Cell biology of spinal cord injury and repair

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Spinal cord injury (SCI) lesions present diverse challenges for repair strategies. Anatomically complete injuries require restoration of neural connectivity across lesions. Anatomically incomplete injuries may benefit from augmentation of spontaneous circuit reorganization. Here, we review SCI cell biology, which varies considerably across three different lesion-related tissue compartments: (a) non-neural lesion core, (b) astrocyte scar border, and (c) surrounding spared but reactive neural tissue. After SCI, axon growth and circuit reorganization are determined by neuron-cell-autonomous mechanisms and by interactions among neurons, glia, and immune and other cells. These interactions are shaped by both the presence and the absence of growth-modulating molecules, which vary markedly in different lesion compartments. The emerging understanding of how SCI cell biology differs across lesion compartments is fundamental to developing rationally targeted repair strategies.

Introduction

Spinal cord injury (SCI) is a major cause of long-term physical impairment. Current treatments are limited mostly to supportive measures. Affected individuals often have life expectancies of decades with permanent disability (1–3). To develop appropriately targeted repair strategies, there is a need for a better understanding of the broad cell biology of SCI and how that cell biology differs in different SCI lesion compartments. Normal function in the CNS requires interactions of many cell types, including neurons, neuroglia, and non-neural cells (4). Similarly, the response to CNS injuries involves complex multicellular interactions, and the activities of diverse cell types can influence SCI outcome (5). Here, we review current information and highlight critical gaps in knowledge about (a) the cellular organization of SCI lesions, (b) mechanisms that regulate circuit reorganization and axon regrowth after SCI, and (c) specifically targeted repair strategies.

Formation of SCI lesions

Most human SCI lesions result from mechanical traction and compression forces secondary to acute crush, penetrating injury, or long-term compression, caused by displaced bone, bone fragments, or disc material (1, 6). Hemorrhage and ischemic damage are prominent features. Lesions can be grouped into major subtypes: (a) microscopic damage with normal gross anatomy, (b) contusions with multifocal areas of hemorrhage and necrosis that convert to connective tissue scar, (c) lacerations from penetrating injury by bony fragments or foreign bodies, (d) crush and compression injuries with large areas of non-neural connective tissue scar, and (e) fluid-filled cysts that replace lost tissue (6).

Compartmental organization of SCI lesions

Regardless of cause or size, mature SCI lesions can be divided into three tissue compartments that each have a unique cell biology: (a)

a central non-neural lesion core, often referred to as fibrotic scar (also mesenchymal or connective tissue scar); (b) an astroglial scar border that intimately surrounds the lesion core; and (c) a surrounding zone of viable neural tissue that is spared and functional but reactive and is demarcated by the presence of reactive glia (Figure 1 and ref. 5). These three compartments exhibit markedly different cellular composition and functional interactions.

Non-neural lesion core. Cell damage after SCI is mediated by physical forces, metabolic damage, and ischemia. Cellular debris can be toxic (7). Multiple mechanisms balance debris clearance and sparing of potentially functional adjacent neural tissue. Specific molecular cues label damaged cells for clearance or label healthy cells for sparing (7). Innate inflammatory responses implement debris clearance. Microglia and astrocytes are local tissue resident cells that are early responders with limited phagocytic capacity and the ability to elaborate growth factors, cytokines, and chemokines to recruit professional blood-borne inflammatory cells to assist with debris clearance. These mechanisms must be balanced because too little inflammation can lead to accumulation of cytotoxins, whereas too much inflammation can destroy tissue (5). Perivascular fibroblast-derived stromal cells, meningeal fibroblasts, and pericytes (8–10) proliferate markedly in lesion cores. As cellular debris are cleared and lesion cores mature, they become composed primarily of these stromal non-neural cells intermingled with endothelial progenitors and newly formed blood vessels, as well as with extravasated blood-borne cells such as fibrocytes and diverse immune cells, including hematogenous macrophages, neutrophils, lymphocytes and other leukocytes (which change over time), as well as substantial extracellular matrix components (Figure 1, A and B, and ref. 5). Over time, blood-borne inflammatory cells normally recede; however, their prolonged persistence can be associated with increased tissue damage (11, 12).

