MEDS Clinical Medicine (2022)
Clausius Scientific Press, Canada

DOI: 10.23977/medsc.2022.030801
ISSN 2616-1907 Vol. 3 Num. 8

Advances in Mesenchymal Stem Cell Derived Exosomes in the Repair Mechanism of Spinal Cord Injury

Heshan Zhou[1,a], Longwang Tan[2,b,*]

[1]Shaanxi University of Chinese Medicine, Xianyang, Shaanxi, 712046, China
[2]Department of Spinal Orthopaedics, Affiliated Hospital of Shaanxi University of Chinese Medicine, Xianyang, Shaanxi, 712000, China
[a]945522807@qq.com, [b]longwangtan@sina.com
[*]Corresponding author

Keywords: Mesenchymal Stem Cells, Exosomes, Spinal Cord Injury

Abstract: Once a spinal cord injury occurs, it can place a tremendous emotional, physical, and financial burden on the patient and their family. Mesenchymal stem cell transplantation has shown promising efficacy in spinal cord injury, and its paracrine production of exosomes may be the main component exerting therapeutic effects. Exosomes are small vesicles that function similarly to source cells and are capable of participating in a variety of cellular processes. Current studies have demonstrated that MSC-derived exosomes can achieve repair of spinal cord injury by inhibiting the inflammatory response, reducing apoptosis, promoting axonal regeneration, angiogenesis, neuroprotection, and other mechanisms. This paper reviews the mechanism of mesenchymal stem cell-derived exosomes in spinal cord injury, which helps to further explore the feasibility of mesenchymal stem cell-derived exosomes in treating spinal cord injury and provides new methods and strategies for the follow-up study of spinal cord injury.

1. Introduction

Spinal cord injury (SCI) refers to the damage to the structure and function of the spinal cord caused by various factors, which can lead to impairment of sensory, motor, sphincter, and autonomic functions below the corresponding injury level[1]. Mesenchymal stem cells (MSCs), with their potential for self-renewal and multidirectional differentiation, easy to isolate and preserve, and avoiding ethical issues, have received more attention compared to other cells. The efficacy of MSCs transplantation therapy in SCI has been demonstrated, but has the disadvantage of low homing rate and high tumorigenicity[2]. Exosomes (Exoes) are tiny particles produced by cells during their life cycle and contain a variety of bioactive substances that can be used in the diagnosis and treatment of diseases[3]. The use of MSCs-derived exo (MSCs-Exoes) alone has been found to produce therapeutic effects similar to those of cell transplantation, and with the increasing research targeting MSCs-Exoes in the field of SCI in recent years, it plays a great role in several aspects such as axonal growth, glial scar formation, inflammatory response, and apoptosis[4]. Therefore, how to make MSCs-Exoes better applied to SCI treatment is a new research hotspot and direction at present.

2. Overview of Spinal Cord Injury

After SCI occurs, it can be divided into primary and secondary stages according to the mechanism of injury. The primary stage is mainly primary trauma caused by external forces that lead to direct injury to the spinal cord tissue by fracture fragments, intervertebral discs, ligaments, etc. This mechanical injury can damage local vascular and cellular structures, causing damage to the nerve parenchyma and axons, resulting in a series of consequences such as vasospasm, hemorrhage, ischemia, edema, and ion imbalance[5].

The secondary stage is dominated by secondary damage, mainly damage to adjacent tissues and cells by cascade reactions brought about by primary injury. At the initial stage of secondary injury, inflammatory cells spread and release inflammatory factors such as interleukins[6], which will manifest as a strong inflammatory response, while local ischemia caused by vascular injury will induce early reperfusion and release large amounts of reactive oxygen and reactive nitrogen species, causing oxidative stress, exacerbating damage to the cytoskeleton and organelles, further disrupting cellular homeostasis, causing mitochondrial dysfunction, and eventually activating apoptotic pathways, while a rapid increase in glutamate concentration to neurotoxic levels will also disrupt the normal ionic internal environment and lead to neuronal death[7].

Later in the secondary stage, glial scarring is the main pathological mechanism. In the case of injury, astrocytes are activated and proliferate reactively, forming a dense glial scar whose physical barrier effect prevents axonal regeneration, while axonal growth and neurological recovery are further hampered by the massive production of axonal growth inhibitory factors such as chondroitin sulfate proteoglycans[8]. The mechanisms and treatments of spinal cord injury are summarized below(Figure 1).

