Neurosci Bull August 1, 2013, 29(4): 421–435. http://www.neurosci.cn
DOI: 10.1007/s12264-013-1358-3

·Review·

The glial scar in spinal cord injury and repair

Yi-Min Yuan, Cheng He

Institute of Neuroscience and Key Laboratory of Molecular Neurobiology of the Ministry of Education, Neuroscience Research Center of Changzheng Hospital, Second Military Medical University, Shanghai 200433, China

Corresponding author: Cheng He. E-mail: chenghe@smmu.edu.cn

Glial scarring following severe tissue damage and inflammation after spinal cord injury (SCI) is due to an extreme, uncontrolled form of reactive astrogliosis that typically occurs around the injury site. The scarring process includes the misalignment of activated astrocytes and the deposition of inhibitory chondroitin sulfate proteoglycans. Here, we first discuss recent developments in the molecular and cellular features of glial scar formation, with special focus on the potential cellular origin of scar-forming cells and the molecular mechanisms underlying glial scar formation after SCI. Second, we discuss the role of glial scar formation in the regulation of axonal regeneration and the cascades of neuro-inflammation. Last, we summarize the physical and pharmacological approaches targeting the modulation of glial scarring to better understand the role of glial scar formation in the repair of SCI.

Keywords: glial scar; spinal cord injury; axonal regeneration; astrocyte activation; reactive astrogliosis; neuro-inflammation

Introduction

Spinal cord injury (SCI) is a common and devastating central nervous system (CNS) insult that results in disruption of cord microstructure and is followed by limited neuronal regeneration and insufficient functional recovery in adult mammals. After severe SCI, and in response to changes in the local microenvironment, astrocytes, the most abundant glial cells in the CNS, transform into reactive astrocytes and undergo dramatic morphological changes[1] as well as massive variations in gene expression[2]. Reactive astrocytes, the major component of glial scars, along with other cells in the spinal cord and blood-borne cells leaking from the damaged blood-spinal cord barrier, participate in the process of scar formation[3]. From the historical perspective, a glial scar is recognized as an impediment to the regeneration of axons in the cord because of the irregular rearrangement of hypertrophic astrocytic processes, as well as major components of the axonal inhibitory extracellular matrix (ECM), including chondroitin sulfate proteoglycans (CSPGs) that are secreted by the reactive astrocytes[4]. Currently, increasing evidence is advancing our knowledge of the mechanism of the formation of glial scars after a lesion and the roles of glial scars in the regulation of neuro-inflammation and repair processes[5,6].

This article reviews the following: (1) the molecular and cellular properties of glial scar formation following SCI; (2) the roles of glial scar formation in axonal regeneration and neuro-inflammation; and (3) the current status of scar-modulating therapeutic strategies for spinal cord repair after injury.

Molecular and Cellular Properties of Glial Scars

Characterization of Glial Scars

Traumatic damage of the spinal cord can result in seal-like scar tissue in the lesion zone, which is filled with dense cellular components and a connective matrix. Generally, the scar tissue is classified into two parts, fibrotic and glial.

The fibrotic scar, primarily composed of invading fibroblasts derived from perivascular and meningeal cells, occupies the epicenter of the lesion area, with sedimentation of collagen matrix[7]. Close to the outer layer of the fibrotic scar, a specialized structure is found, the glial limiting membrane, which marks the interface between the fibrotic scar and glial scar tissue[8]. The glial scar (mainly astrocytic) evolves from the dynamic process of reactive gliosis, which is characterized by complicated and mesh-like processes extended by reactive astrocytes converging on the peri-lesion zone, thus building a physical barrier to axonal growth after SCI. It is noteworthy that the glial scar has a spatial orientation. Magnetic resonance imaging has revealed that the mean thickness of the glial scar rostral/caudal to the cavity is thicker than that in the region lateral to the cavity formed in rat spinal cord[9].

Another important feature of the glial scar is the increased expression of ECM components, which are predominantly secreted by reactive astrocytes. Among the ECM components, the CSPGs and the nature of their inhibition of axonal regeneration and restriction of plasticity have been studied extensively[10-12]. After injury to the rat spinal cord, there is increasing expression of members of the CSPG family, including neurocan, versican, brevican and NG2[13,14]. In humans, after SCI, the CSPG family members NG2 and phosphacan are both detectable in the evolving astroglial scar, while neurocan and versican exist exclusively in the lesion epicenter[15]. Mediated by Rho kinase, CSPGs inhibit neurite growth *in vitro* and *in vivo*[16]. Recently, Siebert and Osterhout found that CSPGs directly suppress the process outgrowth from oligodendrocyte progenitor cells (OPCs) and their differentiation into mature oligodendrocytes *in vitro*[17]. Interestingly, CSPGs also control the activity of resident microglia and infiltrating blood-borne monocytes *via* the CD44 receptor, thus playing a beneficial role in the acute phase of SCI[18].