Astrocyte scar border. Starting 1 to 2 days after SCI and continuing for about 7 to 10 days, astrocytes proliferate and organize around the edges of severely damaged and unsalvageable tissue (13). These newly proliferated astrocytes migrate, intertwine their pro-
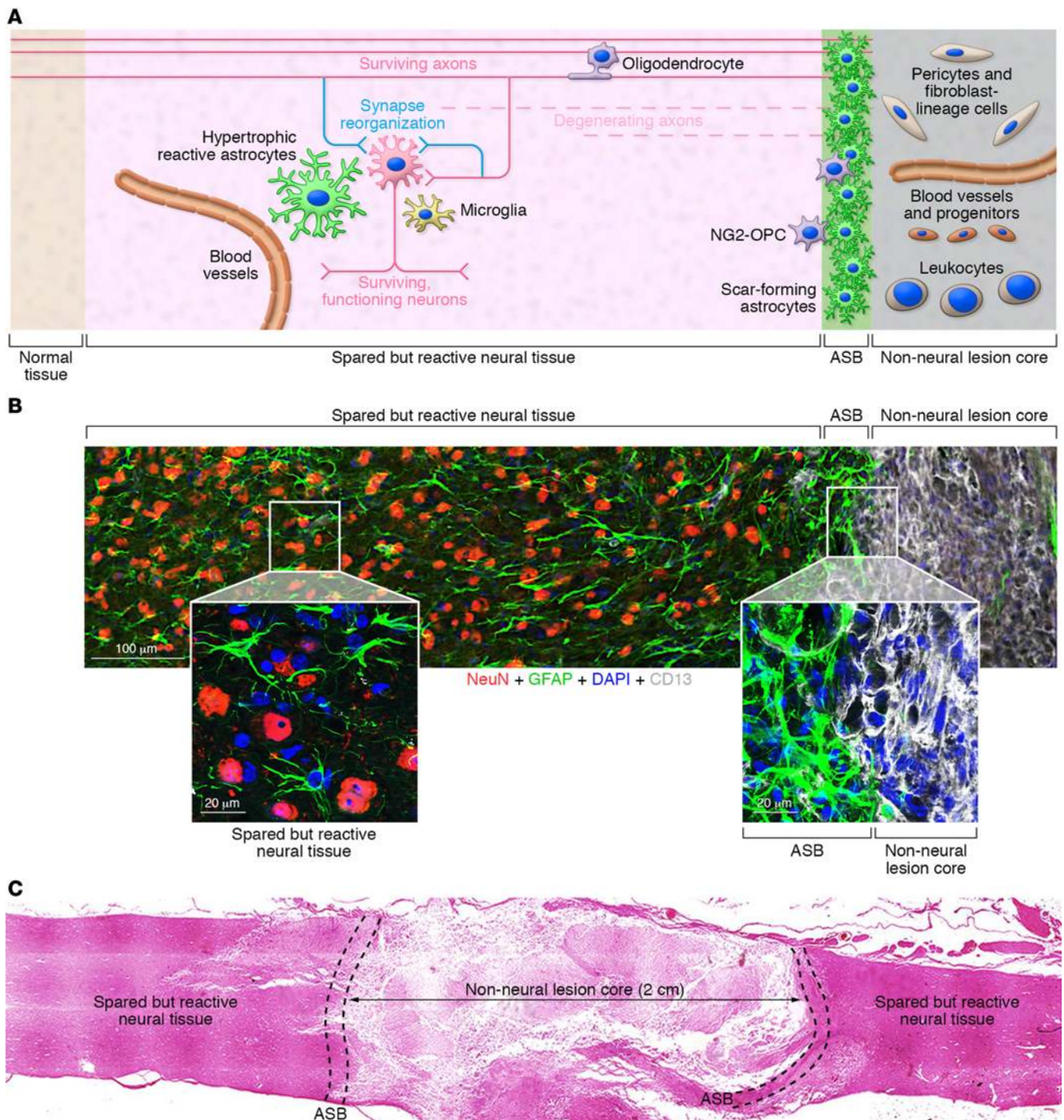
Figure 1. SCI lesions exhibit discrete tissue compartments. (**A**) Schematic of SCI lesion compartments composed of different neural and non-neural cells. (**B**) Photomicrograph showing different cellular components in discrete tissue compartments of a mouse severe crush SCI (T9–T10) lesion. Boxed areas are enlarged to show details. Astrocytes are stained green using glial fibrillary acidic protein (GFAP). Neurons are stained red using NeuN. Pericytes and fibroblast-lineage cells in lesion core are stained white using CD13. Cell nuclei are stained blue using DAPI. (**C**) Survey photomicrograph of human severe SCI (C5–C7) showing relative proportions of lesion compartments (reproduced with permission from *Brain*, ref. 11). OPC, oligodendrocyte progenitor cell; ASB, astrocyte scar border.

