Review

DOI: 10.5582/bst.2013.v7.5.202

Genetic modification of mesenchymal stem cells in spinal cord injury repair strategies

Xiaoyan Cui[1,*], Lei Chen[2], Yilong Ren[2], Yazhong Ji[3], Wei Liu[3], Jie Liu[1], Qiao Yan[1], Liming Cheng[2], Yi E. Sun[1,*]

[1] *Translational Center for Stem Cell Research, Tongji Hospital, Department of Regenerative Medicine, Tongji University School of Medicine, Shanghai, China;*
[2] *Department of Spine Surgery, Tongji Hospital, Tongji University School of Medicine, Shanghai, China;*
[3] *Department of Obstetrics and Gynecology, Tongji Hospital, Tongji University School of Medicine, Shanghai, China.*

Summary **Spinal cord injury (SCI) is a serious injury of the central nervous system and up until now there is no evident effective treatment for SCI. Axonal regeneration is the only way to restore functions after serious SCI that interrupt the long tracts mediating motor and sensory function. The hurdles for axonal regeneration in SCI include: glial scar tissue and molecular barriers, the inhibiting microenvironment, and the lack of sufficient neurotrophic support. Therefore, the key point of applying stem cells to treat SCI is to build a microenvironment conducive to the survival and differentiation of stem cells and regulate neurotrophic factor expression. Adult mesenchymal stem cells (MSCs) have been applied in experimental animal models and clinical trials of SCI. Genetic modification of MSCs can increase secretion of peptides or total length proteins with potential to repair SCI and promote survival of themselves and survival or regeneration of neurons. There are many proteins that have been applied to modified MSCs, such as neurotrophic factors (neurotrophin 3, brain-derived neurotrophic factor, glial cell line-derived neurotrophic factor, nerve growth factor, and MNTS1), receptor tyrosine kinases (tropomyosin-related kinase C), and hepatocyte growth factor. In the future, there will be more molecules acting as transgenes in MSCs for treatment of SCI.**

Keywords: Spinal cord injury, mesenchymal stem cells, neurotrophic factors, neurotrophin 3, brain-derived neurotrophic factor

1. Introduction

Spinal cord injury (SCI) is a serious injury of the central nervous system and its main clinical manifestations include movement, sensory, and sphincter dysfunction below the level of injury, which lead to a consequent reduction of the quality of life. The injury mainly results from contusion, compression or stretch of the spinal cord. An epidemiological study based on a nationwide database reported that SCI accounted for 16.87% of spinal trauma in Mainland China and the incidence of SCI increased annually during the study period (*1*). Operative treatment and conservative treatment are employed in the management of SCI. However, up until now there is no evident effective treatment for SCI due to this injury`s complicated pathophysiology (*2*). The focal mechanical insult disrupts tissue homeostasis during the acute phase that induces secondary injury processes. Multiple destructive cascades in the secondary injury processes cause the necrotic and apoptotic death of neurons, astrocytes, and oligodendrocytes, which spreads beyond the initial injury site and leads to irreversible axonal damage and demyelination (*3*). It has been recognized that axonal regeneration is the only way to restore functions for decades after serious SCI that interrupt the long tracts mediating motor and sensory function

**Address correspondence to:*
Dr. Xiaoyan Cui and Dr. Yi E. Sun, translational Center for Stem Cell Research, Tongji Hospital, Department of Regenerative Medicine, Tongji University School of Medicine, 389 Xincun road Shanghai 200065, China.
E-mail: cuixiaoyan.tu@gmail.com

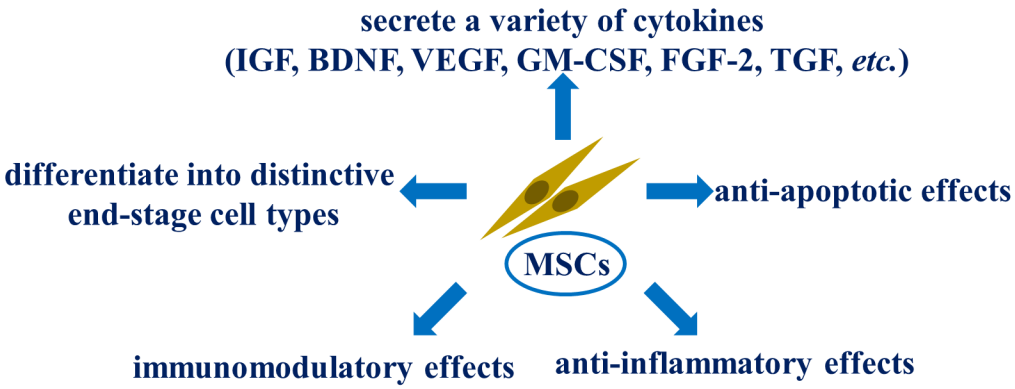

Figure 1. MSCs possess properties directed to the hurdle for axonal regeneration in SCI.

