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·Review·

# Scar-modulating treatments for central nervous system injury

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Traumatic injury to the adult mammalian central nervous system (CNS) leads to complex cellular responses. Among them, the scar tissue formed is generally recognized as a major obstacle to CNS repair, both by the production of inhibitory molecules and by the physical impedance of axon regrowth. Therefore, scar-modulating treatments have become a leading therapeutic intervention for CNS injury. To date, a variety of biological and pharmaceutical treatments, targeting scar modulation, have been tested in animal models of CNS injury, and a few are likely to enter clinical trials. In this review, we summarize current knowledge of the scar-modulating treatments according to their specific aims: (1) inhibition of glial and fibrotic scar formation, and (2) blockade of the production of scar-associated inhibitory molecules. The removal of existing scar tissue is also discussed as a treatment of choice. It is believed that only a combinatorial strategy is likely to help eliminate the detrimental effects of scar tissue on CNS repair.

**Keywords:** CNS injury; glial scar; fibrotic scar; inhibitory molecules; scar modulation

## Introduction

The treatment of central nervous system (CNS) injury is always a major challenge worldwide. Traumatic injury to the adult CNS (either brain or spinal cord) may cause life-long disability, impact quality of life, and become a great burden for the family and society. It has long been held that the adult mammalian CNS has little ability to spontaneously regenerate beyond the lesion site, and the formation of scar tissue acts as one of the largest barriers to CNS regeneration. The scar tissue forming at the lesion site after CNS injury consists of predominantly two types, glial and fibrotic[1].

The molecular changes within glial and fibrotic scars are closely linked to the process of CNS regeneration. Following traumatic CNS injury, bleeding occurs and the blood-brain barrier (BBB) is broken. The infiltration of blood proteins triggers the inflammatory reaction. Hematogenous cells (including leukocytes, macrophages, and lymphocytes) invade the surrounding neural tissue and secrete various cytokines and chemokines, which induce inflammatory reactions in the injured CNS and result in local neural degeneration and cystic cavity formation. Under the action of inflammatory factors, astrocytes and other glial cells are activated to form a glial scar around the lesion site. Several days post-injury, fibroblasts intrude into the lesion site from adjacent meninges and perivascular spaces, proliferate, and secrete extracellular matrix (ECM) molecules, such as type IV collagen, fibronectin, and laminin, to form a fibrotic scar. Actually, a rapid post-injury response of astrocytes to CNS injury mainly aims to protect the integrity of the BBB. Subsequently, there is a fibroblastic reaction around astrocytes, and astrocytes and fibroblasts interact extensively in an organized manner to form the glial scar around a CNS lesion, as demonstrated in rat models of spinal cord injury (SCI)[2]. A more detailed description of the progression of glial and fibrotic scar formation after CNS injury can be found in a previous review[3], in which a schematic is provided to depict the time course of lesion scar formation in the mouse brain.

Although the scar functions to restore the BBB, prevent neuronal degeneration, and limit the spread of cellular damage[4, 5], it is generally recognized as a major

obstacle for CNS repair both by the production of inhibitory molecules and by the physical impedance of axon regrowth. Accordingly, it is of utmost importance to develop scar-modulating treatments for CNS injury. Nowadays, these treatments are mainly aimed at inhibition of scar formation and blockade of the production of scar-associated inhibitory molecules. In addition, the removal of existing scar tissue is a remedy of choice. This review covers both established strategies and emerging therapeutic perspectives for the scar-modulating treatment of CNS injury.

## Inhibition of Glial Scar Formation

Reactive astrocytes are the major cellular component of glial scars formed after CNS injury. A variety of therapeutic strategies targeting reactive astrocytes have been developed (Table 1).

### *Suppression of Astrocyte Activation*

Following a CNS lesion, quiescent astrocytes are activated. Hypertrophic reactive astrocytes undergo proliferation into the major component of the glial scar, and meanwhile secrete many chemicals to inhibit axon regeneration. To control the excessive reactivity of astrocytes and thus to suppress glial scarring, many strategies have been developed. Early in 1997, it was reported that transfection of cultured astrocytes with antisense glial fibrillary acidic protein (GFAP) mRNA successfully block astroglial morphological changes and inhibit astrocyte proliferation, while neurons persist in the vicinity of the lesion site with neurite outgrowth[6]. After hemisection of the spinal cord, GFAP- and vimentin-knockout mice present with reduced astroglial reactivity associated with increased plastic sprouting of supraspinal axons[7]. As another example, introduction of GFAP and vimentin siRNAs suppresses the over-proliferation of reactive astrocytes and improves acute urinary dysfunction in an SCI rat model[8]. More approaches to suppressing the astrocyte activation are described below.

**Calpain inhibitors** The upregulation of calpain, a water-soluble $Ca^{2+}$-activated cysteine protease, has been implicated in apoptosis and tissue degeneration in SCI animals. Research shows that an increase in GFAP is accompanied by upregulation of calpain after SCI[7]. Administration of E-64-d, a calpain-specific inhibitor, decreases reactive astrogliosis and significantly reduces glial scar formation in SCI rats[9]. Another study showed that intrathecal administration of another calpain-specific inhibitor, MDL28170, improves neurological function after SCI, attenuates the ratio of pro-apoptotic Bax/anti-apoptotic Bcl-2 mRNA expression in the lesion site, and reduces the glial scar by quenching astrocyte activation[10].

**Cyclin-dependent kinase inhibitors** Cell proliferation is accomplished through the cell cycle. Cyclins and cyclin-dependent kinases (CDKs) play important roles in cell cycle regulation. To minimize the contribution of astrocyte over-proliferation to glial scar formation, inhibition of CDK activity has proven to be efficient. So far, several CDK inhibitors have successfully quenched the over-proliferation of astrocytes, including p27 (kip1)[11], flavopiridol[12, 13], olomoucine[12, 14], and CR8[15].