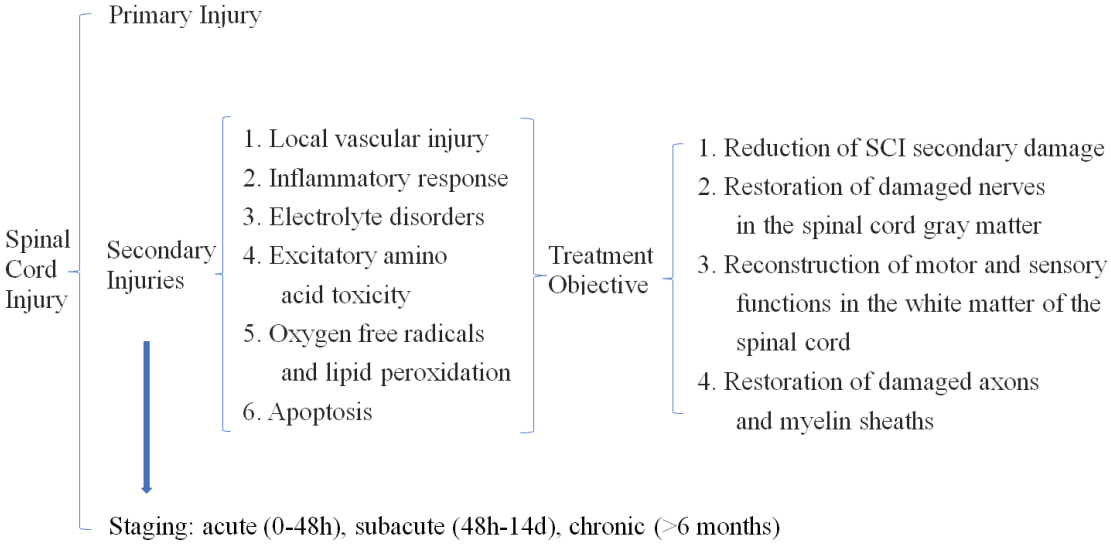

Figure 1: Mechanisms and treatment points of spinal cord injury

3. Overview of Mesenchymal Stem Cell-derived Exosomes

Mesenchymal stem cells, as a class of pluripotent stem cells from a wide range of sources (including bone marrow, umbilical cord, placenta, adipose, etc.), have extremely strong self-renewal ability and multidirectional differentiation characteristics, and have shown good efficacy in central nervous system diseases[9]. Numerous experimental studies have demonstrated that MSCs transplanted into animal models of spinal cord injury can effectively improve animal symptoms[10]. There are clinical trials on MSCs transplantation for SCI in an organized manner.

Junseok et al[11]. injected autologous adipose-derived MSCs into SCI patients by intrathecal injection, and no serious adverse events occurred and the patients showed some improvement in neurological function during the eight-month follow-up. However, MSCs transplantation is also gradually found to have certain drawbacks, and there are related reports that MSCs have the ability to migrate to tumor sites and can promote tumor growth and metastasis[12]. This may be related to the immune status of the experimental animals themselves, which requires us to refine the experimental design and further explore the potential tumorigenicity of MSCs.

Extracellular vesicles (EVs) are nanoscale particles with phospholipid bilayers produced by eukaryotic cells during their life cycle, which were considered as cellular waste in the early days, but the functions of EVs have been gradually explored in recent years as research continues[13]. EVs can be classified into three subtypes (Table 1), such as micro vesicles, apoptotic vesicles, and exosomes, based on size and formation mode, and exosomes are generally 30-150 nm in diameter. Cells form intracellular vesicles by invagination of the plasma membrane, which then develop into endosomes that bud inward through the cell membrane to form multi-vesicular bodies containing multiple intracellular vesicles, and the nanoscale particles that fuse with the cell membrane and are released outside the cell are exosomes[14, 15].

Table 1: Composition and typing of Evs

Classification	Size	Formation method	Main active ingredients
Microvesicles	150-1000 nm	Direct budding and shedding of cell membranes	Lipids and proteins similar to the source cell membrane
Exosomes	30-150 nm	Fusion of intracellular vesicles with early endosomes	Transmembrane transport-related proteins, heat shock proteins, TSPAN protein superfamily, etc., lipids, nucleic acids
Apoptotic vesicles	1000-5000 nm	Produced by apoptotic cells, a product of the apoptotic process	DNA material and organelles

Table 2: Advantages and disadvantages of common exocrine preparation methods

Extraction Method	Production	Purity	Time	Advantages	Disadvantages
Ultracentrifugal method	High	High	Long	High number of withdrawals, gold standard	Time-consuming process, unstable recovery rate, repeated centrifugation affects quality
Ultra-filtration centrifugal method	Medium	Low	Medium	Simple operation and low sample requirements	Filter membranes easily affect the extraction rate and purity of exosomes
Sedimentation method	Medium	Medium	Medium	Simple process, no special equipment required	Low purity, chemical additives may destroy exosome activity
Immunomagnetic bead method	Low	High	Long	High specificity and high purity of exosomes	Low efficiency, expensive, not conducive to universal access
Reagent kit method	Medium	Medium	Short	Simple operation, low equipment requirements	Purity needs to be further improved

Exosomes contain a variety of bioactive substances, such as proteins (transmembrane transport-associated proteins, heat shock proteins, TSPAN, protein superfamily, etc.), lipids

(cholesterol, diglycerides, sphingomyelin, etc.), nucleic acids (DNA, miRNA, lncRNA, mRNA, tRNA, etc.), etc[16]. Their surface markers vary depending on their origin and secretion mechanism, and their markers change significantly in different pathological conditions, whereby they can be used for disease diagnosis[17]. Exosomes as the smallest subtype of EVs, exhibit good transmissibility (can cross the blood-tissue barrier), precise targeting for loading drugs for therapeutic action, and inherently function similarly to the source cells, thus demonstrating good therapeutic effects in a variety of diseases[18]. At present, the common separation and purification methods of exosomes include the following: ultracentrifugation, density gradient centrifugation, ultrafiltration centrifugation, multimer precipitation, immunomagnetic bead method, flow sorting method, and kit extraction method[19], as shown in Table 2.

It was demonstrated in animal experiments that MSCs and MSCs-Exoes have the same physiological functions, and MSCs-Exoes avoids the complications of MSCs transplantation, while the strong secretory capacity and high secretion of MSCs make MSCs-Exoes more advantageous than other cell-derived exosomes in the study[20]. Compared to MSCs, MSCs-Exo has the following advantages.(1) the source is widely available and easier to collect; (2) the structure is stable and easy to store, and its activity can be maintained for a long time under appropriate conditions[21]; (3) it is safer, avoiding the potential carcinogenic risk of MSCs and reducing the possibility of immune rejection; (4) the therapeutic effect is better, as MSCs-Exo can penetrate BSCB and play a better therapeutic role[22]. It is because of the many advantages of MSCs-Exo and its wide range of physiological functions that the current field of regenerative medicine is gradually shifting from MSCs-dependent cellular therapy to MSCs-Exo-dependent cell-free therapy.