(*4*). The hurdle for axonal regeneration in SCI include: glial scar tissue and molecular barriers, inhibiting microenvironment (such as chondroitin sulphate proteoglycans and myelin-associated inhibitors), and the lack of sufficient neurotrophic support (*5,6*). Therefore, the key point of applying stem cells to treat SCI is to build a microenvironment conducive to the survival and differentiation of stem cells and regulate neurotrophic factor expression. Genetic modification of adult mesenchymal stem cells (MSCs) is expected to overcome the hurdle for axonal regeneration and has been applied in experimental animal models of SCI.

2. Mesenchymal stem cells

Embryonic stem cells (ESCs) and neural stem cells (NSCs) have been used to repair SCI. These stem cells survived, differentiated into astrocytes, oligodendrocytes, and neurons, and promoted neural functional recovery (*7*). However, these stem cells can also differentiate into inappropriate cells, resulting in tumor formation (*8*). Moreover, NSCs have a tendency to differentiate into glial cells after they are transplanted into an impaired central nervous system. Therefore, NSCs could promote astrogliosis and the extension of a glial scar (*9*).

At present, adult MSCs have been applied in experimental animal models and clinical trials of SCI (*10*). MSCs are multipotent nonhematopoietic cells with the potential to differentiate into osteoblasts, chondrocytes, adipocytes, as well as myogenic and neuronal cells (*11*). MSCs are a heterogeneous population that can be isolated from several tissues, such as bone marrow, adipose, umbilical cord blood, Whartons jelly, amnion, *etc*. MSCs possess many properties directed to the hurdle for axonal regeneration in SCI (Figure 1). Once MSCs arrive at an injury, they can secrete a variety of cytokines, such as insulin-like growth factor (IGF), brain-derived neurotrophic factor (BDNF), vascular endothelial growth factor (VEGF), granulocyte-macrophage colony stimulating factor (GM-CSF), fibroblast growth factor (FGF)-2, and transforming growth factor (TGF) (*12*). MSCs down-regulate apoptotic molecules and up-regulate anti-apoptotic molecules in SCI animal models. In addition, MSCs increase serum interleukin (IL)-10 and decrease tumor necrosis factor (TNF)-α. T cells change from pro-inflammatory Th1 cells to anti-inflammatory Th2 cells and macrophage phenotypes change from M1 (immune surveillance) to M2 (down-regulating immune response) in the presence of MSCs. The immunophenotype of MSCs are major histocompatibility (MHC) I positive and MHC II negative and MSCs also lack costimulatory molecules CD40, CD80, and CD86. Therefore, MSCs have an immunomodulatory effect (*13*).

Multifunctional therapies seem to be extremely promising because they counteract multiple injury mechanisms and combine both neuroprotective and neuroregenerative agents (*14*). Although MSCs secret some cytokines, the levels of these cytokines are not enough for SCI repair. Genetic modification of MSCs can increase secretion of peptides or total length proteins with potential to repair SCI and promote the survival of themselves and the survival or regeneration of neurons. There are many proteins that have been applied to modified MSCs, such as neurotrophic factors (neurotrophin 3, brain-derived neurotrophic factor, glial cell line-derived neurotrophic factor, nerve growth factor, and MNTS1), receptor tyrosine kinases (tropomyosin-related kinase C), and hepatocyte growth factor (Table 1).

