Research Progress on Stem Cell Regulation of Macrophage Polarization in Promoting Spinal Cord Injury Recovery in Stem Cell Transplantation

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Abstract. With the advancement of spinal cord injury (SCI) research, stem cell therapy has garnered increasing attention. However, the field encompasses numerous research topics and branches, necessitating further organization and focus. This review aims to provide a coherent summary of the mechanisms and roles of stem cells in SCI treatment, addressing three key aspects: the role of macrophage polarization in SCI, the regulatory mechanisms by which stem cells, specifically mesenchymal stem cells (MSCs) and neural stem cells (NSCs), influence macrophage polarization, and the signaling pathways involved in stem cell-regulated macrophage polarization. Correct macrophage polarization is crucial for effective SCI treatment, with MSCs and NSCs contributing through chemical mediators, modulation of macrophage polarization, and interactions with signaling pathways. Ultimately, the review will explore how these signaling pathways enhance SCI treatment through diverse forms of signal transduction and regulation. In conclusion, this review will summarize current insights into stem cell therapy for SCI, integrating the three interconnected aspects discussed.

Keywords: Spinal cord injury; stem cell; mesenchymal stem cells; neural stem cells.

1. Introduction

Spinal cord injury (SCI) is a debilitating trauma to the central nervous system that leads to the loss of motor, sensory, and autonomic functions, profoundly impairing patients' quality of life and social participation [1]. The pathogenesis of SCI is multifaceted, involving both primary mechanical damage and secondary pathophysiological processes such as inflammation, oxidative stress, and apoptosis [2]. A key feature of secondary injury is the rapid onset of an inflammatory response at the injury site. While this inflammation aids in the clearance of necrotic tissue and pathogens, excessive or prolonged inflammation exacerbates tissue damage and hinders neural repair.

Stem cell therapy, with its regenerative potential and multilineage differentiation capabilities, has emerged as a promising strategy for SCI treatment [3]. Stem cells can differentiate into cell types needed by damaged tissues, facilitating neural regeneration and functional recovery [4]. Mesenchymal stem cells (MSCs), characterized by self-renewal, low immunogenicity, and multilineage differentiation potential, are considered particularly suitable for SCI treatment. Similarly, neural stem cells (NSCs), which are directly involved in the development and repair of the nervous system, contribute to the reconstruction of damaged neural circuits when transplanted.

Macrophage polarization refers to the differentiation of macrophages into distinct functional phenotypes in response to specific microenvironmental cues. M1 macrophages exhibit pro-inflammatory properties, playing a key role in pathogen clearance and inflammatory responses, while M2 macrophages possess anti-inflammatory and tissue-reparative functions that support regeneration. After SCI, the polarization state of macrophages critically influences the extent of tissue damage and the effectiveness of repair. Excessive M1 activation exacerbates tissue injury, whereas M2 macrophages alleviate inflammation and promote tissue repair.

Stem cells can modulate macrophage polarization through the secretion of cytokines, exosomes, and other bioactive molecules. This modulation reduces the inflammatory response following SCI,

thereby promoting neural repair and functional recovery. Notably, signaling pathways such as PI3K/Akt and TLR4/NF-κB are implicated in stem cell-mediated regulation of macrophage polarization, influencing the broader pathophysiological processes of SCI.

This review will first provide an overview of the research landscape surrounding SCI and the therapeutic potential of stem cell therapy. It will then explore the role of macrophage polarization in SCI and examine the mechanisms through which stem cells regulate macrophage polarization, with an emphasis on their implications for SCI recovery.

2. The Function of Polarization in Macrophages in SCI

Macrophages are key inflammatory immune cells found throughout the body, including in the central nervous system, where they originate from both circulating monocytes and resident microglia. In the context of SCI, Pro-inflammatory M1 macrophages and anti-inflammatory M2 macrophages are the two main phenotypes of macrophages that can be polarized , each playing distinct roles in the injury and repair processes.

M1 macrophages are primarily involved in the secondary injury phase of SCI, where they contribute to tissue damage through several mechanisms. These include the phagocytosis of apoptotic and necrotic cells, clearance of cellular debris, and the discharge of pro-inflammatory cytokines, reactive oxygen species (ROS), proteases, and other cytotoxic molecules. While these functions are crucial for clearing damaged cells and pathogens, prolonged M1 activation exacerbates neuroinflammation and impedes tissue repair.

Conversely, M2 macrophages exhibit anti-inflammatory and tissue-reparative properties. By secreting anti-inflammatory cytokines and facilitating the recruitment of immune cells across the compromised blood-spinal cord barrier[1], M2 macrophages help limit lesion expansion. They also stimulate axonal growth and nerve regeneration, thereby promoting wound healing and tissue remodeling after SCI [2].

