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Transdifferentiation of bone marrow stromal cells into cholinergic neuronal phenotype: a potential source for cell therapy in spinal cord injury

Majid Naghdi[1]*, Taki Tiraihi[1], Seyed Alireza Mesbah Namin[2] and Jalil Arabkheradmand[3]

[1]Department of Anatomical Sciences, [2]Department of Clinical Biochemistry, Tarbiat Modares University, Tehran, Iran, and [3]Neuroscience Center, Khatam Al- Anbia hospital, Tehran, Iran

Background aims

Cholinergic neurons are very important cells in spinal cord injuries because of the deficits in motor, autonomic and sensory neurons. In this study, bone marrow stromal cells (BMSC) were evaluated as a source of cholinergic neurons in a rat model of contusive spinal cord injury.

Methods

BMSC were isolated from adult rats and transdifferentiated into cholinergic neuronal cells. The BMSC were pre-induced with β-mercaptoethanol (BME), while the induction was done with nerve growth factor (NGF). Neurofilament (NF)-68, -160 and -200 immunostaining was used for evaluating the transdifferentiation of BMSC into a neuronal phenotype. NeuroD expression, a marker for neuroblast differentiation, and Oct-4 expression, a marker for stemness, were evaluated by reverse transcriptase (RT)-polymerase chain reaction (PCR). Choline acetyl transferase (ChAT) immunoreactivity was used for assessing the cholinergic neuronal phenotype. Anti-microtubule-associated protein-2 (MAP-2) and anti-synapsin I antibodies were used as markers for the tendency for synptogenesis. Finally, the induced cells were transplanted into the contused spinal cord and locomotion was evaluated with the Basso-Beattie-Bresnahan (BBB) test.

Results

At the induction stage, there was a decline in the expression of NF-68 associated with a sustained increase in the expression of NF-200, NF-160, ChAT and synapsin I, whereas MAP-2 expression was variable. Transplanted cells were detected 6 weeks after their injection intraspinally and were associated with functional recovery.

Conclusions

The transdifferentiation of BMSC into a cholinergic phenotype is feasible for replacement therapy in spinal cord injury.

Keywords

Bone marrow stromal cells, cholinergic neurons, regeneration, spinal cord injury, transplantation.

Introduction

Choline acetyltransferase (ChAT) immunoreactive neurons have been evaluated in the postnatal life of rats, where ChAT-immunoreactive neurons were subdivided into two main types: somatic motoneurons and sympathetic pre-ganglionic cells. Small ChAT-immunoreactive neurons were also noticed around the central canal, while immunoreactive neurons were detected at birth in the laminae of the dorsal horn. Each type of ChAT-immunoreactive neurons was reported to achieve adult levels of staining intensity at different times during development [1]. In adult rats, ChAT-immunoreactive motoneurons are located in the medial, central and lateral motor columns of the ventral horn; small ChAT-immunoreactive neurons are clustered around the central canal at the central gray matter, whereas intermediate

Correspondence to: **Taki Tiraihi**, Department of Anatomical Sciences, School of Medical Sciences, Tarbiat Modares University, PO Box 14155-4838, Tehran, Iran. E-mail: ttiraihi@yahoo.com, takialtr@modares.ac.ir

*Present address: Shiraz University of Medical Sciences (SUMS), Namazi Hospital.

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gray matter consists of partition neurons, which are medium to large ChAT-immunoreactive multipolar cells. At autonomic spinal levels, ChAT-immunoreactive preganglionic sympathetic or parasympathetic neurons are intermingled with extensions of the partition neurons. Moreover, ChAT-immunoreactive neurons have been noticed in the dorsal horn [2].

The spinal cord contusion model in rats has been documented as a useful model for human spinal cord injury, from functional, electrophysiologic and high-resolution magnetic resonance imaging aspects [3], as well as histology [4,5]. While cell death has been reported in spinal motoneurons following the spinal cord contusion model in rats, dysfunction of the cholinergic neurons of the autonomic nervous system with blood pressure dysregulation, disorders in voiding, defecation and reproduction have also been documented [6,7]. These problems are caused by the destruction of brain pathways that control spinal autonomic neurons lying caudal to the lesion [8], resulting in loss of preganglionic neurons [9] with progressive retrograde death [10].

Cell-based therapy for neurologic diseases with neuronal loss has been carried out in different animal models [11]. Several sources of cells have been suggested for this modality of treatment, including embryonic stem cells and adult stem cells, which hold tremendous promise for replacement therapy for a variety of neurodegenerative diseases [12]. Webber & Minger [13] revealed the possible differentiation of stem cells into a wide range of cell types, making stem cells a valuable source for cell-based replacement therapy. For example, regarding Parkinson's disease, dopaminergic neurons derived from stem cells resulted in improvement in diseased animals [14]. The differentiation of spinal neural progenitors into a cholinergic phenotype has been documented, and the survival of transplanted progenitor cells in the host tissue reported [15]. Human fetal neural stem cells have also improved behavioral test in rats with spinal cord injury [16]; moreover, cholinergic neurons derived from human neural stem cells innervated the muscle of motoneuron-deficient rats [17]. However, we have found no report regarding transdifferentiation of bone marrow stromal cells (BMSC) into a cholinergic phenotype.

The purpose of this study was to develop a protocol for transdifferentiating BMSC into cholinergic neurons and evaluate the functional recovery of animals treated with the transdifferentiated cells.

Methods

Cell preparation and cell characterization

The femurs and tibias from 6–8-week-old Sprague–Dawley rats were removed and dissected. The proximal and distal ends were cut, and the bone marrow (BM) flushed out with 5 mL alpha-modified Eagle medium (αMEM; Gibco, Paisley, UK) supplemented with 10% fetal bovine serum (FBS; Gibco). The whole marrow cells were cultured for 24 h in αMEM medium supplemented with 10% FBS as well as penicillin and streptomycin (Gibco), then the non-adherent cells were removed and washed with phosphate-buffered saline (PBS). As confluency of the cells reached about 80%, the cells were detached with a 0.25% trypsin, 0.04% EDTA solution and reseeded (at a density of 8000 cells/cm^2) in plastic flasks. Anti-fibronectin antibody (Ab) was used for characterizing the BMSC [18]. The cells were split 1:3 and passaged up to five times for subsequent experiments, and the culture medium was changed every other day until the cells became confluent. The viability of the cultured cells, which was about 95%, was assessed before seeding.

Cell transdifferentiation

A two-stage induction protocol (1/6), where the initial stage (pre-induction, 1 day) proceeded to the next stage (induction, 6 days), was used. In order to select an optimal pre-induction method, two pre-inducers were examined: β-mercaptoethanol (BME) and dimethyl sulfoxide (DMSO). The selection of the pre-inducer was based on the results of the expression of four sets of genes. The first set was Oct-4 gene, a stemness marker, which was detected by reverse transcriptase–polymerase chain reaction (RT-PCR). The second set was the neuronal differentiation genes, including neurofilament (NF)-68, NF-160 and NF-200, which were detected by immunocytochemistry. NeuroD gene was evaluated by RT-PCR. The third set was synaptogenesis genes, including microtubule-associated protein-2 (MAP-2) and synapsin I, which were detected by immunocytochemistry. The fourth set was the cholinergic neuron marker (ChAT), which was detected by immunocytochemistry as well. The culturing medium was changed with serum-free media containing either 1 mM BME or 2% DMSO, which were incubated for 24 h. Based on the findings