**High-molecular-weight hyaluronan (HA)** HA is a long-chain polysaccharide widely distributed in the mammalian body, and as the chief component of ECM, it participates in the regulation of many cellular functions. Under normal conditions, HA exists in the high-molecular-weight (MW) form (~$2.0\times10^6$ Da); after injury, it degrades to the low-MW form[16]. The two forms have significantly different biological activities. A high-MW HA-containing gel inhibits astrocyte responses[17], while the low-MW HA elicits cell proliferation and inflammatory responses[18, 19], and stimulates angiogenesis *via* an increased proliferation of endothelial cells[19-21]. High-MW HA added to astrocyte culture reduces the proliferation of astrocytes[16]. Based on this finding, implantation of pre-formed gels made from high-MW HA into transected rat spinal cord attenuates the astrocytic response and decreases glial scar formation[22].

**Interferon-β (IFN-β)** SCI mice receiving IFN-β exhibit a decrease in glial scar formation and functional improvement[23]. IFN-β inhibits the proliferation of reactive astrocytes mainly *via* activation of toll-like receptor (TLR)-4[23], suggesting that stimulation of TLR-4 signaling might be an effective strategy for treating CNS injury.

**Inhibitors of the TGF-β/Smad signaling pathway** The BBB is disrupted immediately by CNS injury, and the leakage of the blood protein fibrinogen induces reactive astrocytosis *via* an interaction between fibrinogen-carried TGF-β and astrocytes, and concurrently promotes deposits of chondroitin sulfate proteoglycans (CSPGs) that block axonal reconnection. This process is believed to be mediated by TGF-β/Smad signaling pathways[24]. Therefore, some researchers have attempted to inhibit this

**Table 1. Strategies for inhibiting glial scar formation**

| Strategy and/or agent | Example | Injury model | Effect |
|---|---|---|---|
| Suppression of astrocyte activation | | | |
| Calpain inhibitor | E-64-d[9] | SCI rats | Reduced astrogliosis |
| | MDL28170[10] | Spinal cord hemisection in rats | Quenched microglia and astrocyte activation |
| CDK inhibitor | P27 (kip1)[11] | Scratch wound model and cortical stab wound in mice | Inhibited astrocyte proliferation |
| | Flavopiridol and olomoucine[12-14] | Brain traumatic injury in rats | Attenuated proliferation/ activation of astrocyte and microglia |
| | CR8[15] | Rat SCI contusion | Reduced astrogliosis and chronic inflammation |
| High-MW HA | High-MW HA[16] | SCI rats and primary rat astrocyte culture | Astrocytes in a state of quiescence |
| | High-MW HA-containing gel[17] | A rat model of spinal dorsal hemisection injury | Reduced glial scar formation |
| INF-β | INF-β and cytosine deaminase[23] | SCI mice | Reduced astrocyte proliferation |
| TGF-β/smad inhibitor | Decorin[25] | SCI rats | Decreased glial scar formation |
| | Anti-TGF-β1 antibody [26] | Thoracic spinal cord contusion in rats | Decreased the number of reactive astrocytes |
| | HGF-MSCs [27] | Spinal cord hemisection in rats | Reduced astrocyte activation |
| | Taxol[28] | SCI rodents | Diminished astrocyte activation |
| JAK2/STAT3 inhibitor | Triptolide[30] | SCI rats | Attenuated glial scaring and inflammation |
| | Nes-Stat3–/– or Nes-Socs3–/–[31] | SCI mice | Rapid migration of reactive astrocytes |
| EGF inhibitor | PD168391 and AG1478[34] | SCI rats | Functional and structural outcome |
| Endothelin receptor antagonist | BQ788[37] | Brain stab wound in rats | Attenuated astrocyte activation |
| | Bosentan and BQ788[38] | Cultured astrocytes in vitro | Prevented astrocyte proliferation |
| Elimination of excessive reactive astrocytes | L-α-aminoadipate[39] | Normal rats | Disrupted astrocytic network |
| | Transgenic expression of HSV-TK[40] | Forebrain stab injury in mice | Inhibited reactive astrocytes |
| Induction of linear alignment | GRP-derived astrocytes[41] | SCI rats | Linear alignment of host astrocytes |
| Regulation of NF-κB signaling | Proinflammatory factors, CXCL10 and CCL2[44] | SCI mice | Reduced glial scar formation |
| | Transgenic inhibition of NF-κB[45] | SCI mice | Reduced astrogliosis and functional improvement |

signaling pathway in order to suppress glial scar formation. Injection of decorin, a small, leucine-rich, TGF-β-binding proteoglycan, into SCI rats reduces glial scar formation, inhibits CSPG synthesis, and alleviates inflammation[25].

Another similar study demonstrated that intrathecal administration of an anti-TGF-β1 antibody into SCI rats decreases the number of reactive astrocytes, inhibits glial scar formation, and enhances locomotor recovery[26].

Hepatocyte growth factor (HGF), originally identified only as a mitogen for hepatocytes, has now been suggested to participate in nerve injury *via* regulation of TGF-β. Transplantation of HGF-overexpressing mesenchymal stem cells (HGF-MSCs) into the hemisected spinal cord of rats diminishes the level of TGF-β isoform, reduces the extent of astrocytic activation, increases axonal growth beyond the glial scar, and improves the recovery of forepaw function[27].

A recent study demonstrated that treatment with taxol diminishes astrocyte activation as well as glial scar formation. Taxol also improves axonal regeneration and functional recovery in animal models. The underlying mechanism may be that taxol causes Smad2/3 to localize persistently to microtubules and inhibits 70% of its translocation to the nucleus, thus abolishing TGF-β signaling[28].