4. Mechanism of MSCs-Exo for Spinal Cord Injury

4.1. Immunomodulatory Effect

Depending on the stimulus signal during injury, macrophages/microglia are polarized and exhibit different phenotypes. Macrophage/microglia type M1, which is usually activated in the first phase after SCI, shows a strong inflammatory response, which gradually reaches its peak through the release of proinflammatory mediators. Macrophage/microglia type M2, which reduces the secretion of proinflammatory factors and increases the expression of anti-inflammatory factors, also has phagocytic activity, resulting in a positive immunomodulatory effect. [23] prepared a rat model of SCI to evaluate the therapeutic effect of MSCs-Exoes. The treatment group showed a decrease in M1 type macrophages/microglia, an increase in anti-inflammatory M2 type, and a decrease in NK cells and leukocytes, and MSCs-Exo was effective in reducing inflammation during CNS injury. [24] Demonstrated the same results for MSCs-Exoes in the treatment of SCI. Cord-derived MSCs-Exoes effectively promoted the polarization of macrophages/microglia from the M1 phenotype to M2 phenotype and significantly decreased the levels of proinflammatory factors TNF-α, IL-6, IFN-γ, G-CSF, MCP-1, MIP-1α and increased the levels of anti-inflammatory cytokines IL-4 and IL-10.

Recent studies have revealed that reactive astrocytes can be classified into A1 and A2 types according to their phenotype, similar to the polarization of macrophages/microglia. Type A1 is induced by neuroinflammation and exerts a proinflammatory effect, while type A2 is activated in neural ischemia and exerts a neuroprotective effect. [25] investigated the application of MSCs-Exoes in SCI treatment by immunodetection of activated microglia by CD68+, using C3 (A1 astrocyte marker, not expressed in A2 astrocytes.) and GFAP (astrocyte marker) to identify A1 astrocytes, and found that the expression levels of IL-1β, IL-6, and TNF-α in the group treated with MSCs-Exo were significantly lower than those in the SCI group, and the number of CD68+

microglia and C3-positive astrocytes at the injury site were also significantly lower than those in the SCI group, demonstrating that MSCs-Exo can treat the SCI after neuroinflammatory response, the mechanism of which may be to inhibit the activation of A1 neurotoxic responsive astrocytes. [26] administered MSCs and equal amounts of homologous MSCs-Exoes intravenously to SCI rats to observe their potential effects on A1 astrocytes. The results showed that both MSCs and MSCs-Exoes inhibited A1 astrocytes, not only downregulated the general responsiveness of GFAP, but also reduced the proportion of A1 in the whole astrocytes. Moreover, in the study, PKH26-labeled MSCs-Exo was detected at the lesion site, while transplanted MSCs were not detected at the lesion site. This further supports our conclusion in the previous paper that the proliferative differentiation capacity of MSCs is not the primary mechanism mediating the therapeutic effects of SCI and that MSCs-Exo plays a major role in the therapeutic function of intravenously injected MSCs.

The inflammatory response after SCI is a multifaceted and complex continuum, and MSCs-Exoes can reduce inflammation and promote SCI recovery by interfering with a variety of inflammatory cells and inflammatory factors. An in-depth study of inflammation-related mechanisms can improve the inflammatory environment in the early stages of SCI, thus influencing the progression of secondary damage and laying the foundation for later repair.

4.2. Maintain BSCB Integrity

SCI causes damage to the microvasculature and disruption of the blood-spinal cord barrier (BSCB), which allows the exchange of harmful elements in the blood and tissues and affects the normal function of the nervous system. Therefore, maintaining the integrity of the BSCB after SCI can have a therapeutic effect on the injury. [27] found that MSCs-Exo could significantly inhibit pericyte migration and improve pericyte coverage by inhibiting the NF-κB signaling pathway. This improves the structural integrity of the BSCB and promotes functional recovery after SCI. [28] directly used Exo of pericyte origin to treat SCI. Pericytes are closely related to endothelial cells, and their secreted Exo is more easily taken up by endothelial cells. Studies have shown that hypoxia-inducible factor-1α (HIF-1α) is activated under hypoxic conditions, leading to increased permeability, edema, and tissue damage in the BSCB. In addition, vascular rupture caused by SCI, which is the main cause of loss of spinal cord perfusion, increases the permeability of the BSCB and subsequently exacerbates tissue edema. Moreover, the activation of matrix metalloproteinases (MMPs) is necessary for inflammatory cell infiltration and BSCB destruction after SCI. By using pericyte-derived Exoes, it significantly downregulated HIF-1α, improved microcirculatory impairment, restored blood perfusion, promoted neural tissue survival, and promoted BSCB integrity during SCI by regulating tight junction proteins. It also reduced MMP2 expression and significantly alleviated the degree of edema after SCI. In summary, it is clear that pericyte-derived Exo protects endothelial cells through the PTEN/Akt pathway and improves endothelial barrier function under hypoxic conditions, leading to the treatment and intervention of SCI.

4.3. Reduces Apoptosis

Cell death is an important process affecting physiological functions after SCI, mainly including two mechanisms of necrosis and apoptosis, and in recent years, cell autophagy has also been extensively studied. After SCI occurs, the neuronal and glial cell necrosis caused by primary injury is irreversible and persists to the next stage. Apoptosis includes both endogenous and exogenous apoptotic pathways, which are mainly regulated by the Bcl-2, Bax, and caspase families, indicating that apoptosis can be controlled by a series of chain reactions leading to apoptosis during the secondary injury phase.

By comparing protein changes in SCI rats before and after treatment with MSCs-Exo, [29] identified several differential proteins associated with apoptosis and inflammation. Bax/Bcl-2 and caspase-3/casepase3 ratios were significantly lower in the MSCs-Exo treated group. And the beneficial effects of high miR-21-5p expression in MSCs-Exoes on motor function, recovery and apoptosis were verified, indicating that miR-21-5p achieves antiapoptotic effects through endogenous regulatory mechanisms. FasL gene is one of the direct target genes of miR-21-5p, and binding to Fas can induce neuronal apoptosis. MSCs-Exoes can reduce the expression of FasL gene by targeting miR-21-5p, thus exerting a therapeutic effect on SCI. Li et al.[30] designed a rat SCI model to investigate the mechanism of SCI repair by bone marrow-derived MSCs-Exoes and found that the apoptosis rate was significantly reduced in the MSCs-Exo treatment group. MSCs-Exo significantly reduced the expression levels of Bax protein and caspase3 and caspase-9, increased the expression levels of Bcl-2 protein, and promoted the recovery of spinal cord physiological functions by inhibiting neuronal apoptosis. Meanwhile, in vitro experiments also showed that Wnt/β-catenin signaling pathway has an important role in stimulating axonal regeneration and inhibiting neuronal apoptosis, and MSCs-Exo attenuated tissue damage after SCI by activating Wnt/β-catenin signaling pathway.