In general, the formation of the glial scar depends on the severity of the lesion, as severe trauma can give rise to a permanent glial scar in the affected region. In addition, the disparity of astrocytic location within the spinal cord can also influence the development of glial scars. Astrocytes proximal to the lesion site densely overlap newly-proliferated astrocytes and those that have migrated from distal areas, and undergo isomorphic gliosis, leading to an established glial scar, while reactive astrocytes far from the site of injury eventually reacquire a quiescent state with normal morphological and functional features[19]. Therefore, the highly heterogeneous state of astrocytes that have been altered to respond to the specific injury appears to be a critical determinant of glial scar formation[20].

Cellular Origin of Glial Scars

Glial scar formation after SCI is generally referred to as reactive astrogliosis. Most reactive astrocytes in a glial scar have two origins: those proximal to the lesion zone that experience dramatic up-regulation of cytoskeletal proteins [such as glial fibrillary acidic protein (GFAP), vimentin and nestin], and those that acquire stem-cell properties to form new astrocytic progeny[21]. The other source is astrocytes that have migrated from a distal area to the lesion site[22]. In addition to the astrocytic origin of the astrocytes localized in the spinal cord[23], glial scar formation also involves the activation of cells including ependymal cells, NG2-positive cells, and meningeal cells surrounding the lesion site[24–26]. More importantly, several studies have shown that the various aforementioned cells, which are able to change their phenotypes, are eventually fated to become reactive astrocytes and are located in the glial scar[25,27,28].

In the adult rodent spinal cord, stem/progenitor cells exist at two main locations: the zone proximal to the central canal and the outer circumference below the pial surface of the cord[29]. *Via* genetic fate mapping, ependymal cells have been identified as the dominant proliferating cell population that act as neural stem cells and give rise to new cells in the intact adult spinal cord[30] (Fig. 1).

In response to SCI, ependymal cells lining the central canal are induced to generate migratory progeny that differentiate into astrocytes and participate in glial scar formation[31]. Most ependymal cell-derived astrocytes are GFAP-negative but express other markers and have ultrastructural characteristics of astrocytes. The ependymal cell-derived astrocytes form the core of the glial scar, and the astrocytes at the periphery arise by self-duplication[26].

NG2, a membrane-spanning CSPG, is not only expressed in a population of fate-restricted OPCs but also exists on many cellular surfaces after damage to the spinal cord[32]. Previous work has provided evidence that the $NG2^+$ cell population includes OPCs[33], pericytes[34], non-myelinating Schwann cells[35], meningeal cells[36],

