ABSTRACT

Xerostomia or severe dry mouth, is the most common long-term side effect of radiotherapy (RT) in patients with head and neck cancer (HNC). RT-damaged salivary glands fail to produce saliva, resulting in recurrent oral infections, dental decay, speech and swallowing dysfunction, leading to an overall deterioration in quality of life. Current treatment strategies for xerostomia are inefficient in delivering long-lasting and effective relief to patients. We and others have demonstrated that salivary glands harbor adult stem/progenitor cells (SSPCs), which are important for regeneration and maintaining homeostasis. In pursuit of new promising therapeutic agents, we focus on the possible role of exosomes in rescuing saliva function post RT. Exosomes regulates unidirectional flow of information and have been shown to regulate epithelial progenitor expansion during normal development of salivary glands. Previous studies have revealed that treatment with extracellular vesicles (EVs), including exosomes and microvesicles, isolated from human cardiomyocyte-derived cells (CDC) led to reduced inflammation, less fibrosis and enhanced regeneration in murine cardiac tissues after ischemic damage (Ibrahim et al., 2014). In this study, we evaluate whether CDC-EVs can stimulate SSPC self-renewal and improve saliva function post RT. CDC-EVs treatment of murine and human SSPCs significantly increased saliva formation, which reflected in vitro self-renewal ability of SSPC. More importantly, the CDC-EVs treatment enhanced saliva formation in culture post RT when compared to either vehicle control or treatment with fibroblast derived EVs. This effect appeared to be specific to SSPCs, as treatment with CDC-EVs did not enhance proliferation, migration and colony formation of head and neck cancer cells. Work are ongoing to evaluate CDC-EV’s ability to rescue saliva function in vivo when administered locally to the murine submandibular gland either before or after radiation treatment. Since EVs from other sources are being tested in clinical trials, it has the potential to rapidly translate to human study as a novel therapy for the management of radiation-induced xerostomia in HNC patients.

BACKGROUND

Xerostomia/Dry mouth in Head and Neck cancer patients

- HNC is the 6th most common type of Cancer
- Treatment primarily includes either surgery + radiotherapy (RT) or RT + chemotherapy
- Serious side effects such as Xerostomia is associated with RT treatment
- Loss of salivary function -> difficulty in chewing, eating, food digestion, dental decay, bone infection & deterioration of quality of life
- Our group has demonstrated the effect of specific growth factors and activator molecules in protecting salivary gland stem/progenitor cells (SSPCs) from the radiation damage

RESULTS

CDC derived EVs improves the sphere formation efficiency of mouse as well as human SSPCs

CDC derived EVs protect the murine primary as well as cultured SSPCs from the harmful effects of irradiation

CDC derived EVs treatment results in downregulation of pro-apoptotic genes in vitro

CDC derived EVs does not exert any influence on proliferation, migration and colony formation of HNC cells in vitro

SUMMARY OF KEY FINDINGS

Murine and human SSPCs can be isolated and maintained in culture in vitro

Figure 1: (A) Graph representing the cost per treatment versus effectiveness of current treatment modalities for Xerostomia (Sasportas et al, 2013); (B) Improvement in salivary function and acute side effects in human patients receiving CDC-EV treatment during RT (Xiao et al. 2014). (C) Effect of CDC-EVs on myocardi al inflammation model (Capricor Therapeutics, LA, Ibrahim et al. 2014)

Figure 2: (A) Representative scheme of isolation of murine SSPCs. (B) Representative images of murine salivary spheres in 2D and 3D culture at day 10. (C) FACs analysis of murine SSPCs grown in 2D culture showing maintenance of stem cell phenotype represented by EpCAM+CD20+ population

Figure 4: (A) Schematic showing in vitro irradiation of murine SSPCs with 15 Gy IR. The glands were isolated 7 days post irradiation and analyzed for proliferation. The graph represents the doubling time of murine SSPCs normalized to the vehicle control. (B) Relative mRNA expression of pro-apoptotic genes bax, puma and noxa in murine SSPCs analyzed 6 hours post exosome treatment in vitro (N=3). Error bars represent SEM

Figure 5: (A) Proliferation assay results of 5C200 and 5C223 cells post treatment with EVs and vehicle (N=3); (B) Colony formation assay showing 5C200 and 5C223 colonies at day 10 post EV treatment fixed and stained with crystal violet (N=3); (C) Graph displaying average of 5C200 cells migrated 24 hours post EV and vehicle treatment. Error bars represent SEM

REFERENCES:

CDCs-EVs show immunomodulatory capabilities in vitro and improve survival and clinical score in a GVHD mouse model

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**ABSTRACT**

Cardiosphere derived cells (CDCs) have immunomodulatory capabilities, limit fibrosis and promote tissue regeneration. Previous studies showed that extracellular vesicles from CDCs (CDC-EVs) recapitulate most of the activities mediated by CDCs both in vitro and in vivo. Here we show that:

- CDC-EVs modulate the immune response of in vitro activated T cells. The results suggest the implication of PD-L1/PD1 pathway in the immunomodulation observed.
- CDC-EVs upregulate the expression of anti-inflammatory genes in activated mouse macrophages in a dose dependent manner.
- Repeated systemic dosing of CDCs-EV in a GVHD model reverted the weight loss observed in vehicle treated mice, increased survival and reduced GVHD score.

**INTRODUCTION**

- CDCs are derived from heart tissue.
- CDCs improve cardiac function and exercise capabilities in a Duchenne muscular dystrophy (DMD) mouse model¹.
- 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.

**MATERIALS AND METHODS**

- EVs isolation from CDCs and MSCs.
  - CDCs: Cardiopulmonary perfusion of donor heart (OPO) followed by explant formation, Cardiosphere formation, followed by CDCs isolation.
  - MSCs: Isolation from bone marrow, followed by expansion and isolation.
- CDCs are derived from heart tissue.
- CDCs improve cardiac function and exercise capabilities in a Duchenne muscular dystrophy (DMD) mouse model¹.
- 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.

**RESULTS**

**CDCs-EVs immuno-modulatory activity on T-Cells**

**CDCs-EVs improve survival in a GVHD model**

**CONCLUSIONS**

- CDCs-EVs have strong immunomodulatory capabilities on human activated T-lymphocytes.
- EVs from CDCs showed a stronger upregulation of anti-inflammatory genes in activated mouse macrophages when compared to MSC.
- Repeat dosing of CDC-EVs is effective increasing survival in a GVHD mouse model and reducing GVHD score.
- These data support the use of CDC-EVs for the treatment of inflammatory indications.

**REFERENCES**