Biomaterials 109 (2016) 40–54

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

Transplantation of tissue engineering neural network and formation of neuronal relay into the transected rat spinal cord

Bi-Qin Lai [a], Ming-Tian Che [a], Bao-Ling Du [b], Xiang Zeng [a], Yuan-Huan Ma [a], Bo Feng [a], Xue-Chen Qiu [a], Ke Zhang [b], Shu Liu [f], Hui-Yong Shen [c, g], Jin-Lang Wu [h], Eng-Ang Ling [i], Yuan-Shan Zeng [a, b, c, d, e, *]

[a] *Key Laboratory for Stem Cells and Tissue Engineering (Sun Yat-sen University), Ministry of Education, Guangzhou, 510080, China*
[b] *Department of Histology and Embryology, Zhongshan School of Medicine, Sun Yat-sen University, Guangzhou, 510080, China*
[c] *Institute of Spinal Cord Injury, Sun Yat-sen University, Guangzhou, 510120, China*
[d] *Co-innovation Center of Neuroregeneration, Nantong University, Nantong, 226001, China*
[e] *Guangdong Provincial Key Laboratory of Brain Function and Disease, Zhongshan School of Medicine, Sun Yat-sen University, Guangzhou, 510080, China*
[f] *Department of Human Anatomy, Zhongshan School of Medicine, Sun Yat-sen University, Guangzhou, 510080, China*
[g] *Department of Orthopedics of Sun Yat-sen Memorial Hospital, Sun Yat-sen University, Guangzhou, 510120, China*
[h] *Department of Electron Microscope, Zhongshan School of Medicine, Sun Yat-sen University, Guangzhou, 510080, China*
[i] *Department of Anatomy, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, 117597, Singapore*

A R T I C L E I N F O

Article history:
Received 27 May 2016
Received in revised form
13 July 2016
Accepted 2 August 2016
Available online 4 August 2016

Keywords:
Neurotrophin-3
TrkC pathway
Synaptogenesis
Tissue engineering neural network
Neuronal relay
Spinal cord injury

A B S T R A C T

Severe spinal cord injury (SCI) causes loss of neural connectivity and permanent functional deficits. Re-establishment of new neuronal relay circuits after SCI is therefore of paramount importance. The present study tested our hypothesis if co-culture of neurotrophin-3 (NT-3) gene-modified Schwann cells (SCs, NT-3-SCs) and TrkC (NT-3 receptor) gene-modified neural stem cells (NSCs, TrkC-NSCs) in a gelatin sponge scaffold could construct a tissue engineering neural network for re-establishing an anatomical neuronal relay after rat spinal cord transection. Eight weeks after transplantation, the neural network created a favorable microenvironment for axonal regeneration and for survival and synaptogenesis of NSC-derived neurons. Biotin conjugates of cholera toxin B subunit (b-CTB, a transneuronal tracer) was injected into the crushed sciatic nerve to label spinal cord neurons. Remarkably, not only ascending and descending nerve fibers, but also propriospinal neurons, made contacts with b-CTB positive NSC-derived neurons. Moreover, b-CTB positive NSC-derived neurons extended their axons making contacts with the motor neurons located in areas caudal to the injury/graft site of spinal cord. Further study showed that NT-3/TrkC interactions activated the PI3K/AKT/mTOR pathway and PI3K/AKT/CREB pathway affecting synaptogenesis of NSC-derived neurons. Together, our findings suggest that NT-3-mediated TrkC signaling plays an essential role in constructing a tissue engineering neural network thus representing a promising avenue for effective exogenous neuronal relay-based treatment for SCI.

© 2016 Elsevier Ltd. All rights reserved.

