Exosomes and miRNAs Effects Spinal Cord Injury

Subjects: Neurosciences

Contributor: Jinsu Hwang , Sujeong Jang , Choonghyo Kim , Sungjoon Lee , Han-Seong Jeong

Neurological disorders represent a global health problem. Current pharmacological treatments often lead to short-term symptomatic relief but have dose-dependent side effects, such as inducing orthostatic arterial hypotension due to the blockade of alpha receptors, cardiotoxic effects due to impaired repolarization, and atrioventricular block and tachycardia, including ventricular fibrillation. Mesenchymal stem cells (MSCs) are pluripotent cells with anti-inflammatory, anti-apoptotic, and immunomodulatory properties, providing a promising alternative due to their ability to differentiate, favorable culture conditions, in vitro manipulation ability, and robust properties.

spinal cord injury mesenchymal stem cell exosome microRNA

1. Introduction

Spinal cord injury (SCI) is a severe traumatic disease that often leads to severe and permanent paralysis and carries a heavy burden for individuals, families, and society [1]. SCI occurs in a population of approximately 250,000 to 500,000 worldwide each year. In addition, according to the World Health Organization (WHO), the costs of healthcare, in both time and money, are expected to increase in the coming years [2][3]. When SCI develops, patients' normal sensory, motor, or autonomic function is greatly affected. They often have numerous multisystem complications, such as motor deficits, gastric dysmotility, cardiac arrest, and bladder dysfunctions [4][5][6]. Based on many early primary injuries, progressive secondary injuries can exacerbate the clinical situation [7]. SCI is primarily caused by spinal misalignment and damage, resulting in immediate neuronal cell death, rupture of blood vessels, and damage to the blood–spinal-cord barrier (BSCB). In addition, the wound microenvironment triggers neuronal cell death, inflammatory responses, and vascular changes. Problems like these cause additional nerve damage and dysfunction, further prolonging SCI [8][9]. Due to the complex pathophysiology of SCI, there still remains no effective, definitive treatment or functional recovery strategy for SCI. Exosomes and microRNAs secreted from stem cells can freely pass through the blood–spinal-cord barrier. Once they reach the spinal cord, they may play a role in promoting nervous system repair, including reducing neuronal cell death, promoting vascular remodeling and neurogenesis, reducing neuroinflammation, and promoting axonal remodeling.

2. Effects of Exosomes in SCI

After SCI, a large number of neuronal cells are damaged following axonal loss, ischemia, inflammation, and apoptosis [10]. Neuronal cell death has been reported to occur only in the early stages of SCI, which involves blood

vessel rupture, resulting in bleeding and hypoxia [3][11]. Therefore, many studies have shown that using exosomes for treatment in the early stages after SCI can successfully attenuate the apoptosis of neurons [1][5][12][13][14]. There are several types of therapy SCI using exosomes that act by attenuating neuronal apoptosis, promoting neurogenesis, and improving axonal remodeling. For this, in the laboratory, exosomes are isolated using various methods for various purposes and applications. For example, it can be isolated using ultracentrifugation techniques [15][16][17], size-based isolation techniques [18][19], polymer precipitation [20][21], and immunoaffinity capture techniques [22][23]. Although various methods have been developed for the isolation and purification of exosomes, a combination of different isolation methods may be better than the isolation effect of a single method. Therefore, in order to obtain ideal exosomes by improving isolation efficiency and enrichment, many research teams have combined different methods of isolating and purifying exosomes to improve yield and purity [24][25].

Apoptosis, which is known as programmed cell death, can be attenuated by intervening with certain factors, such as exosomes, and reducing the ATP-driven process of cell death [26][27]. Apoptotic cell death occurs and contributes to many diseases, such as cancer, restenosis of tissues, stroke, heart failure, neurodegeneration, and AIDS, with cell accumulation and loss [27]. There are two major pathways for caspase activation: the extrinsic pathway, which is induced by the TNF family of cytokine receptors, such as TNFR1 and Fas, and the intrinsic pathway, which is induced by cytochrome c and elevations in the pro-apoptotic Bcl-2 family proteins such as Bax [27].

Exosomes and exosomal miRNAs enhance recovery from SCI by attenuating neuronal cell death. Cell apoptosis can be attenuated under the intervention of certain factors such as exosomes [26]. In preclinical experiments, MSC exosomes have been shown to increase Bcl-2 expression and decrease Bax levels after systemic administration in an SCI mouse or rat model as well as to promote functional improvement and reduce disrupted endothelial cells at the contusion site [1][13]. Another preclinical experiment demonstrated that MSC exosomes could effectively activate the Wnt/β-catenin signaling pathway with anti-apoptotic effects [14]. Other groups have also reported that MSC exosomes improved the expression of autophagy-related proteins, such as LC3IIB and Beclin-1, and induced autophagosome formation [5][12]. Liu et al. found that bone-MSC-derived exosomes had the potential to reduce lesion size, neuronal apoptosis, and glial scar formation and induced blood vessel density and axonal regeneration in SCI rats by suppressing the activation of astrocytes [12].

