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Research Report

Bone marrow mesenchymal stem cells promote cell proliferation and neurotrophic function of Schwann cells in vitro and in vivo

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ABSTRACT

The use of bone marrow-derived mesenchymal stem cells (MSCs) in nerve tissue engineering leads to an improved functional outcome of peripheral nerve repair. Schwann cells (SCs) are primary structural and functional cells in peripheral nervous system and play a crucial role in peripheral nerve regeneration. We hypothesize that MSCs promote peripheral nerve regeneration not only via their direct release of neurotrophic factors, but through indirect modulation of cellular behaviors of SCs. To test this hypothesis we investigated the influences of MSCs on proliferation of and neurotrophic factor expression by SCs using an in vitro co-culture model and an in vivo system of rat sciatic nerve regeneration. The data from cell viability assay and flow cytometry, bromodeoxyuridine/Hoechst 33342 double staining, immunocyto/histochemistry, RT-PCR and quantitative real-time RT-PCR, as well as Western blot analysis collectively confirmed the effects of MSCs on the biological characteristics of SCs, especially during the period of peripheral nerve regeneration. Our results help to elucidate the mechanisms by which MSCs function as a cell therapy agent in peripheral nerve repair.

1. Introduction

Bone marrow mesenchymal stem cells (MSCs) are pluripotent stem cells that localize in the stromal compartment of the bone marrow, where they support hematopoiesis and differentiate into mesenchymal lineages (Abdallah and Kassem, 2008; Phinney and Prockop, 2007; Bianco et al., 2001; Deryugina and Muller-Sieburg, 1993; Johnson and Dorshkind, 1986). Since they were first identified by the pioneering work of Friedenstein et al. (1968, 1970), MSCs have attracted much research interest owing to their potential use in cell-based therapies for various disorders including neural injury and degeneration.

In peripheral nerve repair, bridging large neural gaps with autologous nerve grafts remains the gold standard of therapy despite its inherent drawbacks that are difficult to overcome. Great efforts have been devoted to the development of tissue-engineered nerve grafts as a promising alternative to autologous nerve grafts (Evans, 2000; Navarro et al., 2001). Such artificial grafts are typically composed of a structural element named nerve conduit, and an internal matrix serving as a scaffold for inclusion of cellular implants, cytokines, and/or

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growth factors. Recently, introduction of MSCs into nerve graft scaffolds represents a novel approach of constructing tissue-engineered nerve grafts. A number of basic and clinical trials in rodent, primate and human models provide accumulating evidence that functional outcome of peripheral nerve repair is improved by MSC-based nerve tissue engineering (Lu et al., 2008; Wang et al., 2008; Chen et al., 2006a; Dezawa et al., 2005). More intriguingly, the autologous origin of MSCs eliminates the risk of immune rejection, and the adult cell features of MSCs reduce the possibility of tumorigenesis (Brehm et al., 2002; Prockop and Petrie, 2000). These considerations further increase the significance of MSC implantation in peripheral nerve regeneration.

In order to exploit MSC-based therapies in a responsible and safe manner, it is required to determine how the implanted MSCs encourage peripheral nerve regeneration. To date, despite many explanations that can be found in the literature, experimental evidence is still lacking to support a convincing conclusion about the mechanisms by which MSC-based therapy works. For example, MSCs have been shown to be induced in vitro to differentiate into neural lineages including neurons, astrocytes, oligodendrocytes, microglia and Schwann cells (SCs)-like (Lu et al., 2008; Chen et al., 2006a, b; Munoz-Elias et al., 2003; Suzuki et al., 2004; Wislet-Gendebien et al., 2005; Woodbury et al., 2000), and the in vivo experiments have also reported that the implanted MSCs generate neural phenotypes specific to the lesion site (Coyne et al., 2006; Lu et al., 2006; Kocsis et al., 2002; Kopen et al., 1999). These studies lead to a transdifferentiation mechanism of MSCs, which, however, are also believed to be due to spontaneous fusion of MSCs with host cells rather than real transdifferentiation (Weimann et al., 2003a and b). Otherwise, an alternative explanation about the mechanisms holds that MSCs, behaving as small molecular factories, produce many different cytokines and growth factors that may positively impact neural cell survival and neuritogenesis (Caplan and Dennis, 2006; Liu and Hwang, 2005; Neuhuber et al., 2005; Zhong et al., 2003; Chen et al., 2002; Chopp and Li, 2002).