SCI also causes autophagy dysregulation, autophagy is an essential process for regulating cellular homeostasis, and the autophagic vesicle localization proteins LC3 and p62 are commonly used to monitor the autophagic activity of cells after SCI. [31-32]. Found that MSCs-Exoes could upregulate the expression of autophagy-related proteins LC3IIB and Beclin-1, decrease the expression of p62, and promote the formation of autophagic vesicles after SCI through rat SCI model and in vitro experiments, demonstrating that MSCs-Exo could effectively inhibit neuronal apoptosis and improve behavioral functions in SCI rats by promoting autophagy.

In recent years, research on cell death has been intensified, and death mechanisms such as iron death, cell scorching, and necrotic apoptosis have received increasingly attention, and the cell death process can be effectively improved through the intervention of target proteins and related pathways. However, there are no studies on how the above mechanisms are regulated during MSCs-Exo treatment of SCI. Moreover, different cell death modes are interdependent and do not exist in isolation; therefore, further research on mechanisms such as cell scorching and iron death can provide new targets and directions for the treatment of SCI.

4.4. Axon Regeneration

Axonal regeneration at the site of injury is important for functional recovery after SCI, but repairing axons after injury is very difficult for the mature CNS. After SCI occurs, not only inhibitory factors such as CSPG are produced, but also accompanying glial cell scar formation constitutes a physical barrier to axonal extension[33].

Liu et al.[34] constructed a rat spinal cord contusion model, and after injection of bone marrow-derived MSCs-Exoes, it was found that the deposition of CSPG at the SCI injury site was significantly reduced, and the number of regenerated axons was also significantly improved compared to the blank group. MSCs-Exoes loaded with specific MiRNAs could better promote axon growth compared to normal MSCs-Exoes. Li et al.[35] packaged miR-133b into bone marrow-derived MSCs-Exoes. miR-133b has been shown to play an important role in neuronal and axonal regeneration. After tail vein injection of miR-133b-loaded MSCs-Exoes, the phosphorylation levels of STAT3 and CREB were increased, RhoA expression was inhibited, so myelin inhibitory factors were significantly downregulated and the levels of neural repair-related factors (NF, GAP-43, GFAP and MBP) were increased, which enhanced the in vivo regeneration of spinal axonal proteins and significantly promoted axonal regeneration after SCI. Beneficial to the recovery

of neurological function in SCI animals. The study by Ren et al.[36] similarly demonstrated that miR-133b-modified MSCs-Exoes significantly promoted axon regeneration in SCI rats by affecting axon regeneration-related signaling pathways.

Studies have shown that axon regeneration and synapse formation are closely related to PTEN/AKT/mTOR, and PTEN negatively regulates the PTEN/AKT/mTOR pathway. Knockdown of PTEN can upregulate the endogenous axon regeneration ability of neurons and play a negative role in neuronal regeneration. Thus, inhibition of PTEN action can affect the recovery of neuronal axons after SCI[37]. Guo et al.[38] constructed MSCs-Exo carrying small interfering RNAs of phosphatase and tensin homologs, and administered them nasally to SCI rats, and found that the total axon length, number of axons, branching points and maximum branching levels of neurons in the treated group were higher than those in untreated neurons. It was demonstrated that MSCs-Exoes, carrying PTEN-siRNA, could cross the blood-brain barrier and home to the injured spinal cord region, significantly promoting vascular neovascularization and axonal growth and improving motor and sensory functions in rats by inhibiting PTEN expression in spinal cord injury. Huang et al.[39] also demonstrated in a similar experiment that silencing PTEN expression at the lesion site significantly promoted the regeneration of damaged axons and retained more neurons.

The recovery of neurological function after SCI is the main direction of current research, and axons, as the key structure for maintaining neuronal function, are the focus of SCI intervention and treatment. In contrast, MSCs-Exo not only antagonizes axonal regeneration inhibitors, but also activates endogenous signaling pathways to drive neural signal transduction, effectively promoting axonal growth and myelin remodeling. We need to further clarify the way MSCs-Exo regulates nerve regeneration and precisely intervene in its upstream and downstream signaling axes to induce axonal regeneration and nerve function reconstruction for SCI repair.

4.5. Fibrous Scar

After SCI, glial scars begin to form in the acute phase, and the scars formed during this period limit the spread of immune cells and serve as neuroprotection. In the subacute and chronic phases, the glial scar restricts the regeneration of neurons and axons by means of physical barriers, and at the same time, the glial scar secretes large amounts of chondroitin sulfate (CS) and CSPG and other axonal growth inhibitory factors, which affect the repair and growth of neurons and axons[8].

Liu et al.[40] found that MSCs-Exo exhibited significant therapeutic effects on SCI rats and could effectively promote functional behavioral recovery after SCI through in vitro and in vivo experiments. By detecting the expression of CSPG in the injury area, it was found that CSPG deposition was significantly reduced, the number of neovascularization was significantly increased, and glial scar formation was reduced by nearly 75% in the exo-treated group, demonstrating that MSCs-Exoes could inhibit glial scar formation. Luo and Guo et al.[38, 41] demonstrated the same conclusion in a similar experiment, where MSCs-Exoes effectively reduced CSPG deposition at SCI sites and inhibited A1 neurotoxic reactive astrocyte activity, suggesting an inhibitory effect of Exo on glial scarring. Romanelli et al.[42] used umbilical cord-derived MSCs-Exoes to treat traumatic SCI and found that application of MSCs-Exoes reduced reactive hyperplasia of AS by approximately 40%, as well as reduced type I collagen and NG2 deposition at the injury site, both of which demonstrate the anti-scarring effect of MSCs-Exo.

