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Review Article

Stem Cells for the Treatment of Neuropathic Pain

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ABSTRACT

Aim of review: Neuropathic pain induced by injury to the somatosensory system is a great clinical problem. Despite multiple therapeutic strategies, the medical community still faces a challenge to treat neuropathic pain in a complete and definitive way, since the pathogenesis of this hypersensitive state is very complex. Stem cell transplantation may be an important approach for the treatment of neuropathic pain. This article aimed to review important and illustrative results from recent stem cell studies under various neuropathic pain conditions and to interpret their clinical implications for stem cell transplantation.

Method: We reviewed recent articles and literatures about stem cells for the treatment of neuropathic pain, in order to identify the types of stem cells, delivery approaches and the advances of stem cells for the treatment of peripheral nerve injury induced neuropathic pain, painful diabetic peripheral neuropathy and spinal cord injury (SCI) induced chronic pain.

Recent findings: Recently, the successful use of stem cell for the treatment of a diverse spectrum of diseases in animals has attracted more attentions from pain scientists. Accumulating evidence has shown that stem cell transplantation has a therapeutic effect on neuropathic pain. Stem cell transplantation can effectively relieve neuropathic pain under different pathological conditions. However, it is interesting to point out that peripheral neuropathic pain seems to be more responsive to stem cell therapy than SCI-induced chronic pain. Moreover, stem cell treatment does not always exert positive results in SCI-induced chronic pain (e.g. aggravating pain above the lesion spinal cord segment).

Summary: The analgesic effect of stem cells depends on the capacity to offer a multipotent cellular source for replacing injured neural cells and delivering trophic factors to lesion sites. Stem cell researches should focus on both experimental and clinical studies of neuropathic pain in the future.

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Neuropathic pain triggered by multiple insults to the somatosensory system is a clinical problem and the pathogenesis of this hypersensitive state is very complex, involving structural and neurophysiological changes. Currently, there are no drugs that can treat neuropathic pain in a complete and definitive way. Therefore, there is an overwhelming need to develop a novel drug or approach for the treatment of neuropathic pain. Recently, stem cells transplantation has been recognized as an important approach for the treatment of a variety of diseases in the future. Neuroscientists have also been beginning to realize the potential application of stem cells for the treatment of neuropathic pain. This review will characterize the types of stem cells, delivery approaches, and highlight the recent advances of stem cells for the treatment of various

neuropathic pain states.

Types of Stem Cells for the Treatment of Chronic Pain

Stem cells are defined as cells that possess the potential of self-replication and multipotent differentiation. According to the stage of development, they are classified as embryonic stem cells and adult stem cells. Because embryonic stem cell researches have some ethical limitations, adult stem cells such as neural stem cells (NSCs), mesenchymal stem cells (MSCs) and bone marrow mononuclear cells (BM- MNCs) are more widely used in experimental and clinical studies. NSCs present in the hippocampal dentate gyrus, olfactory bulb, subventricular zone (SVZ) surrounding the ventricles, subcallosal zone underlying the corpus callosum, and the spinal cord of the embryonic, neonatal, and adult rodent central nervous system (CNS) (1), as well as human fetal CNS (2). Under particular conditions, they can differentiate into neurons, astrocytes and oligodendrocytes (3). The most commonly used cells for pain relief are MSCs such as those from bone marrow (bone marrow MSC, BMSC) and adipose tissues (adipose tissue derived MSC, ASCs), excluding hematopoietic cells. Moreover, BM-MNCs have also been extensively evaluated for the treatment of chronic pain. They are bone marrow-derived cells which contain various kinds of cell lineages (e.g., hematopoietic cells, fibroblasts, osteoblasts, myogenic cells and endothelial lineage) (4). In addition, endothelial progenitor cells (EPCs) are progenitor cells of mature endothelial cells that show potential for their endothelial repair and vasculogenesis. They are being explored as therapeutic cell types for chronic ischemic pain, especially painful diabetic peripheral neuropathy. Recently, the therapeutic effects of human umbilical cord blood-derived mesenchymal stem cells (hUCB-MSCs) and amniotic epithelial stem cells (hAESCs) on chronic pain attract researchers' attention. HUCB-MSCs are derived from the umbilical vessels (i.e., two umbilical arteries and one umbilical vein) which are embedded in Wharton's jelly (WJ); hAESCs are derived from amniotic epithelium. They have emerged as alternative cell types for attractive advantages such as low risk of infection and teratoma formation, multipotency and low immunogenicity (5).