When SCI occurs, the injured spinal column is hypoxic to reduce endogenous neural tissue repair and tissue regeneration with abnormal angiogenesis. Vascular endothelial cells show increased uptake of exosomes to activate protein kinase A (PKA) signaling and promote vascular endothelial growth factor (VEGF) expression as a component of the blood vessel wall [28]. Cao et al. found that exosomes derived from human urine stem cells stimulated angiogenesis through the PI3K/AKT signaling pathways to enable SCI recovery in the damaged area [29]. In addition, MSC exosomes have been shown to enhance angiogenesis and axon regeneration by reducing PTEN expression in the damaged spinal cord region and to reduce microglia and astrocyte proliferation in SCI rats [30]. Exosomes have also been confirmed to promote axon regeneration by regulating the NF-κB p65 signaling pathway in pericytes [31].

Exosomes are known to contain various proteins including growth factors, such as NGF, which regulates neuronal survival and the release of neurotransmitters and facilitates the plasticity of axons in the adult central and peripheral nervous systems [4][32]. One group reported that exosomes derived from NGF-overexpressed MSCs promoted SCI repair in a mouse model, leading to the regeneration of neuronal axons and differentiation into neurons [4].

3. Effects of miRNAs in SCI

miRNAs are 21–23 nucleotides in length and are endogenous nonprotein-coding RNA molecules. Many researchers have predicted that the administration of miRNAs could easily reach a target region that has experienced neuronal death or a damaged organ [3][33][34][35][36][37][38]. Numerous studies have shown that miRNAs have important roles in some autoimmune diseases and are essential in the development and homeostasis of the immune system [39][40][41]. Recently, miRNAs have been identified as potential new targets for SCI treatment [42][43][44][45]. Accumulating evidence indicates that exosomes with a bilayer membrane structure can be used as valuable carriers for targeting miRNAs at SCI sites. In addition, exosomes can cross the BBB to enhance the therapeutic effect of miRNAs [46]. MSCs are pre-transfected with specific miRNA plasmids to secrete exosomes containing high levels of specific miRNAs [37]. Extensive studies have shown that exosomes from MSCs carrying miRNAs exhibit efficient repair effects in SCI.

Lin's group reported that increased miR-126 could promote angiogenesis, inhibit inflammation, and improve functional recovery after SCI [43]. However, there remains a limitation regarding the delivery of miRNAs into the damaged tissue. Similar to siRNAs, miRNAs are highly unstable in the local extracellular environment and are delivered to the target tissues by an effective carrier system. In this regard, there are two delivery systems: viral and non-viral systems. The viral system is the most efficient delivery system but has toxic side effects, and the non-viral system contains physical and chemical components that are safe for clinical application [47][48][49][50]. Therefore, exosome-derived miR-126-treated MSCs were used as a non-viral delivery system and were found to promote angiogenesis and neurogenesis and attenuate apoptosis after SCI in a rat model [47]. Similarly, there are a few studies regarding exosomes of miRNA-modified MSCs as a therapeutic delivery vehicle to the CNS. Another group found that exosome-derived miR-133b-MSCs contributed to neurite remodeling and improved functional recovery poststroke [51]. In addition, miR-146b-transferred exosomes were found to have the potential to inhibit tumor growth in brain tumor models [52]. Zhao et al. demonstrated that exosomes from miR-25-overexpressed-MSCs exerted neuroprotective effects in an ischemic SCI model [33]. miR-25-enriched exosomes were also shown to inhibit NADPH oxidase 4 expression and increase superoxide dismutase activity and exhibited lower levels of IL-1β and TNF-α.

miR-19b and miR-21 exosomes have been found to enhance neuronal cell viability and inhibit neuronal cell death by inhibiting PTEN/PDCD4 expression. These two miRNAs are found in bone-marrow-derived MSCs, and they inhibit neuronal cell death and promote neuronal differentiation in SCI. When the number of exosomes increases, phosphatase and tensin homolog (PTENC) is inhibited preventing neuronal cell death and promoting neuronal differentiation [53][54]. BMSC-derived exosomes have been shown to have neuroprotective effects in the ischemic

SCI. This effect may be due to the pre-transfection of BMSCs to secrete exosomes with high expression of miR-25, thus indicating that miR-25 enhances neuroprotection [33]. Exosomes secreted from miR-29b-rich MSCs and human neuroepithelial stem cells can exert therapeutic effects on SCI by downregulating PTEN/caspase-3 expression and subsequently inhibiting neuronal apoptosis [55][56]. Injecting exosomes secreted from BMSCs modified with miR-29b into a mouse model showed that these exosomes not only promote nerve regeneration but also accelerate motor function recovery in mice with SCI and reduce pathological damage to spinal cord tissue. This mechanism may also be related to the regulation of the expression of nerve-regeneration-related proteins such as NF200, GAP-43, and GFAP [57].