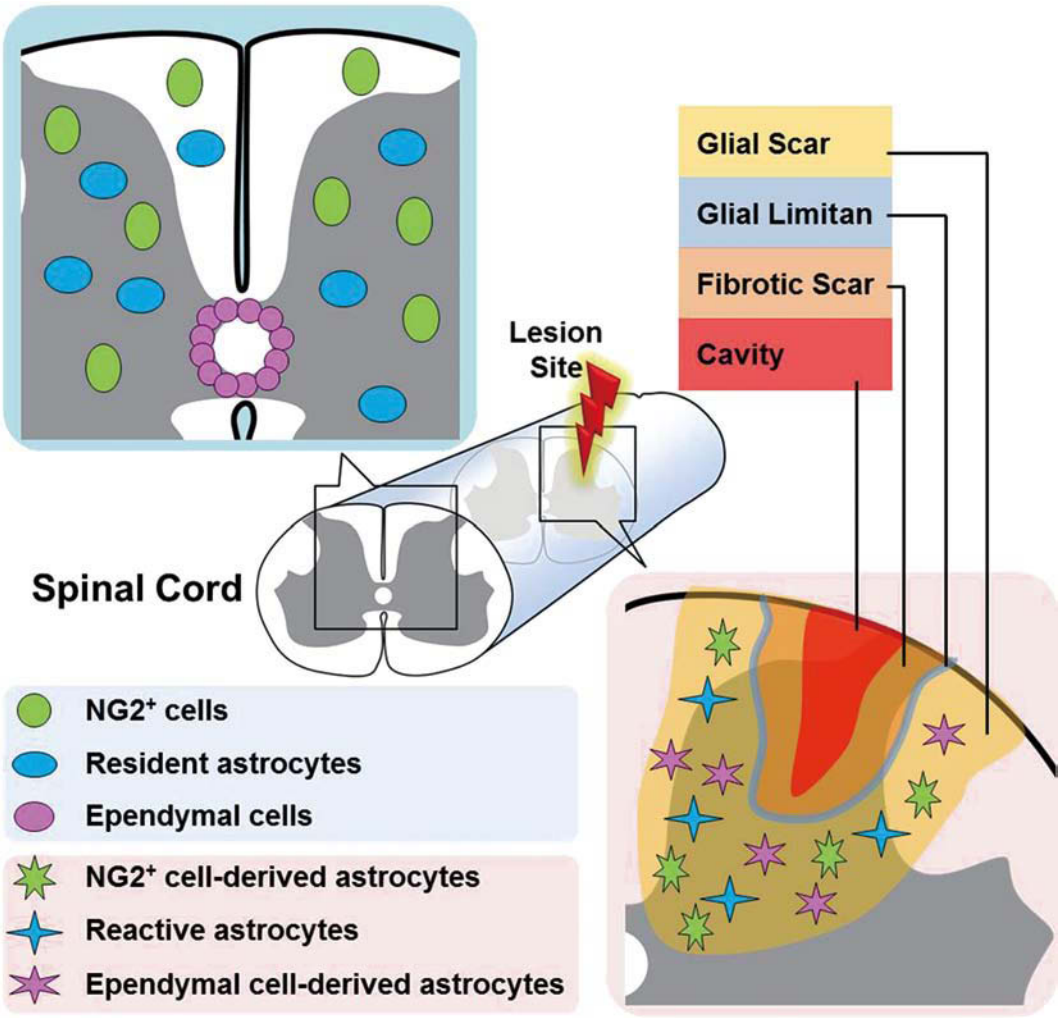

Fig. 1. Schematic of the cellular origin of glial scar formation following spinal cord injury (SCI). Astrocytes (blue), ependymal cells (rose) and NG2$^+$ progenitor cells (green) can give rise to a heterogeneous scar-forming astrocyte population within the glial scar established after SCI.

macrophages[37] and microglia[38], suggestive of a heterogeneous cell population within the injured spinal cord. Sellers and colleagues have demonstrated that NG2 progenitors have a diverse differentiation profile, producing oligodendrocytes, expressing myelin basic protein in processes that wrap axons 7 days after injury, and differentiating into scar-forming astrocytes during 24 h post-lesion[28]. Evidence has also been presented that nestin- and double cortin-positive meningeal cells may be a new source of multipotential adult stem/precursor cells in the spinal cord parenchyma, and these cells can differentiate and migrate to participate in glial scar formation[27]. In addition, Nestin$^+$ and Nkx6.1$^+$ vascular muscle cells[39], which are characteristic of mineralization, can give rise to scar-forming stromal cells, but their interaction with glial scar-forming astrocytes is not fully established. Further studies are required to clarify the features and the fate of NG2$^+$ infiltrating macrophages and resident microglia around the scar[40,41].

Mechanisms of Glial Scar Formation

Considerable research on the signaling pathways and molecular mediators involved in the triggering and modulation of reactive astrocytes has advanced our understanding of the important role of reactive astrogliosis

in CNS injury. However, the molecular mechanisms underlying glial scar formation after SCI have not been fully elucidated. Here, we briefly summarize some molecules associated with the initiation and maintenance of the glial scar in different animal models of SCI (Table 1).

Bone morphogenetic proteins (BMPs) Traumatic injury to the spinal cord results in the up-regulation of BMPs 2/4/7 and noggin in reactive astrocytes at the lesion site[42]. Neurosphere cultures from the cord of the adult mouse show that BMP-4 promotes astrocyte differentiation from NSCs while suppressing the production of neurons and oligodendrocytes[43]. Moreover, genetic ablation of BMPR1b, a subtype of the type 1 BMP receptor, leads to an attenuated glial scar in the chronic stages following compression injury, indicative of a vital role in glial scar progression[44]. Interestingly, BMPR1a and BMPR1b signaling have opposite effects on the early beneficial phases of astrocytic hypertrophy, while an apparent disruption of reactive astrocytic gliosis at the acute stage following SCI occurs in BMPR1a conditional knockout animals. Collectively, these findings reveal that BMPs are deeply involved in the initial establishment and progressive maintenance of glial scar formation.

Matrix metalloproteinases (MMPs) MMP-2 and MMP-9, members of the MMP family implicated in ECM remodeling, are spatially and temporally correlated with glial scar formation[45]. By using specific gene-deficient mice, MMP-2 and MMP-9 have been demonstrated to play a synergistic role in glial scar formation. An overt role of limiting extensive glial scarring has been found in the MMP-2-null mouse[46], while MMP-9 is instrumental in the development of an inhibitory glial scar by increasing the migration of reactive astrocytes surrounding the lesion site[47]. Furthermore, infusion of a recombinant extracellular MMP inducer adenovirus into the contused animal spinal cord, and, consequently, increasing the secretion of MMP-3, reduces CSPG deposition in the glial scar and improves locomotor recovery[48].