Multiple Approaches for Stem Cells Delivery in the Treatment of Neuropathic Pain

Stem cells can be transplanted by local delivery, intrathecal or intracerebroventricular administration, intravenous injection, intranasal delivery and endogenous mobilization by drugs for chronic intractable pain treatment. Hofstetter et al. (6) performed local delivery of neurogenin-2 expressing-NSCs directly into the damaged spinal segments in rats subjected to spinal cord injury, and found that it could reduce allodynia and improve motor function. However, the transplantation manner has some disadvantages such as local bleeding and tissue injury, which restrict its use (7). Hence, intravenous injection is more attractive for clinical application given the broad distribution. Surprisingly, following intravenous administration, stem cells can migrate from blood vessels into the central nervous system, crossing the blood-brain barrier (8-10), and might therefore home and integrate themselves into the DRG and spinal cord dorsal horn. It has been demonstrated that human BMSC either injected in the mouse lateral cerebral ventricle (11) or systemically into the caudal vein (12) were able to home into the spinal cord and prefrontal cortex of SNI-induced neuropathic mice to repair the damage. Another animal study of chronic constriction injury (CCI) models in rats has also shown that stem cells have a capacity of specifically reaching the damaged nerve (13) after a systemic injection to exert an analgesic effect. However, systemic delivery has a pulmonary trapping effect and only a few cells can reach the injured sites (7), which restrict its clinical application. Intrathecal administration is a common drug-given approachof pain research. The intrathecal administration of stem cells allows the target for DRGs and spinal cord in the pain pathway and helps to clarify the underlying mechanisms (14). Recently, intranasal delivery is emerging as a noninvasive option for delivering stem cells with minimal peripheral exposure (15). Finally, the endogenous stem cells in the bone marrow might be mobilized by drugs (e.g.,

granulocyte-colony stimulating factor and plerixafor) into the blood flow, therefore home to the lesion sites (16, 17).

Peripheral Nerve Injury-Induced Neuropathic Pain

Neuropathic pain induced by peripheral nerve injury is challenging to treat and often refractory to current pharmacotherapies. Despite decades of research, information regarding the mechanism of peripheral neuropathic pain is sparse. However, in recent years, some studies have suggested that the uninjured fibers intermingled with degenerating injured nerve fibers play critical roles in peripheral nerve injury-induced neuropathic pain (18). It is well established that nerve damage leads to Wallerian degeneration which causes the formation of neurinomas and alteration of nerve conduction (19) that result in neuropathic pain. In addition, during peripheral nerve injury, the adjacent uninjured nerve fibers develop ectopic and spontaneous discharges which are associated with central sensitization in neuropathic pain development. Hence, providing a favorable milieu for the injured and adjacent uninjured nerve fibers may ease neuropathic pain. About providing a protective microenvironment, neurotrophic factors are known to induce neuroprotection by maintaining functional integrity, promoting regeneration, regulating neuronal plasticity, and repairing the damaged nerves (20). Furthermore, recent studies have demonstrated that the activation of immune system also contributes to peripheral neuropathic pain pathology (21). Immune cells can penetrate into peripheral and central nervous system, activate glial cells and initiate a series of neuroinflammatory cascade which are known to facilitate pain signaling. In turn, the cascade of neuroinflammation-related events may maintain and worsen the original lesions and subsequently result in a more generalized immune response (22, 23). Consequently, protecting the injury microenvironment and balancing the pro- and anti-inflammatory cytokines may open new avenues for neuropathic pain treatment.