There are several studies of BMSC-derived exosomal miR-124-3p, which has been shown to attenuate neurological damage, such as in Parkinson's disease and spinal cord ischemia-reperfusion injury [36][57]. miR-124-3p and miR-125 are responsible for regulating M2 macrophages. M2 macrophages are key effector cells of the inflammatory response to SCI, and the repair of SCI is based on macrophage activation. miR-125 promotes M2 macrophage polarization and negatively regulates IRF5 to ameliorate SCI. In addition, miR-124-3p promotes M2 macrophage polarization and negatively regulates Ern1 to ameliorate SCI [36][38]. miR-124-3p has also been found to suppress A1 astrocytes by inhibiting the activation of M1 microglia and microglia-induced neuroinflammatory responses through the MYH9/PI3K/AKT/NF-κB signaling pathway [58]. Exosomes derived from MSCs transformed with miR-126 have been shown to reduce neuronal death and promote functional regeneration after SCI. BMSC-exosome-derived miR-126 promotes angiogenic migration by repressing the expression of SPRED1 and PIK3R2 to promote SCI recovery [47]. miR-216a-5p has been found to affect the activation of NF-κB signaling by regulating TLR4. Exosomal miR-216a-5p also transforms microglia from an M1 pro-inflammatory phenotype to an M2 anti-inflammatory phenotype, increasing its therapeutic potential by inhibiting TLR4/NF-κB and activating the PI3K/Akt signaling pathway [59][60]. Exosomes secreted from BMSCs containing miR-145-5p improved functional recovery and reduced histopathological damage and inflammation in SCI mice. Exosomes promoted miR-145-5p expression in spinal cord tissue, which specifically targeted TLR4 and inhibited TLR4/NF-κB pathway activation in SCI rats [61]. MSC-derived miR-26a exosomes increased phosphorylation of PI3K, AKT, and mTOR proteins, promoting neurofilament production and nerve regeneration in neurons. miR-26a exosomes were injected into the tail vein of SCI rats to promote functional recovery in rats and induce neuronal and axonal regeneration by targeting the PTEN and mTOR pathways [62].

miR-199a-3p/145-5p, which is highly expressed in exosomes secreted from human umbilical cord mesenchymal stem cells, showed an anti-apoptosis effect in vivo. These exosomes could be an effective therapeutic strategy in neuronal injury by affecting TrkA ubiquitination and promoting the NGF/TrkA signaling pathway [63].

NSC-derived miRNA-124, which is involved in nerve regeneration, ameliorates nerve loss and reduces astrocytes in mice with SCI while increasing neurofilament-200 (NF-200) expression. It has been shown that the expression of nuclear-enriched abundant transcript 1 (Neat1) induces neuron-specific differentiation of neural stem cells by miR-124. It can also activate Wnt/β-catenin signaling to promote neuronal differentiation and migration ability [64]. NSC-derived MiR-615, which is involved in neuronal survival and axonal regeneration, inhibits LINGO-1 by directly targeting LRR and Ig domain-containing NOGO receptor-interacting protein 1 (LINGO-1), a potent negative

regulator of neuronal survival and axonal regeneration, and may contribute to neuronal differentiation through the LINGO-1/RhoA or EGFR signaling pathways. Intrathecal administration of miR-615 to SCI rats suppressed LINGO-1, increased neuronal survival, enhanced axon extension and myelination, and enhanced motor function [65].

When exosomes encapsulated with miR-133b were injected into SCI rats, STAT3, ERK1/2, and CREB were found to be activated, damaged neurons were shown to be protected, and the recovery of hind limb motor function was demonstrated to be improved. RhoA is a direct target of miR-133b found in exosomes of adipose-derived stem cells. miR-133b protects neurons from apoptosis by downregulating RhoA and regulating Rho-associated kinase (ROCK) to promote ERK phosphorylation. In addition, miR-133b promotes axonal regeneration after SCI by promoting CREB and STAT3 phosphorylation [66]. Neural stem cell-derived miR-219a-2-3p has also been found to inhibit inflammation by downregulating the YY1 gene and inhibiting NF-κB [67]. miR-388-5p has been found to downregulate the expression of its target, cannabinoid receptor 1 gene (Cnr1), which increases cAMP accumulation through miR-338-5p, which activates Rap1. In turn, this activates the PI3K/AKT pathway. This pathway inhibits apoptosis and enhances neuronal survival [68].

Another exosomal miRNA with neuroprotective effects is miR-544. Rat BMSCs were transfected with miR-544 mimics to obtain exosomes highly expressing miR-544, and these exosomes were intravenously injected into a rat model of SCI [69]. The results of this study showed that miR-544 accelerated the recovery of neuronal function after SCI. In addition, overexpression of miR-544 in BMSC exosomes ameliorated histological defects and neuronal loss due to SCI [59].