**Blockade of the JAK2/STAT3 signaling pathway** Signal transducer and activator of transcription 3 (STAT3) is a member of the JAK-STAT family that transduces signals for many cytokines and growth factors. The JAK2/STAT3 pathway is one of the triggers for reactive astrogliosis. STAT3 knockout mice exhibit attenuated upregulation of GFAP and marked suppression of astrocyte hypertrophy[29]. Triptolide, one of the major active ingredients of a traditional Chinese herb *Tripterygium wilfordii Hook F*, significantly inhibits inflammation and astrogliosis in SCI rats by blocking the JAK2/STAT3 pathway[30]. Okada *et al.* also analyzed the impact of conditional ablation of suppressor of cytokine signaling 3 (SOCS3, a target of STAT3 transcriptional activation) on reactive astrocytes after SCI. They found that knockout of SOCS3 in mice leads to the rapid migration of reactive astrocytes to seclude inflammatory cells, enhances the contraction of the lesion area, and thereby improves functional recovery[31].

**Epidermal growth factor inhibitors** Activation of epidermal growth factor receptor (EGFR) triggers quiescent astrocytes into reactive astrocytes[32], and stimulates the secretion of CSPGs, thus contributing to the formation of glial scars[33]. Therefore, inhibition of EGFR may reduce the extent of astrocyte activation and abate the glial scarring. Erschbamer *et al.* showed that direct delivery of the potent EGFR inhibitors PD168391 and AG1478 to the injured area in SCI rats improves the functional and structural outcomes[34].

**Endothelin receptor antagonists and JNK/c-Jun and ERK/c-Jun signaling pathway inhibitors** Endothelin is a potent vasoconstrictor peptide that mainly acts on blood vessels. The endothelin level is, however, markedly upregulated after CNS injury, inducing reactive astrogliosis[35, 36]. Infusion of BQ788, a selective endothelin-B receptor antagonist, into the cerebral ventricle of rats with brain injury attenuates the activation and proliferation of astrocytes[37]. Recently, researchers provided *in vitro* evidence that endothelin-1 induces the activation and proliferation of astrocytes *via* the JNK/c-Jun signaling pathway[38]. The two endothelin receptor antagonists Bosentan and BQ788 prevent astrocyte proliferation and JNK phosphorylation, while c-jun siRNA prevents the endothelin-1-induced proliferation of astrocytes, confirming the involvement of the ERK-dependent pathway in the regulation of reactive astrogliosis. They proposed that after CNS injury, the increased level of endothelin activates the JNK- and ERK-dependent pathways *via* binding to endothelin receptors on astrocytes, and both pathways activate c-Jun and trigger astrocyte proliferation[38]. These findings indicate that molecular targeting of both the JNK/c-Jun and ERK/c-Jun pathways could be an approach to reducing reactive astrogliosis after brain injury[38].

### *Elimination of Reactive Astrocytes*

Besides inhibiting astrocyte activation, researchers have been concerned about how to remove reactive astrocytes to prevent scar formation. Early in 1996, Khurgel *et al.* reported that a focal injection of L-α-amino adipate, an astroglial toxin, directly into intact amygdala of adult rats creates an astrocyte-free region for 2 days[39]. In another study, given that the transgenic expression of *Herpes simplex* virus thymidine kinase (HSV-TK) in reactive astrocytes renders them sensitive to ganciclovir, Bush *et al.* found that reactive astrocytes adjacent to a forebrain stab injury are selectively ablated by ganciclovir treatment in adult mice expressing HSV-TK from the *Gfap* promoter[40].

### *Induction of Linear Alignment of Astrocytes*

Glial-restricted precursors (GRPs) are precursors of oligodendrocytes and astrocytes, and GRP-derived

astrocytes (GDAs) are a new glial type generated by GRPs *via* MBP4. Transplantation of GDAs not only promotes axon regeneration and protects locomotor function, but also induces the linear alignment of host astrocytes. This provides an ideal tract for axon regeneration and growth, accompanied by reduced over-proliferation of astrocytes and reduced scar formation[41].

### *Regulation of Nuclear Factor κB in Astrocytes*

Nuclear factor κB (NF-κB) plays a key role in inflammation and secondary lesions after traumatic CNS injury[42, 43]. In SCI animal models, the expression of NF-κB-dependent genes is upregulated. Selective inactivation of NF-κB in astrocytes in contusive SCI mice reduces the expression of pro-inflammatory factors, such as CXCL10 and CCL2, suppresses GFAP expression and CSPG secretion, and reduces scar formation[44]. Similarly, spared and sprouting spinal tracts are increased, together with improved functional recovery after SCI in GFAP-inhibitor of κB dominant-negative mice, as transgenic inhibition of astroglial NF-κB-dependent cascades reduces the reactive astrogliosis[45]. Collectively, these findings suggest that treatments targeting NF-κB may have an indirect inhibitory effect on glial scar formation.

## Inhibition of Fibrotic Scar Formation

Several days after CNS injury, fibroblasts originating from meningeal and perivascular cells[46, 47] invade and proliferate at the lesion site to secrete ECM components, such as type IV collagen, fibronectin, and laminin. The contact between fibroblasts and astrocytes enables ECM to form a continuous basal membrane between the two types of cells, and the membrane ultimately creates the fibrotic scar. In different types of CNS injury, most SCI cases are characterized as contusions that leave the meninges intact. The latest research indicates that the fibrotic scar formed after contusive or penetrating SCI is predominantly composed of collagen1α1 cells that migrate to the injury site from a perivascular source *via* a dynamic temporospatial process[47].

Local injection of α,α'-dipyridyl, both an iron chelator and an inhibitor of collagen triple helix synthesis, significantly reduces collagen formation, and thus suppresses the formation of fibrotic scars[48-50]. The expression of TGF-β1 and its receptors (types I and II) increases during the migration of fibroblasts to the injury site, and the increased TGF-β1 directly activates meningeal fibroblasts[50]. Accordingly, fibrotic scar formation is promoted by exogenous TGF-β1 and prevented by anti-TGF-β1 antibody in injured rat brain[51, 52], and more recently, researchers have found that blocking TGF-β function with cyclic adenosine monophosphate in combination with a collagen synthesis inhibitor transiently reduces fibrotic scar formation and promotes axonal regeneration in the injured spinal cord[53].

