Human mesenchymal stem cell-derived neural progenitors (MSC-NPs) exhibit trophic properties that may influence repair in MS

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INTRODUCTION

Multiple sclerosis (MS) is associated with irreversible disability in a significant proportion of patients. At present, there is no treatment to halt or reverse the progression of established disability. In an effort to develop cell therapy-based strategies for progressive MS, we have investigated bone marrow mesenchymal stem cell-derived neural progenitors (MSC-NPs) as an autologous source of stem cells with neural progenitor and immunoregulatory properties. Previously, we found that MSC-NPs were found to promote neurological recovery after intrathecal injection into mice with chronic experimental autoimmune encephalomyelitis (EAE). Injected MSC-NPs migrated to lesion areas where they were associated with reduced demyelination and immune cell infiltration. Although the mechanism of action of MSC-NPs in the CNS is unknown, we hypothesize that immunoregulatory and/or trophic properties of MSC-NPs may influence the lesion environment to promote endogenous repair mechanisms.

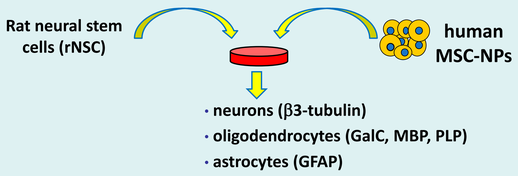
OBJECTIVE

In the current study, we tested whether MSC-NPs express and secrete bioactive factors that might exert trophic effects on host progenitors.

DESIGN AND METHODS

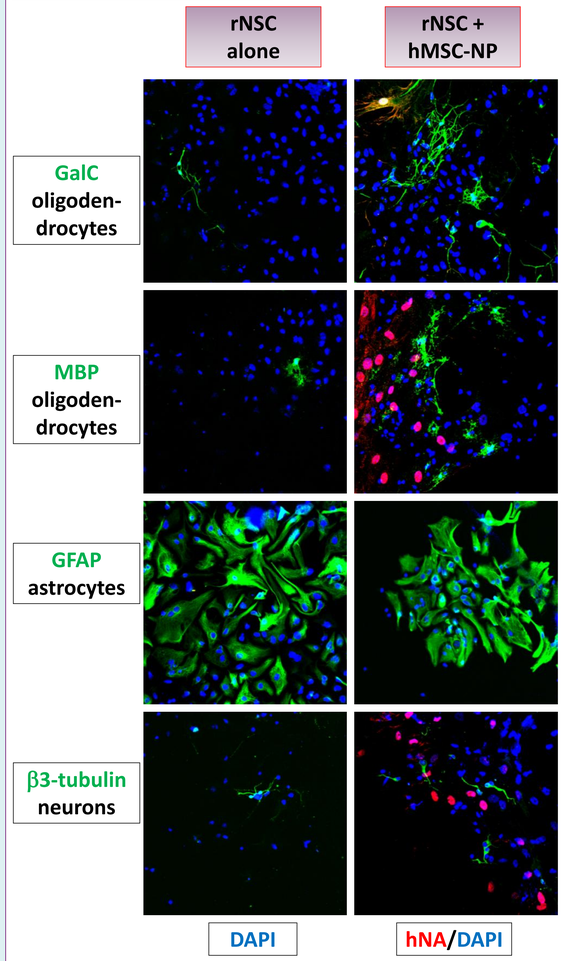
MSC-NPs were isolated and characterized from a panel of bone marrow samples from both control and MS patients. MSCs were first expanded by standard methods. MSC-NPs were derived by culturing MSCs in neural progenitor maintenance media (+EGF/bFGF) for three weeks. All cells were characterized by flow cytometry and gene expression.

To study their in vitro trophic properties, MSC-NPs were co-cultured along with rat brain-derived neural stem cells (rNSCs, Stem Cell Technologies), which differentiate into oligodendrocytes, neurons, and astrocytes upon growth factor withdrawal (see diagram below). Differentiation was assayed by immunocytochemistry and quantitative PCR, and secreted factors were measured by Luminex or ELISA. In vivo trophic effects of MSC-NPs were tested by injecting intrathecally into EAE mice during the chronic phase of EAE. Levels of endogenous progenitors were analyzed by immunofluorescence of Nestin positive sites in fixed spinal cord tissue.