The abovementioned study results demonstrate that exosome-mediated miRNA transport is a new treatment method for SCI because exosomes increase neuron activity through miRNA transport and promote functional recovery by attenuating apoptosis at an early stage during SCI. **Table 1** shows the various mechanisms by which miRNAs exert a role after SCI. In addition, **Table 2** shows the summary of the function of miRNAs. miRNAs can be observed to modulate processes such as neuroinflammation and apoptosis, can exert neuro-regenerative effects by targeting various molecular mechanisms, and can help recovery from SCI.

Table 1. Studies on the treatment of SCI with miRNA derived from MSCs.

miRNA	Model	Mechanism	Ref.
miR-126	Rat, Contusion model, Intrathecal injection	VEGF, SPRED1, PIK3R2	[43]
	Rat, Contusion model, Intraperitoneal injection	ERK, AKT	[47]
miR-133b	Rat, Hemorrhage model, Intravenous injection	RhoA, ERK1/2, CREB	[51]
	Rat, Compression model, Intravenous injection	ERK1/2, STAT3, CRE	[66]
miR-146b	Rat, Tumor implantation model, Intratumoral injection	EGFR	[52]
miR-25	Rat, Ischemia model, Intrathecal injection	NOX2, NOX4	[33]

miRNA	Model	Mechanism	Ref.
miR-19b	Rat, Contusion model, Intravenous injection	PTEN	[54]
miR-21	Rat, Contusion model, Intravenous injection	PTEN, PDCD4	[53]
miR-29b	Rat, Contusion model, Intravenous injection	NF200, GAP-43, GFAP	[55]
	Rat, Contusion model, Intravenous injection	PTEN, Caspase-3	[56]
miR-124-3p	Rat, Ischemia model, Intravenous injection	Ern1, Arg1, Ym1, Fizz	[36]
	Mouse, Contusion model, Intravenous injection	MYH9, PI3K, AKT, NF-κB	[57]
miR-125	Rat, Contusion model, Intrathecal injection	IRF5, Arg1, Ym1, Fizz	[38]
miR-145-5p	Rat, Transection model, Intravenous injection	TLR, NF-κB	[61]
miR-26a	Rat, Contusion model, Intravenous injection	PTEN, mTOR	[62]
miR-199a-3p	Rat, Contusion model, Intravenous injection	NGF/TrkA	[63]
MiR-124	Mouse, Transection model, Intrathecal injection	Neat1, Wnt/β-catenin	[64]
miR-615	Rat, Transection model, Subdural injection	LINGO-1, RhoA, EGFR	[65]
miR-219-a-2-3p	Rat, Contusion model, Intravenous injection	YY1, NF-κB	[67]
miR-216-5p	Mouse, Contusion model, Intravenous injection	TLR4/NF-κB/PI3K/AKT	[59]
miR-388-5p	Rat, Contusion model, Intravenous injection	cAMP, PI3K/Akt	[68]
miR-544	Rat, Contusion model, Intravenous injection	IL-1α, TNF-α, IL-17B, IL-36β	[69]

Table 2. Summary of the effects of miRNAs in SCI.

Effect/Function	miRNAs	Refs.
Neurogenesis	miR-126, miR-133b, miR-19b, miR-21, miR-216-5p, miR-544, miR-124, miR-615, miR-26a	[43][47][51][53][54][59][62][64][65][66][69]
Neuroprotection	miR-124-3p, miR-125	[36][38][57]
Apoptosis	miR-126, miR-133b, miR-19b, miR-21, miR-25, miR-124-3p, miR-199a-3p	[33][36][43][47][51][53][54][57][63][66]
Neuroinflammation	miR-126, miR-219-a-2-3p, miR-544, miR-145-5p	[43][47][61][67][69]

References

1. Yuan, X.; Wu, Q.; Wang, P.; Jing, Y.; Yao, H.; Tang, Y.; Li, Z.; Zhang, H.; Xiu, R. Exosomes derived from pericytes improve microcirculation and protect blood-spinal cord barrier after spinal cord injury in mice. Front. Neurosci. 2019, 13, 319.