Epidermal growth factor receptor (EGFR) EGFR, which is undetectable in astrocytes in normal adult CNS, is upregulated in astrocytes after contusive damage to the spinal cord[49]. Further study has demonstrated that EGFR induces reactive astrocytes to migrate, undergo hypertrophy, and form a glial scar through activation of the Rheb–mTOR signal pathway[50]. Deprivation of EGFR activity in mice results in malformed glial borders and larger lesions after SCI[51]. In addition, EGFR ligands, such as EGF and transforming growth factor-alpha (TGF-alpha), stimulate astrocytes to secrete CSPGs to further form the glial scar[52]. EGFR also favors the activation of microglia *via* the phosphorylation of MAPK and downstream mediators[53]. Hence, EGFR at the lesion area is a critical player responsible for glial scar formation during the acute stage after SCI.

Eph/Ephrins The Eph family of receptor tyrosine kinases is well known for its role in guiding axonal growth during development. After damage to the adult spinal cord, members of the Eph family, along with their ligands (ephrins), accumulate in the proximal axon stump and reactive astrocytes and are thus implicated in glial scar formation[54]. The studies of the effects of ephrin-B2 on reactive astrocytes and EphB2 on meningeal fibroblasts demonstrate that cellular interplay mediated by ephrin-B2 and EphB2 is an early event that results in the development of a glial scar in the injured spinal cord[55]. In addition, the up-regulation of EphA4 in injured axons leads to the retraction of corticospinal axons and inhibition of axon regeneration in the weeks after a spinal lesion[56]. Notably, a study by Turnley's team showed that astrocytic gliosis and glial scars are greatly reduced in EphA4-deficient mice after a lateral spinal cord hemisection[57]. In contrast, the genetic deletion of EphA4 does not significantly alter the astroglial response or cause the development of an astroglial fibrotic scar after dorsal hemisection SCI in mice[58]. This discrepancy cannot be simply explained by the lesion models used. Turnley's team re-examined astrocytic gliosis following injury in EphA4-null mice and revealed that these mice no longer show attenuation of astrocyte reactivity, while other phenotypes, such as decreased size of the dorsal funiculus, are unaltered. They demonstrated that the change in the EphA4-mediated astroglial phenotype is associated with long-term breeding of the EphA4 colony but does not appear to be influenced by the background mouse strain[59].

Cytoskeleton Vimentin and GFAP are the main cytoskeletal intermediate filaments of astrocytes expressed during development and adulthood, respectively. After SCI, both vimentin and GFAP are up-regulated in reactive astrocytes[60]. An analysis of mice that are genetically deficient in vimentin or GFAP showed that glial scar

Table 1. Triggers and mediators involved in the formation of glial scar

Molecules	Manipulation	Injury model	GS formation	Outcome	Ref
BMPs 4&7	↑By injection into spinal cord	Local demyelination by lysolecithin	↑CSPGs such as neurocan and aggrecan	↓Axonal regeneration, ↓functional recovery	[42]
BMPR1b	BMPR1b KO mouse	Compression	↓Gial scar in chronic stages	↓lesion size; ↑compaction of inflammatory cells	[44]
MMP-2	MMP-2-null mouse	Contusion	↑CSPGs and extensive astrocytic scar	↓Serotonergic fibers, ↓white matter sparing, ↓locomotor performance	[46]
MMP-9	MMP-9-null mouse	Contusion	↓CSPGs and glial scar formation	Abnormalities in actin cytoskeletal organization, impaired migration	[47]
EGFR	C57BL/6J- $Egfr^{(Vel)}$/J mouse	Contusion at T9	Malformed glial borders	↑Lesion area; ↓recovery of function	[51]
	↓By specific EGFR inhibitor (AG1478)	Contusion at T10	↓Accumulation of CSPGs	↓Demyelination; ↑GAP-43 expression, hindlimb function	[53]
EphA4	EphA4 (-/-) KO mouse	Lateral hemisection at T12	↓GS formation	↑Axonal regeneration	[57]
	EphA4 (-/-) KO mouse	Dorsal hemisection at T8	No formation of astroglial-fibrotic scar	No change in lesion size, neuronal survival, inflammation marker expression	[58]
VIM & GFAP	VIM & GFAP (-/-) double KO mouse	Hemisection	↓GS formation	↑Density of serotonergic fibers	[63]
TGF-beta1	↓By antibody	Contusion at T9/10	↓GS formation, CSPG expression	↑Accumulation and activation of microglia/macrophages; ↑growth and/or preservation of serotonergic axons	[67]
Stat3	Gfap-Stat3 (-/-) CKO mouse	Crush injury at L1/L2	↓GS formation	↑Inflammation and lesion volume; ↓motor recovery	[71]
IL-6	↓By IL-6 receptor monoclonal antibody	Contusion	↓GS formation	↓Connective scar formation, ↓inflammation; ↑functional recovery	[78]
S100A4	S100A4 (-/-) KO mouse	Focal demyelination by ethidium bromide	↓GS formation	↑Astrocyte migration into demyelinated area	[79]
SOX9	Sox9 (-/-) CKO mouse	Contusion at T9	↓CSPG deposition	↑Hindlimb function, ↑locomotor recovery; ↑NF^+ and 5-HT^+ fibers	[80]
PKC	↓By Gö6976	Dorsal hemisection	↓Inhibitory activity of CSPGs	↑Regeneration of dorsal column axons	[81]

