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Microenvironment Imbalance of Spinal Cord Injury

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Abstract

Spinal cord injury (SCI), for which there currently is no cure, is a heavy burden on patient physiology and psychology. The microenvironment of the injured spinal cord is complicated. According to our previous work and the advancements in SCI research, 'microenvironment imbalance' is the main cause of the poor regeneration and recovery of SCI. Microenvironment imbalance is defined as an increase in inhibitory factors and decrease in promoting factors for tissues, cells and molecules at different times and spaces. There are imbalance of hemorrhage and ischemia, glial scar formation, demyelination and re-myelination at the tissue's level. The cellular level imbalance involves an imbalance in the differentiation of endogenous stem cells and the transformation phenotypes of microglia and macrophages. The molecular level includes an imbalance of neurotrophic factors and their pro-peptides, cytokines, and chemokines. The imbalanced microenvironment of the spinal cord impairs regeneration and functional recovery. This review will aid in the understanding of the pathological processes involved in and the development of comprehensive treatments for SCI.

Keywords

spinal cord injury, microenvironment, imbalance, regeneration

Introduction

Spinal cord injury (SCI), a serious damage to the central nervous system, has historically been considered an incurable impairment worldwide. Patients with SCI suffer a lot both in terms of physiology and psychology[1,2], and simultaneously, SCI has been a major burden on the society with increasing prevalence[1].

Currently, the prevalence of SCI is approximately 180,000 cases worldwide, with numbers still rising. Our study found that the annual incidence was 23.7 cases per million population, and SCI was more common in older individuals in Tianjin, China[3]. Individuals with SCI had a higher rate of death than controls[4]. According to an epidemiological investigation of SCI, the most common causes of SCI are falls and traffic accidents[5]. Current treatments of SCI include traditional drug therapy[1], surgery[6,7], cell therapy[8–10], gene therapy and tissue engineering[11–13]. However, these strategies cannot fully repair SCIs but can only improve symptoms and reduce complications.

The spinal cord consists of the gray and white matter which contains nerve cell bodies and ascending and descending tracts. Thus, the different locations and the extent of SCI can cause varying degrees of disability, from partial loss of sensory or motor function to complete paralysis below the injured location, as well as acute and chronic complications[14]. The poor prognosis of SCI is associated with the extremely weak regenerative capacity of the spinal cord; although there is some inherent regenerative capacity of the central nervous system, it is inadequate. The poor regenerative capacity of the spinal cord is further complicated by the fact that SCI is often accompanied by various

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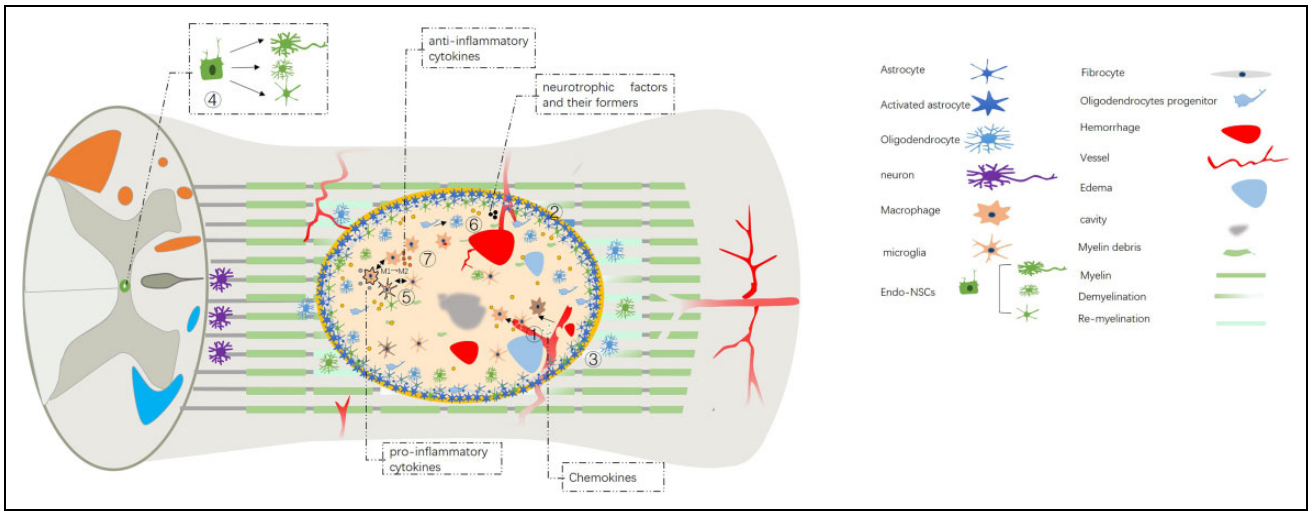

Fig. 1. Microenvironment imbalance of spinal cord injury.
① Hemorrhage and ischemia.
② Scar formation.
③ Demyelination and re-myelination.
④ Differentiation balance of endogenous neural stem cells.
⑤ Transformation of the phenotypes of microglia and macrophages.
⑥ Imbalance of neurotrophic factors and their pro-peptides.
⑦ Imbalance of the cytokines and chemokines.
Endo-NSC: endogenous neural stem cell.

molecular pathology cascades that interact with each other. Traditionally, the pathophysiology of SCI is divided into two phases: primary injury and secondary injury[6,7,15,16]. Recent studies have provided a more detailed description of these phases based on different times after injury reviewed in Rowland et al.[17] and Hayta et al.[18], and which have demonstrated the high complexity of SCI at different levels. The complicated pathophysiology of SCI includes cell death, axonal collapse and demyelination, glial scar formation, inflammation and other pathological defects. However, there is no systematic theory that can define these complicated pathophysiological processes and guide the development of therapies for SCI.