Prolyl 4-hydroxylase (P4H), a key enzyme for catalyzing protocollagen proline hydroxylation in fibroblasts and other type IV collagen-producing cells, plays a critical role in the synthesis of type IV collagen[54]. Local injection of P4H inhibitors to the injury site suppresses fibrous scar formation after CNS injury[55]. On the other hand, since the functioning of P4H requires its co-factors such as $Fe^{2+}$ ion, ascorbate, and α-ketoglutarate, inhibition of these cofactors also suppresses the function of P4H. α,α'-dipyridyl is one of the most common cofactor inhibitors reported so far. A recent study[53] demonstrated that combined use of 2,2'-bipyridine-5, 5'-dicarboxylic acid, a potent iron chelator, and cyclic adenosine monophosphate[56], an inhibitor of fibroblast proliferation and collagen biosynthesis, significantly reduces fibrotic scar formation in SCI rats.

In some less-frequent cases of CNS injury involving disruption of the meninges, repair of the meninges has been suggested to reduce the number of fibroblasts that invade the injury site and suppress the scar formation. For instance, dural repair was used to reduce connective tissue scar invasion and cystic cavity formation after acute spinal cord laceration in adult rats[57]. Besides, in a study by Li *et al.*, topical glucocorticoids applied immediately after cerebral cortical stab wounds in adult rats modulate the lesion interface and attenuate the scarring process, suggesting that the inhibition of injury-induced inflammation might also suppress fibrotic scar formation[58].

In addition, cell proliferation is associated with the formation of either glial or fibrotic scars. Therefore, inhibition of the over-proliferation of either reactive astrocytes or fibroblasts after CNS injury may significantly reduce the scarring. Previous research reported that an optimal dose of X-irradiation eliminates connective tissue scars as well as glial scars in a rat model of SCI[59, 60]. More physical interventions targeting scar formation after SCI have been described in a previous review[61].

Cell transplantation is a widely-used strategy to treat CNS injury. Transplantation of different cell types, including neural stem cells, bone marrow stromal cells, and Schwann cells, has shown potential to promote axon regeneration because they can either differentiate into neural cells or secrete various neurotrophic factors. Recent studies have demonstrated that after CNS injury, cell transplantation inhibits fibrotic scar formation at the lesion cite. For example, combined transplantation of olfactory-ensheathing cells and olfactory nerve fibroblasts or single transplantation of meningeal fibroblasts into the lesion site of SCI rats is able to suppress fibrotic scar formation, the former performing better on axonal regeneration. The mechanism by which cell transplantation inhibits fibrotic scar formation remains to be elucidated. However, it seems likely that cell transplantation into the lesion site disturbs the linkage of fibroblasts and reactive astrocytes, thus preventing the formation of fibrotic scars[62].

## Blockade of the Production of Scar-Associated Inhibitory Molecules

The inhibitory molecules that go along with scar formation present chemical barriers to successful axon regeneration[63, 64]. These molecules have been a matter of concern in eliminating the scar-induced chemical suppression of axon regeneration. They are mainly derived from glial and fibrotic scars, and myelin debris (Fig. 1). Diverse interventions are required to tackle different inhibitory molecules (Table 2).

### *CSPGs*

Among the various inhibitory molecules, CSPGs from the glial scar[65, 68] are considered the major component[65, 69-71]. The CSPG family includes neurocan, brevican, phosphacan, aggrecan (produced by astrocytes), NG2, and versican (produced by oligodendrocyte precursors or meningeal cells)[68]. The protein core and one or more sulfated glycosaminoglycan (GAG) side-chains constitute an intact CSPG[72]. Chondroitinase ABC (ChABC), an enzyme that degrades GAG side-chains, when delivered to the site of axotomy in rat brain injury models promotes axon regeneration. These results confirm that CSPGs have inhibitory effects on axon regeneration *via* their GAG side-chains[73]. Since the mechanism of action of ChABC does not clash or overlap with many other treatment strategies, several studies have examined the feasibility of combining it with other treatments for SCI repair, such as myelin-inhibitory molecule blockers, cell implantation, growth factors, and ion channel expression[74]. The polymerization of GAG chains onto CSPG core proteins occurs through the action of an enzyme complex consisting of chondroitin synthase[75] and chondroitin polymerizing factor (ChPF)[76]. So RNA interference can be used to decrease the mRNA expression of ChPF and so reduce the inhibitory effect of CSPG on axon regeneration[77].

Besides the GAG side-chains, CSPG core proteins also inhibit axon regeneration[78, 79]. Accordingly, it is of great importance to degrade these proteins. Both plasmin and plasminogen lead to the degradation of various kinds of CSPGs with protein cores as the target of action[25, 80-82]. However, the degradation of core proteins is limited due to the existence of GAG chains. Therefore, the GAG must be removed in order to efficiently degrade the core proteins.

Tissue plasminogen activator (tPA) is markedly upregulated after SCI in C57BL/6 mice[83]. tPA is a serine protease that mediates the proteolytic conversion of plasminogen to plasmin. After administration of ChABC to tPA knockout mice and intact C57BL/6 mice, the latter exhibit pronounced downregulation of CSPGs and axon regeneration compared to the former. This result can be ascribed to the fact that ChABC cleaves GAG, exposing the core proteins, and thereby allows their degradation by plasmin. Furthermore, tPA may tightly bind to the exposed core protein of CSPGs, thus facilitating the production of plasmin[83]. Consequently, compared to single treatment, co-treatment with both ChABC and tPA/plasmin is more suitable for SCI treatment.