The polarization of macrophages is driven by the microenvironment, which can be influenced by pathogenic microorganisms, chemokines, or other physicochemical stimuli. Following SCI, neutrophil infiltration occurs within 24 hours, which is followed by macrophage and microglia hyperactivity. This hyperactivity leads to the release of neurotoxic substances that contribute to neuronal apoptosis and tissue damage. The spontaneous regeneration of axons is limited, as M1 macrophages and microglia at the injury site hinder axonal regrowth.

M2 macrophage polarization has been demonstrated to serve an essential role in immune defense, anti-inflammatory responses, and tissue remodeling. These macrophages not only promote axonal growth but also contribute to wound healing and regeneration following SCI. For instance, M2 macrophage transplantation, induced by olfactory ensheathing cells, inhibits Signal transducer and activator of transcription/Janus kinase (JAK/STAT) signaling pathway, improving neuronal survival and promoting axon regrowth. In the later phases of M2 macrophage polarization, arginase-catalyzed metabolites such as ornithine support tissue repair and angiogenesis.

In SCI treatment, M1 macrophages are implicated in neuroinflammation, CNS demyelination, and neuronal death. These neurotoxic M1 macrophages prevent the clearance of myelin phospholipid fragments through macrophage surface receptors and the complement system, prolonging the inflammatory environment [6]. Furthermore, direct interactions between M1 macrophages and damaged neurons lead to the release of neurotoxic substances like ROS and reactive nitrogen species, causing further neuronal damage [7].

In contrast, promoting the polarization of M2 macrophages mitigates inflammation, enhances angiogenesis, and prevents the formation of fibrotic scarring. However, persistent M2 macrophage activity post-SCI may also have detrimental effects by secreting pro-fibrotic factors that accelerate scar formation, thereby inhibiting axonal regeneration [8]. As such, increasing the proportion of M2 macrophages and extending their presence within the local injury microenvironment represent promising therapeutic strategies for SCI.

3. Mechanism of Stem Cell Regulation of Macrophage Polarization

3.1. MSCs

In a nutshell, at the level of chemicals, MSCs-derived extracellular vesicles contain various proteins, nucleic acids and other substances, which have anti-inflammatory and growth-promoting functions. Additionally, the migration of MSCs and the inhibition of T cell, B cell, and NK cell-related inflammatory behaviors by MSCs are significant reasons for the ability to alleviate inflammation. By extension, bone marrow mesenchymal stem cell-derived exosomes are also associated with signaling pathways and one possible pathway is the inhibition of the PTEN-PI3K pathway, which promotes axonal regeneration.

3.2. NSCs

Overall, exogenous NSCs are able to activate endogenous NSPCs to produce neurotrophin-3 (NT-3), which can achieve anti-inflammatory effects, and more importantly, their secretion of factors that have something to do with the promotion of growth promotes the survival and growth of damaged neurons [2,8,9]. In addition, NSCs can also contribute to the polarization to the M2 type of macrophages by modulating the inflammatory and immune microenvironment[9]. Similarly, in terms of linkage signaling pathways, NSC secretion of the cytokine Interleukin (IL)4 helps induce macrophages to polarize to M2, inhibit the signaling process of NF-κB/p65 and prevent it from polarizing to M1.

NSCs make an obvious contribution to neuroprotection and the promotion of axonal growth. Exogenous NSCs not only contribute directly to neurogenesis but also activate endogenous neural stem/progenitor cells (NSPCs), stimulating them to secrete neurotrophin-3 (NT-3) and other related factors. These factors help reduce inflammation and help the damaged neurons survive and regenerate [2, 8, 9].

Moreover, NSCs are actively involved in regulating the immune microenvironment following SCI. They induce the polarization of macrophages toward the M2 phenotype by secreting cytokines such as interleukin-4 (IL-4), which inhibits the activation of the NF-κB/p65 signaling pathway. This mechanism prevents macrophages from adopting the pro-inflammatory M1 phenotype, further supporting tissue repair and functional recovery.

3.3. Key Cytokines and Growth Factors in Macrophage Polarization

Stem cells' control over macrophage polarization and involves a complex interplay of cytokines, growth factors, and miRNAs. MSCs secrete various potent factors, including hepatocyte growth factor (HGF), IL-10, IL-13, and IL-27, all of which have demonstrated anti-inflammatory potential [10]. Additionally, certain miRNAs secreted by MSCs, such as miR-132-3p, miR-213p, and miR-22-3p, exhibit anti-apoptotic properties, thus helping to inhibit neuroapoptosis and reduce cell death after SCI.