Abbreviations: "-", deficient; "↓", down-regulation; "↑", upregulation; 5-HT, 5-hydroxytryptamine (serotonin); CSPGs, chondroitin sulfate proteoglycans; GAP-43, growth-associated protein-43; GFAP, glial fibrillary acidic protein; GS, glial scar; IL-6, interleukin-6; KO, knockout; NF, neurofilament; Ref, reference; VIM, vimentin.

formation appears normal after spinal cord or brain lesions. However, less dense scars frequently accompanied by bleeding occur in mice double-deficient in vimentin and GFAP[61], most likely due to the impaired astrocytic motility resulting from the ablation of intermediate filaments[62]. Moreover, the genetic absence of vimentin and GFAP also improves axonal sprouting[63] and facilitates functional recovery in the context of a spinal cord hemisection model[64].

Transforming growth factor-beta TGF-beta expression increases immediately after SCI in the injured segment[65]. Both TGF-beta1 and TGF-beta2 are detectable in macrophages and astrocytes, while TGF-beta1 is also neuron-associated[66]. The cellular localization and temporal expression pattern of TGF-beta after SCI suggest that early induction of TGF-beta1 at the point of SCI modulates acute inflammatory responses and glial scar formation[67], while the later induction of TGF-beta2 indicates a regulatory role in the maintenance of the scar[68].

Signal transducer and activator of transcription and interleukin (STAT/IL) family STAT3, which contributes to neuro-inflammation[69] and promotes neuronal survival and regeneration[70] after SCI, is one of the most important transducers in the process of glial scar formation. A marked reduction of glial scarring and a significant improvement of locomotion occur in animals with conditional knockdown of STAT3 compared with their wild-type counterparts[71]. Notably, the post-injury inhibition of STAT3 nuclear translocation mitigates the proliferation and migration of spinal astrocytes, suggesting that regulating STAT3 is an effective avenue to control the development and maintenance of glial scars[72,73]. A wide spectrum of extracellular triggers is able to activate intracellular STAT3, particularly type I and II interferons, neurocytokines, and growth factors including EGF and platelet-derived growth factor[74]. In the case of the interleukin-6 (IL-6) family, cytokines such as IL-6, leukemia inhibitory factor and ciliary neurotrophic factor, which undergo dimerization of the gp130 signal transduction subunit with other specific receptors, direct the activation of STAT3 and elicits changes in reactive astroglia and astrocytic scar formation[75–77]. Emerging lines of evidence indicate that neutralization of pro-inflammatory signals such as IL-6 during the acute phase of SCI significantly suppresses the astrocytic differentiation-promoting effect of endogenous neural stem/progenitor cells, implying a cellular impact on glial scar formation by IL-6[78].

Moreover, using knock-out mice, S100A[79] and SOX9[80] are also reported to be directly involved in glial scar formation by promoting reactive astrocytic migration and targeting CSPG deposition, respectively. In addition, a study of PKC disruption by the inhibitor Gö6976 has linked an increase in the regeneration of dorsal column axons with the inactivation of CSPGs after dorsal spinal cord hemisection[81]. More interestingly, Bhalala *et al*. recently reported that miR-21 regulates astrocytic hypertrophy and glial scar progression after SCI, which implicates epigenetic regulation as a potential therapeutic target for manipulating glial scar formation and improving functional performance[82].

Roles of Glial Scars in SCI

In a relative sense, there are two facets of glial scar function in the injured spinal cord. At the acute and subacute stages after SCI, the glial scar serves to seclude the lesion area from healthy tissue by limiting disruption of the blood-spinal cord barrier, the amplification of an overwhelming inflammatory response[83] and massive cellular degeneration[11]. Gradually, the glial scar has its detrimental effect, which is characteristically a long-lasting physical and chemical barrier to axonal regrowth during the chronic period[84,85].