The biosynthesis of CSPGs requires the participation of many enzymes, including xylosyltransferase-1 (Xt-1) and chondroitin 4-O-sulfotransferase-1 (C4st-1). Xt-1 is a critical enzyme that catalyzes glycosylation of the CSPG protein backbone, whereas Xt-1 inhibition suppresses CSPG synthesis. Application of a DNA enzyme against Xt-1 to the injured spinal cord significantly promotes neurite outgrowth[84]. Similarly, injection of 4-methylumbelliferyl-β-D-xylopyranoside to the injured mouse spinal cord reduces CSPG synthesis and improves remyelination[85].

Bioinformatics has been used to identify putative binding sites for the transcription factor SOX9 in the promoter regions of the Xt-1, Xt-2, and C4st-1 genes in humans, rats, and mice. Researchers have suggested that

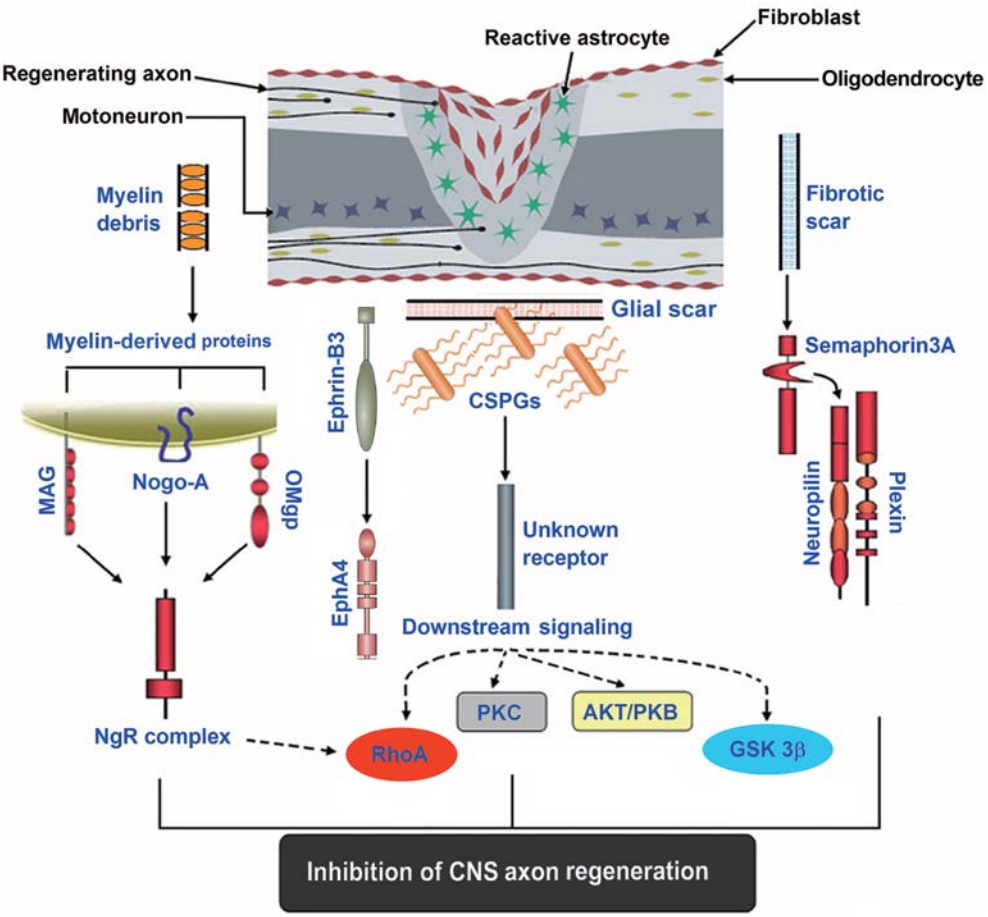


**Fig. 1. Schematic of the molecular inhibitors of CNS axon regeneration, which mainly include glial scar-based CSPGs and their downstream signaling pathways (RhoA, PKC, AKT/PKB, and GSK 3β); myelin-derived proteins (such as NogoA, MAG, and OMgp) and their receptors: the NgR complex; and fibrotic scar-based Semaphorin3A and its receptors (Plexin and Neuropilin). Also shown is the appearance of a variety of cells (reactive astrocytes, fibroblasts, oligodendrocytes, and neurons; oligodendrocyte precursors and microglia/macrophages are omitted) around the lesion site after CNS injury. This figure was drawn by referring to previous review articles[65-67].**

SOX9 regulates the expression of Xt-1, Xt-2, and C4st-1, and *in vitro* experiments have confirmed this hypothesis[86]. CSPG deposition and collagenous scarring are reduced in the SCI lesion of SOX9 conditional knockout mice, suggesting that inhibition of SOX9 activity may become a novel therapeutic strategy for SCI[87].

Fibrinogen is a soluble blood protein. After CNS injury, it leaks from the disrupted blood-brain barrier or damaged blood vessels, and enters the lesion site. Since fibrinogen works as a carrier of latent TGF-β to induce the phosphorylation of Smad2, it promotes the activation of astrocytes and deposition of CSPGs *via* the TGF-β/Smad signaling pathway. Genetic knockout or pharmacologic inhibition of fibrinogen reduces TGF-β activation, Smad2 phosphorylation, glial cell activation, and neurocan deposition after cortical injury in mice. Given these results, fibrinogen-targeting therapies might be beneficial for the repair of CNS injury[24].

Although it has been long known that CSPGs inhibit axon regeneration, the underlying mechanism remains poorly understood. Recent research has revealed that CSPGs may inhibit axon regeneration through four receptors, including PTPσ, leukocyte common antigen-related (LAR) phosphatase, Nogo receptor 1 (NgR1), and NgR3[88-90].

