EXOSOMES RELEASED BY MESENCHYMAL STEM CELL POPULATIONS PROMOTE DIFFERENTIATION AND MATURATION OF OLIGODENDROCYTES

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INTRODUCTION

MSCs and MSC-NPs

- Novel cell therapy-based treatments for MS aim to promote CNS repair and regeneration in order to halt or reverse the progression of established disability.
- Mesenchymal stem cells (MSCs) and MSC-derived neural progenitors (MSC-NPs) have been shown to promote neurological recovery in animal models of MS through both immunoregulatory and trophic mechanisms which alter the lesion environment to promote endogenous repair.
- Early clinical trials are underway to investigate the safety and efficacy of MSCs and MSC-NPs in MS patient populations.
- The specific mechanisms by which MSCs and MSC-NPs exert proximal influence on oligodendroglial and neuronal differentiation from progenitor populations remains unknown.

Exosomes

- Exosomes are microvesicles, 40-100 nm in diameter, secreted by all cell types, and are present in body fluids such as blood and CSF, as well as in the spent media of cultured cells.
- Exosomes contain a cargo of proteins, miRNAs and mRNAs, which are known to change with the disease state of the secreting tissue.
- Although their precise role remains elusive, exosomes are thought to be important for intercellular communication.

OBJECTIVE

To determine whether the release of exosomes (microvesicles) by MSCs and MSC-NPs can mediate trophic effects on oligodendrocyte differentiation from neural stem cells and glial progenitor cells.

DESIGN & METHODS

- Three MSC lines were isolated and expanded from bone marrow aspirates from MS patients; control MSC line (StemPro BM MSC) was purchased from Life Technologies
- After 3-4 days of culture with exosome-free FBS, cell supernates were collected and treated with Exoquick-TC (SBI) to precipitate endogenous exosomes
- Exosomes were identified and characterized by Western blotting, then added to cultures of rat neural stem cells (rNSCs) and glial progenitor cells (rGPCs) on day zero of differentiation. Differentiation was induced by growth factor withdrawal for 10(days (rNSC) or 3 days (rGPC).

RESULTS

Table 1. MS Donors from which bone marrow MSCs were derived. All MSC cell lines were characterized by growth rate, cell surface marker expression, and adipogenic/osteogenic differentiation potential and found to be similar to control MSCs (StemPro).

Patient ID	Gender	Age	MS Subtype	EDSS
112-SF	M	39	SPMS	7.0
076-FF	M	44	PPMS	2.5
147-MB	M	46	SPMS	6.5

Figure 1. Western blot analysis. After precipitation, the presence of exosomes was confirmed by western blot for exosome surface markers, including Hsp70 and Flotillin-1

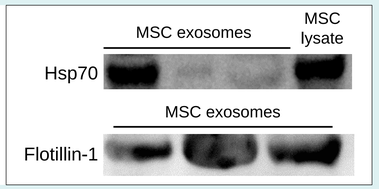
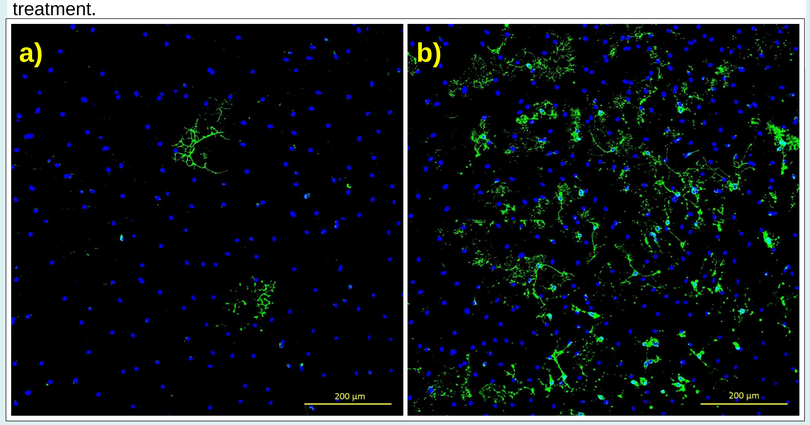
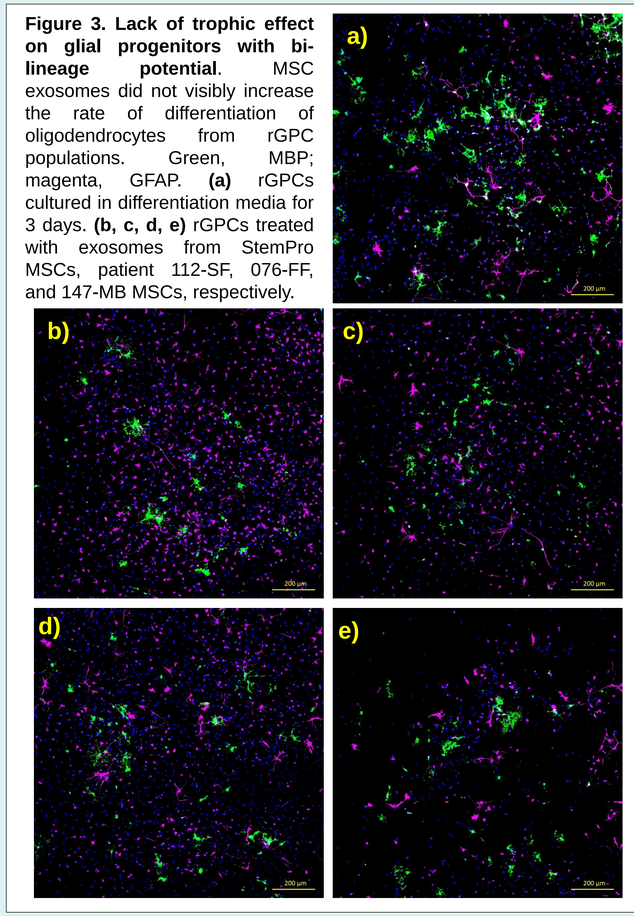


Figure 2. MSC-exosomes exert a trophic effect on oligodendroglial differentiation from neural stem cells with tri-lineage potential. StemPro MSC-derived exosomes were added to cultures of rNSCs on day 0 of differentiation. After 10 days in culture, immunocytochemistry was performed, and mature oligodendrocytes were detected by staining for galactosylceramidase (GALC, green). **(a)** Spontaneous differentiation of rNSCs after growth factor withdrawal. **(b)** Oligodendroglial differentiation increases with MSC-derived exosomes treatment.



RESULTS

Figure 3. Lack of trophic effect on glial progenitors with bi-lineage potential. MSC exosomes did not visibly increase the rate of differentiation of oligodendrocytes from rGPC populations. Green, MBP; magenta, GFAP. **(a)** rGPCs cultured in differentiation media for 3 days. **(b, c, d, e)** rGPCs treated with exosomes from StemPro MSCs, patient 112-SF, 076-FF, and 147-MB MSCs, respectively.



CONCLUSIONS

- **The trophic effects of MSC/MSC-NPs on oligodendroglial cells may be mediated by exosome release**
- **Optimization of exosome release by MSC/MSC-NPs may have therapeutic implications in influencing the rate of CNS repair and remyelination and in developing cell-free treatments in MS**
- **Preliminary results suggest that MSC-derived exosomes exert their trophic effect at the neural stem cell stage rather than the glial progenitor stage of differentiation.**

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