3. Proteins with potential to repair SCI

3.1. *Neurotrophic factors*

3.1.1. *Neurotrophin 3 (NT-3)*

NT-3 has been shown to act as a neuroprotective agent (*15*). NT-3 can promote axonal growth and the differentiation of sensory neurons, motor neurons, dopaminergic neurons, and other neurons. The effect of NT-3 promoting neuron growth is due largely to activating tropomyosin-related kinase (Trk) C (*16*). The co-expression of NT-3 and BDNF had an anti-apoptotic

Table 1. Activities of Genetic modification of MSCs in SCI animal models

Transduced genes	Animals	SCI models	Modified MSCs	Time point and dose	Gene carriers	Outcome	*Ref.*
NT-3	60 female SD rats	Compression of at L1 level	HUMSCs	Seven days after injury, 1×10^6 per rat	Adenovirus vector	Significant improvement of locomotor function	*19*
NT-3	36 female SD rats	Complete transection at T10 level	Rat BMSCs	Immediately, 5×10^5 per rat	Adenovirus vector	Some improvement (both functionally and structurally)	*20*
NT-3	25 female SD rats	1 μL EB (0.1 mg/mL) into the T10 thoracic cord	Rat BMSCs	Three days after EB injection, 1×10^5 per rat	Adenovirus vector	Significant improvement of locomotor function and restoration of electro-physiological properties	*21*
BDNF	66 female SD rats	Transection at T9 level	Human BMSCs	Immediately, 1.2×10^5 cells per rat	Adenovirus vector	Locomotor recovery improvement	*26*
GDNF	18 female SD rats	Contusion injury at T9 level	Rat BMSCs	Seven days after surgery, 2×10^5 cells per rat	Retrovirus vector	Limited capacity for the replacement of neural cells lost	*32*
MNTS1	48 female Fischer rats	Moderate contusion at T8 level	Rat BMSCs	Seven days after injury, 4×10^5 cells per rat	Lentivirus vector	Axonal growth increase and cutaneous hypersensitivity prevention	*39*
TrkC	80 female SD rats	Complete transection of the spinal cord at T10	Rat BMSCs	Immediately, 5×10^5 cells per rat	Adenovirus vector	Improvement in conduction of cortical MEPs and hindlimb locomotor function	*43*
HGF	51 female SD rats	Hemisection injury at C4 level	Human BMSCs	Immediately, 2.0×10^5 cells per rat	Lentivirus vector	Anti-glial scar, axonal growth increase and improvement in recovery of forepaw function	*47*

effect in a cellular SCI model of rat spinal cord neurons (*17*). Moreover, transduction of spinal motoneurons with adenoviral vector (Adv) carrying the NT-3 gene induced growth of axons from the intact corticospinal tract (CST) across the midline to the denervated side in animals with a CST lesion (*18*).

Implantation of genetically modified MSCs with NT-3 can improve locomotor function, structure, and electrophysiological properties. Sprague–Dawley (SD) rats in a NT-3-human umbilical cord MSCs (HUMSCs) group had significantly improved locomotor function recovery and more than the control group in a rat model for clipped SCI (*19*). The NT-3-HUMSCs group achieved better functional recovery, more intensive 5-HT fibers, a larger volume of spared myelination, and a smaller area of cystic cavity than the HUMSCs group at the end of 12 weeks after SCI. Bone marrow-derived MSCs (BMSCs) overexpressing NT-3 also can promote locomotor function and structure recovery. After NT-3 modified BMSCs were implanted into the transected spinal cord of rats, the animals obtained some improvement (both functionally and structurally), including the recovery of hindlimb locomotor function, dramatically reduced cavity volume, clear axonal regeneration, and more neuronal survival (*20*). In contrast, simple MSC implantation was not a very effective therapy for spinal transection. Moreover, implantation of NT-3 gene-modified BMSCs resulted in significant improvement of locomotor function and restoration of electrophysiological properties in rats via a recombinant adenoviral vector (Adv) into a region of ethidium bromide (EB)-induced demyelination in the spinal cord (*21*). The morphological basis of this recovery was evidenced by robust myelin basic protein (MBP) expression and extensive remyelination and these results may be due to participating directly in myelination of the endogenous remyelinating cells.

3.1.2. *BDNF*

BDNF was discovered in the early 1980's (*22*). An intensive interest in exploring BDNF's potential in treating SCI have been spurred because of its role as a promoter of cell survival and neurite outgrowth. BDNF can enhance plasticity and regenerative growth in tracts, such as the CST (*23*). It can specifically interact with the high affinity TrkB receptor inducing most of the desirable effects of BDNF in SCI. Furthermore, BDNF can also interact with the low-affinity pan-neurotrophin receptor p75, leading to signaling effects that often counteract TrkB activation (*24*).

Although MSCs continuously produce BDNF and significantly rescue avulsed motoneurons (*25*), gene-modified human BMSCs overexpressing BDNF can further increase the potential therapeutic effect of BDNF in SCI (*26*). At 5 weeks after transplantation of modified

BMSCs for SCI, locomotor recovery improvement was observed for the BDNF-BMSC group, but not in the BMSC group. Structurally there was increased sprouting of the injured corticospinal tract and an increased cell survival of corticospinal tract neurons in the primary motor cortex.