PTPσ, a transmembrane protein tyrosine phosphatase,

**Table 2. Strategy examples for blocking scar-associated inhibitory molecules**

| Inhibitory molecules | Strategy | Example | Model |
|---|---|---|---|
| CSPGs | Inhibition of the GAG side chains of CSPGs | Transfection with siRNA against ChPF[77] | Astrocyte culture |
| | | Combined use of chondroitinase ABC with cell implantation, growth factors, myelin-inhibitory molecule blockers, or ion channel expression[74] | SCI models |
| | Degradation of CSPG core proteins | Use of decorin to promote plasminogen/plasmin expression[25, 80] | SCI rats and cultured adult spinal cord microglia |
| | Clearance of CSPG core proteins | ChABC and tPA/plasmin system[83] | SCI mice |
| | Disruption of GAG-chain initiating enzyme | DNA enzyme against Xt-1[84] | Astrocyte culture and SCI mice |
| | | 4-methylumbelliferyl-β-D-xylopyranoside[85] | SCI mice |
| | | Conditional knockout of SOX9[87] | SCI mice |
| | Inhibition of fibrinogen | Genetic or pharmacologic depletion of fibrinogen[22] | Cortical SWI mice |
| | Blockade of CSPGs binding to receptors | Knockout of PTPσ[88, 91] | Neuronal culture and SCI mice |
| | | Knockout or blockade with peptides of LAR[89] | Neuronal culture and SCI mice |
| | | Knockout of NgR1 and NgR3 [90] | Neuronal culture and mice with retro-orbital optic nerve crush injury |
| | Stimulation of downstream pathways of CSPGs | Activation of Akt by amphotericin B[98] | Cerebellar neuron culture |
| | | Inhibition of RhoA by 17β estradiol, ibuprofen or indometacin[100-102] | SCI rats |
| | | Blockade of PKC activity by Gö6976[96] | SCI rats |
| | | Use of GSK-3 inhibitor, lithium[92] | SCI rats |
| Myelin-derived proteins | Inhibition of Nogo-A | Use of anti-Nogo-A antibody IN-1[107] | SCI monkeys |
| | | Use of antibodies against rat Nogo-A (7B12 and 11C7)[108] | SCI rats |
| | Targeting receptors of Nogo-A and other myelin-derived proteins | Use of NgR antagonist NEP1-40[121] | Rats with lateral funiculus injury |
| | Inhibition of related signaling | Blocking Rho function by C3 transferase from Clostridium botulinum[129] | SCI mice |
| Slit proteins | Inhibition of slit-glypican interactions | Use of heparan sulphate proteoglycan glypican-1[136] | Glypican binding to Slit model |
| Semaphorin3A, Plexins, and Neuropilins | Inhibition of semaphoring/plexin signaling | Use of anti-Semaphorin 3A[141] | Optic nerve axotomy-injured retinal ganglion cells |
| | | PlexinA2 deletion[144] | PlexinA2 –/– cultures; PlexinA2 –/– mice subjected to unilateral pyramidotomy |
| | | Blockade of Neuropilin 2[146] | Interfaces of astrocyte/meningeall cell |
| EphA4 and Ephrin-B3 | Inhibition of EphA4 and Ephrin-B3 | Use of EphA4 antagonist[148] | Astrocyte and neuronal culture; SCI mice |
| Tenascin-R | Inhibition of tenascin-R | Use of antibody against tenascin-R[154] | SCI rats |

ChPF, chondroitin polymerizing factor; ChABC, chondroitinase ABC; CSPGs, chondroitin sulfate proteoglycans; GAG, glycosaminoglycan; LAR, leukocyte common antigen-related phosphatase; NgR, Nogo receptor; SWI, stab wound injury; tPA, tissue plasminogen activator; Xt-1, xylosyltransferase-1.

is functionally involved in the inhibitory effects of CSPGs on neurons *via* an interaction between the conserved, positively-charged region on the surface of the first immunoglobulin-like domain of PTPσ and the GAG chain of CSPG[88]. In culture, PTPσ-null neurons show reduced inhibition by CSPG[88], while in PTPσ-null mice, dramatic regeneration of corticospinal tract axons has been reported after dorsal spinal injury[91].

LAR is a widely expressed protein in various neurons in the brain and spinal cord. DRG neurons derived from adult LAR-null mice exhibit increased neurite length when cultured on CSPG substrates, while blockade of LAR by extracellular LAR peptide (ELP) and intracellular LAR peptide (ILP) overcomes the neurite growth restriction by CSPGs in neuronal cultures[89]. To further confirm the efficacy of ELP and ILP, it was found in SCI mice treated with ELP that the density of 5-5-hydroxytryptamine fibers in the spinal cord 5–7 mm caudal to the lesion epicenter is significantly increased, and axon regeneration is also enhanced[89].

NgR1 is the common receptor of Nogo66, oligodendrocyte myelin glycoprotein (OMgp), and myelin-associated glycoprotein (MAG), whereas NgR2 is the receptor of MAG. In contrast, the related molecule, NgR3, is poorly characterized and no functional ligands have yet been identified. A recent report indicated that NgR1 and NgR3 bind with high affinity to the glycosaminoglycan chain of CSPGs and serve as CSPG receptors. The combined loss of NgR1 and NgR3 enhances axon regeneration[90].

Although PTPσ, LAR phosphatase, NgR1, and NgR3 all contribute to the restriction of axon regeneration, deep insights are required into the actions of these receptors. It is believed that there will be in depth studies on this topic in future.

Then, how do CSPGs inhibit axon regeneration after binding their receptors on neurons? So far, four downstream pathways have been proposed: the Akt/PKB, RhoA, PKC, and glycogen synthase 3β (GSK 3β) pathways[92-96].

Akt is the key factor in the mTOR pathway[97], and mTOR regulates protein translation, for example, cytoskeleton formation during axon regeneration. CSPGs inhibit Akt activity and restrict axon regeneration indirectly. Amphotericin B, identified from a natural product screen, activates Akt and suppresses GSK 3β activity. The compound counters the CSPGs-induced inhibition of axonal growth possibly *via* a mechanism involving the activation of Akt[98].

RhoA, a small GTPase, leads to cytoskeleton collapse and inhibits axon regeneration though ROCK activation[99]. Production of CSPGs after CNS injury activates RhoA, and so inhibition of RhoA with 17β-estradiol[100] or some non-steroidal anti-inflammatory drugs[101, 102], such as ibuprofen and indomethacin, can be used for treating CNS injury.