Thus, according to the developments in SCI research[7,19–21] and our previous work, we defined 'microenvironment imbalance after SCI' as the imbalance of tissue-, cell- and molecule-promoting and inhibiting factors at different times and sites that aggravate and accelerate the course of SCI.

Microenvironment Imbalance After SCI

SCI can be divided into two categories: traumatic and non-traumatic spinal cord injury. Traumatic SCI is much more common and is typically caused by external physical impact[1]. Non-traumatic SCI is often caused by compression of tumor, vascular ischemia or congenital disease[22]. This review will focus on traumatic SCI. Following contusion injury, the balance of the spinal cord microenvironment is disrupted, which leads to a series of pathophysiological changes; beneficial factors become downregulated, and harmful factors become upregulated after SCI. The microenvironment imbalance consists of three levels at different times and sites: molecules, cells and tissues. The cellular level involves the activation of astrocytes, the differentiation of endogenous neural stem cells, oligodendrocyte progenitors and microglia, the infiltration of macrophages, etc. The tissue level involves hemorrhage and ischemia, glial scar formation, demyelination and re-myelination, etc. The molecular level involves the expression of neurotrophic factors and their pro-peptides, cytokines, chemokines, etc. These imbalances impair regeneration and functional recovery (Figs. 1 and 2).

Tissue Imbalance

Hemorrhage and Ischemia

Primary mechanical damage from SCI leads to the disruption of the topical capillaries and the blood–brain-spinal cord barrier (BSCB), which provides a specialized microenvironment for the spinal cord parenchyma. The imbalance of hemorrhage and ischemia was broken. (Fig. 1 ①) A direct rupture of the local capillaries induces bleeding into the parenchyma of the spinal cord, especially into the gray matter[23], which could cause increased release of cytokines and chemokines from macrophages, microglia and astrocytes into the extracellular space. And the presence of red blood cells/heme in parenchyma, a rich source of iron, is likely to induce free radicals and