Similarly, NSCs contribute to the regulation of macrophage polarization through the secretion of neurotrophic factors. Notable examples include brain-derived neurotrophic factor (BDNF) and insulin-like growth factor-1 (IGF-1), both of which help maintain neural integrity. NSCs also release NT-3, basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF), which encourage angiogenesis, tissue remodeling, and axonal regeneration in the damaged spinal cord.

3.4. Role of Exosomes in SCI Repair

Exosomes, a subtype of extracellular vesicles, have emerged as key players in SCI repair due to their small size (40–100 nm) and ability to penetrate biological barriers, such as the blood-spinal cord barrier. Exosomes derived from MSCs and NSCs exhibit enhanced stability and immune tolerance compared to stem cells themselves, making them more effective in treating SCI [11].

BMSC-derived exosomes contain miR-125a, which regulates interferon regulatory factor 5 (IRF5) to reduce inflammatory responses associated with SCI. This modulation protects neurons and reduces

tissue damage. Similarly, NSC-derived exosomes (NSC-Exos) have been shown to locally modulate the CNS microenvironment, affecting both microglia and neurons. On one hand, NSC-Exos deliver specific molecular signals that mitigate SCI pathology by reducing inflammation. On the other hand, they act as neuroprotective agents, promoting peripheral nerve regeneration and enhancing the repair of damaged neural tissues [12]..

4. Relevant Signaling Pathways Regulating Macrophage Polarization by Stem Cells

Current research has identified several key signaling pathways involved in macrophage polarization following SCI, including the JAK/STAT, NF-κB, and PI3K/Akt pathways, among others [1, 13]. These mechanisms are critical in deciding whether macrophages adopt a pro-inflammatory M1 or anti-inflammatory M2 phenotype, hence impacting the outcome of SCI recovery.

4.1. JAK/STAT

JAK/STAT pathway is pivotal in regulating macrophage polarization in SCI. JAK1, JAK2, JAK3, and tyrosine kinase 2 (TYK2) interact with the STAT family, which comprises STAT1, STAT2, STAT3, STAT4, STAT5A, STAT5B, and STAT6.[14] . Upon cytokine binding to their receptors, JAK kinases become activated and phosphorylate tyrosine residues on the receptors. STAT proteins, characterized by their SH2 domains, then recognize and bind to these phosphorylated residues, leading to their activation. Activated STAT proteins either form dimers or enter the nucleus as monomers to bind DNA promoter regions, thereby regulating gene transcription related to various cellular processes, including inflammation and immune responses.

In macrophage polarization, STAT1 is crucial for promoting the M1 phenotype. Inhibiting the JAK/STAT1 pathway reduces M1 macrophage activation [15]. However, combining JAK/STAT1 with the TLR4/NF-κB pathway increases inflammatory mediator production and worsens M1 polarization [1]. This underscores the role of STAT1 in mediating M1 polarization and highlights the pathway's complex interactions with other signaling networks.

Furthermore, the JAK/STAT pathway also influences M2 macrophage polarization. MSC-derived exosomes (MSC-Exos) modulate macrophage polarization towards the M2 phenotype through the JAK/STAT pathway. Interleukins IL-4 and IL-13 promote M2 polarization by activating the JAK1/3-STAT6 signaling axis, emphasizing the role of STAT6 in driving the M2 phenotype [16]. Additionally, IL-10 reprograms macrophages towards M2 polarization via the JAK1/STAT3 pathway [17]. These findings illustrate the versatility of the JAK/STAT pathway in regulating macrophage polarization and its potential as a therapeutic target in SCI.

4.2. NF-κB

The NF-κB pathway regulates cellular anti-apoptosis and pro-survival processes. The mammalian NF-κB family consists of five proteins: RelA (p65), RelB, c-Rel, and the processed forms of precursor proteins, NF-κB1 p50 and NF-κB2 p52[18]. This protein family is integral in balancing cell survival and apoptosis. NF-κB, in its inactive state, is bound to inhibitory proteins from the IκB family and remains sequestered in the cytoplasm. When exposed to pathogens, cytokines, or stress signals, IκB is phosphorylated and ubiquitinated, leading in the release of NF-κB and its translocation to the nucleus. Within the nucleus, NF-κB binds to specific DNA sequences to initiate or enhance the expression of genes involved in inflammation, immunity, and cellular homeostasis.

Following SCI, the NF-κB pathway becomes activated, triggering an inflammatory response that exacerbates neural damage. Inhibiting the NF-κB pathway promotes macrophage polarization towards the M2 phenotype, which reduces neuroinflammation and supports functional recovery after SCI [19]. Lipopolysaccharide (LPS) can activate the NF-κB signaling pathway through its binding to the TLR4 receptor on macrophages, leading to macrophage polarization towards the M1 phenotype and the initiation of an inflammatory response [20]. This process involves the activation of the MAPK

pathway, which results in the phosphorylation and degradation of IκB, releasing the p50-p65/RelA heterodimer.NF-κB translocates to the nucleus and regulates gene transcription [21]. Akt isoforms can regulate NF-κB upstream, leading to inflammation and macrophage polarization [13].