Both CSPGs and myelin-associated proteins activate PKC. Blocking PKC activity by pharmacological or genetic manipulation attenuates the ability of CSPGs and myelin to activate Rho and promotes neurite regeneration[96].

GSK-3β is highly expressed in neurons, and CSPGs suppress axon regrowth possibly through activation of GSK-3β. In consequence, treatment with GSK-3 inhibitors including a clinical dose of lithium to rats with thoracic spinal cord transection or contusion injury decreases corticospinal and serotonergic axon sprouting in the caudal spinal cord and promotes locomotor functional recovery[92].

### *Myelin-Derived Proteins*

Myelin-derived proteins consist mainly of Nogo-A, MAG, and OMgp[103].

Nogo-A was discovered in myelin as a potent inhibitor of neurite growth[104]. A monoclonal antibody against Nogo-A (IN-1) was produced soon after its discovery. Implantation of IN-1-producing hybridoma cells into SCI rats enhances axon regeneration, improves functional recovery, and increases the plasticity of intact CNS fibers[105]. Therefore, the application of Nogo-A inhibitors has therapeutic effects in CNS injury, which suggests a possibility of clinical trials[106, 107]. IN-1, an IgM-type antibody to Nogo-A, has long been a research focus, but recently two IgG-type monoclonal antibodies to Nogo-A (7B12 and 11C7) have gained much attention. These two antibodies have been delivered separately into the cerebrospinal fluid of SCI rats, and they dramatically enhance axon sprouting and improve recovery of motor function[108].

Although *in vitro* and *in vivo* models have pointed out the importance of Nogo-A in limiting regeneration and recovery after CNS injury, data from Nogo-knockout mutants, generated by three groups independently[109-111], are less simple to interpret[112]. Subsequent studies have demonstrated that age, strain, and type of lesion also modulate the Nogo-null phenotype, and thus the roles of Nogo in CNS axon repair may be more intricate than previously envisioned[113-115].

MAG is known to inhibit neurite outgrowth *in vitro*[116],

and MAG-knockout mice have been used to assess its *in vivo* function in axonal regeneration, but the results from different labs are contradictory. One group reported that after SCI, more axons extend long neurites across the lesion site in MAG-null mice[117], while another group reported no difference in axonal regeneration between MAG-null and wild-type mice after SCI or optic nerve injury[118]. This discrepancy may be due to the difference in genetic background between the two independently-generated MAG-null mouse lines, and/or variations in the methods of regeneration assessment. Therefore, although MAG is an inhibitory molecule that actively suppresses axonal regeneration *in vitro* and *in vivo*, its deletion is not necessarily associated with robust regeneration after CNS injury[119].

The studies using OMgp-knockout mice indicate that these mice exhibit elevated collateral sprouting from the CNS node of Ranvier[120], and that OMgp-null mice show greater functional and anatomical regeneration after SCI, both suggesting that OMgp plays a role in restricting axonal sprouting.

Since Nogo-A, MAG, and OMgp share the neuronal membrane-bound NgR complex, which includes NgR1, NgR2, NgR co-receptors (p75NTR, Troy, and Lingo-1), ganglioside GT1b, and PirB[119], researchers turned their attention toward NgRs. Various approaches to targeting NgRs, including the NgR antagonist NEP1-40[121], genetic deletion of NgR[122], transfection with siNgR199[123], and DNA vaccine against NgR[124], have been reported. However, some researchers failed to demonstrate the maintenance of neuroregeneration in NgR-knockout mice after SCI[111], suggesting that neuronal receptors for Nogo-A, MAG, and OMgp are not limited to NgRs. Blockade of leukocyte immunoglobulin-like receptor subfamily B member 2 (LILRB2) was noted to improve neurite outgrowth[125], and this might be another neuronal receptor for Nogo-A, MAG, and OMgp, so it will attract more attention in future.

Besides NgR and LILRB2, other co-receptors for Nogo-A, such as p75NTR, LINGO-1, and Troy, have also been studied. After SCI, p75NTR-deficient mice do not show enhanced axon regeneration due to the limited expression of p75NTR in a very small subset of ascending sensory axons[126]. Unlike p75NTR, LINGO-1 and Troy are broadly expressed in neurons. Neurons in Troy-deficient mice are less sensitive to the reduction of neurite outgrowth by myelin inhibitors[127], while administration of the LINGO-1 antagonist LINGO-1-Fc dramatically enhances axonal sprouting in SCI rats[128].

In addition, inhibition of signaling pathway is effective for blocking the activity of myelin-derived proteins. Both CSPGs and myelin-derived proteins (Nogo-A, MAG, and OMgp) inhibit neurite outgrowth through the Rho/Rho kinase pathway, and *Clostridium botulinum* C3 transferase has been used to inhibit Rho, and achieve good outcomes of CNS regeneration[129]. Now this therapy has been launched in clinical trials[130].

### *Slit Proteins*

Slit, a group of proteins that are chemically repulsive to growing axons, was first identified in *Drosophila melanogaster* mutants[131]. During neural development, slit proteins, expressed by midline glial cells, bind Robo receptors on growth cones and function as repellents of axon outgrowth[132, 133]. So far, three types of slit proteins have been identified in vertebrates: slit1, slit2, and slit3.

One study showed that all slit family members are expressed at the lesion, and slit2 mRNA is most intensely expressed in the cells surrounding the necrotic tissue. This study also indicated that slit and glypican-1 mRNAs are co-expressed in the reactive astrocytes of the injured adult brain[134]. Glypican-1, a high-affinity ligand of slit2 protein, binds to the C-terminal fragment of slit proteins mainly *via* heparan sulfate chains. The tight interaction between slit2 and glypican-1 in reactive astrocytes promotes the binding of slit2 to Robo-1, and suppresses axonal outgrowth.