Considering that SCs are primary structural and functional cells in peripheral nervous system and play a crucial role in peripheral nerve regeneration, we hypothesize that MSCs might exert their efficacy not only via direct release by themselves of growth factors, chemokines and cytokines, but through indirect modulation of the cellular behavior of SCs. To test this hypothesis we investigated the influences of MSCs on proliferation and neurotrophic function of SCs using an in vitro co-culture model and an in vivo system of rat peripheral nerve regeneration.

2. Results

2.1. *In vitro co-culture of SCs and MSCs*

To determine whether an interaction between MSCs and SCs could take place, we first investigated in vitro effects of rat MSCs on rat SC proliferation. As shown by labeling with Hoechst 33342, SCs co-cultured with MSCs exhibited an increase in their proliferation as compared to SCs cultured alone. The average number of SCs was counted by Hoechst labeling, and plotted against culture/co-culture time (Fig. 1a). From the resulting cell proliferation curve, the cell doubling time for SCs co-cultured with and without MSCs was calculated to be 3±0.22 and 8±0.64 d (mean±SD) respectively. MTT assay indicated that the cell viability of SCs treated with MSC-conditioned medium for 24 and 48 h was significantly higher than that treated with plain medium for 24 and 48 h, respectively (Fig. 1b). BrdU immunostaining revealed that DNA synthesis and cell proliferation rate of SCs co-cultured with MSCs for 48 h were significantly higher than that cultured alone for 48 h. Flow cytometric analysis on cell cycle provided further evidence that MSCs encouraged the cell proliferation of SCs (Figs. 1c–e). Collectively, the data suggest the favorable effects of MSCs on the survival and proliferation of SCs in vitro.

Next, we investigated in vitro influences of rat MSCs on expression of various trophic factors or receptors in rat SCs. RT-PCR and Western blot analysis showed that expressions at the mRNA or protein levels of nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), high-affinity NGF receptor (TrkA) or low affinity NGF receptor (LNGFR) in SCs co-cultured with MSCs for 48 h were significantly higher than those in SCs cultured alone for 48 h, respectively (Figs. 2a and b). Immunocytochemistry shared the consistent results with Western blot analysis, confirming in vitro enhancing effects of MSCs on expression of the above molecules in SCs (Fig. 2c).

2.2. *In vivo interaction of SCs with MSCs in the regenerating nerve*

To further determine the cell-interaction between MSCs and SCs in vivo, we investigated the process of peripheral nerve regeneration after bridging a 10-mm-long neural gap in rat sciatic nerve with a nerve graft into which rat MSCs (for MSC group) or IMDM medium (for control group) was administered respectively. Rat sciatic nerve injuries induced a great cascade of cellular and molecular events in the nerve stumps. We examined the regenerating nerve by immunohistochemistry against neurofilament heavy (NF-H) or S100. At 3 d post nerve grafting, nerve regeneration demonstrated few differences between two groups. At 7 and 14 d after nerve grafting, poor regeneration was still seen in control group, while in MSC group modest axonal growth into nerve conduits was notable and larger numbers of SCs were found to be in close contact with axons. This observation implied that administration of MSCs into nerve conduits might stimulate the generation of SCs (Fig. 3a). Furthermore, quantitative real-time RT-PCR was accomplished to measure the mRNA level of growth-associated protein-43 (GAP43), NF-H, NGF, BDNF, TrkA or LNGFR in regenerating nerves. The mRNA levels of these molecules for MSC group were noted to increase in a time-dependent manner. At 7 d after nerve grafting, a significant difference in the mRNA level of GAP43 or LNGFR appeared between MSC group and control group, while at 7 and 14 d after nerve grafting, a significant difference in the mRNA level of NF-H, NGF, BDNF, or TrkA appeared between MSC group and control group (Fig. 3b). These data suggest that