1. Introduction

Severe spinal cord injury (SCI) causes ongoing cell death, axonal loss and demyelination, and disrupts neuronal connections between brain and periphery, resulting in a devastating loss of function [1,2]. Endogenous nerve regeneration fails after SCI due to multiple factors, including extrinsic signaling molecules and intrinsic growth state of injured neurons [2,3]. The pathophysiological features and the dearth of available treatment options for severe SCI have made it an attractive target for exploring of potential exogenous neuronal relay-based therapies to repair injured spinal cord. Therefore, therapeutic strategies to replace loss neurons and re-establish new neuronal relay circuits after SCI are urgently needed [4,5].

It is encouraging that neuron-based transplantation has made remarkable progress in the past 20 years in creating a novel neuronal relay between injured axons and denervated targets [6,7].

* Corresponding author. Department of Histology and Embryology, Zhongshan School of Medicine, Sun Yat-sen University, 74# Zhongshan 2nd Road, Guangzhou, 510080, China.

E-mail address: zengysh@mail.sysu.edu.cn (Y.-S. Zeng).

http://dx.doi.org/10.1016/j.biomaterials.2016.08.005
0142-9612/© 2016 Elsevier Ltd. All rights reserved.

The simplest neuronal relay is made up of 3 neurons: an injured neuron, a transplant-derived neuron and a target neuron [1,4]. Anatomical analysis of the neuronal relay requires the ability to identify the 3 component neurons. The development of the neuronal relay can be divided into 3 discrete steps: 1.) synapse formation between regenerating axons and grafted neural stem cell (NSC)-derived neurons; 2.) appropriate neurotransmitters secreted by grafted NSC-derived neurons at the injury/graft site of spinal cord; 3.) synapse formation between axons derived from the grafted neurons and host target neurons [8]. Recent study by Lu and colleagues has raised hope for stem cell-mediated relay formation of the spinal cord [9]. In this connection, neural stem cells (NSCs) were transplanted into the complete transection site of thoracic spinal cord. The cells were treated with a growth factor cocktail and transplanted in a fibrin matrix. The grafted cells survived and expanded to completely fill the lesion cavity. There was robust outgrowth of axons extending for a long distance into the host spinal tissue where they formed numerous synapses with host neurons, leading to electrophysiologically active relays across the lesion site [9]. However, in a similar recent study, it was reported a likewise long-distance axon outgrowth but the locomotor and bladder function improvement was insignificant [10]. Furthermore, several studies have suggested that therapeutic *in vivo* application of stem cells for spinal cord injury might face a series of challenges including the lack of neuronal differentiation or abnormal phenotype differentiation [11]. The possibility of continued stem cell proliferation may result in space-occupying masses or teratoma formation [12,13]. Accordingly, despite robust axonal regeneration, the small functional improvements reported in these stem cell and growth factor cocktail studies suggest that, in order to have function, the transplanted stem cells must at lease meet the following conditions: 1.) they must be able to survive yet do not over-proliferate in the host spinal cord; 2.) they must be able to differentiate into the right types of cells, neurons or neuroglial cells; 3.) they must make right connections with the host neural network [4,13,14].

We have demonstrated that co-culture of TrkC overexpressing NSCs (TrkC-NSCs) and neurotrophin-3 overexpressing Schwann cells (NT-3-SCs) in a gelatin sponge scaffold can constitute tissue engineering neural network with excellent potential of synaptic transmission. This *in vitro* method to prepare neural network obviates the risk of differentiation uncertainty and abnormal proliferation when stem cells are implanted directly, which therefore may serve as an effective and safe neuronal relay. We reported recently that the pre-constructed functional neural network was integrated into host neural network in the injured spinal cord. More importantly, it could stimulate functional axonal regeneration and remyelination, and mediate functional recovery as evidenced by improvement of hindlimb movement and cortical motor evoked potential (CMEP) [15]. However, there was still lack of a concrete anatomical evidence to prove whether the donor-derived neuron plays a critical role as interneuron to relay segmental or even some descending and ascending nerve signals to mediate recovery. It also remains uncertain whether the interaction between NT-3 and TrkC plays a major role in the formation of a large number of synaptic contacts between the neurons differentiated from TrkC-NSCs. Some studies have shown that NT-3/TrkC interaction may activate MAPK signaling pathway to promote the differentiation of NSCs into neurons with synaptic functions [16]. However, this study was carried out in a 2 dimensional culture system without overexpressing NT-3 or TrkC in NSCs. As far as can be ascertained, there are no available reports on signaling pathways by which NT-3 is involved in the formation of synapses in a 3 dimensional culture system of NSC-derived neuron overexpressing TrkC (TrkC-NSC-derived neuron).

