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Research Report

Down-regulation of Nogo-A by collagen scaffolds impregnated with bone marrow stromal cell treatment after traumatic brain injury promotes axonal regeneration in rats

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ABSTRACT

Nogo-A is a major form of growth inhibitory molecule (growth-IM) which inhibits axonal regeneration and neurite regrowth after neural injury. Bone marrow stromal cells (MSCs) have been shown to inhibit Nogo-A expression in vitro and in cerebral ischemic animal models. The present study was designed to investigate the effects of treatment with human MSCs (hMSCs) impregnated into collagen scaffolds on the expression of Nogo-A and axonal plasticity after traumatic brain injury (TBI). Adult male Wistar rats were injured with controlled cortical impact and treated either with saline, hMSCs-alone or hMSCs impregnated into collagen scaffolds (scaffold+hMSC) transplanted into the lesion cavity 7 days after TBI. Rats were sacrificed 14 days after TBI and brain tissues were harvested for immunohistochemical studies, Western blot analysis, laser capture microdissections and qRT-PCR to evaluate axonal density and Nogo-A protein and gene expressions. Our data showed that treatment of TBI with scaffold+hMSC significantly decreased TBI-induced Nogo-A protein expression and increased axonal density compared to saline and hMSC-alone treatments. In addition, scaffold+hMSC transplantation decreased Nogo-A transcription in oligodendrocytes after TBI. Scaffold+hMSC treatment was superior to hMSC-alone treatment in suppressing Nogo-A expression and enhancing axonal regeneration after TBI.

Abbreviations: CNS, central nervous system; ECM, extracellular matrix; Growth-IMs, growth inhibitory molecules; MSCs, marrow stromal cells; LCM, laser capture microdissection; MAG, myelin associated glycoproteins; OMgp, oligodendrocytic myelin glycoprotein; PNS, peripheral nervous system

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Our data suggest that transplanting hMSCs with scaffolds down-regulates Nogo-A transcription and protein expression which may partially contribute to the enhanced axonal regeneration after TBI.

1. Introduction

The neurons in the adult central nervous system (CNS) fail to regenerate their axons after an injury. In contrast, neurons in the peripheral nervous system (PNS) as well as neurons in lower-vertebrate CNS can survive an injury and regenerate their axons leading to functional recovery (Goldberg and Barres, 2000). However, it has been observed that many types of CNS neurons are able to regenerate their axons through fragments of peripheral nerve grafts after injury (David and Aguayo, 1981), suggesting that CNS neurons can regenerate if provided with a facilitatory milieu. The significance of the extrinsic environment in promotion or inhibition of neurite outgrowth is well recognized (Bovolenta and Fernaud-Espinosa, 2000; Condic and Lemons, 2002). In the PNS as well as olfactory tract the presence of agents such as laminin and heparin sulfate proteoglycans in extracellular matrix (ECM) is able to promote axon growth (Bovolenta and Fernaud-Espinosa, 2000). However, the same agents fail to induce neurite regeneration in the CNS because of the presence of competing growth inhibitory molecules (growth-IMs) (Condic and Lemons, 2002). Growth-IMs have been identified both in myelin surrounding the CNS axons as well as in the glial scar which forms at the injury site (Properzi et al., 2003). Myelin contains growth-IMs such as Nogo-A, myelin-associated glycoproteins (MAG) and oligodendrocytic myelin glycoprotein (OMgp) (Lee et al., 2003). These growth-IMs are exposed to regenerating axons following disruption of myelin due to injury (Goldberg and Barres, 2000). The discovery and characterization of growth-IMs were recognized as a major breakthrough in our understanding of neuroplasticity (Buchli and Schwab, 2005), and growth-IMs are potential treatment targets whose suppression may be used to enhance neural restoration. Nogo-A was the first growth-IM to be described and the most well-studied to date (Buchli and Schwab, 2005; Caroni and Schwab, 1988a; Lenzlinger et al., 2005). Our present research focuses on studying the effects of treatment of human bone marrow stromal cells (hMSCs) on inhibiting Nogo-A to promote axonal regeneration after traumatic brain injury (TBI).