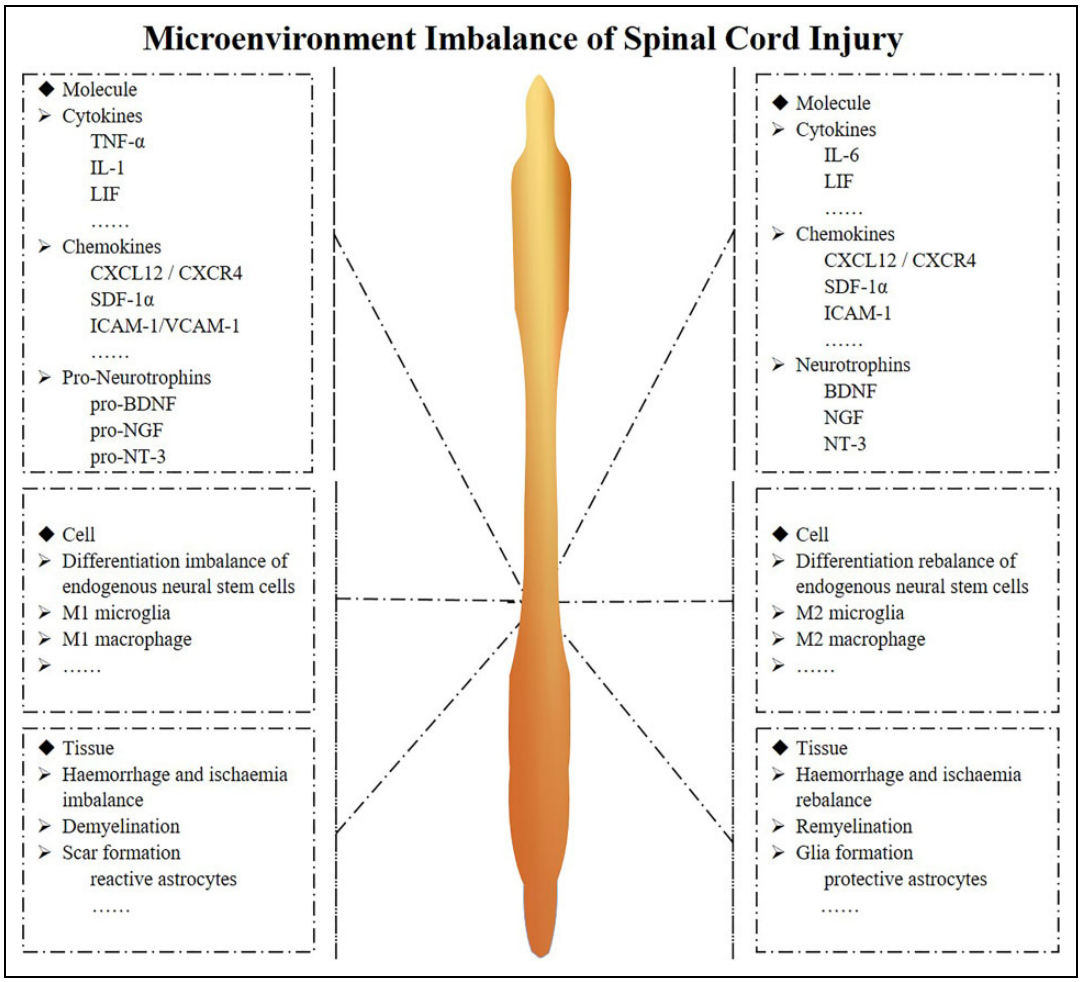

Fig. 2. Microenvironment imbalance of spinal cord injury at different level.
The microenvironment at the molecule, cell and tissue level is shown separately. The tissue level imbalance includes hemorrhage and ischemia, glial scar formation, demyelination and re-myelination; the cellular level imbalance involves an imbalance in the differentiation of endogenous stem cells and the transformation phenotypes of microglia and macrophages; the molecular level includes an imbalance of neurotrophic factors and their pro-peptides, cytokines, and chemokines.
BDNF: brain-derived neurotrophic factor; CXCR4: C-X-C chemokine receptor type 4; CXCL12: C-X-C motif chemokine 12; ICAM1: intercellular adhesion molecule; IL: interleukin; LIF: leukocyte inhibitory factor; NGF: nerve growth factor; NT-3: neurotrophin-3; SDF-1: stromal cell-derived factor 1; TNF-: tumor necrosis factor alpha; VCAM1: vascular cell adhesion protein.

be toxic[24], which is a potential mechanism for ferroptosis. Venous stasis and distension would further cause the accumulation of proteinaceous fluid in the tissues, leading to edema[25]. On the other hand, neural tissue edema can also increase interstitial pressure, which would compress the surrounding vessels and subsequently cause ischemia[26]. In addition, damage to the BSCB can increase its permeability, which would cause macrophages infiltrated from injured blood vessels and accumulate in the microenvironment of the spinal cord, and the macrophages would express more cytokines and chemokines; in turn, this would further increase permeability of the BSCB. The lack of adenosine triphosphate (ATP) caused by ischemia and ion channel defects would result in an ion imbalance. Moreover, the accumulation of water in cells and the extracellular compartment worsens the neural tissue edema[27].

Scar Formation

Glial scar formation is a vital part of the pathology of SCI, which includes fibrous and glial components. The fibrous component contains stromal cells at the center of the scar[28], which are derived from the vascular-related type A pericytes; the glial component consists of astrocytes, which are derived from self-replicating astrocytes and endogenous neural stem cells (endo-NSCs), and the astrocytes from the endo-NSCs reinforce the glial scar[29]. The glial scar is also composed of microglia, macrophages and extracellular matrix and astrocytes. The extracellular matrix is mainly composed of chondroitin sulfate proteoglycans (CSPGs). From 2 days to 2 weeks after injury, astrocytes proliferate at the injured area, and their large cell bodies and protrusions closely link together to form glial scars; this separates the nerve tissue

from the inflammatory cells and reduces the early stage of the neuroinflammatory response. From 2 weeks to 6 months after injury, the astrocyte scar is considered mature. Due to the presence of a glial scar and other inhibitory factors, such as CSPGs and myelin-associated protein, axon regeneration is limited. At 6 months after injury, the scar is continuously reinforced as cysts and cavities are gradually formed[17,30]. The scar forms a physical and molecular barrier, limiting the spread of inflammation; however, this also hinders axon regeneration and outgrowth.

Dual aspects of astrocytes. Damage to the BSCB causes macrophage infiltration and microglial activation, which could trigger the activation of local astrocytes. Furthermore, the resulting lack of oxygen and glucose and the increase in albumin would stimulate astrocyte accumulation in the center of the injury site. This would simultaneously alter the protein expression pattern of the astrocytes. Pekny et al. reviewed that glial fibrillary acidic protein (GFAP), a kind of III intermediate filamentous protein, was highly expressed in the reactive astrocytes, and vimentin, nestin, S100β were also upregulated, which would lead to cellular hypertrophy[31]. In addition, these astrocytes express inhibitory proteins that contribute to the formation of the glial scar[31]. The most important of these proteins, chondroitin sulfate proteoglycans (CSPGs)[32], hinder axon outgrowth. Researchers at the Department of Cell Biology and Program in Neuroscience at Harvard Medical School, USA have reported that the tyrosine phosphatase receptor σ (PTPσ) is distributed on the surface of neurons. PTPσ is a CSPG receptor, and the PTPσ–CSPG interaction prevents axonal growth cone movement, thus inhibiting axons from passing through the glial scar[33]. This suggests that PTPσ functions in the spinal cord injury microenvironment as a 'molecular switch' to directly define the regenerative capacity of the axon. Our lab demonstrated that axons could bypass CSPG by inhibiting PTPσ[34]. The inhibitory proteins secreted by astrocytes combine with other cells to form a physical and molecular wall to prevent the expansion of the injury site into the intact area, and to inhibit the regenerative axons from passing through this barrier. Our team successfully established an isolation, culture and purification protocol for spinal cord-derived astrocytes in vitro, used small interfering RNA against PTPσ[35] and conducted photodynamic therapy using upconverting nanoparticles to inhibit astrocytes[36]. These studies suggested that the inhibition of activated astrocytes at the subacute phase could be used as an effective repair strategy to rebalance the microenvironment in SCI patients.