3.1.3. *Glial cell line -derived neurotrophic factor (GDNF)*

GDNF exists in embryonic limb and muscle at high levels at the time of innervation and is necessary for normal neuromuscular development (*27*). It has been shown to protect motor neurons in a number of different animal models (*28*). GDNF can increase neural sprouting and prevent cell death (*29*). The heterodimer receptor system of GDNF includes GDNF receptor alpha (GFRα) and c-Ret expressed by healthy motor neurons. These neurons can bind, internalize, and transport GDNF in both antero- and retrograde directions in a receptor-dependent manner (*30*). GDNF administration may stimulate the survival of injured motor neurons and promote axonal regeneration (*31*). GDNF-transduced MSCs can survive and express the therapeutic gene after 6 weeks of transplantation to the site of SCI, while maintaining an undifferentiated phenotype. However, they provide excellent opportunities for local delivery of neurotrophic factors into the injured spinal cord (*32*).

3.1.4. *Nerve growth factor (NGF)*

NGF can promote survival and axonal growth of sensory and sympathetic neurons. The functions of NGF are mediated by its binding to TrkA and the p75 neurotrophin receptor (p75 NTR) (*33*). This NGF-receptor complex undergoes endocytosis and retrograde transport to the neuronal soma where it regulates gene expression (*34*).

NGF expression significantly increased in the spinal cord injured tissue 3 days after MSC graft (*35*). Moreover, secreted NGF from genetically modified MSCs induced neurite outgrowth from PC12 cells (*36*). Combination of MSC transplantation with NGF promoted axonal regeneration and further functional improvement compared with single MSC transplantation or NGF on the repair of SCI in adult rats (*37*).

3.1.5. *MNTS1*

MNTS1 contains only seven amino acid changes from multineurotrophin NT-3/D15A. It can bind all receptors of the Trk family and induce autophosphorylation of TrkA, TrkB, and TrkC (*38*). MSCs transduced with a multineurotrophin are effective in cell growth promotion and sensory function improvement after SCI. Kumagai *et al*. reported that transplantation with MSC-MNTS1 and MSC-MNTS1/p75$^-$ enhanced axonal growth and significantly prevented cutaneous hypersensitivity after SCI (*39*). Furthermore, transplantation with MSC-MNTS1/p75$^-$ increased angiogenesis and decreased glial scar formation.

3.2. *TrkC*

The effects of mature neurotrophins on neuronal survival are mediated by members of the Trk family of receptor tyrosine kinases (*40*) and are modulated by the common neurotrophin receptor p75 NTR (also known as NGFR) (*41*). The Trk family of receptor tyrosine kinases which neurotrophins bind to includes TrkA (NGF), TrkB (BDNF and NT-4/5) and TrkC (NT-3).

Chen *et al*. showed that *in vivo* transplanted MSCs overexpressing TrkC migrated into the NT-3 enriched area. Moreover, the migrating incidence as well as migration distance of MSCs was significantly higher than the control (*42*). The results indicated that TrkC acts as a chemokine receptor with its high affinity for NT-3 and may play a role in MSC homing. TrkC gene-modified MSCs transplantation combined with electroacupuncture treatment not only increased MSC survival and differentiation into neuron-like cells but also promoted CST regeneration across injured sites to the caudal cord and functional improvement in SCI (*43*). In addition, the conduction of cortical motorevoked potentials (MEPs) and hindlimb locomotor function increased as compared to controls. These results are perhaps due to an increase of NT-3 levels, upregulation of laminin and GAP-43, and downregulation of GFAP and chondroitin sulphate proteoglycan (CSPG) proteins.

3.3. *Hepatocyte growth factor (HGF)*

HGF is primarily produced by cells of mesenchymal origin. It is a pleiotropic cytokine which promotes angiogenesis and cell survival (*44*). Injection of HGF has been demonstrated to enhance kidney and liver regeneration (*45*). In addition, systemic treatment with HGF significantly accelerated remyelination in lysolecithin-induced rat dorsal spinal cord lesions and in slice cultures (*46*). Moreover, HGF has anti-glial scar effects and could be used to ameliorate functional deficits following SCI. Transplantation of HGF overexpressing MSCs (HGF-MSCs) into hemisection spinal cord lesions at C4 markedly decreased TGFβ isoform and neurocan levels and reduced the extent of astrocytic activation and glycosaminoglycan chain deposition around hemisection lesions. Furthermore, animals treated with HGF-MSCs showed axonal growth promotion beyond glial scars and recovery improvement of forepaw function (*47*).