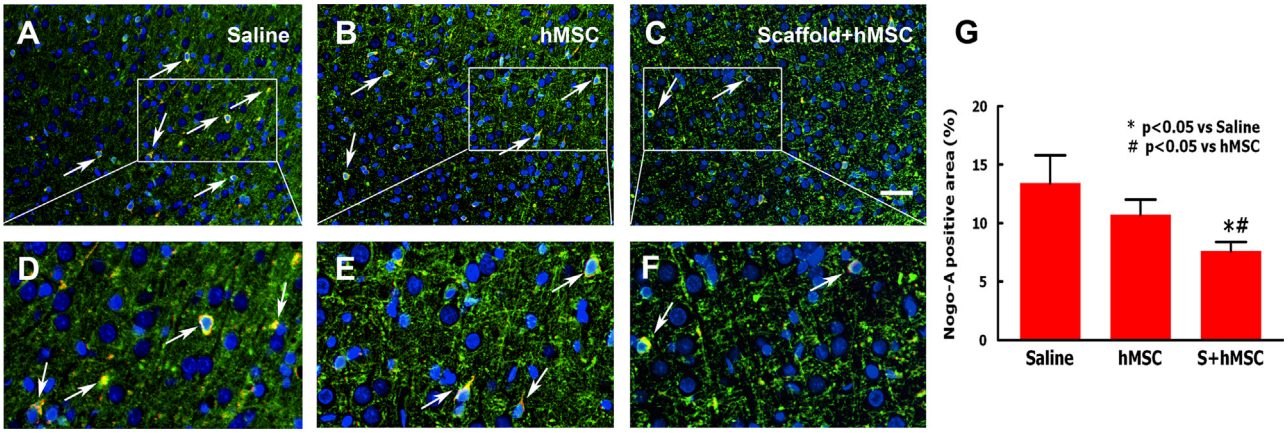

Fig. 1 – Nogo-A expression in the lesion boundary zone. Double immunostaining with CNPase (green) and Nogo-A (red) shows the Nogo-A expression by oligodendrocytes (A–C). Enlarged photomicrographs (D–F) show colocalized Nogo-A/CNPase signals (yellow, arrows). Quantitative data of immunostainings (G) demonstrate the effect of scaffold+hMSC treatment on Nogo-A expression ($n=8$). Data represent mean±SD. Scale bar=50 μm, $^{*}P<0.05$, vs. saline group; $^{\#}P<0.05$ vs. hMSC-alone group.

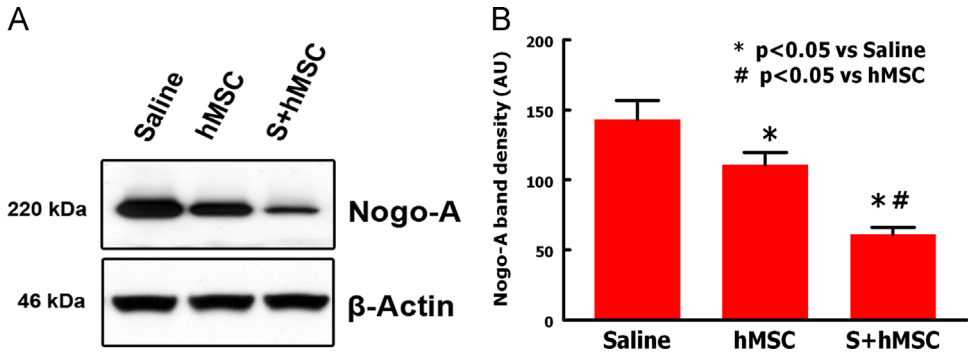

Fig. 2 – Representative Western blot (A) and densitometry measurement (B) of Nogo-A in the injured area confirmed the effect of scaffold+hMSC treatment ($n=4$). Data represent mean±SD. $^{*}P<0.05$, vs. saline group; $^{\#}P<0.05$ vs. hMSC-alone group.