Influence on Axonal Growth

Over the past decade, increasing evidence has attributed the failure of axonal regrowth after SCI to limited intrinsic neuronal plasticity and the local non-permissive microenvironment including myelin-associated growth inhibitors as well as the glial scar[86,87]. Inhibition of glial scar formation by deleting key signals that mediate the process has shown an overt facilitation of the affected axon regrowth. Of note, MRL/MpJ mice are a genetically inbred strain that exhibit decreased scarring and fibrosis with a rapid regenerative healing response in several somatic tissue wounds[88,89]. *Bona fide* axonal regrowth, including sprouting and regeneration, has been observed in MRL/MpJ mice with dorsal hemi-section and contusion[90,91]. However, there are many differences between the two studies. In the MRL/MpJ mice with mid-thoracic spinal contusion, poor locomotor recovery is accompanied by

ongoing degeneration both within and surrounding the chronic lesion site[89]. On the contrary, MRL/MpJ mice that undergo a dorsal hemisection show improved recovery of motor function, a reduced astrocytic response, and fewer micro-cavities at the injury site[90]. Although a spectrum of axonal growth-promoting molecules, such as laminin[92] and fibronectin[93], is induced by scar tissue after damage to the spinal cord, the prominent chemical constituents secreted by reactive astrocytes, CSPGs, stimulate axonal regrowth in the inhibitory milieu[94]. Currently, the two predominant receptor protein tyrosine phosphatase (RPTP) receptors, RPTP-sigma[95] and leukocyte common antigen-related receptor (LAR)[85] have been identified as functional receptors of CSPGs. In RPTP-sigma-deficient mice, the regrowth of axons across the CSPG-rich glial scar has been reported in many SCI models, including those of dorsal column crush, spinal hemisection and contusion[96]. Fisher *et al.* demonstrated that the LAR-CSPG complex triggers the inhibition of axonal growth, partially *via* inactivating Akt and activating RhoA signals; furthermore, the deletion of LAR in knock-out mice or the blockade of LAR with sequence-selective peptides significantly overcomes the growth restriction of serotonergic fibers by CSPG and promotes functional locomotor recovery[97].

Regulation of Neuro-inflammation

The neuroinflammatory response includes the activation of tissue-resident microglia and the infiltration of blood-borne macrophages, further contributing to secondary damage in the spinal cord, along with glial scarring and progressive cavitation[98,99]. In the acute stage after SCI, the single-layer perivascular basement membrane in the specialized blood-spinal cord barrier often separates into an inner endothelial basement membrane and an outer parenchymal basement membrane. Numerous mononuclear phagocytes accumulate in the space between the membranes and infiltrate into the surrounding parenchyma. Attenuation of the inflammatory cascade exerted by infiltrating monocytes occurs during the subacute period following SCI and coincides with the appearance of the glial scar and glia limitans, suggesting the potential regulation of inflammation by glial scarring[100]. Moreover, disruption of astroglial scar formation using astrocytic STAT3-CKO mice to augment the spread of inflammation after SCI underscores the beneficial effect of glial scars on inflammatory restriction[70]. From a historical perspective, neuro-inflammation has a negative reputation in the context of SCI. However, increasing evidence over the past decade has changed this perception due to the existence of alternative anti-inflammatory monocytes[5]. The alternative activation of mononuclear (IL-10 producing) phagocytes, in contrast to the classic phenotype of pro-inflammatory features, has a strong matrix-resolving property to degenerate the glial scar depending on the expression of the matrix-degrading enzyme MMP-13[101,102]. More interestingly, CSPGs, the predominant component of glial-scar ECM, directly activate microglia/macrophages *via* the CD44 receptor and modulate neurotrophic factor secretion by these cells during the first two days after injury[18]. These studies advance our knowledge of the interaction between glial scar formation and the neuro-inflammatory response after SCI and emphasize the synergistic impact on the reparative process, suggesting a promising strategy for controlling neuro-inflammation by manipulating glial scarring.

Targeting Glial Scars to Promote Repair after SCI

SCI, with high morbidity and poor prognosis, including paraplegia, hemiplegia and quadriplegia, is a worldwide medical problem. Clinically, in addition to surgical decompression and spinal stabilization[103], the administration of methyl-prednisolone has been used to prevent secondary neuronal damage[104]. However, these interferences have not translated into significant functional recovery or sparing of neuronal tissue. In the past two decades, great progress has been made on the development of remedial interventions targeting the glial scar formed after traumatic damage to the spinal cord.

Physical Interventions

Low-power laser irradiation, a modified physical therapy, is used in the treatment of severely injured peripheral nervous system and CNS[105]. In SCI, regardless of whether the damage is contusive or compressive, the irradiated spinal cord reveals a prominent reduction in the extent of the astrocyte response, manifesting attenuated CSPG-matrix accumulation, glial scar and syringomyelia[106]. In addition, the conversion of a normally non-permissive environment into a friendly milieu induced by low-dose irradiation is conducive to motor recovery after SCI[107].

Based on a study of the injured mammalian spinal cord, the instructive effect of an imposed electrical field

may be attributed to a shift in the arrangement of reactive astrocytes in undamaged white matter near the lesion and the significantly reduced number of astrocytes possessing oriented cell processes within the injury site, which potentially has an influence on glial scar formation after SCI[108]. More importantly, in patients with complete SCI, implantation of an oscillating field stimulator for 15 weeks allows the recording of upper-extremity somatosensory-evoked potentials at 6 weeks, 6 months, and 1 year[109].