LPS induces IKK activation, leading to phosphorylation of IκBα and its subsequent ubiquitination and degradation. This step allows NF-κB dimers to enter the nucleus, promoting the M1 macrophage phenotype [4]. Exosomes from bone marrow mesenchymal stem cells (BMSCs) block the LPS-dependent NF-κB signaling pathway, leading to increased polarization of M2 macrophages and decreased polarization of M1 macrophages [13]. Exosomes of the UC-MSCs can prevent SCI by reducing inflammation via the NF-κB/MAPK signaling pathway [4]. MSCs lower CARD9 expression, reducing inflammation from proinflammatory cytokines and decreasing NF-κB phosphorylation in macrophages. MSCs also suppress M1 macrophage polarization by downregulating the CARD9-NF-κB signaling pathway [22].

4.3. PI3K/Akt

PI3K family is essential for catalyzing the phosphorylation of the 3'-hydroxy position of phosphatidylinositol, producing biologically active phosphatidylinositol-3-phosphate (PI3P) and its further phosphorylated derivatives. Akt, a serine/threonine protein kinase, functions downstream of the PI3K signaling pathway and acts as a critical mediator of PI3K signal transduction. Upon activation of PI3K, lipid second messengers such as PIP3 are generated, which then recruit and activate Akt. Activated Akt further phosphorylates various downstream substrates, influencing cellular processes such as proliferation, survival, and metabolism, thereby affecting cell fate and function.

The PI3K/Akt signaling pathway is pivotal in regulating macrophage polarization. When AKT1 is activated, it facilitates the differentiation of macrophages towards the M2 phenotype. Conversely, inactivation of AKT1 promotes M1 macrophage polarization [23]. In contrast, AKT2 primarily mediates the polarization of M1 macrophages. Activation of AKT2 is closely associated with M1 macrophage differentiation, while the absence of AKT2 encourages M2 polarization and inhibits M1 activation [23, 24]. Various cytokines, such as IL-10 and TGF-β, as well as exosome-derived signals, can activate the PI3K/Akt pathway, promoting M2 macrophage polarization [1, 4, 25].

The stimulation of the the signaling pathway involving in MSCs drives M2 macrophage polarization [4]. Furthermore, exosomes produced from iPSC-Exos have been found to activate the PI3K/Akt pathway and enhance M2 macrophage polarization [1]. Cytokines like IL-10 and TGF-β also activate the pathway, contributing to M2 macrophage polarization [26]. UC-MSCs activate the PI3K/Akt pathway to enhance M2 macrophage polarization, which helps attenuate inflammatory responses and reduce LPS-induced neuronal apoptosis in spinal cord injury [4]. Furthermore, exosomes from hypoxia-preconditioned MSCs activate the PI3K/Akt pathway while inhibiting the TLR4/NF-κB pathway, leading to the polarization of macrophages/microglia towards an anti-inflammatory M2 phenotype and aiding in the repair of traumatic SCI [25]. LPS-primed bone marrow stem cell-derived exosomes (L-Exo) significantly activate AKT1 phosphorylation, promoting M2 macrophage polarization while inhibiting AKT2 phosphorylation and reducing M1 macrophage polarization [13].

5. Conclusion

Primary spinal cord injury results in axonal and neuronal damage, triggering neuroinflammation and leading to secondary injuries such as spinal cord edema, cavity formation, and glial cell proliferation. Macrophages, as key components of the innate immune defense system, play a crucial role in the progression of secondary damage and tissue repair following spinal cord injury through their M1/M2 phenotypic transformation. Stem cell transplantation therapy can effectively repair SCI by modulating macrophage phenotype and function, demonstrating notable benefits. NSCs have

shown considerable promise in SCI treatment due to their capacity to generate primitive nerve cells, reduce tumor risk, shrink lesions, and promote functional recovery in preclinical studies.

Moreover, the regulation of macrophage polarization and its connection to signaling pathways actively contribute to therapy, enhancing the treatment of spinal cord injuries through various signal transduction and modulation mechanisms. Future studies should explore the effects of stem cells on macrophage polarization and immune system dynamics during SCI treatment, revealing interactions among different immune cells to improve nerve injury outcomes. Additionally, researchers should investigate the application strategies of MSCs and NSCs from the perspective of signaling pathways that regulate macrophage polarization, and consider integrating pharmacological treatments and other strategies to further enhance therapeutic efficacy.

Authors Contribution

All the authors contributed equally and their names were listed in alphabetical order.

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