Stem cells have been shown to release neurotrophic and anti-neuroinflammatory cytokines. An interesting note is that stem cells, regardless of their sources, can secrete neurotrophic factors. For example, human mesenchymal stem/stromal cells (hMSCs) produce at least 84 trophic factors including epidermal growth factor (EGF), brain derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), ciliary neurotrophic factor (CNTF), basic fibroblast growth factor (bFGF/FGF-2), hepatocyte growth factor (HGF), and vascular endothelial growth factor (VEGF) (24). Further, these neurotrophic factors have also been released by ASCs and other stem cells (25). Neurotrophic factors released by stem cells have neuroprotective and neuroregenerative effects (26, 27). Additionally, both in vitro and in vivo studies have shown that stem cells can balance pro- and anti-inflammatory cytokines via paracrine effect (28). In vitro experiments, stem cells present an immunosuppressive effect by interacting with immune cells and regulating soluble factors such as cytokine interleukin-1β (IL-1β) and IL-10 (29, 30). Other studies have shown that stem cells can inhibit the neuroinflammatory cascade through bystander effect in vivo (12, 13, 31). Besides, other mechanisms of stem cells including opioids may also be involved in relieving peripheral nerve injury-induced neuropathic pain (32). Together, these data clearly indicate that, stem cells can produce both neurotrophic factors and neuromodulators following administration and therefore exert an analgesic effect.

Because stem cells of different origin all release neurotrophic factors and neuromodulators, various kinds of stem cells exhibit therapeutic effect on peripheral neuropathic pain. Considering the homologous development of the lesion in the peripheral and central nervous system, NSCs seem to be the most appropriate cell type to prompt physiological repair of the lesion. Xu et al. (33) considered that intrathecal administration of NSCs significantly attenuated mechanical and thermal hyperalgesia via marked increasing protein and mRNA levels of glial cell line derived neurotrophic factor (GDNF) in the spinal dorsal horn and dorsal root ganglia of nerve-injured rats. Yet, another experiment in rats subjected to neuropathic pain from CCI demonstrated that the effect contributing to NSCs-induced pain relief depends upon the reduction of proinflammatory cytokines (e.g., IL-1

and IL-6) and the production of anti-inflammatory IL-10 mRNA (13). However, different injection strategies may be responsible for the distinct mechanisms. Recently, ASCs have emerged as an attractive cell type for the treatment of chronic pain because it can be acquired by low invasive procedures. Like NSCs, by the use of ASCs isolated from female donors undergoing plastic surgery, recently published data showed that intravenous administration of ASCs induced a rapid, long lasting and dose dependent antihyperalgesic and antiallodynic effect (31). Importantly, ASCs-induced thermal hyperalgesia seems to be more potent and the level of anti-inflammatory IL-10 is higher compared to NSCs transplantation, suggesting that ASCs produce an increased analgesia in response to peripheral nerve injury (18). Moreover, these studies also showed that NSCs and ASCs both exert dose-dependent analgesia by repeated administration. Analogously, MSCs also offer a prominent cell type for the treatment of peripheral neuropathic pain as ASCs, because they have no marked phenotypic differences. In neuropathic pain mice, Siniscalco et al. (12) reported that systemic administered hMSCs can permeate the blood-brain barrier to home in the spinal cord and prefrontal cortex, where they can reduce the protein levels of pro-inflammatory cytokines (i.e., IL-1β and IL-17) and increase protein levels of anti-inflammatory cytokines (i.e., IL-10) that participate in pain formation. Besides, BM-MNCs, another type of stem cells from bone marrow, are beginning to yield encouraging results. Klass et al. (34) reported the beneficial effect of BM-MNCs in attenuating neuropathic pain in a CCI rat model although the mechanisms were not involved. Together, these data suggest that the neurotrophic factor-releasing nature coupled with the neuroinflammation regulatory capacity of stem cells may contribute to the relief of peripheral neuropathic pain. However, more extensive researches are still needed in this area to uncover the beneficial effects of stem cells before additional clinical trials are conducted.