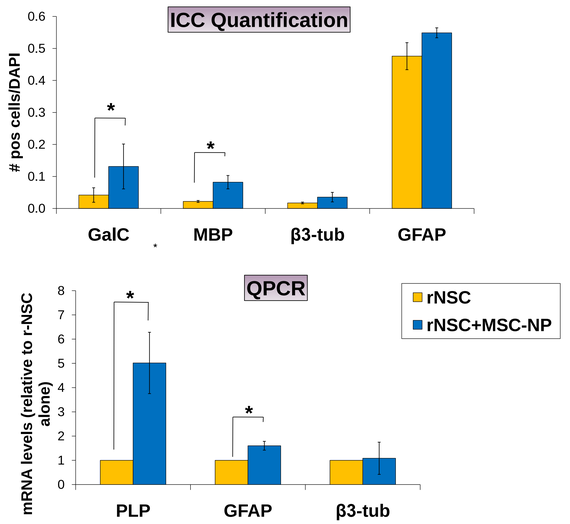
RESULTS

Figure 1. MSC-NP co-culture promotes oligodendroglial differentiation from rNSCs.



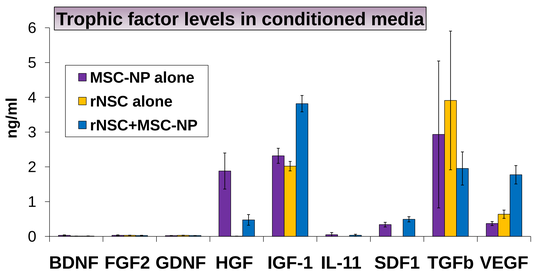
➢ ***Co-culture with MSC-NPs results in increased number of GalC+ and MBP+ oligodendrocytes in differentiating rNSCs.***
➢ ***MSC-NPs have no apparent effect on the number of astrocytes or neurons.***

Figure 2. Quantification of trophic effects of MSC-NPs in rNSC co-cultures



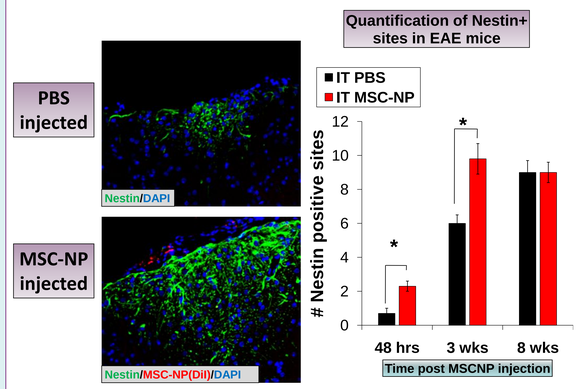
➢ ***Quantization of ICC combined from two separate co-culture experiments showed a significant increase in the number of oligodendrocytes, and no difference in neurons or astrocytes.***
➢ ***MSC-NP co-culture resulted in a significant increase in PLP mRNA expression in rNSCs, along with a slight increase in GFAP.***
➢ ***These results confirm that MSC-NPs promote oligodendroglial differentiation from rNSCs.***

Figure 3. Levels of candidate trophic factors present in conditioned media of co-cultures



➢ ***Hepatocyte growth factor (HGF) and SDF1 (stromal derived factor-1) were secreted at significant levels by MSC-NPs, both alone or when co-cultured with rNSCs, and were not detected in rNSCs alone. Levels of both factors correlated with trophic effects on rNSCs.***
➢ ***IGF-1, TGFb, and VEGF were secreted by both MSC-NPs and rNSCs. Levels of IGF1 and VEGF increased additively in the co-culture, correlating with increased oligodendrocyte differentiation.***

Figure 4. Intrathecal injection of MSC-NPs into EAE mice increases the number of Nestin+ sites in the spinal cord.



➢ ***Endogenous Nestin+ progenitors were increased in the spinal cords of mice during EAE.***
➢ ***We noted a significant increase in the number of Nestin+ sites in MSC-NP-injected mice, which correlated with amelioration of disease (data not shown).***
➢ ***The MSC-NP-mediated increase in Nestin+ sites was transient, returning to same levels as PBS controls 8 weeks after injection.***
➢ ***These results suggests that intrathecally-injected MSC-NPs may have trophic effects on endogenous progenitors, thereby increasing the rate of CNS repair.***

CONCLUSIONS

- **MSC-NPs promote oligodendrocyte differentiation and maturation from brain derived NSCs.**
- **Candidate trophic mediators secreted by MSC-NPs include HGF, IGF-1, SDF1, and VEGF.**
- **Intrathecal injections of MSC-NPs during chronic EAE results in transient increase in the number of endogenous progenitor cells in the spinal cord.**
- **These results suggest that MSC-NPs may influence the rate of repair through trophic effects on host progenitors in the brain and spinal cord.**
- **This study supports the use of autologous MSC-NPs in MS patients as a means of promoting CNS repair.**

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