ABSTRACT ISEV 2017

Title: **Activity assays for evaluation of clinical grade MSC-EVs anti-inflammatory properties for use in treatment of drug-resistant epilepsy.**

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Introduction

MSCs exert their biological effects through secretion of extracellular vesicles (EV). We previously showed that MSC-EV have significant immunomodulatory properties. MSC-EV inhibit B cells proliferation/differentiation upon PBMC CpG stimulation, similarly to parent MSCs. Furthermore, MSC-EV induce T *reg* proliferation/apoptosis and IL-10 secretion, following antiCD3/CD28 PBMC stimulus. In this study we show that clinical grade (CG) EV exert similar immunomodulation to research grade (RG) counterparts. CG EV could be produced with higher efficiency if compared to RG EV and MSCs manufacturing. Currently our group is preparing MSC-derived EV for clinical tests in treatment of epilepsy, a disorder resistant to antiepileptic drugs in 40% of children due to neuroinflammation. A novel anti-inflammatory strategy, based on CG EV, is proposed.

Methods

A method of CG EV production is based on human umbilical cord derived (UC) MSCs cultured in a closed, scalable stirred-tank bioreactor system in fully defined GMP culture media. EVs are purified by sequential filtration/sterilization. The final product is analyzed by NTA to evaluate size and quantity, and EVs are characterized by MACSPlex immunophenotyping (FACS), to identify specific CD markers. The immunomodulatory activity of the CG product is evaluated in comparison with RG EVs and MSCs by specific in vitro B and T cells assays.

Results

The CG EV isolation method, has been optimized to obtain at least 1.5 * 10^9 EV/mL in 24 h from 0.1 * 10^6 MSCs. EV diameter cut off is 300 nm. MACSPlex exosome assay revealed that EV are CD9, CD63 and CD81 positive, but HLA-ABC and HLA-DRPQ negative. T and B potency assays, performed on PBMC, indicate immunosuppression by CG EV, similarly to the RG EV obtained from the same MSCs. This effect is revealed by T *reg* increase, counteracting T *eff*, upon T cells activation, and by reduction of B cells proliferation and plasma cell differentiation, following B cells activation.

Conclusions

We have developed and standardized a reproducible method for the production, quantification and immunophenotyping of CG EV, starting from human UC MSCs, with similar immunomodulation if compared to RG EV counterparts. Our data indicate that the use of CG EV could be effective in the treatment of a wide range of immunological diseases, and provide a more accessible alternative for allogenic MSCs.