Aside from the detrimental function, astrocytes play a critical role in the restriction of inflammation and the lesion area and contribute to endogenous neuroprotection. Sabelström et al. generated the FoxJ1-CreER mouse strain and demonstrated that astrocytes derived from endogenous stem cells are necessary to reinforce the scar and restrict the area of damaged tissue[37]. They further demonstrated that astrocytes derived from endogenous stem cells could express ciliary neurotrophic factor, hepatocyte growth factor, and insulin-like growth factor-1 (IGF-1)[37]. In addition, another study showed that astrocytes could also express brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), glial cell-derived neurotrophic factor (GDNF), basic fibroblast growth factor (FGF-2) and laminin and fibronectin[38]. In contrast, Anderson et al. reported that astrocytes promote axon regeneration, while fibroblasts inhibit axon passage through the glial scar[39]. Thus, the astrocytes from the endo-NSCs play a protective role in axon regeneration.

Altogether, the imbalance of dual aspects of astrocytes can be regulated by inhibiting the overactivation of astrocytes and maintaining the protective aspects to repair SCI (Fig. 1 ②).

Demyelination and re-myelination. In the central nervous system, each oligodendrocyte is responsible for generating and maintaining myelin segments of 30–80 distinct axons[40,41]. Myelin is essential to maintain the integrity of axons and could facilitate axon signal conduction. After SCI, direct damage and the imbalance of local microenvironment factors leads to demyelination (Fig. 1 ③). However, the mechanisms of demyelination are unclear. The necrosis and apoptosis of oligodendrocytes are potentially the leading causes of axonal demyelination. The level of oligodendrocyte apoptosis at the epicenter of a lesion peaks within a week of contusion injuries to the spinal cord[42]. This results in demyelination of the most injured axon; however, uninjured axons around the lesion remain myelinated[43]. Apoptosis of oligodendrocytes after SCI lasts for approximately 3 months, and then injured axons appear to become remyelinated. The continued loss of oligodendrocytes in the chronic phase of SCI is a major impediment to functional recovery[44]. Mechanical injury, ischemia, proinflammatory cytokines, oxidative stress, glutamate- and ATP-mediated excitotoxicity and autophagy[45,46] can all potentially cause the death of oligodendrocytes due to the resulting imbalance of demyelination and re-myelination. Molecules involved in demyelination are potent inhibitors of axon regeneration, such as neurite outgrowth inhibitor A (Nogo-A), oligodendrocyte-myelin glycoprotein (OMgp) and myelin-associated glycoprotein (MAG)[47], which cause growth cone collapse, neurite retraction and increases the risk of apoptosis. Thus, the process of demyelination inhibits the regeneration of axons.

Re-myelination naturally occurs after SCI. The process of re-myelination is mainly the process of replacement of oligodendrocytes[45]. The new oligodendrocytes have two sources: progenitor oligodendrocytes and endogenous neural stem cells. Progenitor oligodendrocytes become activated and convert to an immature state; following increased proliferation, these oligodendrocytes differentiate into myelinating oligodendrocytes, thus re-myelinating the spared and regenerated axons[45]. Endo-NSCs remain quiescent in the normal spinal cord, and become activated upon spinal cord damage; these cells primarily differentiate into astrocytes but

also differentiate into oligodendrocytes to a lesser degree. This suppression of differentiation into oligodendrocytes is mainly due to the lack of growth factors that shift the balance to favor differentiation into oligodendrocytes. Epidermal growth factor (EGF), bFGF, and platelet-derived growth factor-AA (PDGF-AA) are important for oligodendrocyte differentiation, and Neuregulin-1 could promote oligodendrocyte progenitor cell (OPC) differentiation into mature myelinating oligodendrocytes. However, re-myelination-inhibiting factors are also present in the microenvironment[44]. Oligodendrocyte apoptosis causes the disruption of myelin, and the prolonged presence of myelin debris inhibits re-myelination. Wnt[48] and LINGO1 signaling also inhibits re-myelination. As a result, the extent and quality of re-myelination are limited. Although spared and regenerated axons are myelinated, the conductive function of the axons does not change[49]. Thus, many studies demonstrated SCI repair and recovered spinal cord function through the restoration of the myelin sheath[50–52]. Our team transplanted autologous activated Schwann cells into the spinal cord, and we found relatively complete restoration of the myelin sheath and improved microenvironment balance[53], and another study demonstrated similar results when co-transplanting human umbilical cord mesenchymal stem cells and human Schwann cells[54]. In addition, our clinical experiment showed functional recovery of the spinal cord following autologous activated Schwann cell transplantation[55].