Pharmacological Strategies

Following SCI, the specialized microstructure of the blood-spinal cord barrier is damaged, and the permeability between spinal cord tissue and blood vessels is unrestricted until the subacute phase[110]. Thus, by harnessing this window of barrier impairment, the delivery of pharmacological agents is a feasible approach to treat SCI. Agents that have been reported to promote axonal regeneration or/and functional recovery after SCI by manipulating glial scar formation are listed in Table 2.

Many studies have provided evidence of the growth-inhibitory nature of CSPG-containing glial scars, which are primary targets for therapeutic strategies after SCI[92]. It is well-established that chondroitinase ABC (ChABC) significantly ameliorates the hostile-matrix microenvironment following SCI by enzymatically digesting the glycosaminoglycan side-chains of CSPGs and promotes functional recovery[4,111,112]. In accord with these findings, a recent study showed that the infusion of ChABC is also successful in restoring neuronal glycosylation to normal[113]. However, the clinical translation of ChABC may be set back by two major challenges: (1) its poor thermostability and (2) its synthesis from an *Escherichia coli* origin, which may carry immunological risk[114,115]. In addition to ChABC, there is evidence that MMPs can degrade the protein backbone of some CSPGs to restrict the formation of a glial scar[116], and the process is associated with mitigation of a functional deficit; however, axonal dieback is an unpleasant secondary response[117]. Grimpe and Silver showed that a newly-designed DNA enzyme against xylosyltransferase-1, a critical enzyme that initiates glycosylation of the protein core of CSPGs, blocks the formation of intact glycosaminoglycan-bearing CSPGs in the glial scar and allows micro-transplanted dorsal root ganglia axons to regenerate around the core of the lesion[118].

Decorin, a small leucine-rich proteoglycan containing only one glycosaminoglycan chain, is a versatile player in regulating glial scar formation following SCI. Mini-pump administration of decorin into acute stub injuries of the adult rat spinal cord inhibits neurocan, brevican, phosphacan and NG2 expression[119]. In addition, decorin is reported to attenuate glial scar formation in the rat cerebral hemisphere[120]. In accord with these findings, decorin treatment in acute SCI induces the synthesis of plasminogen/plasmin by microglia, and these are known to play major roles in degrading the inhibitory components of the glial scar and promoting CNS plasticity[121]. More interestingly, a recent study showed that decorin has the ability to directly stimulate neurons to extend axons within CSPG- or myelin-rich environments, suggestive of a coherent pharmacological base that suppresses scar-formation to facilitate axonal regrowth after SCI[122].

It is well established that anaerobic metabolism at the lesion site due to the loss of high-energy metabolites is an important contributor to secondary damage and neurological deficits[123]. Before undergoing a moderate spinal cord contusion, rats pretreated with creatine, a potent intracellular energy buffer, score better than controls in the BBB open-field locomotor rating scale and have significantly less scar tissue surrounding the cavity[124]. Our recent study found that ethyl pyruvate, which is able to compensate for cellular ATP synthesis[125], improves functional recovery by evoking a significant amelioration of the abnormal glial microenvironment after SCI[126]. In the damaged cord, glial scar formation is markedly reduced, whereas the number of proliferative astrocytes decreases in an astrocyte culture scratch injury model after ethyl pyruvate administration. Moreover, ethyl pyruvate affects activated microglia and CD11b-positive inflammatory cells in an anti-inflammatory fashion.

A recent study showed that the delayed expression of cell-cycle proteins contributes to astroglial scar formation and chronic inflammation after rat spinal cord contusion, suggesting that pharmacological agents against cell-cycle proteins ameliorate the non-permissive microenvironment[127]. Specifically, the use of olomoucine to inhibit cell cycle kinases (CDKs) 2 and 5 results in the persistent down-regulation of astroglia[128] and microglia[129] proliferation within the glial scar around the site of a spinal