4. Gene carriers

Effective gene transduction is the basis of genetic modification of MSCs in SCI repair. Viral vectors are characterized by high transduction efficiency and stable transgene expression. Viral vectors mediating genetic

modification of MSCs include retroviral, adeno-associated viral, adenoviral, and lentiviral vectors. They have their own advantages and disadvantages (*48*). Lentiviral vectors have the unique ability to integrate into the genome of non-dividing cells and enable their relatively long and stable transgene expression. On the contrary, other retroviral vectors only transduce dividing cells. Moreover, the immunogenicity of lentiviral vectors is significantly reduced (*49,50*). Adenoviral vectors are able to transduce dividing and non-dividing cells (*51*). They are relatively safe to the host due to no integration function. Meanwhile, adeno-associated virus is also considered non-pathogenic to humans because it is a defective virus (*52*).

Non-viral vector systems have many advantages compared to viral vector systems, including, significantly lower toxicity/immunogenicity and potential tumorigenicity, unlimited transgene size (range is from oligonucleotides to artificial chromosomes), simple quality control, and simple requirements for drugs and management (*53*). Non-viral vectors may be applied to transduce exogenous genes into MSCs in SCI repair.

5. Conclusions

Continuous development of new strategies to treat SCI is urgently needed because, to date, there is no evident effective treatment for SCI. More information is needed regarding genetic modification of MSCs, including transgene expression level and stabilization, elaborate gene regulation, and safety. Further experimental and clinical investigations will allow a better understanding of mechanisms of action, therapeutic effects, and the safety profile. Many molecules have been recognized for their promising and potent activities of rescuing SCI. Besides the above-mentioned neurotrophic factors, TrkC, and HGF, other cytokines and anti-apoptosis molecules can also be used to modify MSCs, such as D15A (with NT-3 and BDNF activity) (*54*), ciliary neurotrophic factor (CNTF) (*55*), and survivin (*56*). In the future, more molecules acting as overexpressing genes in MSCs and treating SCI will be recognized.

Acknowledgements

This project was supported by a grant (2012M520933) from the National Science Foundation for Post-doctoral Scientists of China, a grant (20124y147) from the Shanghai Municipal Health Bureau Youth Projects, a grant (2012KJ016) from the Scientific Research Foundation for Training Excellent Youth Teacher of Tongji University.