Cellular Imbalance

Differentiation Balance of Endogenous Neural Stem Cells

Traditionally, stem cells were thought to be absent from the mature central nervous system, especially from the spinal cord. In the mature spinal cord, OPCs and astrocytes are the main dividing cells[29]; SCI results in increased proliferation of OPCs and activation of astrocytes[56]. However, OPCs and activated astrocytes are not stem cells, as both lack pluripotency. Recent studies have revealed that in the central canal of the normal spinal cord, ependymal cells remain quiescent but have the ability to differentiate into astrocytes and oligodendrocytes. Johansson et al. reported that cells derived from ependymal cells could migrate to the olfactory bulb and differentiate into neurons, as well as migrate to the injured spinal cord and differentiate into astrocytes. Thus, the use of endo-NSCs of the spinal cord for the treatment of SCI attracted public attention[57]. Barnabe-Heider et al. further demonstrated that new glial cells were derived from ependymal cells using the construction of genetic fate mapping[29]. SCI caused a strong, persistent, long-distance proliferation of ependymal cells[58], which peaked after 3–7 days, and there were 2 million new cells produced within 1 month at the injured site[59]. Ependymal cells rapidly divide, produce large amounts of astrocytes, and contribute to scar formation and the small amounts of oligodendrocytes. However, the activation of the ependymal stem cells is not sufficient to promote functional recovery due to the lack of neuronal differentiation.

As the imbalance in endo-NSC differentiation leads to the overall cellular imbalance in SCI, the rebalance of the cellular microenvironment of the injured spinal cord would improve SCI recovery (Fig. 1 ④).

The differentiation of neurons. The loss of neurons is the main reason for the limited recovery after SCI. The ratio of endo-NSCs to neurons directly impacts SCI recovery. The differentiation of endogenous stem cells can be impacted by inhibiting factors that are present in the microenvironment after SCI, which promote endogenous stem cell differentiation into more astrocytes. Thus, the population of neurons derived from endogenous stem cells is inadequate to reconstruct the synapses and nerve circle. Cell reprogramming technology is currently the principal strategy used to promote neuron differentiation via enhancing growth factors and decreasing inhibitors of the imbalance microenvironment. Recent studies have used reprogramming technology to convert endogenous glial cells into functional neurons within the brain and spinal cord[60,61]. The differentiation of endogenous neural progenitor cells into motor neurons is insufficient because the ratio of Ngn2/Olig2 for neural progenitor cells is 10 times lower than that for embryonic stem cells (ESCs)[62]. The ratio of Ngn2/Olig2 determines the differentiation of motor neurons and oligodendrocytes[63]. In the brain, the single transcription factor SOX2 was sufficient to reprogram the local astrocytes to neuroblasts, and these cells could further differentiate into functional neurons when combined with BDNF[64]. To decrease the impact of the inhibitors in the microenvironment, Fan et al. used a modified scaffold with a collagen-binding epidermal growth factor receptor (EGFR) antibody Fab fragment to neutralize myelin inhibitory molecules and repair SCI; they found that enhanced neurogenesis of endo-NSCs and neurons could reconnect the injured gap[65]. After transplantation into the injured spinal cord, an NT-3 chitosan biomaterial, which slowly releases NT-3, improved the local NT-3 concentration and attracted endo-NSCs to migrate towards the lesion epicenter and differentiate into neurons[66]. And there was study demonstrated that melatonin combined with exercise could also promoted endogenous stem cell differentiation into neurons after SCI[67]. Furthermore, our team used small molecules, valproic acid (VPA), combined with all-trans retinoic acid to promote neural stem cell differentiation into neurons in vitro. These results showed the promotion of neuron differentiation and the suppression of astrocyte differentiation[68]. For the imbalance of endo-NSC differentiation, the selective use of small molecules can effectively achieve its differentiation rebalance.

The differentiation of oligodendrocytes. Contusion or crushing injury of the spinal cord results in the loss of oligodendrocytes. After SCI, oligodendrocyte apoptosis at the center of

the injury peaks within a week[42], which leads to the demyelination of the most injured axon. Thus, the imbalance of differentiation of endo-NSCs can be caused by promoting oligodendrocyte differentiation to improve the remyelination. Currently, there are limited studies focused on the differentiation of oligodendrocytes from endo-NSCs. The Nrg-ErbB network is essential for oligogenesis. Gauthier et al. demonstrated that Nrg-1 could enhance the differentiation of neural progenitor cells into oligodendrocytes in vitro. Administering rhNrg-1b1 *in vivo* increased the number of new oligodendrocytes and promoted the preservation of axons, whereas inhibiting its receptor, ErbB, had the opposite effect[69]. Insufficient oligodendrocyte differentiation was associated with the lack of neurotrophic factors in the microenvironment following SCI. A previous study transplanted human umbilical cord blood-derived mesenchymal stem cells into injured spinal cords and showed that cell transplantation enhanced the proliferation of endogenous neural stem cells and increased new oligodendrocytes[70]. This study suggested that the neuroprotective trophic factors secreted from graft cells contributed to the differentiation of oligodendrocytes. In addition, Karimi-Abdolrezaee et al. utilized chondroitinase and growth factors (EGF, bFGF and PDGF-AA) to repair SCI and demonstrated that this strategy promoted endogenous oligodendrocyte replacement and improved the microenvironment[71]. In addition, electroacupuncture was shown to promote the proliferation of endo-NSCs and oligodendrocytes[72].