Painful Diabetic Peripheral Neuropathy

Diabetic peripheral neuropathy (DPN) is the most common complication of diabetes mellitus, affecting up to 60% of diabetic patients (35). Symmetric spontaneous pain, hyperalgesia, allodynia and paresthesia are early symptoms of DPN (36). Especially, painful diabetic peripheral neuropathy (pDPN) which presents in 3% to over 20% of diabetic patients (37) is often refractory to current pharmacotherapies. It is well established that long-term diabetes leads to the destruction of peripheral blood vessels, particularly the vasa nervorum, and this destruction can cause microcirculation transformation and neurotrophin reduction in peripheral nerves that result in pDPN (38). In addition, deficiency of neurotrophic factors in the development and progress of pDPN results in distal axonal degeneration, axonal loss, and demyelination (39), which may attribute to the distal pre-dominant nerve pathology. Taken together, because the mechanism involves both vascular and neurotrophic deficiency, using a therapeutic agent that has dual angioneurotrophic activities may prove beneficial for the treatment.

For many decades, most researches on stem cells have revolved around neurotrophic mechanism. However, recent studies have demonstrated that stem cells also have paracrine properties of angiogenic cytokines. For example, EPCs produce multiple angiogenic factors such as VEGF, insulin-like growth factor-1 (IGF-1), and fibroblast growth factor-2 (FGF-2) (40); other stem cells (e.g., BM-MNCs and MSCs) have also been shown to release angiogenic ligands (41, 42). Angiogenic factors have been reported to play a crucial role in neovascularization (43). Furthermore, most angiogenic factors released by stem cells also exert neurotrophic effects. Thus, stem cell transplantation may exhibit therapeutic effects encompassing both supplying neurotrophic cytokines through direct effects on functional recovery of peripheral nerves and inducing neovascularization to crease nerve blood flow. Different mechanisms of stem cell transplantation involved in different pathological conditions were shown in figure.

Application of stem cells in preclinical studies and clinical trials for therapeutic purposes is beginning to yield encouraging results. EPCs are putative progenitor cells of endothelial cells that contribute to postnatal neovascularization. For example, cord blood-derived endothelial progen-