References

1. Liu P, Yao Y, Liu MY, Fan WL, Chao R, Wang ZG, Liu YC, Zhou JH, Zhao JH. Spinal trauma in mainland China from 2001 to 2007: An epidemiological study based on a nationwide database. Spine (Phila Pa 1976). 2012; 37:1310-1315.
2. Sakai K, Yamamoto A, Matsubara K, Nakamura S, Naruse M, Yamagata M, Sakamoto K, Tauchi R, Wakao N, Imagama S, Hibi H, Kadomatsu K, Ishiguro N, Ueda M. Human dental pulp-derived stem cells promote locomotor recovery after complete transection of the rat spinal cord by multiple neuro-regenerative mechanisms. J Clin Invest. 2012; 122:80-90.
3. Rowland JW, Hawryluk GW, Kwon B, Fehlings MG. Current status of acute spinal cord injury pathophysiology and emerging therapies: promise on the horizon. Neurosurg Focus. 2008; 25:E2.
4. Tuszynski MH, Steward O. Concepts and methods for the study of axonal regeneration in the CNS. Neuron. 2012; 74:777-791.
5. Blits B, Kitay BM, Farahvar A, Caperton CV, Dietrich WD, Bunge MB. Lentiviral vector-mediated transduction of neural progenitor cells before implantation into injured spinal cord and brain to detect their migration, deliver neurotrophic factors and repair tissue. Restor Neurol Neurosci. 2005; 23:313-324.
6. Löw K, Blesch A, Herrmann J, Tuszynski MH. A dual promoter lentiviral vector for the *in vivo* evaluation of gene therapeutic approaches to axon regeneration after spinal cord injury. Gene Ther. 2010; 17:577-591.
7. Karimi-Abdolrezaee S, Eftekharpour E, Wang J, Morshead CM, Fehlings MG. Delayed transplantation of adult neural precursor cells promotes remyelination and functional neurological recovery after spinal cord injury. J Neurosci. 2006; 26:3377-3389.
8. Erdö F, Bührle C, Blunk J, Hoehn M, Xia Y, Fleischmann B, Föcking M, Küstermann E, Kolossov E, Hescheler J, Hossmann KA, Trapp T. Host-dependent tumorigenesis of embryonic stem cell transplantation in experimental stroke. J Cereb Blood Flow Metab. 2003; 23:780-785.
9. Vroemen M, Aigner L, Winkler J, Weidner N. Adult neural progenitor cell grafts survive after acute spinal cord injury and integrate along axonal pathways. Eur J Neurosci. 2003; 18:743-751.
10. Caplan AI. Adult mesenchymal stem cells for tissue engineering versus regenerative medicine. J Cell Physiol. 2007; 213:341-347.
11. Sun T, Ma QH. Repairing neural injuries using human umbilical cord blood. Mol Neurobiol. 2013; 47:938-945.
12. Azari MF, Mathias L, Ozturk E, Cram DS, Boyd RL, Petratos S. Mesenchymal stem cells for treatment of CNS injury. Curr Neuropharmacol. 2010; 8:316-323.
13. Chidgey AP, Layton D, Trounson A, Boyd RL. Tolerance strategies for stem-cell-based therapies. Nature. 2008; 453:330-337.
14. Rossi F, Ferrari R, Papa S, Moscatelli D, Casalini T, Forloni G, Perale G, Veglianese P. Tunable hydrogel-nanoparticles release system for sustained combination therapies in the spinal cord. Colloids Surf B Biointerfaces. 2013; 108:169-177.
15. Beutner C, Lepperhof V, Dann A, Linnartz-Gerlach B, Litwak S, Napoli I, Prinz M, Neumann H. Engineered stem cell-derived microglia as therapeutic vehicle for experimental autoimmune encephalomyelitis. Gene Ther. 2013; 20:797-806.
16. Novikova LN, Novikov LN, Kellerth JO. Survival effects of BDNF and NT-3 on axotomized rubrospinal neurons depend on the temporal pattern of neurotrophin

