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Human ciliary neurotrophic factor–overexpressing stable bone marrow stromal cells in the treatment of a rat model of traumatic spinal cord injury

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Abstract

Background aims. Traumatic injury to the central nervous system (CNS) often causes motor dysfunctions. However, because of the CNS complexity and variability in the clinical presentations, efforts to repair damaged CNS tissue and restoring its functions are particularly demanding. On the other hand, recent progress in the regenerative therapy field have led to novel approaches for the treatment of traumatic CNS injury and renewed hopes to overcome the obstacles. It appears that the balance between neurite re-growth–inhibiting and neurite re-growth–inducing molecules determines the axonal re-growth fate. Neurotrophic factors can tilt this balance and indeed promote cell survival and axonal re-growth over neuro-degeneration. One of the promising neurotrophic factors in this field is ciliary neurotrophic factor (CNTF). *Methods.* We transfected rat bone marrow stromal cells with a mammalian expression vector–inserted human *CNTF* gene through the use of a non-viral method to prepare human CNTF-overexpressing stem cells under *ex vivo* conditions. We transplanted these modified cells to the rat model of spinal cord traumatic injury to explore functional recovery after contusion induction. *Results.* Our data from immunocytochemistry and behavioral tests showed that such cells can act as a powerful potential approach to treat traumatic CNS injuries because these modified cells improved the behavioral test scores in the rat model of spinal cord injury. *Conclusions.* CNTF-overexpressing bone marrow stromal cells can ameliorate spinal cord traumatic injury and can be used in the treatment of traumatic CNS injuries in the near future.

Key Words: *BMSCs, CNS regeneration, CNS traumatic injury, CNTF*

Introduction

Traumatic injury to the central nervous system (CNS) has devastating effects on the quality of life and causes an economic burden to the suffering patient [1]. Damaged CNS caused by trauma can lead to impairment of many parts of the body or even cause paralysis in many cases. CNS traumatic injury often causes motor dysfunctions such as loss of ambulation, strength, coordination, balance, fine motor skills, endurance and cognitive impairments [2]. Because the structure-function relationships within the CNS are extremely complicated, any damage to a specific area of it can trigger a loss of homeostasis within the system and result in increasing entropy. However, because of the complexity and variability in the clinical presentations, efforts to repair damaged CNS tissue and restoring its functions are particularly demanding. These complications prompt the demand for developing novel and more effective treatments. Ideally, the therapeutic approach for CNS traumatic injury must minimize secondary necrosis and apoptosis significantly by targeting a variety of mechanisms and enhance regeneration processes as well. Indeed, it also must promote neuronal plasticity for recovering recently lost circuit connections among them and neuronal re-growth. Dampening of cell damage propagation is the main aim of the clinical responses; however, pharmacological agents that have been developed for routine clinical applications only decrease secondary CNS damage [3]. On the other

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hand, recent progress in the regenerative therapy field have led to novel approaches, and researchers put their efforts into two main options: (i) neurotrophin therapy that acts on regeneration-associated genes to stimulate axonal sprouting and re-growth and (ii) delivering molecules to neutralize the inhibitory cues present in the traumatically injured CNS. One of the promising neurotrophic factors in this field is ciliary neurotrophic factor (CNTF). CNTF has neuroprotective effects on a variety of central and also peripheral nervous system neurons [4] because it promotes differentiation and maturation of oligodendrocyte precursor cells to oligodendrocytes under *in vitro* conditions [5] and thus improves re-myelination. Importantly, it also increases the survival of mature oligodendrocytes [5,6]. In this regard, we transfected rat bone marrow stromal cells (BMSCs) with a mammalian expression vector–inserted human *CNTF* gene to prepare human CNTF-overexpressing stem cells under *ex vivo* conditions and transplanted these modified cells to a spinal cord traumatic injury rat model to explore functional recovery after contusion induction. Our results showed that such cells act as a powerful and potential approach to treat traumatic injuries of the spinal cord.

Methods

Subcloning

After amplification of pCMV-SPRT6-CNTF vector (Open Biosystems) as the carrying vector, this plasmid was subjected to polymerase chain reaction (PCR) amplification by use of two primers containing *Hind III* and *EcoR V* restriction enzyme (Fermentas) sites in the forward and reverse primers, respectively (Table I). The amplification product was separated by low-melting-point agarose gel electrophoresis (Sigma). The DNA fragment (*CNTF* gene) was extracted from the gel and purified; it was subsequently subcloned into the *Hind III*, *EcoR V* pre-digested and purified pSecTag2/HygroA (Invitrogen) mammalian expression vector, with the use of T4 DNA ligase (Fermentas). Finally, this expression vector was subjected to colony PCR as well as DNA sequencing with the use of universal primers on the vector for accuracy assessments of the subcloning processes of the human *CNTF* gene.

Ethical approval

Female Sprague-Dawley rats (Razi Institute, Tehran, Iran), weighing 200 to 250 g, were housed under standard conditions; the experimental procedures were approved by the Ethical Committee for Laboratory Animal of Shahid Beheshti University of Iran.

BMSC cell culture

After the adult Wistar rats were euthanized, the tibia and femur bones were dissected, and, after removal of proximal and distal ends, the central canal of the bones was aspirated by use of 5 mL of α-mimimum essential medium (MEM) (Invitrogen) cell culture medium to extrude the marrow cells. The whole marrow cells were seeded on the 25-cm^2 plastic flasks in the α-MEM supplemented with 10% fetal bovine serum (Gibco), 100 U/mL of penicillin (Invitrogen) and 100 mg/mL of streptomycin (Invitrogen). The flasks were incubated in a humidified atmosphere with 5% CO_2 at 37°C. After 24 h, non-adherent cells were removed by means of washing steps with phosphate-buffered saline (PBS, Gibco), and the medium was changed every other day until the isolated BMSCs became confluent. At confluence, the cells were detached with the use of 0.25% trypsin containing ethylene diamine tetra-acetic acid (EDTA) (Gibco) by incubation for 5 min at 37°C, and thus a single-cell suspension was obtained and replated until passage 4.

Immunocytochemistry

The immunoreactivity of BMSC markers was evaluated on the purified cells at the fourth passage. After trypsinization, the cells were seeded on the gelatin-coated glass coverslips and then were washed with PBS and fixed with 4% paraformaldehyde in PBS for 15 min. The fixed cells were washed two times with PBS before the permeabilization stage. The permeabilization and blocking non-specific antigen reactions were carried out in the blocking buffer consisting 0.1% of Triton X-100, 10% goat serum in PBS for 1 h. For cell-surface antigens, the permeabilization stage was excluded from the procedures, but blocking non-specific antigen reactions were carried out in the blocking buffer. The primary antibodies were used in the blocking buffer overnight

Table I. Sequence of primers for amplification of the *CNTF* gene on the carrier vector.

Human *CNTF*	Sequence	Annealing temperature
Forward	TATGGTACCGTTGAGTTAAGGGATGGC	58°C
Reverse	GCGGATATCGAAGGGACTAACTGCTACA	

Restriction sites are underlined in the primer sequences.