Here, we show that CDC-EVs administered systemically also recapitate the beneficial therapeutic effects of CDCs in a mouse preclinical model of DMD. mxd mice treated with CDC-EVs have a significant increase in exercise capacity compared with mice treated with placebo. CDC-EVs also elicit therapeutic effects in models of radiation dermatitis and graft-versus-host disease.

INTRODUCTION

Cardiosphere-derived cells (CDCs), a cell product currently in clinical trials (Regress-HFpEF, HOPE 2, ALPHA PAH) secrete extracellular vesicles. CDC-EVs contain known EV markers (Figure 1A) and are <200 nm in size (Figure 1B). CDC-EVs show a unique miRNA expression profile when compared with fibroblast (NHDF) EVs (Figure 1C).

CDC-EVs recapitulate the regenerative properties of CDCs².

CDC-EVs improve radiation dermatitis score in irradiated mice

A) Study timeline. Radiation was given to mice on days 0, 1, 2, 5, 6, and 7. Mice were injected with either CDC-EVs or vehicle control on days 7, 22, 29, and 36. B) Mean derm score for each group ± SEM. The score reflects the severity of the radiation dermatitis and the groups were blinded for score assessment.

CDC-EVs improve exercise capacity in mouse model of DMD

A) Study timeline. Mice were exercised on a treadmill prior to the start of the study to assess baseline exercise capacity. 1x10⁶ CDC-EVs or vehicle control were administered weekly for 3 weeks by systemic injection. Exercise capacity was assessed again 1 week after the final injection. B) Exercise capacity of mice given either vehicle control or CDC-EVs before administration (week 0) and one week after 3 administrations (week 3). C) Exercise capacity on weeks 0 and 3 represented as a percent change from baseline.

REFERENCES