cesses, and assemble into a scar border around inflamed non-neural lesion core tissue (Figure 1, A and B, and refs. 5, 13). This scar border formation is complete by 2 to 3 weeks after SCI and consists almost entirely of newly proliferated astrocytes (13, 14). Astrocyte

scar borders have structural and functional similarities to the glia limitans borders formed by astrocytes along all meningeal surfaces and blood vessels in the healthy CNS, which also separate neural from non-neural tissue (15). Functionally, astrocyte scar borders

corral inflammatory cells within areas of damaged tissue (13, 15) and protect adjacent viable neural tissue, such that their loss or dysfunction leads to increased spread of destructive inflammation, resulting in larger lesions and worse functional outcome (13, 15–18). Like glia limitans borders along meninges, astrocyte scar borders are narrow and only several cell layers thick (Figure 1, A and B) (13, 15). For this reason, the proportion of astrocyte scar to lesion core volume is often small, particularly in human SCI lesions (Figure 1C) (6). Astrocyte scar borders are intermingled with reactive oligodendrocyte progenitor cells (OPC) that express chondroitin sulfate proteoglycan 4 (also known as neuron glial antigen 2 [NG2]), and these cells are often referred to as NG2-OPC (Figure 1A). The lineage derivation of newly proliferated scar-forming astrocytes is not completely defined, but many if not most appear to derive from proliferation of local astrocytes (17) and not from putative ependymal progenitor cells (14). Genomic profiles of astrocytes and non-astrocyte cells in mature SCI lesions have recently been evaluated by RNA sequencing (19), and data for individual genes are available in a searchable open-access website (https://astrocyte.rnaseq.sofroniewlab.neurobio.ucla.edu).

Spared but reactive neural tissue. Astrocyte scar borders are directly continuous with, and are surrounded by, an outer zone of spared but reactive neural tissue containing all elements found in normally functioning neural tissue (Figure 1, A and B, and ref. 5). This tissue extends away from the lesion core and its astrocyte scar border in all directions and can be surprisingly large (Figure 1, B and C). It is characterized by the presence of reactive glia, including astrocytes, microglia, and NG2-OPCs, whose hypertrophy and other reactive changes taper in a graded fashion that diminishes with distance from the lesion core (Figure 1B and ref. 5). Notably, hypertrophic reactive astrocytes in spared but reactive neural tissue retain their interactions with functioning neurons. These hypertrophic reactive astrocytes are fundamentally different, both phenotypically and functionally, from newly proliferated astrocytes that form the narrow scar borders around non-neural lesion cores (Figure 1B and ref. 20). This spared but reactive tissue is undergoing substantial synapse turnover and circuit reorganization as discussed below.

Anatomically complete or incomplete SCI lesions

SCI lesions and their different compartments present diverse challenges for repair strategies. Anatomically complete lesions will require restoration of neural connectivity across large and hostile non-neural lesion cores (Figure 1C), whereas anatomically incomplete injuries may benefit from augmentation of spontaneous circuit reorganization in spared but reactive neural tissue (Figure 1A). Anatomically complete SCI can be caused by single large lesions or multiple small lesions that span the entire spinal cord and result in the complete absence of neural connectivity across the lesion site (Figure 2A). Anatomically complete SCI is functionally complete, in that no voluntary motor control or sensory perception is conveyed across the lesion. Anatomically incomplete SCI can be formed by one or more smaller lesions that spare sufficient neural tissue so as to preserve or allow the spontaneous re-formation of some neural connectivity across the lesion site, either directly from supraspinal sources or indirectly via propriospinal neurons (Figure 2A). Anatomically

incomplete SCI can be associated with varying degrees of preserved or restored functions, or can be functionally complete in situations where neural connections may be present but are insufficient to mediate functions.

Spontaneous circuit reorganization occurs after all SCI

Fifty years ago, Raisman first showed that after injury-induced loss of one set of synaptic inputs to a forebrain region, new synapses are spontaneously formed by surviving local terminals derived from a different set of inputs (21). It is now clear that this type of synapse and circuit reorganization occurs spontaneously after all forms of CNS injury, including after SCI, and that it can be associated with either adaptive or maladaptive functional changes. After anatomically complete or incomplete SCI, synapse loss is prominent in spared but reactive neural tissue, and this synapse loss spontaneously leads to new synapse formation that can derive from different sources, including immediately local surviving terminals or sprouting of more distant axons (Figure 1A; Figure 2, A and B; and refs. 22–24). In some cases, spontaneous synapse turnover and circuit reorganization can lead to maladaptive consequences such as muscle spasticity (25), autonomic dysreflexia (26), or neuropathic pain (27, 28). In other cases, spontaneous circuit reorganization can be adaptive and restore function after incomplete SCI, as in the spontaneous bilateral locomotor recovery that occurs after unilateral hemisection SCI (Brown-Séquard syndrome) in spite of permanent loss of descending supraspinal connections on the injured side (23, 24, 29–32). Dissecting underlying cellular and molecular mechanisms and learning how to beneficially modulate spontaneous adaptive or maladaptive circuit reorganization are important targets for SCI research.