Table 2. Pharmacological agents that affect glial scar formation

Compound	Injury model	Mechanism	Effect on GS	Outcome	Ref
ChABC	Moderate contusion	Intracellular energy supplement in maintaining cellular energy homeostasis	↓Scar tissue surrounding cavity	Better BBB open field locomotor score than control	[4]
Decorin	Stub lesion at C1/2	Elevated plasminogen/ plasmin to digest CSPGs	↓CSPGs and inflammation	↑Ability of axons from microtransplanted adult sensory neurons to enter, grow within lesion area	[119] [121] [122]
Ethyl pyruvate	Hemisection at T8	Supplement of energy substrate and anti-inflammation signal pathway	↓Reactive astrogliosis and CSPG deposits	↑Survival of neurons, regrowth of CST and functional locomotor recovery	[125] [126]
Creatine	Moderate contusion	Intracellular energy supplement in maintaining cellular energy homeostasis	↓Scar tissue surrounding cavity	Better BBB open field locomotor score than control	[124]
Olomoucine	Hemisection at T12–T13	Selective inhibition of cell cycle kinase (CDK) 2 and 5	↓Astroglia and microglia proliferation; accumulation of CSPGs	↑Expression of GAP-43 and functional recovery	[128] [129]
Flavopiridol	Moderate contusion at T9	Reduction of Cyclin D1 and G1 protein	↓Proportional area of GFAP-labeled tissue around lesion epicenter	↑BBB and CBS	[130]
CR8	Moderate contusion at T8	Inhibition of CDKs 1, 2, 5, 7 and 9	↓Astrogliosis and inflammatory protein expression	↑BBB and CBS	[131] [132]
Rapamycin	Ischemia	Selective inhibition of Rheb-mTOR pathway	↓Reactive astrogliosis	↑Anti-inflammation; ↓neural tissue damage and locomotor impairment	[50]
Triptolide	Hemisection at T8	Blocking JAK2-STAT3 pathway	↓Astrocytic gliosis and CSPG expression	↑Regrowth of CST and functional locomotor recovery	[134]
Rolipram	Hemisection at C3/4	Inhibition of phosphodiesterase	↓Reactive gliosis; formation of GS	↑Axon regrowth into embryonic spinal transplant; significant improvement in motor function	[135]
Rose Bengal	Contusive injury	Photochemical irradiation	Ablation of astrocytes in scar tissue, partial spared tissue	No change in behavioral score	[136]
7 beta-OH cholesteryl-oleate	Hemisected at T8/9	Not mentioned	↓Astrocyte hyperplasia and hypertrophy	↑Regrowth of axons in denervated dorsal horn, below hemisection originating from contralateral side	[137]

Abbreviations: "↓", reduction or decrease; "↑", promotion or enhancement; BBB, Basso Beattie and Bresnahan locomotor rating scale; CBS, combined behavioral score; CR8, N6-biaryl-substituted derivative of roscovitine; CSPGs, chondroitin sulfate proteoglycans; CST, corticospinal tract; GAP-43, growth-associated protein-43; GFAP, glial fibrillary acidic protein; GS, glial scar; Ref, reference; SCI, spinal cord injury.

lesion, and this, in turn, results in successful neuronal sprouting and functional recovery. Similarly, flavopiridol, a non-selective CDK inhibitor that also inhibits the transcription of cyclin D1, reduces glial scar formation and mitigates the functional deficits from 21 days post-injury[130]. Recently, CR8, a potent and selective roscovitine-derived inhibitor of CDKs that has been reported to provide neuroprotection in experimental traumatic brain injury[131], was found to have a beneficial effect after impact contusive SCI in rats[132]. The systemic and continuous administration of CR8 limits the sustained elevation of cell-cycle proteins related to delayed astroglial scar formation and chronic inflammation, thus significantly improving the functional recovery.

There is strong evidence that rapamycin is neuroprotective and promotes functional recovery by overcoming glial-scar inhibition through inhibition of a specific signal pathway[50]. Rapamycin, an mTOR-selective inhibitor, promotes autophagy and anti-inflammation, thereby playing a neuroprotective role[133]. Based on a screen of the active ingredients of *Tripterygium wilfordii* Hook. F., we found that triptolide reduces glial scar formation by inactivating the JAK2–STAT3 signal pathway[134]. Moreover, observations from sporadic studies suggest that compounds such as rolipram[135], rose Bengal[136] and 7 beta-OH cholesteryl-oleate[137] may be beneficial in treating SCI by manipulating glial scarring.

Concluding Remarks

Glial scar formation is an important biological event after SCI. Research in recent years has highlighted the pivotal and complex roles played by the glial scar in secondary damage in SCI. We have discussed recent progress in our understanding of the glial scar: its molecular and cellular properties; its role in regulating axonal regeneration and neuro-inflammation; and its manipulation by physical and pharmacological approaches. However, many important questions remain to be answered, and the key implications of the glial scar need to be further explored. Basic research that focuses on investigating the mechanisms underlying glial scar formation, especially the role of endogenous stem/progenitor cells localized in the parenchyma of the injured spinal cord, as well as developing pharmacological approaches to manipulate glial scar formation and promote axonal regeneration, will be helpful in achieving successful therapeutic explorations for SCI.

ACKNOWLEDGEMENTS

This review was supported by grants from the National Basic Research Development Program of China (2011CB504401) and the National Natural Science Foundation of China (31130024, 31070922 and 81261130313).

Received date: 2012-12-18; Accepted date: 2013-05-03

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