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

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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.
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 secrete 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
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) implementing 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

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

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

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