In another part of the study, the role of glial scarring was reconceptualized. Anderson et al.[43] found that astrocyte scar formation contributes to CNS axon regeneration and that spontaneous axon regeneration is not promoted by preventing or attenuating astrocyte scar formation. Glial scarring inhibits axonal growth mainly by producing CSPGS, but it also promotes activation of the intrinsic axonal growth program by neurons and is a necessary bridge for axonal regeneration.

With further research, the role played by glial scarring in SCI is more fully understood, and the negative effects of its physical and chemical barriers have been demonstrated. We want to impede the production of axonal inhibitory factors through relevant measures, while promoting axonal sprouting by reducing the formation of glial scarring. However, the positive role of glial scarring cannot be ignored, and the neuroprotective effect in the early stages of SCI, as well as the activation of the intrinsic neuronal growth program in the later stages, are effective targets in SCI treatment, for which interventions can further promote post-SCI recovery.

4.6. Neural Protection

MSCs have strong neuroprotective effects, promoting the repair and regeneration of injured neurons by secreting several neurotrophic factors. MSCs-Exo, which is homologous to MSCs, also plays a neuroprotective role through several mechanisms, which can reduce neuroinflammation after SCI in animal models and significantly promote their functional recovery[44].

Chang et al.[45] used bone marrow-derived MSCs-Exoes to treat SCI rats and showed a significant reduction in the number of damaged neurons in spinal cord tissue after the application of MSCs-Exoes. MSCs-Exo inhibited the expression of IRF5 in post-SCI tissues by carrying miR-125a, a gene that may be a target of miR-125a, while enriched in M1 macrophages. Reduced expression of IRF5 attenuated the inflammatory response induced by M1 phenotype macrophages in rat spinal cord tissue, promoted the polarization of M2 phenotype macrophages, and significantly inhibited the apoptosis, degeneration and inflammatory response of neurons after SCI, thus exerting a neuroprotective effect. Zhao et al.[46] established a rat spinal cord ischemia model and found that MSCs-Exoes could achieve neuroprotective effects on the ischemic spinal cord by, promoting neural and vascular regeneration and preventing post-ischemic immunosuppression. Further, transfection of MSCs-Exoes with miR-25 significantly increased the level of miR-25 in MSCs-Exoes, and miR-25-enriched MSCs-Exoes could effectively transfer miR-25 to the spinal cord, providing better neuroprotection to damaged tissues.NOX4 may be a target protein of miR-25, which directly affects the sensitivity of neural tissues to ischemic injury. miR-25-enriched MSCs-Exoes could affect the enhancement of NOX4, attenuate oxidative stress after spinal cord ischemia, and mediate additional neuroprotection against SCI. Fan et al.[47] evaluated the neuroprotective effect of MSCs-Exoes using a GLU-induced excitotoxicity model and found that the number of TUNEL-positive neuronal cells was significantly reduced in the MSCs-Exo-treated group. Further detection using flow cytometry showed that Exo attenuated the apoptotic effect of GLU, with reduced expression levels of proapoptotic Bax protein and caspase3 and caspase-9 and increased expression levels of anti-apoptotic Bcl-2 protein, suggesting a protective effect of MSCs-Exo against GLU-induced neuronal apoptosis.

Through the above aspects, the mechanism of MSCs-Exoes for the treatment of SCI has been initially discussed (Figure 2). While seeing the superiority of MSCs-Exoes, we also need to reasonably examine the disadvantages. The current preparation process cannot achieve large-scale extraction while ensuring the purity of MSCs-Exoes. At the same time, MSCs-Exoes cannot yet completely achieve the reconstruction of neural function after SCI[48], and the efficacy of Exo alone is limited, and its efficacy and targeting can be further improved by constructing engineered Exoes by means of pretreatment. To summarize and organize the articles related to the mechanism of MSCs-Exo for SCI in recent years (Table 3).