administration. Eur J Neurosci. 2000; 12:776-780.
17. Wang Y, Gu J, Wang J, Feng X, Tao Y, Jiang B, He J, Wang Q, Yang J, Zhang S, Cai J, Sun Y. BDNF and NT-3 expression by using glucocorticoid-induced bicistronic expression vector pGC-BDNF-IRES-NT3 protects apoptotic cells in a cellular injury model. Brain Res. 2012; 1448:137-143.
18. Zhou L, Baumgartner BJ, Hill-Felberg SJ, McGowen LR, Shine HD. Neurotrophin-3 expressed in situ induces axonal plasticity in the adult injured spinal cord. J Neurosci. 2003; 23: 1424-1431.
19. Shang AJ, Hong SQ, Xu Q, Wang HY, Yang Y, Wang ZF, Xu BN, Jiang XD, Xu RX. NT-3-secreting human umbilical cord mesenchymal stromal cell transplantation for the treatment of acute spinal cord injury in rats. Brain Res. 2011; 1391:102-113.
20. Zhang W, Yan Q, Zeng YS, Zhang XB, Xiong Y, Wang JM, Chen SJ, Li Y, Bruce IC, Wu W. Implantation of adult bone marrow-derived mesenchymal stem cells transfected with the neurotrophin-3 gene and pretreated with retinoic acid in completely transected spinal cord. Brain Res. 2010; 1359:256-271.
21. Zhang YJ, Zhang W, Lin CG, Ding Y, Huang SF, Wu JL, Li Y, Dong H, Zeng YS. Neurotrophin-3 gene modified mesenchymal stem cells promote remyelination and functional recovery in the demyelinated spinal cord of rats. J Neurol Sci. 2012; 313:64-74.
22. Barde YA, Edgar D, Thoenen H. Purification of a new neurotrophic factor from mammalian brain. EMBO J. 1982; 1:549-553.
23. Hiebert GW, Khodarahmi K, McGraw J, Steeves JD, Tetzlaff W. Brain-derived neurotrophic factor applied to the motor cortex promotes sprouting of corticospinal fibers but not regeneration into a peripheral nerve transplant. J Neurosci Res. 2002; 69:160-168.
24. Weishaupt N, Blesch A, Fouad K. BDNF: the career of a multifaceted neurotrophin in spinal cord injury. Exp Neurol. 2012; 238:254-264.
25. Rodrigues Hell RC, Silva Costa MM, Goes AM, Oliveira AL. Local injection of BDNF producing mesenchymal stem cells increases neuronal survival and synaptic stability following ventral root avulsion. Neurobiol Dis. 2009; 33:290-300.
26. Sasaki M, Radtke C, Tan AM, Zhao P, Hamada H, Houkin K, Honmou O, Kocsis JD. BDNF-hypersecreting human mesenchymal stem cells promote functional recovery, axonal sprouting, and protection of corticospinal neurons after spinal cord injury. J Neurosci. 2009; 29:14932-14941.
27. Wang CY, Yang F, He XP, Je HS, Zhou JZ, Eckermann K, Kawamura D, Feng L, Shen L, Lu B. Regulation of neuromuscular synapse development by glial cell line-derived neurotrophic factor and neurturin. J Biol Chem. 2002; 277:10614-10625.
28. Acsadi G, Anguelov RA, Yang H, Toth G, Thomas R, Jani A, Wang Y, Ianakova E, Mohammad S, Lewis RA, Shy ME. Increased survival and function of SOD1 mice after glial cell-derived neurotrophic factor gene therapy. Hum Gene Ther. 2002; 13:1047-1059.
29. Deshpande DM, Kim YS, Martinez T, Carmen J, Dike S, Shats I, Rubin LL, Drummond J, Krishnan C, Hoke A, Maragakis N, Shefner J, Rothstein JD, Kerr DA. Recovery from paralysis in adult rats using embryonic stem cells. Ann Neurol. 2006; 60:32-44.
30. von Bartheld CS, Wang X, Butowt R. Anterograde axonal transport, transcytosis, and recycling of neurotrophic factors: The concept of trophic currencies in neural networks. Mol Neurobiol. 2001; 24:1-28.
31. Henderson CE, Camu W, Mettling C, Gouin A, Poulsen K, Karihaloo M, Rullamas J, Evans T, McMahon SB, Armanini MP, Berkemeier L, Phillips HS, Rosenthal A. Neurotrophins promote motor neuron survival and are present in embryonic limb bud. Nature. 1993; 363:266-270.
32. Rooney GE, McMahon SS, Ritter T, Garcia Y, Moran C, Madigan NN, Flügel A, Dockery P, O'Brien T, Howard L, Windebank AJ, Barry FP. Neurotrophic factor-expressing mesenchymal stem cells survive transplantation into the contused spinal cord without differentiating into neural cells. Tissue Eng Part A. 2009; 15:3049-3059.
33. Schor NF. The p75 neurotrophin receptor in human development and disease. Prog Neurobiol. 2005; 77:201-214.
34. Ginty DD, Segal RA. Retrograde neurotrophin signaling: Trk-ing along the axon. Curr Opin Neurobiol. 2002; 12:268-274.
35. Quertainmont R, Cantinieaux D, Botman O, Sid S, Schoenen J, Franzen R. Mesenchymal stem cell graft improves recovery after spinal cord injury in adult rats through neurotrophic and pro-angiogenic actions. PLoS One. 2012; 7:e39500.
36. Rooney GE, Moran C, McMahon SS, Ritter T, Maenz M, Flügel A, Dockery P, O'Brien T, Howard L, Windebank AJ, Barry FP. Gene-modified mesenchymal stem cells express functionally active nerve growth factor on an engineered poly lactic glycolic acid (PLGA) substrate. Tissue Eng Part A. 2008; 14:681-690.
37. Huang F, Wang J, Chen A. Effects of co-grafts mesenchymal stem cells and nerve growth factor suspension in the repair of spinal cord injury. J Huazhong Univ Sci Technolog Med Sci. 2006; 26:206-210.
38. Urfer R, Tsoulfas P, Soppet D, Escandón E, Parada LF, Presta LG. The binding epitopes of neurotrophin-3 to its receptors trkC and gp75 and the design of a multifunctional human neurotrophin. EMBO J. 1994; 13:5896-5909.
39. Kumagai G, Tsoulfas P, Toh S, McNiece I, Bramlett HM, Dietrich WD. Genetically modified mesenchymal stem cells (MSCs) promote axonal regeneration and prevent hypersensitivity after spinal cord injury. Exp Neurol. 2013; 248:369-380.
40. Huang EJ, Reichardt LF. Neurotrophins: roles in neuronal development and function. Annu Rev Neurosci. 2001; 24:677-736.
41. Lee KF, Davies AM, Jaenisch R. p75-deficient embryonic dorsal root sensory and neonatal sympathetic neurons display a decreased sensitivity to NGF. Development. 1994; 120:1027-1033.
42. Chen YF, Zeng X, Zhang K, Lai BQ, Ling EA, Zeng YS. Neurotrophin-3 stimulates migration of mesenchymal stem cells overexpressing TrkC. Curr Med Chem. 2013; 20:3022-3033.
43. Ding Y, Yan Q, Ruan JW, Zhang YQ, Li WJ, Zeng X, Huang SF, Zhang YJ, Wu JL, Fisher D, Dong H, Zeng YS. Electroacupuncture promotes the differentiation of transplanted bone marrow mesenchymal stem cells overexpressing TrkC into neuron-like cells in transected spinal cord of rats. Cell Transplant. 2013; 22:65-86.
44. Sulpice E, Ding S, Muscatelli-Groux B, Bergé M, Han ZC, Plouet J, Tobelem G, Merkulova-Rainon T. Cross-