2. Fehlings, M.G.; Tetreault, L.A.; Wilson, J.R.; Kwon, B.K.; Burns, A.S.; Martin, A.R.; Hawryluk, G.; Harrop, J.S. A clinical practice guideline for the management of acute spinal cord injury: Introduction, rationale, and scope. Global Spine J. 2017, 7 (Suppl. S3), 84S–94S.
3. Feng, J.; Zhang, Y.; Zhu, Z.; Gu, C.; Waqas, A.; Chen, L. Emerging exosomes and exosomal MiRNAs in spinal cord injury. Front. Cell Dev. Biol. 2021, 9, 703989.
4. Li, S.; Liao, X.; He, Y.; Chen, R.; Zheng, W.V.; Tang, M.; Guo, X.; Chen, J.; Hu, S.; Sun, J. Exosomes derived from NGF-overexpressing bone marrow mesenchymal stem cell sheet promote spinal cord injury repair in a mouse model. Neurochem. Int. 2022, 157, 105339.
5. Gu, J.; Jin, Z.S.; Wang, C.M.; Yan, X.F.; Mao, Y.Q.; Chen, S. Bone marrow mesenchymal stem cell-derived exosomes improves spinal cord function after injury in rats by activating autophagy. Drug Des. Dev. Ther. 2020, 14, 1621–1631.
6. Eckert, M.J.; Martin, M.J. Trauma: Spinal cord injury. Surg. Clin. N. Am. 2017, 97, 1031–1045.
7. Witiw, C.D.; Fehlings, M.G. Acute spinal cord injury. J. Spinal Disord. Technol. 2015, 28, 202–210.
8. Tran, A.P.; Warren, P.M.; Silver, J. The biology of regeneration failure and success after spinal cord injury. Physiol. Rev. 2018, 98, 881–917.
9. Silva, N.A.; Sousa, N.; Reis, R.L.; Salgado, A.J. From basics to clinical: A comprehensive review on spinal cord injury. Prog. Neurobiol. 2014, 114, 25–57.
10. Wong, K.R.; Mychasiuk, R.; O'Brien, T.J.; Shultz, S.R.; McDonald, S.J.; Brady, R.D. Neurological heterotopic ossification: Novel mechanisms, prognostic biomarkers and prophylactic therapies. Bone Res. 2020, 8, 42.
11. Singh, R.; Letai, A.; Sarosiek, K. Regulation of apoptosis in health and disease: The balancing act of BCL-2 family proteins. Nat. Rev. Mol. Cell Biol. 2019, 20, 175–193.
12. Liu, W.; Wang, Y.; Gong, F.; Rong, Y.; Luo, Y.; Tang, P.; Zhou, Z.; Zhou, Z.; Xu, T.; Jiang, T.; et al. Exosomes derived from bone mesenchymal stem cells repair traumatic spinal cord injury by suppressing the activation of A1 neurotoxic reactive astrocytes. J. Neurotrauma 2019, 36, 469–484.
13. Huang, J.H.; Yin, X.M.; Xu, Y.; Xu, C.C.; Lin, X.; Ye, F.B.; Cao, Y.; Lin, F.Y. Systemic administration of exosomes released from mesenchymal stromal cells attenuates apoptosis, inflammation, and promotes angiogenesis after spinal cord injury in rats. J. Neurotrauma 2017, 34, 3388–3396.
14. Li, C.; Jiao, G.; Wu, W.; Wang, H.; Ren, S.; Zhang, L.; Zhou, H.; Liu, H.; Chen, Y. Exosomes from bone marrow mesenchymal stem cells inhibit neuronal apoptosis and promote motor function recovery via the Wnt/β-catenin signaling pathway. Cell Transplant. 2019, 28, 1373–1383.
15. Thery, C.; Amigorena, S.; Raposo, G.; Clayton, A. Isolation and characterization of exosomes from cell culture supernatants and biological fluids. Curr. Protoc. Cell Biol. 2006, 30, 3.22.1–

3.22.29.