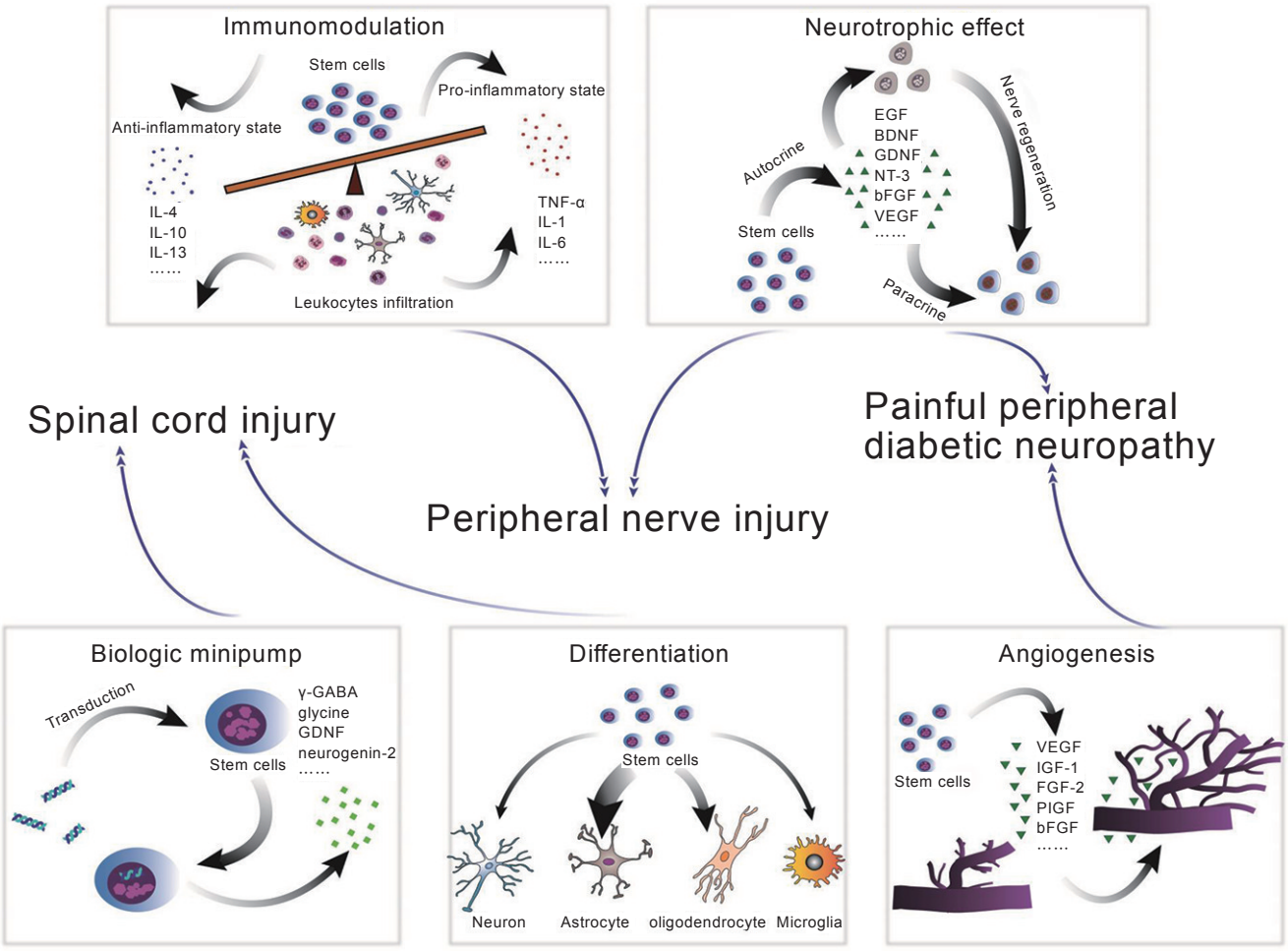

Figure. Different Mechanisms of Stem Cell Transplantation Involved in Different Pathological Conditions.
The major analgesic mechanisms of stem cell transplantation under the conditions of peripheral nerve injury, painful peripheral diabetic neuropathy and spinal cord injury are now believed to be different and they can be divided into five main categories: immunomodulation, neurotrophic effect, biologic minipump, differentiation and angiogenesis. Although knowledge of stem cell-dependent mechanisms known to mediate the relief of chronic pain increases every year, these mechanisms are depicted here for illustrative purposes. The immunomodulatory effects of stem cells consist of inhibition of the pro-inflammatory cytokines (e.g., TNF-α, IL-1 and IL-6) and upregulation of anti-inflammatory cytokines (e.g., IL-4, IL-10 and IL-13). Stem cells and stem cell-stimulated resident cells also release neurotrophic factors (e.g., EGF, BDNF, GDNF, NT-3, bFGF and VFGF), which are responsible for neuroprotective and neuroregenerative effects. Neurotrophic factors are acted through autocrine effect on differentiated cells and paracrine effect on resident neurons and glial cells. In addition, stem cells can be genetically modified and then transplanted as a biological minipump to release neurotransmitters and neurotrophins (e.g., γ-GABA, glycine, GDNF and neurogenin-2). Moreover, the special characteristics of stem cells are associated with their differentiation into neuron, astrocyte, oligodendrocyte and microglia. Finally, stem cells stimulate local angiogenesis by secretion of extracellular matrix molecules, VEGF, IGF-1, FGF-2, PIGF and bFGF. However, after stem cell transplantation, immunomodulation and neurotrophic effects are mainly responsible for peripheral nerve injury induced neuropathic pain, neurotrophic effect and angiogenesis for painful peripheral diabetic neuropathy; and biologic mini-pump and differentiation are primarily associated with spinal cord injury induced chronic pain. Meanwhile, these mechanisms may interact with each other under different pathological conditions.