Transformation of the phenotypes of microglia and macrophages. Microglia are the resident macrophages of the central nervous system, and with regard to their cytokine production and immune function, they remain quiescent to a certain extent[73]. After SCI, the damaged neurons, astrocytes and other injured cells release cytokines and other factors such as interleukin (IL)-1β, tumor necrosis factor alpha (TNFα)[74,75], signals of damage associated molecular patterns (DAMPs)[76], interferon gamma (IFN-γ)[77], ATP[78,79], nitric oxide (NO)[80], and growth factors[81]. The release of these cytokines induces the activation of microglia and, consequently, increases the proliferation of microglial cells. The number of activated microglia becomes elevated on the first day after SCI, and continues to increase within 7 days, until the cell population plateaus between 2–4 weeks[82]. In the central nervous system, activated microglia release trophic factors for the survival and proliferation of infiltrating cells as well as the growth and regeneration of axons in the lesion site during earlier stages of SCI[83–85]; moreover, microglial activation serves a protective role by limiting the expansion of the lesion site[86]. However, activated microglia can also express various proinflammatory cytokines, such as IL-1α, IL-1β and TNFα[75]. At 2–3 days after injury, microglia can induce macrophages from the peripheral circulation to infiltrate the injured site and trigger the inflammatory response through these cytokines. Macrophages can reach maximum numbers 7–10 days after SCI[87] and persist in the lesion area for up to 42 days[88,89]. Macrophages, which are crucial for the inflammatory response in the spinal cord[90,91], can be derived from two cell types: the resident microglial cells and the peripherally circulating macrophages. The latter originate from the bone marrow and infiltrate the injured site after SCI. However, the appropriate activation of macrophages can also aid in the repair and regeneration of the injured central nervous system[87].

Macrophages and microglia both have the ability to become polarized[92–94]. There are two main polarization phenotypes, M1 and M2 (Fig. 1 ⑤); additionally, the M2 phenotype can be divided into M2a, M2b and M2c. The ratio of M1 to M2 determines the homeostasis of the local microenvironment. During the acute response to trauma, high levels of reactive oxygen species (ROS) are detectable. With the stimulation of these factors, the M1 macrophage/microglia in SCI occupy a predominant state, which is detrimental to the repair of SCI[95]. This ratio results in the production of proinflammatory cytokines, such as IL-6, IFN-γ, IL-12, IL-23, IL-1β, and TNFα[96]. M1 macrophages are converted to the M2 phenotype with the phagocytosis of myelin phenotype. M2 macrophages are anti-inflammatory cells that exhibit tissue repair properties (i.e. high production of IL-10 and transforming growth factor beta (TGFβ)), exhibit defective nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) activation, upregulate arginase 1 and downregulate the expression of proinflammatory cytokines[94]. Although apoptotic neutrophils and red blood cells (RBCs) can transform the phenotype of macrophages from M1 to M2, the number of M2 macrophages was low at the early stage of SCI and further decreased after 7 days[93,97]. The microenvironment post-SCI is unfavorable for M2 macrophages, such that the high expression of TNF would inhibit the transformation of M1 to M2[91].

Molecular Imbalance

Imbalance of Neurotrophic Factors and Their Pro-Peptides

There is an imbalance between growth promoting molecules and growth inhibiting molecules among the SCI microenvironment, where growth inhibitors occupy the dominant position (Fig. 1⑥). This results in the death of neurons and oligodendrocytes as well as the degeneration of axons. Among the growth promoting molecules, neurotrophic factors play a critical role in the development, maintenance and survival of cells in the central and peripheral nervous system[98,99]. Neurotrophic factors significantly promote the survival and proliferation of different cells and axon regeneration after SCI[100,101]. The neurotrophic factor family consists of BDNF, NGF, and neurotrophin-3 (NT-3), neurotrophin-4/5 (NT-4/5). Recently, several studies have showed that the pro-neurotrophins of NGF, BDNF and NT-3 are also present and play a vital role in the cell death[102]. NGF, BDNF and NT-3 are synthesized as uncleaved pro-peptides

(proNGF, proBDNF and proNT-3), which are either secreted from cells or cleaved intracellularly into mature neurotrophic factors[103,104]. The balance of proneurotrophins and neurotrophins is disrupted after SCI, and the resulting elevated expression of proneurotrophins accelerates apoptosis, reduces synaptic plasticity, increases the inflammatory response and induces degeneration[105,106].

BDNF versus proBDNF. BDNF is mostly involved in the repair of SCI. BDNF was first extracted from the brain by Barde et al. in 1982[107]. BDNF can combine with the receptor of tropomyosin receptor kinase (Trk)B and promote the outgrowth of axons and survival of dorsal root ganglion neurons,[108,109] as well as promote the regeneration of axons of corticospinal tract. In addition, BDNF can promote myelination, regulate synaptic plasticity and affect synaptic transmission[110]. Several studies have used BDNF to repair SCI by direct administration, transplantation of cells overexpressing BDNF and release by scaffold[111]; these studies demonstrated a neuroprotective role of BDNF. Our team used BDNF, NGF genetically modified Schwann cells and fetal spinal cord cell suspension to repair SCI in rats. This combination treatment elicited a robust growth response of corticospinal axons and significant functional recovery[112]. Following SCI, Wong et al. found that proBDNF levels were upregulated in the spinal cord 1 to 3 days after injury but downregulated after 7 days[105]. Moreover, the inhibition of proBDNF promoted an increase in the number of neurons and improved the functional recovery of the animals. Our team used proBDNF-specific antibodies to antagonize the inhibitory effect of proBDNF. This resulted in increased proliferation of OPCs and cell division activity and promoted the function of the animal[113]. These results suggested that proBDNF in the spinal cord microenvironment suppressed the survival of neurons and that, through the use of proBDNF-specific antibodies, microenvironment rebalance can be achieved.