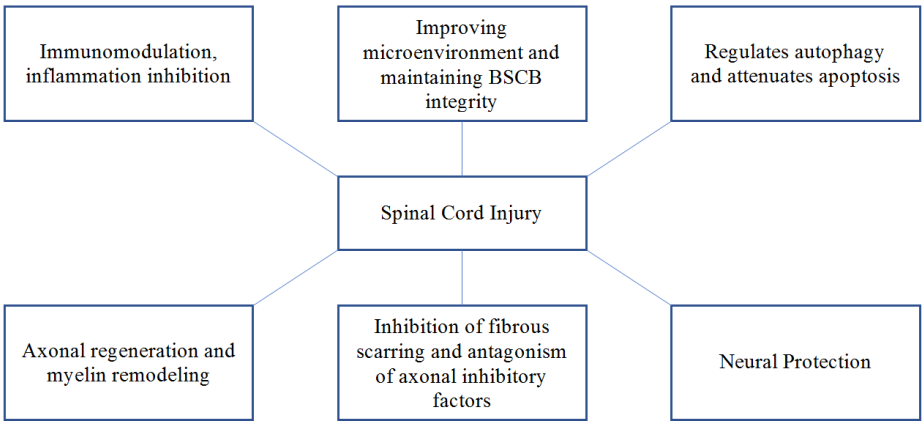

Figure 2: Summary of the mechanism of MSCs-Exoes for SCI treatment

Table 3: Summary of SCI-based studies on MSCs-Exo treatment

Author	MSCs-Exo Source	Research Model	Drug delivery method	Other interventions	Treatment Mechanism
Sun	Human umbilical cord-derived	Spinal cord strike model in mice	Caudal vein injection	/	Promotes M2-type macrophage polarization and reduces inflammatory response
Liu	Bone marrow-derived in rats	Spinal cord strike injury model in rats	Caudal vein injection	/	Inhibits inflammatory response, inhibits glial scar formation, promotes angiogenesis, and promotes axonal regeneration
Wang	Bone marrow-derived in rats	Spinal cord strike injury model in rats	Caudal vein injection	/	Reduces the proportion of A1 astrocytes, anti-inflammatory and neuroprotective
Lu	Bone marrow-derived in rats	Spinal cord strike injury model in rats	Caudal vein injection	/	Inhibits pericyte migration, maintains the integrity of the blood-spinal cord barrier, and promotes neuronal survival and axonal regeneration
Li	Bone marrow-derived in rats	Spinal cord strike injury model in rats	Caudal vein injection	/	Activation of Wnt/β-catenin signaling pathway and inhibition of neural cell apoptosis
Gu	Bone marrow-derived in rats	Spinal cord strike injury model in rats	Caudal vein injection	/	Promotes autophagy and attenuates neuronal apoptosis
Li	Bone marrow-derived in rats	Spinal cord entrapment model in rats	Caudal vein injection	miR-133b	Protects neurons and promotes axonal regeneration
Huang	Bone marrow-derived in rats	Spinal cord strike injury model in rats	Caudal vein injection	PTEN gene small interfering RNA	Neuroprotection, axonal regeneration
Romanelli	Human umbilical cord-derived	Spinal cord contusion model in rats	Caudal vein injection	/	Anti-inflammatory, anti-scar formation
Zhao	Bone marrow-derived in rats	Rat spinal cord hemisection injury model	Caudal vein injection	/	Inhibition of complement mRNA synthesis and release and suppression of activated NF-κB expression

5. Summary

There are no clinical reports on the application of MSCs-Exo in humans, but a series of basic

studies have demonstrated that MSCs-Exo has great therapeutic potential for post-SCI repair by inhibiting the inflammatory response, reducing apoptosis, maintaining the integrity of the blood-spinal cord barrier, inhibiting glial scar formation, promoting axonal regeneration, neuroprotection, and other mechanisms. The current treatment of SCI can alleviate patients' symptoms to a certain extent, but it cannot fundamentally improve patients' quality of life, and there are still many defects[49]. MSCs-Exo has a broad research prospect and may be a new measure and means to solve the problem of SCI, and as a representative of cell-free therapy, it is more promising for research than MSCs[50]. With further scientific research, the mechanism of MSCs-Exo repair of SCI is found to become clearer, providing a more high-grade evidence-based basis for the clinical application of Exo. Meanwhile, along with the continuous progress of high-throughput sequencing technology and network analysis technology, the gene expression profile after SCI will be increasingly improved, and the joint molecular pathological mechanism of SCI will help to explore the key targets affecting the physiological function, to realize the precise intervention of SCI[51]. The repair of SCI by MSCs-Exoes is a comprehensive process, and this paper provides a clearer direction for other researchers by reviewing the relevant mechanisms, so that they can be targeted in subsequent studies.

Although MSCs-Exo has shown good therapeutic results in animal studies, treatment regimens that are effective in rodents and primates may not be effective in humans. Therefore, a large number of case models and clinical trials are needed as a basis for repeated practice and replication, leading to clinical translation and application[52]. However, the use of MSCs-Exoes alone for SCI treatment still suffers from poor organ targeting and insufficient therapeutic effect, which is the key to limit its further application[53]. In-depth study of MSCs-Exo mechanism, while making full use of the good piggybacking and passing properties of Exoes, and constructing Exo drug delivery system[54]. Combining biological scaffolds with hydrogel materials to improve Exo colonization and release[55]. Combined with herbal medicine and electro-acupuncture, to further enhance the therapeutic effect of Exo[56], etc. This is just part of the future direction of Exo, and we still need to keep exploring to make full use of the advantages of tissue engineering to open up new channels for treating SCI.

References

[1] Anjum, A., Yazid, M.D., Fauzi, D.M., Et Al. (2020) Spinal Cord Injury: Pathophysiology, Multimolecular Interactions, and Underlying Recovery Mechanisms. International Journal of Molecular Sciences, 21(20).

[2] Cofano, F., Boido, M., Monticelli, M., Et Al. (2019) Mesenchymal Stem Cells For Spinal Cord Injury: Current Options, Limitations, And Future Of Cell Therapy. International Journal of Molecular Sciences, 20(11).

[3] Ma, Z.J., Yang, J.J., Lu,Y.B., Et Al. (2020) Mesenchymal Stem Cell-Derived Exosomes: Toward Cell-Free Therapeutic Strategies in Regenerative Medicine. World Journal of Stem Cells, 12(8): 814-40.

[4] Ren, Z., Qi, Y., Sun, S., Et Al. (2020) Mesenchymal Stem Cell-Derived Exosomes: Hope for Spinal Cord Injury Repair. Stem Cells and Development, 29(23): 1467-78.

[5] Quadri, S.A., Farooqui, M., Ikram, A., Et Al. (2020) Recent Update on Basic Mechanisms of Spinal Cord Injury. Neurosurgical Review, 43(2): 425-41.

[6] Rouanet, C., Reges, D., Rocha, E., Et Al. (2017) Traumatic Spinal Cord Injury: Current Concepts And Treatment Update. Arq Neuropsiquiatr, 75(6): 387-93.

[7] Zhang, Y., Al, M.A., Yuan, Y., Et Al. (2021) Acute Spinal Cord Injury: Pathophysiology and Pharmacological Intervention (Review). Mol Med Rep, 23(6).

[8] Yang, T., Dai, Y., Chen, G., Et Al. (2020) Dissecting the Dual Role of the Glial Scar and Scar-Forming Astrocytes in Spinal Cord Injury. Frontiers in Cellular Neuroscience, 14(78).

[9] Liau, L.L., Looi, Q.H., Chia, W.C., Et Al. (2020) Treatment Of Spinal Cord Injury with Mesenchymal Stem Cells. Cell Biosci, 10(1): 17.

[10] Shao, A., Tu, S., Lu, J., Et Al. (2019) Crosstalk between Stem Cell And Spinal Cord Injury: Pathophysiology And Treatment Strategies. Stem Cell Research & Therapy, 10(1): 238.