itor cells (CB-EPCs) were reported to improve motor nerve conduction velocity (MNCV) and sciatic nerve blood flow (SNBF) with intramuscular injection in streptozotocin (STZ)-induced diabetic rats through enhancing vascular density in the treated leg muscles (44). Another mechanism by which EPCs contribute to pDPN is up-regulating the expression of mRNA and proteins

of VEGF, bFGF, BDNF and stromal cell-derived factor-1a in the sciatic nerve (40). However, the interaction of these effects may exist at the same time. Recently, mononuclear cells including PB-MNCs and BM-MNCs yield potential beneficial effects for the treatment pDPN in preclinical models. Naruse and Kim (41, 45) both confirmed that BM-MNCs-induced amelioration in experimental pDPN was associated with increased angiogenesis and neurotrophic factors in peripheral nerves. Moreover, MSCs may also be a good candidate for the treatment of pDPN. Shibata et al. (46) found that VEGF and bFGF mRNA expressions were significantly increased in MSCs-injected thigh muscles of STZ-induced diabetic rats. Interestingly, Kim et al. (47) reported that the levels of NGF and NT-3, but not VEGF or bFGF were increased in diabetic animals received BM-MNCs transplantation. Notably, additional researches are required to understand the discrepancy in MSCs-induced neurotrophic actions. However, it is clear that MSCs would be an optimal strategy in the treatment of pDPN. Additionally, intraperitoneal administration of MSC2, a protective MSCs phenotype, was shown to prevent thermal hyperalgesia, alleviate mechanical allodynia, and facilitate the reduction of pro-inflammatory cytokines in the serum level of pDPN mice (48), indicating that other mechanisms (e.g., immunosuppression) might also exist. Alternatively, newly improved induced pluripotent stem cells (iPSCs) have emerged as attractive tools for the treatment of pDPN. Okawa et al. (49) suggested that iPSCs-derived neural crest-like cells can differentiate into Schwann cell-like cells and vascular smooth muscle cells, improve the impaired nerve and vascular functions, and produce growth factors (e.g., NGF, VEGF, NT-3 and bFGF) in mice with pDPN. Based on the preclinical data, Comerota et al. (50) treated a diabetes mellitus type I patient with 15 intramuscular injections of 49-fold increased CD90+ mesenchymal cells in a case report. Dramatic pain relief of this patient was reported two months later, and analgesics were no longer needed and blood perfusion was increased on laser Doppler imaging 6 months later. After 9 months the patient had no pain and all the gangrenous and infected tissues were healed.

Spinal Cord Injury Induced Chronic Pain

Spinal cord injury (SCI) is a serious central nervous system disease because it is associated with catastrophic consequences in patients. Most SCI victims suffer from chronic pain which is difficult to manage or treat (51). Although some studies suggest that various neuroanatomical and neurochemical changes take place in the central nervous system after SCI, little is known about how these changes facilitate pain signaling. However, it has been generally accepted that hypofunction of the inhibitory pathways (e.g., GABAergic inhibitory system) contributes to many pathological conditions including SCI-induced chronic pain (52). Furthermore, the reduction of neurotrophic factors and the production of pro-inflammatory cytokines (e.g. IL-1 and TNF-α) in the injured spinal cord (53, 54) are other mechanisms that contribute to the painful state. These pathological changes inhibit abnormal axon regeneration and nerve remyelination (55) and thereby lead to aberrant regeneration and axonal sprouting.

Multiple types of cells, including stem cells, can be genetically modified and then transplanted as biological agents to release neurotransmitters and neurotrophins for therapeutic purposes (56, 57). Moreover, both in vitro and in vivo studies have shown that the special characteristics of stem cells are associated with their differentiation ability. For example, in vitro studies, NSCs may differentiate into neurons, astrocytes and oligodendrocytes, whereas when transplanted into spinal cord, NSCs give rise to glia (almost exclusively astrocytes and only relatively few oligodendrocytes) and occasional neurons (6). Importantly, the glia cells play important roles in synthesis, release, and uptake neurotransmitters (58). Stem cell derived astrocytes induce anti-nociception by increasing the release of trophic factors (6, 59), which then counteract factors that inhibit axonal regeneration. Stem cell derived oligodendrocytes play a critical role in remyelination of spared axons within the injured white matter tracts (60). Together, stem cell transplantation may be a potential treatment for SCI-induced chronic pain through acting as a biologic minipump and differentiation.

