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Regular Article

Neural progenitor cell transplantation promotes neuroprotection, enhances hippocampal neurogenesis, and improves cognitive outcomes after traumatic brain injury

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ABSTRACT

Transplantation of neural progenitor cells (NPCs) may be a potential treatment strategy for traumatic brain injury (TBI) due to their intrinsic advantages, including the secretion of neurotrophins. Neurotrophins are critical for neuronal survival and repair, but their clinical use is limited. In this study, we hypothesized that pericontusional transplantation of NPCs genetically modified to secrete a synthetic, human multineurotrophin (MNTS1) would overcome some of the limitations of traditional neurotrophin therapy. MNTS1 is a multifunctional neurotrophin that binds all three tropomyosin-related kinase (Trk) receptors, recapitulating the prosurvival activity of 3 endogenous mature neurotrophins. NPCs obtained from rat fetuses at E15 were transduced with lentiviral vectors containing MNTS1 and GFP constructs (MNTS1-NPCs) or fluorescent constructs alone (control GFP-NPCs). Adult rats received fluid percussion-induced TBI or sham surgery. Animals were transplanted 1 week later with control GFP-NPCs, MNTS1-NPCs, or injected with saline (vehicle). At five weeks, animals were evaluated for hippocampal-dependent spatial memory. Six weeks post-surgery, we observed significant survival and neuronal differentiation of MNTS1-NPCs and injury-activated tropism toward contused regions. NPCs displayed processes that extended into several remote structures, including the hippocampus and contralateral cortex. Both GFP- and MNTS1-NPCs conferred significant preservation of pericontusional host tissues and enhanced hippocampal neurogenesis. NPC transplantation improved spatial memory capacity on the Morris water maze (MWM) task. Transplant recipients exhibited escape latencies approximately half that of injured vehicle controls. While we observed greater transplant survival and neuronal differentiation of MNTS1-NPCs, our collective findings suggest that MNTS1 may be superfluous in terms of preserving the cytoarchitecture and rescuing behavioral deficits given the lack of significant difference between MNTS1- and GFP-control transplanted groups. Nevertheless, our overall findings support the potential of syngeneic NPC transplantation to enhance endogenous neuroreparative responses and may therefore be an effective treatment for TBI.

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Introduction

Traumatic brain injury (TBI) is a significant global health problem that often results in long-lasting impairment. Functional deficits result from a combination of pathological events that include selective neuronal loss, damage to the microenvironment, as well as decreased levels of hippocampal neurogenesis, which is crucial for hippocampal-dependent memory (Yu et al., 2008; Zhao et al., 2008).

Neurotrophins contribute to the functional integrity of the CNS through regulation of neuronal survival, differentiation, repair, neurite outgrowth, synaptic plasticity, and apoptosis (Chao, 2003). Each mature neurotrophin has a cognate Trk receptor. Through these specific interactions, neurotrophin–Trk signaling increases the expression of survival-promoting genes, prodifferentiation genes, and other substrates involved in synaptic plasticity (Reichardt, 2006). Neurotrophin–Trk interactions have clinical potential due to intrinsic neurorestorative activity. However, there are some limitations to using neurotrophins therapeutically, such as short half-lives, negligible blood brain barrier permeability, and limited diffusion in CNS parenchyma (Lessmann et al., 2003). Furthermore, cells express Trk receptors differentially and thus may only be responsive to cognate neurotrophins. The generation of multitargeting neurotrophins with broader binding specificities may therefore be an effective treatment for promoting protection and recovery after TBI.

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NPCs possess tropic properties, maintain multipotency, and can be genetically modified to deliver potentially therapeutic molecules (Gage and Temple, 2013). Transplanted NPCs can integrate within existing host circuitry, provide and provoke trophic support, and modulate host immune responses (Cossetti et al., 2012). Important to this study, NPC-mediated trophic secretion can mobilize endogenous stem cells and enhance neuroregenerative responses, such as hippocampal neurogenesis, within the injured milieu (Shetty, 2014).

Abbreviations

BrdU	5-bromo-2′-deoxyuridine
FdUrd	5-fluoro-2′-deoxyuridine
ALDH1L1	aldehyde dehydrogenase 1, member L1
bFGF	basic fibroblast growth factor
BFP	blue fluorescent protein
CsA	cyclosporine A
DG	dentate gyrus
DCX	doublecortin
ELISA	enzyme-linked immunosorbent assay
EGF	epidermal growth factor
GFAP	glial fibrillary acidic protein
GFP	green fluorescent protein
HBSS	Hank's balanced salt solution
H&E	hematoxylin and eosin
IP	intraperitoneal
Iba1	ionized calcium-binding adapter molecule 1
MABP	mean arterial blood pressure
MWM	Morris water maze
MNTS1	multineurotrophin
NPCs	neural progenitor cells
NeuN	neuronal nuclei (for immunohistochemistry)
SGZ	subgranular zone
TBI	traumatic brain injury
Trk	tropomyosin-related kinase
ANOVA	analysis of variance
SEM	standard error of the mean
SNK	Student–Newman–Keuls

Hippocampal neurogenesis occurs continually throughout the life of most mammals (Ming and Song, 2011). Active neural stem cells (NSCs) residing in the subgranular zone (SGZ) of the adult dentate gyrus (DG) give rise to doublecortin (DCX)-positive immature neurons, which make unique contributions to specific aspects of hippocampal-dependent function, including spatial memory (Deng et al., 2010). CNS injury induces acute neurogenic responses, which have been shown to contribute to some degree of recovery after TBI (Blaiss et al., 2011). However, this endogenous neuroreparative response is insufficient as residual cognitive deficits persist. Therapeutic strategies that enhance endogenous neuroreparative responses may augment recovery processes and remain a critical area of study.