Distinguishing axon regeneration, local sprouting, and synapse plasticity

Axon responses to CNS injury are often referred to simply as degeneration or regeneration. However, it is important to delineate multiple forms of potential axon responses (33, 34), which are summarized here as (a) axon degeneration and retraction, (b) axon regeneration attempts across non-neural lesion core, (c) axon regeneration from transected ends through spared reactive neural tissue, (d) axon sprouting of branches that grow through spared neural tissue, and (e) local synaptic plasticity in spared neural tissue (Figure 2B). Effective treatment strategies will need to be based on an understanding of how different forms of axon growth are regulated by different specific molecular mechanisms in different lesion compartments.

Diverse regulation of axon growth and synapse remodeling

Multiple factors regulate axon growth and synapse formation after SCI, including neuron-cell-autonomous mechanisms, effects mediated by other cells, and the presence or absence of various molecular signals. Although the notion of environmental axon growth inhibition has received much recent attention, mounting evidence indicates that axon growth and circuit repair are regulated as much, or more, by neuron-cell-autonomous mechanisms, access to essential growth-promoting factors, and other regulatory mechanisms.
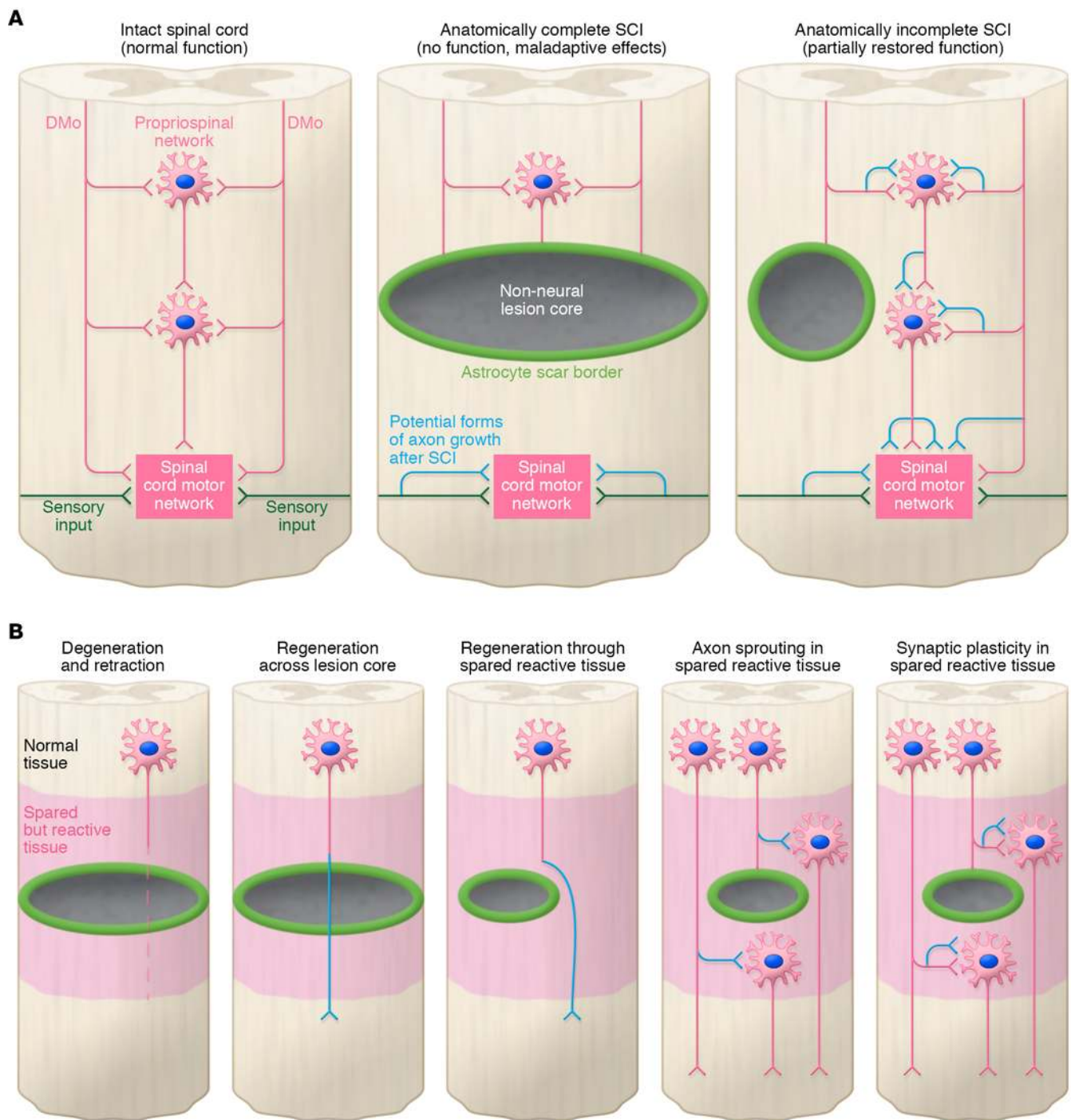
Figure 2. Circuits reorganize after SCI. (**A**) Different types of circuit reorganization after different types of SCI. Anatomically complete SCI is associated with synaptic plasticity that can give rise to maladaptive effects such as muscle spasticity or autonomic dysreflexia. Anatomically incomplete SCI can also give rise to axon growth and synaptic plasticity that can partially restore function. (**B**) Different potential growth responses of axons and synapses after SCI. DMo, descending motor projections.