[11] Hur, J.W., Cho, T.H., Park, D.H., Et Al. (2016) Intrathecal Transplantation Of Autologous Adipose-Derived

Mesenchymal Stem Cells For Treating Spinal Cord Injury: A Human Trial. The Journal of Spinal Cord Medicine, , 39(6): 655-64.
[12] Lee, H.Y., Hong, I.S. (2017) Double-Edged Sword of Mesenchymal Stem Cells: Cancer-Promoting Versus Therapeutic Potential. Cancer Science, 108(10): 1939-46.
[13] Kim, G.U., Sung, S.E., Kang, K.K., Et Al. (2021) Therapeutic Potential of Mesenchymal Stem Cells (Mscs) And Msc-Derived Extracellular Vesicles For The Treatment of Spinal Cord Injury. International Journal of Molecular Sciences, 22(24).
[14] Kalluri, R., Lebleu, V.S. (2020) the Biology, Function, and Biomedical Applications of Exosomes. Science (New York, Ny), 367(6478).
[15] Ha, D.H., Kim, H.K., Lee, J., Et Al. (2020) Mesenchymal Stem/Stromal Cell-Derived Exosomes for Immunomodulatory Therapeutics and Skin Regeneration. Cells, 9(5).
[16] Tsiapalis, D., O'Driscoll, L.. (2020) Mesenchymal Stem Cell Derived Extracellular Vesicles for Tissue Engineering and Regenerative Medicine Applications. Cells, 9(4).
[17] Barile, L., Vassalli, G. (2017) Exosomes: Therapy Delivery Tools and Biomarkers of Diseases. Pharmacology & Therapeutics, 174(63-78).
[18] Familtseva, A., Jeremic, N., Tyagi, S.C. (2019) Exosomes: Cell-Created Drug Delivery Systems. Molecular and Cellular Biochemistry, 459(1-2): 1-6.
[19] Alzhrani, G.N., Alanazi, S.T., Alsharif, S.Y., Et Al. (2021) Exosomes: Isolation, Characterization, and Biomedical Applications. Cell Biology International, 45(9): 1807-31.
[20] Zhao, T., Sun, F., Liu, J., Et Al. (2019) Emerging Role of Mesenchymal Stem Cell-Derived Exosomes in Regenerative Medicine. Current Stem Cell Research and Therapy, 14(6): 482-94.
[21] Rezabakhsh, A., Sokullu, E., Rahbarghazi, R. (2021) Applications, Challenges and Prospects of Mesenchymal Stem Cell Exosomes in Regenerative Medicine. Stem Cell Research & Therapy, 12(1): 521.
[22] Tan, T.T., Toh, W.S., Lai, R.C., Et Al. (2022) Practical Considerations in Transforming Msc Therapy For Neurological Diseases From Cell To Ev. Exp Neurol, 349(113953).
[23] Srivastava, A.K., Ruppert, K.A., Nguyen, T.T., Et Al. (2018) Immunomodulatory Treatment Approach To Cns Injury: Role Of Mesenchymal Stem Cell Derived Extracellular Vesicles. Journal of Extracellular Vesicles, 7(109-10).
[24] Sun, G., Li, G., Li, D., Et Al. (2018) Hucmsc Derived Exosomes Promote Functional Recovery in Spinal Cord Injury Mice Via Attenuating Inflammation. Materials Science & Engineering C, Materials for Biological Applications, 89(194-204).
[25] Liu, W., Wang, Y., Gong, F., Et Al. (2019) Exosomes Derived From Bone Mesenchymal Stem Cells Repair Traumatic Spinal Cord Injury by Suppressing the Activation of A1 Neurotoxic Reactive Astrocytes. Journal of Neurotrauma, 36(3): 469-84.
[26] Wang, L., Pei, S., Han, L., Et Al. (2018) Mesenchymal Stem Cell-Derived Exosomes Reduce A1 Astrocytes via Downregulation of Phosphorylated Nfκb P65 Subunit in Spinal Cord Injury. Cellular Physiology and Biochemistry: International Journal Of Experimental Cellular Physiology, Biochemistry, And Pharmacology, 50(4): 1535-59.
[27] Lu, Y., Zhou, Y., Zhang, R., Et Al. Bone Mesenchymal Stem Cell-Derived Extracellular Vesicles Promote Recovery Following Spinal Cord Injury Via Improvement Of The Integrity Of The Blood-Spinal Cord Barrier. Frontiers in Neuroscience, (2019) 13(209).
[28] Yuan, X., Wu, Q., Wang, P., Et Al. (2019) Exosomes Derived From Pericytes Improve Microcirculation and Protect Blood-Spinal Cord Barrier After Spinal Cord Injury in Mice. Frontiers in Neuroscience, 13(319).
[29] Zhou, X., Chu, X., Yuan, H., Et Al. (2019) Mesenchymal Stem Cell Derived Evs Mediate Neuroprotection After Spinal Cord Injury In Rats Via The Microrna-21-5p/Fasl Gene Axis. Biomedicine and Pharmacotherapy, 115.
[30] Li, C., Jiao, G., Wu, W., Et Al. (2019) Exosomes From Bone Marrow Mesenchymal Stem Cells Inhibit Neuronal Apoptosis and Promote Motor Function Recovery Via the Wnt/B-Catenin Signaling Pathway. Cell Transplantation, 28(11): 1373-83.
[31] Gu, J., Jin, Z.S., Wang, C.M., Et Al. (2020) Bone Marrow Mesenchymal Stem Cell-Derived Exosomes Improves Spinal Cord Function After Injury In Rats by Activating Autophagy. Drug Design, Development and Therapy, 14(1621-31).
[32] Tsai, M.J., Liou, D.Y., Lin, Y.R, Et Al. (2019) Attenuating Spinal Cord Injury by Conditioned Medium from Bone Marrow Mesenchymal Stem Cells. J Clin Med, 8(1): 13.
[33] Fan, B., Wei, Z., Feng, S. (2022) Progression in Translational Research on Spinal Cord Injury Based On Microenvironment Imbalance. Bone Research, 10(1): 35.
[34] Liu, W.Z., Ma, Z.J., Li, J.R., Et Al. (2021) Mesenchymal Stem Cell-Derived Exosomes: Therapeutic Opportunities and Challenges for Spinal Cord Injury. Stem Cell Research & Therapy, 12(1): 102.
[35] Li, D., Zhang, P., Yao, X., Et Al. (2018) Exosomes Derived From Mir-133b-Modified Mesenchymal Stem Cells Promote Recovery After Spinal Cord Injury. Frontiers in Neuroscience, 12(845).
[36] Ren, Z.W., Zhou, J.G, Xiong, Z.K, Et Al. (2019) Effect of Exosomes Derived From Mir-133b-Modified Adscs on