NGF versus proNGF. NGF has historically been thought to function only in the peripheral nervous system. However, recent studies have demonstrated a similar role for NGF in the central nervous system. NGF binds to the Trk receptors and pan-neurotrophin receptors ($p75^{NTR}$) to maintain and promote the survival of neural cells, which was reviewed by Richner et al.[114]. Several studies have demonstrated that NGF promotes the regeneration of axons and improves the functional recovery. Romero et al. used conditional expression of NGF in the adult rat spinal cord and found that the expression of NGF could promote the axonal sprouting of the sensory afferents and achieve better behavioral outcomes[115]. In addition, our team prepared genetically modified Schwann cells overexpressing NGF to repair SCI in rats, which improved hind limb movement[116]. The precursor of NGF could interact with Sortilin and p75 to form a complex to lead to an apoptotic cascade[117,118]. Thus, the balance between NGF and proNGF could determine the balance of cell survival and death. Harrington et al. demonstrated that proNGF was increased in the brain injuries and SCIs[102]. ProNGF is the predominant form of NGF expressed in nearly all brain tissue in mice, rat and human. Beattie et al. reported that the expression of NGF and proNGF were both upregulated in contusion SCIs and that the expression of proNGF was equivalent to or higher than that of NGF. They also demonstrated that proNGF induced the $p75^{NTR}$-mediated decrease in the number of oligodendrocytes[106]. In addition, proNGF was detected in the GFAP-positive cells in the brain. Domeniconi et al. also demonstrated that astrocytes from neonatal spinal cord could express proNGF with stimulation, which resulted in neuron death when cultured in vitro, suggesting that astrocytes are potentially the major source of proNGF[119].

NT-3 versus proNT-3. The neurotrophin NT-3, plays an important role in the development of the nervous system. The mRNA of NT-3 is mainly expressed in the developing brain and motor neurons of the spinal cord, whereas the expression of NT-3 is low in the adult spinal cord. After SCI, the expression of NT-3 dropped rapidly in the first 6 hours and recovered to normal levels by 12 hours[120]. Thus, follow-up studies utilized NT-3 to repair SCI and obtained functional recovery[116,121]. The pro-peptide of NT-3 is proneurotrophin-3 (proNT-3). Tauris et al. demonstrated that proNT-3 induces the neuron death in the inner ear using Sortilin[122]. Furthermore, this study demonstrated that recombinant proNT-3 could induce sympathetic neuron death through a p75NTR- and a Sortilin-dependent mechanism. However, the role of proNT-3 in the process of SCI is unknown. Thus, much remains to be understood about the balance of NT-3 and proNT-3 in the SCI microenvironment.

Imbalance of the Cytokines and Chemokines

Cytokines. Cytokines can be divided into proinflammatory or anti-inflammatory proteins that participate in neuroinflammation, neurodegeneration, neuropathic pain[123,124]. After SCI, neurons in the spinal cord express these cytokines within 30 min, and microglia express these cytokines 5 hours later; however, the expression of both decreases by the second day[125]. In addition, TNFα and IL-6 can be secreted by other cells in the central nervous system (CNS), such as astrocytes and epidermal cells[126]. Several cytokines, such as IL-1, IL-6, TNFα, granulocyte-macrophage colony-stimulating factor (GM-CSF) and leukocyte inhibitory factor (LIF), participate in the dynamic changes of the SCI microenvironment[26,127]. Some proinflammatory cytokines have protective qualities at low concentrations due to their induction of neurotrophin expression as well as the induction of adhesion molecules in the cell surface, which mediates leukocyte activation/recruitment to the injury site[128]. Proinflammatory cytokines also activate endogenous stem cells. However, the main function of these cytokines, as proinflammatory molecules, leads to neuronal damage and

destruction when their concentration exceeds a certain threshold. At higher concentrations, these proinflammatory cytokines activate transcription factors (ATF) as well as factors that stimulate the expression of neurotoxic genes, including cyclooxygenase 2 (COX-2), inducible nitric oxide synthase (iNOS), and proinflammatory proteases such as thrombin in different target cells[129,130]. Accumulation of IL-1 in the spinal cord leads to enhanced vascular permeability and lymphocyte recruitment. Moreover, the release of IL-6 has been found to promote the activation and infiltration of macrophages and microglia. Several studies have revealed that the continuous inhibition of IL-6 is detrimental to functional recovery because it also participates in axonal regeneration and gliosis[131,132]. TNFα is significantly upregulated in neurons, glia, and endothelial cells following SCI[133]. In addition, TNFα could recruit neutrophils to the site of the lesion by the induction of adhesion molecules such as intercellular adhesion molecule (ICAM-1) and vascular cell adhesion protein (VCAM-1)[134]. With the increased level of TNFα, the permeability of endothelial cells is altered, which further resulted in the disabling of the blood–spinal cord barrier. TNFα could induce cell death in oligodendrocytes[135] and lead to demyelination; and the suppression of TNFα resulted in decreased demyelination[136]. Neutralizing antibodies against TNFα improved functional neurological recovery following SCI[137]. However, TNFα signaling has also been demonstrated to have a neuroprotective role in vitro[138] and promote functional recovery following SCI[139].