16. Livshits, M.A.; Khomyakova, E.; Evtushenko, E.G.; Lazarev, V.N.; Kulemin, N.A.; Semina, S.E.; Generozov, E.V.; Govorun, V.M. Isolation of exosomes by differential centrifugation: Theoretical analysis of a commonly used protocol. Sci. Rep. 2015, 5, 17319.
17. Tauro, B.J.; Greening, D.W.; Mathias, R.A.; Ji, H.; Mathivanan, S.; Scott, A.M.; Simpson, R.J. Comparison of ultracentrifugation, density gradient separation, and immunoaffinity capture methods for isolating human colon cancer cell line LIM1863-derived exosomes. Methods 2012, 56, 293–304.
18. Lamparski, H.G.; Metha-Damani, A.; Yao, J.Y.; Patel, S.; Hsu, D.H.; Ruegg, C.; Le Pecq, J.B. Production and characterization of clinical grade exosomes derived from dendritic cells. J. Immunol. Methods 2002, 270, 211–226.
19. Heinemann, M.L.; Ilmer, M.; Silva, L.P.; Hawke, D.H.; Recio, A.; Vorontsova, M.A.; Alt, E.; Vykoukal, J. Benchtop isolation and characterization of functional exosomes by sequential filtration. J. Chromatogr. A 2014, 1371, 125–135.
20. Zeringer, E.; Barta, T.; Li, M.; Vlassov, A.V. Strategies for isolation of exosomes. Cold Spring Harb. Protoc. 2015, 2015, 319–323.
21. Rider, M.A.; Hurwitz, S.N.; Meckes, D.G., Jr. ExtraPEG: A polyethylene glycol-based method for enrichment of extracellular vesicles. Sci. Rep. 2016, 6, 23978.
22. Hong, C.S.; Muller, L.; Boyiadzis, M.; Whiteside, T.L. Isolation and characterization of CD34+ blast-derived exosomes in acute myeloid leukemia. PLoS ONE 2014, 9, e103310.
23. Zarovni, N.; Corrado, A.; Guazzi, P.; Zocco, D.; Lari, E.; Radano, G.; Muhhina, J.; Fondelli, C.; Gavrilova, J.; Chiesi, A. Integrated isolation and quantitative analysis of exosome shuttled proteins and nucleic acids using immunocapture approaches. Methods 2015, 87, 46–58.
24. Ryu, K.J.; Lee, J.Y.; Park, C.; Cho, D.; Kim, S.J. Isolation of small extracellular vesicles from human serum using a combination of ultracentrifugation with polymer-based precipitation. Ann. Lab. Med. 2020, 40, 253–258.
25. Mathivanan, S.; Lim, J.W.; Tauro, B.J.; Ji, H.; Moritz, R.L.; Simpson, R.J. Proteomics analysis of A33 immunoaffinity-purified exosomes released from the human colon tumor cell line LIM1215 reveals a tissue-specific protein signature. Mol. Cell Proteom. 2010, 9, 197–208.
26. Grossman, S.D.; Wolfe, B.B.; Yasuda, R.P.; Wrathall, J.R. Changes in NMDA receptor subunit expression in response to contusive spinal cord injury. J. Neurochem. 2000, 75, 174–184.
27. Reed, J.C. Mechanisms of apoptosis. Am. J. Pathol. 2000, 157, 1415–1430.
28. Rauch, M.F.; Hynes, S.R.; Bertram, J.; Redmond, A.; Robinson, R.; Williams, C.; Xu, H.; Madri, J.A.; Lavik, E.B. Engineering angiogenesis following spinal cord injury: A coculture of neural

progenitor and endothelial cells in a degradable polymer implant leads to an increase in vessel density and formation of the blood-spinal cord barrier. Eur. J. Neurosci. 2009, 29, 132–145.

29. Cao, Y.; Xu, Y.; Chen, C.; Xie, H.; Lu, H.; Hu, J. Local delivery of USC-derived exosomes harboring ANGPTL3 enhances spinal cord functional recovery after injury by promoting angiogenesis. Stem Cell Res. Ther. 2021, 12, 20.
30. Kim, H.Y.; Kumar, H.; Jo, M.J.; Kim, J.; Yoon, J.K.; Lee, J.R.; Kang, M.; Choo, Y.W.; Song, S.Y.; Kwon, S.P.; et al. Therapeutic efficacy-potentiated and diseased organ-targeting nanovesicles derived from mesenchymal stem cells for spinal cord injury treatment. Nano Lett. 2018, 18, 4965–4975.
31. Lu, Y.; Zhou, Y.; Zhang, R.; Wen, L.; Wu, K.; Li, Y.; Yao, Y.; Duan, R.; Jia, Y. Bone mesenchymal stem cell-derived extracellular vesicles promote recovery following spinal cord injury via improvement of the integrity of the blood-spinal cord barrier. Front. Neurosci. 2019, 13, 209.
32. Keefe, K.M.; Sheikh, I.S.; Smith, G.M. Targeting neurotrophins to specific populations of neurons: NGF, BDNF, and NT-3 and their relevance for treatment of spinal cord injury. Int. J. Mol. Sci. 2017, 18, 548.
33. Zhao, L.; Jiang, X.; Shi, J.; Gao, S.; Zhu, Y.; Gu, T.; Shi, E. Exosomes derived from bone marrow mesenchymal stem cells overexpressing microRNA-25 protect spinal cords against transient ischemia. J. Thorac. Cardiovasc. Surg. 2019, 157, 508–517.
34. Pegtel, D.M.; Gould, S.J. Exosomes. Annu. Rev. Biochem. 2019, 88, 487–514.
35. Huang, J.; Gadotti, V.M.; Chen, L.; Souza, I.A.; Huang, S.; Wang, D.; Ramakrishnan, C.; Deisseroth, K.; Zhang, Z.; Zamponi, G.W. A neuronal circuit for activating descending modulation of neuropathic pain. Nat. Neurosci. 2019, 22, 1659–1668.
36. Li, R.; Zhao, K.; Ruan, Q.; Meng, C.; Yin, F. Bone marrow mesenchymal stem cell-derived exosomal microRNA-124-3p attenuates neurological damage in spinal cord ischemia-reperfusion injury by downregulating Ern1 and promoting M2 macrophage polarization. Arthritis Res. Ther. 2020, 22, 75.
37. Chen, L.; Lu, F.B.; Chen, D.Z.; Wu, J.L.; Hu, E.D.; Xu, L.M.; Zheng, M.H.; Li, H.; Huang, Y.; Jin, X.Y.; et al. BMSCs-derived miR-223-containing exosomes contribute to liver protection in experimental autoimmune hepatitis. Mol. Immunol. 2018, 93, 38–46.
38. Chang, Q.; Hao, Y.; Wang, Y.; Zhou, Y.; Zhuo, H.; Zhao, G. Bone marrow mesenchymal stem cell-derived exosomal microRNA-125a promotes M2 macrophage polarization in spinal cord injury by downregulating IRF5. Brain Res. Bull. 2021, 170, 199–210.
39. Haneklaus, M.; Gerlic, M.; O'Neill, L.A.; Masters, S.L. miR-223: Infection, inflammation and cancer. J. Intern. Med. 2013, 274, 215–226.