Despite the limitations and negative effects, NSCs transplantation is a potential treatment for SCI-related chronic pain. It has been generally accepted that genes of regulatory cytokines (e.g., neurotransmitters and neurotrophic factors) that have the desired therapeutic efficacy may be harnessed to provide long-term pain relief. In rats with SCI, a single subarachnoid injection of hNT2.17 cells potently reversed tactile allodynia and thermal hyperalgesia by acting as a "biologic minipump" to synthesize and release inhibitory neurotransmitters γ-aminobutyric acid (GABA) and glycine in the lumbar subarachnoid space (61). In contrast, Melissa et al. (62) suggested that murine embryonic C17.2 NSCs lead to thermal and mechanical forelimb allodynia when transplanted into the injured spinal cord in rats, whereas GDNF-transfected C17.2 NSCs (C17.2/GDNF) exert an analgesic effect on SCI-related pain by inhibiting neuronal sprouting. Moreover, another preclinical study suggested that transduction of NSCs with neurogenin-2 before transplantation can suppress astrocytic differentiation, increase oligodendrocytes conversion, prevent graft-related sprouting and thereby reduce SCI-induced allodynia (6, 63). Yet, a major challenge of developing NSCs-dependent strategies for the treatment of SCI-induced chronic pain is their low transplant efficiency which restricts the therapeutic potential (64). Accordingly, recent efforts have focused on identifying combinatorial strategies to improve grafted cell survival in the host damaged spinal cord. Olfactory ensheathing glial cells (OECs) were reported to promote axonal regeneration, reorganize the glial scar, remyelinate axons and stimulate neural repair after transplantation. Luo et al. (64) found that administion of NSCs and OECs together produce an increase in analgesia by enhancing NSCs survival and down regulating NGF expression. Similarly, neurotrophic factors were also found to act synergistically with neural progenitor cells (NPCs) to optimize their integration into the host spinal cord and facilitate oligodendrocytes differentiation (65). Taken together, these findings suggest that NSCs transplantation has a crucial role in pain remission in response to SCI.

Alternatively, a growing number of preclinical experiments and clinic trials suggest that MSCs may also exhibit therapeutic effects on SCI (66). In early preclinical studies, Yang et al. (67) reported that intraspinal transplantation of HUMSCs derived from Wharton's jelly of the umbilical cord improves locomotor recovery, possibly by increasing axon regeneration in the corticospinal tract and neurofilament-positive fibers around the lesion. Although whether the transplantation relieves pain-related behavior is not known, NT-3 and bFGF produced by hUMSCs indicate a potential therapeutic evaluation for pain relief. Recently, Roh et al. (68) suggested that intraspinal transplantation of hAESCs or hUMSCs reduces mechanical allodynia in T13 spinal cord hemisected rats both by reducing spinal cord microglia activity and NR1 phosphorylation. Together, these data formed the basis for clinical trials to use MSCs in patients with SCI. In a case report, a patient with an incomplete T12-L1 spinal cord injury and a L1 vertebral body crush fracture was treated with local transplantation of several cycles of MSCs and CD34+ cells. Allogeneic transplantation markedly decreased SCI-induced pain (from daily 10/10 to once a week 3/10 visual analogue scale) and significantly improved muscle strength of the patient (69). Overall, both preclinical studies and clinical reports support the therapeutic effect of MSCs in SCI-related chronic pain relief.

These preclinical and clinic data suggest stem cell transplantation would be an alternative strategy in attenuating SCI-induced chronic pain. However, additional researches are required to optimize the function of stem cells and increase their clinical safety before further clinical application.

Summary

Stem cell transplantation can effectively relieve neuropathic pain under different pathological conditions. However, it is interesting to point out that peripheral neuropathic pain seems to be more responsive to stem cell therapy than SCI-induced chronic pain. Moreover, stem cell treatment does not always exert positive results in SCI-induced chronic pain (e.g. aggravating pain above the lesion spinal cord segment). Understanding the molecular mechanisms underlying both positive and negative effects of stem cells on pain processing is very important for the de-

velopment of novel, specific and effective therapeutic modalities for pain relief. Stem cell researches should focus on both experimental and clinical studies of neuropathic pain in the future. In clinical trials, the type and dosage of the infused stem cells, the safety and the grafting efficiency should be further investigated. In animal researches, the analgesic mechanisms of stem cells in different animal models of neuropathic pain should be explored.

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