Slit expression has also been identified in an animal model of SCI, in which slit1 and slit3 were expressed at the lesion site at 8 days after injury, suggesting that they are expressed in activated microglial cells (macrophagic cells) or meningeal fibroblastic cells[135]. These cells are involved in scar formation. Based on these findings, the ability of slit to inhibit axon regeneration has gained considerable attention. For example, PI-88 (a chemically sulfonated yeast-derived phosphomannan), D120 (a sulfated hydrophilic dextran), and a low-MW heparin have been identified as potent inhibitors of glypican–slit interactions[136]. So far, however, there is little experimental evidence on the improving effects of these three agents on axon regeneration.

### *Semaphorin3A, Plexins, and Neuropilins*

Semaphorins are a class of proteins that function to guide

the axon growth cone during neural development[137]. Following CNS injury, fibroblasts migrating to the lesion site show robust expression of meningeal cell-derived Semaphorin 3A, and cause the collapse of growth cones[138-140]. Application of antibodies against Semaphorin 3A protects retinal ganglion cells from optic nerve axotomy-induced cell death[141], suggesting the therapeutic significance of Semaphorin inhibitors. However, there is still controversy over whether inhibition of Semaphorin 3A is an effective treatment for CNS injury.

Plexins are a large family of cell surface receptors. The ability of Semaphorin to cause growth-cone collapse is mainly attributed to an interaction of Semaphorin with Plexins, its neuronal receptors[142]. The binding of Semaphorin to Plexins requires Neuropilins as a co-receptor[143].

The role of Semaphorin/Plexin signaling in axonal growth after adult CNS injury has been studied with different results. For example, after unilateral pyramidal transection in the medulla, PlexinA2-null mice exhibited the sprouting of unlesioned corticospinal fibers across the midline to innervate the contralateral gray matter of the spinal cord, accompanied by improved behavioral recovery[144]. However, another finding suggested limitations of targeting Semaphorin-mediated inhibition for promoting spinal axon regeneration, thus raising an issue of whether Semaphorin 3A modulates injury-induced axonal growth in a less severe injury model and whether receptors other than Plexins may mediate the inhibition of Semaphorin in the adult CNS[145].

Semaphorin receptors consist of the Neuropilin/Plexin complex. In principle, disruption of this receptor complex may enable injured axons to overcome Semaphorin 3A-mediated inhibition. It has been reported that blockade of Neuropilin2 promotes axon growth across the astrocyte–meningeal fibroblast border[146].

### *EphA4 and Ephrin-B3*

Erythropoietin-producing hepatocellular (Eph) receptors and their ephrin ligands are important mediators of cell–cell communication, regulating cell attachment, shape, and mobility[147]. EphA4 is critically involved in the formation of the astroglial scar[67, 148, 149]. After CNS injury, EphA4 not only participates in astrocyte gliosis and glial scar formation, but also contributes to the suppression of axon regeneration[148]. Therefore, injection of an EphA4 antagonist into SCI mice leads to substantial recovery from injury and axon regeneration[150]. EphA4-deficient mice also exhibit less gliosis and significant axonal regeneration in SCI mice[148].

Myelin-derived Ephrin-B3[151] has also been reported to serve as an inhibitor of axon growth[152], but there are few reports on the treatment of CNS injury targeted at Ephrin, possibly because knockout of Ephrin-B3 is the only feasible approach for its inhibition.

### *Tenascin-R*

The expression of tenascin-R, an ECM constituent within the glial scar[153], is dramatically upregulated after CNS injury, and tenascin-R upregulation is also likely to inhibit axon growth[154]. Immunization with an antibody against tenascin-R promotes axonal regeneration and functional recovery in SCI rats[155].

### *Removal of Existing Scar Tissue*

The above strategies have proven effective for the prevention of scar formation and for the restriction of scar-associated inhibitory molecules in CNS injury. To date, however, research has not fully focused on the removal of existing scar tissue that often occurs in cases of chronic CNS injury. The chronic lesion possesses irregularly shaped scar tissue that line the entire perimeter of the cavity. As a result, it is difficult to ablate this tissue by surgery or laser radiation. Interestingly, after injecting rose Bengal, a biological stain, into the cavity at the injury site in rat spinal cord, researchers found that the glial scar tissue is at least partially ablated by illumination[156].

## Concluding Remarks

The scar plays a double-faceted role in CNS injury and repair depending on the phase of recovery. In the acute and subacute phases, the scar and its components may have beneficial effects by sealing the lesion site, remodeling the tissue, mediating the 'SOS' response, restoring homeostasis, providing trophic support, preserving spared tissue, and regulating immune activity. As time passes, however, the scar gradually has detrimental effects, serving as a physical and molecular barrier to axonal regrowth during the chronic phase[157]. In this review, we were concerned only with the destructive features of the scar, especially focusing on therapeutic interventions targeted at the modulation of (1) scar formation, (2) scar-associated inhibitory molecules, and (3) existing scar tissue. Over

the past decades, tremendous efforts have been made to identify the complex interactions during scar formation (mainly during reactive astrogliosis), to elucidate the molecular and cellular basis of scar inhibition, and to further develop therapeutic strategies directed at scar modulation after CNS injury.

The strategies outlined in this review have demonstrated considerable success in animal studies. Their drawbacks in clinical trials, however, suggest that biological and pharmaceutical treatments based on a single mechanism are not efficient enough to allow satisfactory recovery of patients with CNS injury. The great challenge is how to deliberately manipulate the molecular and cellular interactions that lead to scar formation and how to accurately control the signaling pathways engaged by the scar-associated inhibitory molecules. Clearly, this is a challenging task because either the manipulation or the control varies as a function of injury severity, injury site, intervention timing, and other factors. It is most probable that an efficacious treatment of CNS injury is a combinatorial strategy that encourages axon growth under a favorable environment provided by neural cells and ECM molecules without the detrimental effects of the scar.

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