40. Bauernfeind, F.; Rieger, A.; Schildberg, F.A.; Knolle, P.A.; Schmid-Burgk, J.L.; Hornung, V. NLRP3 inflammasome activity is negatively controlled by miR-223. J. Immunol. 2012, 189, 4175–4181.
41. Gris, D.; Ye, Z.; Iocca, H.A.; Wen, H.; Craven, R.R.; Gris, P.; Huang, M.; Schneider, M.; Miller, S.D.; Ting, J.P. NLRP3 plays a critical role in the development of experimental autoimmune encephalomyelitis by mediating Th1 and Th17 responses. J. Immunol. 2010, 185, 974–981.
42. Jee, M.K.; Jung, J.S.; Choi, J.I.; Jang, J.A.; Kang, K.S.; Im, Y.B.; Kang, S.K. MicroRNA 486 is a potentially novel target for the treatment of spinal cord injury. Brain 2012, 135, 1237–1252.
43. Hu, J.; Zeng, L.; Huang, J.; Wang, G.; Lu, H. miR-126 promotes angiogenesis and attenuates inflammation after contusion spinal cord injury in rats. Brain Res. 2015, 1608, 191–202.
44. Hu, J.Z.; Huang, J.H.; Zeng, L.; Wang, G.; Cao, M.; Lu, H.B. Anti-apoptotic effect of microRNA-21 after contusion spinal cord injury in rats. J. Neurotrauma 2013, 30, 1349–1360.
45. Ujigo, S.; Kamei, N.; Hadoush, H.; Fujioka, Y.; Miyaki, S.; Nakasa, T.; Tanaka, N.; Nakanishi, K.; Eguchi, A.; Sunagawa, T.; et al. Administration of microRNA-210 promotes spinal cord regeneration in mice. Spine 2014, 39, 1099–1107.
46. Ding, S.Q.; Chen, J.; Wang, S.N.; Duan, F.X.; Chen, Y.Q.; Shi, Y.J.; Hu, J.G.; Lu, H.Z. Identification of serum exosomal microRNAs in acute spinal cord injured rats. Exp. Biol. Med. 2019, 244, 1149–1161.
47. Huang, J.H.; Xu, Y.; Yin, X.M.; Lin, F.Y. Exosomes derived from miR-126-modified MSCs promote angiogenesis and neurogenesis and attenuate apoptosis after spinal cord injury in rats. Neuroscience 2020, 424, 133–145.
48. Zhang, Y.; Wang, Z.; Gemeinhart, R.A. Progress in microRNA delivery. J. Control Release 2013, 172, 962–974.
49. Nayerossadat, N.; Maedeh, T.; Ali, P.A. Viral and nonviral delivery systems for gene delivery. Adv. Biomed. Res. 2012, 1, 27.
50. Kamimura, K.; Suda, T.; Zhang, G.; Liu, D. Advances in gene delivery systems. Pharmaceut Med. 2011, 25, 293–306.
51. Shen, H.; Yao, X.; Li, H.; Li, X.; Zhang, T.; Sun, Q.; Ji, C.; Chen, G. Role of exosomes derived from miR-133b modified MSCs in an experimental rat model of intracerebral hemorrhage. J. Mol. Neurosci. 2018, 64, 421–430.
52. Katakowski, M.; Buller, B.; Zheng, X.; Lu, Y.; Rogers, T.; Osobamiro, O.; Shu, W.; Jiang, F.; Chopp, M. Exosomes from marrow stromal cells expressing miR-146b inhibit glioma growth. Cancer Lett. 2013, 335, 201–204.
53. Kang, J.; Li, Z.; Zhi, Z.; Wang, S.; Xu, G. MiR-21 derived from the exosomes of MSCs regulates the death and differentiation of neurons in patients with spinal cord injury. Gene Ther. 2019, 26,

491–503.