We sought to enhance the inherent salutary effects of NPCs through genetic modification. We investigated the potential benefits of transplanting NPCs that were transduced to continually secrete MNTS1, a multineurotrophin with multiple neurotrophic specificities. Through the exchange and mutation of 8 amino acid residues on mature human NT-3, Urfer et al. (1994) generated a human multifunctional, multitargeting molecule that retains the capacity to bind all Trk receptors and supports the survival of NGF-, BDNF-, and NT-3-responsive neurons.

The objective of this study was to assess histopathological and functional outcomes with transplantation of control NPCs and MNTS1-expressing NPCs in an experimental model of TBI. We hypothesized that engaging all Trk signaling cascades via MNTS1, together with the intrinsic advantages of NPC transplantation, would result in significant rescue of neuropathological outcomes and augmentation of endogenous reparative responses after moderate TBI.

Materials and methods

Construction of the MNTS1 lentivirus

The MNTS1 molecule was generated via selective point mutations and amino acid residue exchanges on a mature human NT-3 backbone as discussed previously (Urfer et al., 1994). MNTS1 complementary DNA was produced synthetically by GeneArt® Gene Synthesis (Life Technologies, Carlsbad, CA) and subcloned into the lentiviral vector pRRLsinPPT-CMV-MCS-WPRE as previously described (Dull et al., 1998). The lentiviral particles were generated by the Miami Project Viral Vector Core of University of Miami Miller School of Medicine. Lentiviruses were produced using the four-plasmid method (Follenzi and Naldini, 2002). Viral titers, shown as transducing units from 10^7 to 10^8, were determined by a p24 enzyme-linked immunosorbent assay (ELISA; Perkin Elmer, Waltham, MA) used to quantify p24 core protein concentrations. Purified lentiviral particles were stored at −80 °C until use.

Isolation and preparation of neural progenitor cells

NPCs were isolated from Sprague Dawley rat fetuses at embryonic stage E15. Frontal cortical tissue was microdissected in Lebovitz's L-15 medium (GIBCO/Life Technologies, Carlsbad, CA). Cortical tissue was transferred to a conical tube, titrated to obtain a single-cell solution, and counted using trypan blue (Life Technologies). Between 600,000 and 700,000 cells were placed on sterile, polystyrene 10 cm tissue culture dishes (Corning, Corning, NY) in NeuralCult proliferation medium solution (StemCell Technologies, Vancouver, BC) that was selective for neurospheres and supplemented with penicillin–streptomycin, 10 μg/ml of basic fibroblast growth factor (bFGF), 10 μg/ml epidermal growth factor (EGF), and 0.2% Heparin. Two days later, neurospheres were infected with lentiviral vectors containing MNTS1 and GFP (pLV-eGFP) constructs, or GFP and blue fluorescent protein (BFP; pLV-EBFP2-nuc) constructs, and 2–3 ml of fresh medium was added to dishes. After two days of proliferation, NPCs were assessed for positive lentiviral transduction and cell viability.

If infection was positive, cells were plated on 10 cm fibronectin (Sigma-Aldrich, St. Louis, MO)-coated tissue culture dishes and allowed to adhere and colonize for an additional 1–2 days. On the day of transplantation, NPCs were trypsinized with Hank's Balanced Salt Solution (HBSS; GIBCO/Life Technologies, Carlsbad, CA) and centrifuged at 2000 rpm for 4.5 min. Medium was carefully removed and pellet was resuspended in fresh medium. Cells were assessed for viability and counted using trypan blue in a hemacytometer, followed by microcentrifugation at 6500 rpm for 1.5 min. One million cells were resuspended in 10 μl of fresh medium (for a final volume of 100,000/μl) and kept on ice until transplantation (<30 min).

Animals

Adult male Sprague Dawley rats (n = 58) were randomly assigned to 1 of 6 groups: sham/vehicle (n = 12), sham/GFP-NPCs (n = 8), sham/MNTS1-NPCs (n = 7), TBI/vehicle (n = 15), TBI/GFP-NPCs (n = 8), or TBI/MNTS1-NPCs (n = 8). Animal care was in accordance with the guidelines set forth by the University of Miami Animal Care and Use Committee and the NIH *Guide for the Care and Use of Laboratory Animals*. Animals were housed in a temperature-controlled room (22 °C) with a 12-h light/dark cycle and allowed at least 7 days of acclimation before undergoing any experimentation. All animals had access to food and water ad libitum, except for a 24 h fast before the surgical procedure in order to maintain consistent glucose levels.

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