talk between the VEGF-A and HGF signalling pathways in endothelial cells. Biol Cell. 2009; 101:525-539.
45. Anan F, Shimomura T, Imagawa M, Masaki T, Nawata T, Takahashi N, Yonemochi H, Eshima N, Saikawa T, Yoshimatsu H. Predictors for silent cerebral infarction in patients with chronic renal failure undergoing hemodialysis. Metabolism. 2007; 56:593-598.
46. Bai L, Lennon DP, Caplan AI, DeChant A, Hecker J, Kranso J, Zaremba A, Miller RH. Hepatocyte growth factor mediates mesenchymal stem cell–induced recovery in multiple sclerosis models. Nat Neurosci. 2012; 15:862-870.
47. Jeong SR, Kwon MJ, Lee HG, Joe EH, Lee JH, Kim SS, Suh-Kim H, Kim BG. Hepatocyte growth factor reduces astrocytic scar formation and promotes axonal growth beyond glial scars after spinal cord injury. Exp Neurol. 2012; 233:312-322.
48. Hendriks WT, Ruitenberg MJ, Blits B, Boer GJ, Verhaagen J. Viral vector-mediated gene transfer of neurotrophins to promote regeneration of the injured spinal cord. Prog Brain Res. 2004; 146:451-476.
49. Naldini L, Blömer U, Gage FH, Trono D, Verma IM. Efficient transfer, integration, and sustained long-term expression of the transgene in adult rat brains injected with a lentiviral vector. Proc Natl Acad Sci U S A. 1996; 93:11382-11388.
50. Schambach A, Baum C. Clinical application of lentiviral vectors – concepts and practice. Curr Gene Ther. 2008; 8:474-482.
51. Lu CH, Chang YH, Lin SY, Li KC, Hu YC. Recent progresses in gene delivery-based bone tissue engineering. Biotechnol Adv. 2013; pii: S0734-9750(13)00149-3.
52. Ke J, Zheng LW, Cheung LK. Orthopaedic gene therapy using recombinant adeno-associated virus vectors. Arch Oral Biol. 2011; 56:619-628.
53. Kostarelos K, Miller AD. Synthetic, self-assembly ABCD nanoparticles; a structural paradigm for viable synthetic non-viral vectors. Chem Soc Rev. 2005; 34:970-994.
54. Hurtado A, Moon LD, Maquet V, Blits B, Jérôme R, Oudega M. Poly (D,L-lactic acid) macroporous guidance scaffolds seeded with Schwann cells genetically modified to secrete a bi-functional neurotrophin implanted in the completely transected adult rat thoracic spinal cord. Biomaterials. 2006; 27:430-442.
55. Lu Z, Hu X, Zhu C, Wang D, Zheng X, Liu Q. Overexpression of CNTF in Mesenchymal Stem Cells reduces demyelination and induces clinical recovery in experimental autoimmune encephalomyelitis mice. J Neuroimmunol. 2009; 206:58-69.
56. Liu N, Zhang Y, Fan L, Yuan M, Du H, Cheng R, Liu D, Lin F. Effects of transplantation with bone marrow-derived mesenchymal stem cells modified by Survivin on experimental stroke in rats. J Transl Med. 2011; 9:105.

(Received September 22, 2013; Revised October 24, 2013; Accepted October 26, 2013)