54. Xu, G.; Ao, R.; Zhi, Z.; Jia, J.; Yu, B. miR-21 and miR-19b delivered by hMSC-derived EVs regulate the apoptosis and differentiation of neurons in patients with spinal cord injury. J. Cell Physiol. 2019, 234, 10205–10217.
55. Yu, T.; Zhao, C.; Hou, S.; Zhou, W.; Wang, B.; Chen, Y. Exosomes secreted from miRNA-29b-modified mesenchymal stem cells repaired spinal cord injury in rats. Braz. J. Med. Biol. Res. 2019, 52, e8735.
56. Kang, J.; Zhang, C.; Zhi, Z.; Wang, Y.; Liu, J.; Wu, F.; Xu, G. Stem-like cells of various origins showed therapeutic effect to improve the recovery of spinal cord injury. Artif. Cells Nanomed. Biotechnol. 2020, 48, 627–638.
57. Dong, R.F.; Zhang, B.; Tai, L.W.; Liu, H.M.; Shi, F.K.; Liu, N.N. The neuroprotective role of MiR-124-3p in a 6-hydroxydopamine-induced cell model of Parkinson's disease via the regulation of ANAX5. J. Cell Biochem. 2018, 119, 269–277.
58. Jiang, D.; Gong, F.; Ge, X.; Lv, C.; Huang, C.; Feng, S.; Zhou, Z.; Rong, Y.; Wang, J.; Ji, C.; et al. Neuron-derived exosomes-transmitted miR-124-3p protect traumatically injured spinal cord by suppressing the activation of neurotoxic microglia and astrocytes. J. Nanobiotechnol. 2020, 18, 105.
59. Liu, W.; Rong, Y.; Wang, J.; Zhou, Z.; Ge, X.; Ji, C.; Jiang, D.; Gong, F.; Li, L.; Chen, J.; et al. Exosome-shuttled miR-216a-5p from hypoxic preconditioned mesenchymal stem cells repair traumatic spinal cord injury by shifting microglial M1/M2 polarization. J. Neuroinflammation 2020, 17, 47.
60. Zhang, M.; Wang, L.; Huang, S.; He, X. Exosomes with high level of miR-181c from bone marrow-derived mesenchymal stem cells inhibit inflammation and apoptosis to alleviate spinal cord injury. J. Mol. Histol. 2021, 52, 301–311.
61. Jiang, Z.; Zhang, J. Mesenchymal stem cell-derived exosomes containing miR-145-5p reduce inflammation in spinal cord injury by regulating the TLR4/NF-κB signaling pathway. Cell Cycle 2021, 20, 993–1009.
62. Chen, Y.; Tian, Z.; He, L.; Liu, C.; Wang, N.; Rong, L.; Liu, B. Exosomes derived from miR-26a-modified MSCs promote axonal regeneration via the PTEN/AKT/mTOR pathway following spinal cord injury. Stem Cell Res. Ther. 2021, 12, 224.
63. Wang, Y.; Lai, X.; Wu, D.; Liu, B.; Wang, N.; Rong, L. Umbilical mesenchymal stem cell-derived exosomes facilitate spinal cord functional recovery through the miR-199a-3p/145-5p-mediated NGF/TrkA signaling pathway in rats. Stem Cell Res. Ther. 2021, 12, 117.
64. Cui, Y.; Yin, Y.; Xiao, Z.; Zhao, Y.; Chen, B.; Yang, B.; Xu, B.; Song, H.; Zou, Y.; Ma, X.; et al. LncRNA Neat1 mediates miR-124-induced activation of Wnt/β-catenin signaling in spinal cord

neural progenitor cells. Stem Cell Res. Ther. 2019, 10, 400.

65. Wu, H.; Ding, L.; Wang, Y.; Zou, T.B.; Wang, T.; Fu, W.; Lin, Y.; Zhang, X.; Chen, K.; Lei, Y.; et al. MiR-615 regulates NSC differentiation in vitro and contributes to spinal cord injury repair by targeting LINGO-1. Mol. Neurobiol. 2020, 57, 3057–3074.
66. Li, D.; Zhang, P.; Yao, X.; Li, H.; Shen, H.; Li, X.; Wu, J.; Lu, X. Exosomes derived from miR-133b-modified mesenchymal stem cells promote recovery after spinal cord injury. Front. Neurosci. 2018, 12, 845.
67. Ma, K.; Xu, H.; Zhang, J.; Zhao, F.; Liang, H.; Sun, H.; Li, P.; Zhang, S.; Wang, R.; Chen, X. Insulin-like growth factor-1 enhances neuroprotective effects of neural stem cell exosomes after spinal cord injury via an miR-219a-2-3p/YY1 mechanism. Aging 2019, 11, 12278–12294.
68. Zhang, A.; Bai, Z.; Yi, W.; Hu, Z.; Hao, J. Overexpression of miR-338-5p in exosomes derived from mesenchymal stromal cells provides neuroprotective effects by the Cnr1/Rap1/Akt pathway after spinal cord injury in rats. Neurosci. Lett. 2021, 761, 136124.
69. Li, C.; Li, X.; Zhao, B.; Wang, C. Exosomes derived from miR-544-modified mesenchymal stem cells promote recovery after spinal cord injury. Arch. Physiol. Biochem. 2020, 126, 369–375.