the Recovery of Neurological Function After Sci. European Review For Medical And Pharmacological Sciences, 23(1): 52-60.
[37] Tang, B.L. (2018) Promoting Axonal Regeneration through Exosomes: An Update of Recent Findings on Exosomal Pten and Mtor Modifiers. Brain Research Bulletin, 143(123-31).
[38] Guo, S., Perets, N., Betzer, O., Et Al. (2019) Intranasal Delivery of Mesenchymal Stem Cell Derived Exosomes Loaded with Phosphatase and Tensin Homolog Sirna Repairs Complete Spinal Cord Injury. Acs Nano, 13(9): 10015-28.
[39] Huang, W., Zhang, S., Liu, T., Et Al. (2018) Mesenchymal Stem Cells-Derived Exosomes Loaded with Sirna for Spinal Cord Injury Treatment in Rat. Basic and Clinical Pharmacology and Toxicology, 123(7-8).
[40] Liu, W., Rong, Y., Wang, J., Et Al. (2020) Exosome-Shuttled Mir-216a-5p From Hypoxic Preconditioned Mesenchymal Stem Cells Repair Traumatic Spinal Cord Injury By Shifting Microglial M1/M2 Polarization. Journal of Neuroinflammation, 17(1): 47.
[41] Luo, Y., Xu, T., Liu, W., Et Al. (2021) Exosomes Derived From Git1-Overexpressing Bone Marrow Mesenchymal Stem Cells Promote Traumatic Spinal Cord Injury Recovery In A Rat Model. The International Journal of Neuroscience, 131(2): 170-82.
[42] Romanelli, P., Bieler, L., Scharler, C., Et Al. (2019) Extracellular Vesicles Can Deliver Anti-Inflammatory And Anti-Scarring Activities of Mesenchymal Stromal Cells After Spinal Cord Injury. Frontiers in Neurology, 10(1225).
[43] Anderson, M.A., Burda, J.E., Ren, Y., Et Al. (2016) Astrocyte Scar Formation Aids Central Nervous System Axon Regeneration. Nature, 532(7598): 195-200.
[44] Ruppert, K.A., Nguyen, T.T., Prabhakara, K.S., Et Al. (2018) Human Mesenchymal Stromal Cell-Derived Extracellular Vesicles Modify Microglial Response and Improve Clinical Outcomes In Experimental Spinal Cord Injury. Scientific Reports, 8(1): 480.
[45] Chang, Q., Hao, Y., Wang, Y., Et Al. (2021) Bone Marrow Mesenchymal Stem Cell-Derived Exosomal Microrna-125a Promotes M2 Macrophage Polarization In Spinal Cord Injury By Downregulating Irf5. Brain Research Bulletin, 170(199-210).
[46] Zhao, L.L., Jiang, X.J., Shi, J., Et Al. (2019) Exosomes Derived From Bone Marrow Mesenchymal Stem Cells Overexpressing Microrna-25 Protect Spinal Cords against Transient Ischemia. Journal of Thoracic and Cardiovascular Surgery, 157(2): 508-17.
[47] Fan, L., Dong, J., He, X., Et Al. (2021) Bone Marrow Mesenchymal Stem Cells-Derived Exosomes Reduce Apoptosis and Inflammatory Response During Spinal Cord Injury By Inhibiting The Tlr4/Myd88/Nf-Kb Signaling Pathway. Human & Experimental Toxicology, 40(10): 1612-23.
[48] Yang, B., Zhang, F., Cheng, F., Et Al. (2020) Strategies and Prospects of Effective Neural Circuits Reconstruction After Spinal Cord Injury. Cell Death & Disease, 11(6): 439.
[49] Khalatbary, A.R. (2021) Stem Cell-Derived Exosomes as a Cell Free Therapy against Spinal Cord Injury. Tissue & Cell, 71(101559).
[50] Dutta, D., Khan, N., Wu, J., Et Al. (2021) Extracellular Vesicles as an Emerging Frontier In Spinal Cord Injury Pathobiology And Therapy. Trends in Neurosciences, 44(6): 492-506.
[51] Yi, H., Wang, Y. (2021) A Meta-Analysis of Exosome in the Treatment of Spinal Cord Injury. Open Medicine (Warsaw, Poland), 16(1): 1043-60.
[52] Rezaie, J., Nejati, V., Mahmoodi, M., Et Al. (2022) Mesenchymal Stem Cells Derived Extracellular Vesicles: A Promising Nanomedicine for Drug Delivery System. Biochemical Pharmacology, 203(115167).
[53] Yang, Z.L., Rao, J., Lin, F.B., Et Al. (2022) The Role of Exosomes and Exosomal Noncoding Rnas From Different Cell Sources In Spinal Cord Injury. Frontiers in Cellular Neuroscience, 16(882306).
[54] Patil, S.M., Sawant, S.S., Kunda, N.K. (2020) Exosomes as Drug Delivery Systems: A Brief Overview and Progress Update. European Journal of Pharmaceutics and Biopharmaceutics: Official Journal of Arbeitsgemeinschaft Fur Pharmazeutische Verfahrenstechnik Ev, 154(259-69).
[55] Guo, S., Redenski, I., Levenberg, S. (2021) Spinal Cord Repair: From Cells And Tissue Engineering To Extracellular Vesicles. Cells, 10(8):
[56] Lu, Y., Yang, J., Wang, X., Et Al. (2020) Research Progress in Use of Traditional Chinese Medicine for Treatment of Spinal Cord Injury. Biomedicine & Pharmacotherapy = Biomedecine & Pharmacotherapie, 127(110136).