Chemokines. There are complex changes in the levels of a variety of important chemokines at different times and sites after SCI, among which stromal cell-derived factor 1α (SDF-1α) binds to G-protein-coupled C-X-C chemokine receptor type 4 (CXCR4) and plays an important role in the repair of SCI. Kucia et al. reviewed that SDF-1-CXCR4 axis could regulate stem/progenitor cell trafficking and the metastatic behavior of tumor cells[140]. Our team used immunohistochemistry to observe the changes in CXCR4 expression in spinal cord tissue[141]. It was found that the expression of CXCR4 in neurons, glial cells, macrophages and ependymal cells in spinal gray matter was increased on the third day after SCI in rats. The number of CXCR4-positive cells peaked in the gray matter of spinal cord. In addition, a previous study showed that the CXCR4-mediated stem cell migration to the injured area to repair the injured spinal cord tissue[142]. In addition, the expression of SDF-1α in the proximal and distal SCI centers was significantly increased[143]. The number of SDF-1α-positive cells in the spinal cord tissue began to rise at 1 day after SCI, reaching its peak at 2 days after injury. The number of proximal SDF-1α-positive cells at 7 days after injury was significantly higher than the numbers in normal and sham-operated groups. The results showed that proliferative astrocytes could release trophic factors to promote damaged axon repair and regeneration, high levels of expression of SDF-1α can strongly stimulate the proliferation of astrocytes and play a role in repairing the spinal cord. Thus, 48 h after acute SCI, continuous local intrathecal injection of SDF-1α to restore local SDF-1α concentrations to a high level may be a viable option for early treatment of SCI.

Ion Imbalance

In the pathological process of SCI, ion imbalance plays a fundamental role in regulating other pathological changes. The most important ions are K^+, Na^+, and Ca^{2+}. After SCI, the selectivity of the K^+, Na^+, and Ca^{2+} channels is altered due to damage to the membrane of cells and the release of proinflammatory factors by different cells. Subsequently, the cellular and extracellular homeostasis of K^+, Na^+, and Ca^{2+} is disrupted. Finally, the concentrations of Na^+ and Ca^{2+} are upregulated in cells, while the concentrations of K^+ and Mg^{2+} are upregulated extracellularly[144]. With the Na^+ influx into the cell, water gradually accumulates in the cell, which leads to cytotoxic cellular edema. This further leads to the stimulation of intracellular phospholipase activity and promotion of intracellular acidosis[145]. There were studies utilizing Na^+ channel inhibitors, such as tetrodotoxin[146,147], riluzole[148,149], and phenytoin[150,151], to repair SCI, and these study demonstrated that inhibition of the Na^+ channel has a neural protective effect. As Ca^{2+} participates in several pathological processes (e.g. synaptic transmission), Ca^{2+} plays a vital role in responding to injuries of the central nervous system. After SCI, the concentration of Ca^{2+} was increased within 1 min after SCI and reached its peak at 8 hours; moreover, this high concentration of Ca^{2+} persisted for 2 weeks. The high concentration of Ca^{2+} in cells could cause apoptosis or necrosis through increasing the activation of cellular enzymes, mitochondrial damage, acidosis, and the production of free radicals[145], and it could also further impact the white matter after SCI[152]. The K^+ channel is the most extensively studied ion channel in SCI myelination[153]. The myelin sheath of axons in spinal cord is disrupted after SCI, which exposes K^+ channels and disrupts K^+ channel distribution[45]. This could result in a number of detrimental effects, including conduction failure and demyelination. The conduction failure is ascribed to the increased activity of K1 channels. In contrast, the voltage-gated K+ channels are also important for re-myelination. There was a study that injected 4-aminopyridine (4-AP), a K+ channel antagonist, subcutaneously into adult male C57BL/6 mice, and found that 4-AP could decrease re-myelination in the corpus callosum[154].

Recently, iron has been shown to play a vital role in the maintenance of the normal function of the CNS[155,156]. After SCI, the accumulation of iron in the extracellular space is caused by the influx of RBCs due to hemorrhage. Liu et al. demonstrated that the level of iron was increased at 0.5 h[157]. In addition, Liu et al. demonstrated that iron was rapidly increased within 20 min[155]. Iron plays a significant role in glutamate excitotoxicity, the formation of ROSs and the

production of free radicals[158,159], which inhibit the regeneration of SCI. Our team utilized deferoxamine (DFO), an iron chelator, in the repair of SCI. We found that the application of DFO could decrease the total iron ion level, TNFα, IL1-β and caspase-3 expression and glial scar formation after SCI and promote the survival of cells and recovery of motor function[24].

Conclusions

The theory of 'microenvironment imbalance after SCI' describes the imbalance of molecules, cells and tissues in the spinal cord following injury. This theory explains the complicated intercorrelation of each level, which will provide guidance for the understanding of pathological process and treatment of SCI.

Authors' Note

Baoyou Fan and Zhijian Wei contributed equally to this work.

Declaration of Conflicting Interests

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