In view of the above, it is desirable and the aims of this study to determine whether the NSC-derived neurons (donor-derived neurons) would form synaptic contacts with the host neurons, and whether the contacts would effectively deliver motor command from the brain to the spinal cord motor center of hindlimbs. Simultaneously, there is a need to elucidate the mechanism underlying the formation of synaptic contacts in NSC-derived neurons induced by NT-3 in the 3 dimensional culture system. To ascertain and visualize the selected neuronal pathway, cholera toxin B subunit conjugated to biotin (b-CTB), which can be transsynaptically transported, is used to address how the disrupted neuronal circuits are reconstructed by donor-derived neurons serving as a neuronal relay [17]. Additionally, NT-3 and TrkC receptor blockers and downstream signaling pathway blocking agents are used to investigate the signaling pathway affecting the formation of synaptic contacts between NSC-derived neurons induced by NT-3 in the 3 dimensional culture system to understand the underlying mechanism and signal pathway.

2. Materials and methods

2.1. NSCs and SCs preparation and identification

NSCs were isolated as previously described [15] from green fluorescent protein (GFP) transgenic Sprague-Dawley (SD) rats (Osaka University, Osaka, Japan). Briefly, rats (1–3 days old) were anesthetized. The whole hippocampus was dissected and dissociated. Basal medium included DMEM/F12 (1:1), B27 supplement and basic fibroblast growth factor (bFGF). To obtain SCs, SD neonate rats (5–6 days old) were decapitated and sterilized. Sciatic nerves and brachial plexus were dissected. The epineurium and connective tissue were removed under a dissecting microscope. All nerves were cut into small pieces (< 2 mm) and dissociated with 0.16% collagenase (Sigma). Culture medium contained DMEM/F12, 10% FBS, 2 μmol/L forskolin (Sigma) and 20 μg/ml bovine pituitary extract (Sigma). The cells were passaged when 90% confluence was reached and were purified by differential adhesion and differential digestion techniques [18,19].

2.2. NSCs and SCs transfection and seeded in 3 dimensional gelatin sponge scaffold

Recombinant adenoviral (Ad) vectors (Ad-TrkC and Ad-NT-3) were produced as described in our previous study [20]. Neurospheres were infected with Ad-TrkC and SCs were infected with Ad-NT-3. The transfection efficiency with these viral vectors has been well documented [18,19,21]. A 3 dimensional gelatin sponge scaffold was prepared as previously described [15,18,19]. A total of 5×10^5 cells (NSCs and SCs were mixed in 1:1 ratio) in 10 μl culture medium were seeded to each scaffold. The scaffolds were incubated for 14 days.

2.3. Western blot, ELISA and RT-PCR analysis

After 14 days in culture, 3 scaffolds from each group were dissolved yielding intracellular and extracellular proteins; equal amounts of the protein suspension were then loaded on a 10% polyacrylamide gel, separated by gel electrophoresis, and transferred onto a polyvinylidene fluoride (PVDF) membrane. The membranes were incubated with primary antibodies at 4 °C overnight, followed by incubation with anti-rabbit HRP-conjugated IgG. The bands were detected with an enhanced chemiluminescence (ECL) Western blot substrate kit. Since all internal references in the present study were GFP or β-actin protein, following the first acquisition of the ratio of β-actin *vs* GFP in each group, the amount

Download English Version:

https://daneshyari.com/en/article/4752486

Download Persian Version:

https://daneshyari.com/article/4752486

Daneshyari.com