Neuron-cell-autonomous regulation of axon growth. During development, CNS neurons exhibit robust axon growth; but even during development axons do not grow by default and require environmental factors that attract and support growth (35, 36). CNS neurons lose their intrinsic capacity to grow axons as they mature (36, 37) and exhibit poor reactivation of intrinsic programs for axon growth after injury (38). Transected adult CNS

axons exhibit poor spontaneous formation of new growth cones (38, 39). Neuron-intrinsic mechanisms regulating the capacity to regrow injured axons are being dissected. Early studies showed that transected CNS branches of sensory neurons are 100 times more likely to grow into peripheral nerve grafts after SCI if their peripheral branches have been cut previously by so-called priming injuries (40). Such priming injuries also trigger some sensory

axon regeneration through SCI lesions (41). Stimulation of sensory neurons with cAMP mimicked priming injury effects (42–44). Ground-breaking work from Zhigang He and colleagues showed that signaling through PTEN, mTOR, STAT3, and SOCS3 pathways intrinsically regulates the axon-regenerative capacity of mature retinal and corticospinal neurons (38, 45–47). Additional neuron-intrinsic regulators of axon regrowth are depicted in Figure 3A (38, 48–51).

Multicellular influences on axon regrowth. Multiple cell types influence axon regrowth after SCI, including astrocytes, NG2-OPCs, fibroblast-lineage cells, microglia, hematogenous macrophages, and other immune cells. Different cell types are present in different lesion compartments. Many molecular regulators are produced by multiple cell types.

Astrocytes are present in two SCI lesion compartments, scar borders and spared but reactive tissue. Astrocyte phenotype and function differ fundamentally in these two compartments (20). Scar-forming astrocytes are almost all newly proliferated, do not exhibit individual domains, and intertwine to form glia limitans borders that restrict inflammation and separate non-neural lesion core from adjacent functioning neural tissue (refs. 13, 15, 52, and Figure 1, A and B). Scar-forming astrocytes were long thought to be the primary cause for the failure of CNS axons to regenerate (53, 54), but this notion is now strongly challenged by studies showing that appropriately stimulated CNS axons regenerate robustly in spite of astrocyte scar formation in optic nerve (45, 47, 55) and in spinal cord (19). Neither preventing astrocyte scar formation nor removing chronic astrocyte scars leads to spontaneous axon regeneration of descending motor, ascending sensory, or serotonin axons (19). Scar-forming astrocytes express the axon growth–supporting matrix protein laminin (19, 56), and antibody blockade of laminin-integrin binding attenuates stimulated axon regeneration after SCI (19). Mature injured CNS axons regrow along astrocytes after CNS injury when stimulated by appropriate growth factors (57) or by genetic activation (58). Grafts of progenitor-derived astrocytes support axon regeneration though SCI lesion cores (59–61). Targeted disruption of astrocyte scar formation attenuates stimulated axon regeneration after SCI (19). Axons grow along astroglia during development (62–64). Spontaneous axon regeneration after SCI in lower vertebrates occurs along astroglial bridges formed in response to connective tissue growth factor (65). Thus, astrocyte scars may aid rather than hinder axon regeneration (19).

Reactive astrocytes in spared tissue of SCI lesions differ fundamentally from scar-forming astrocytes. They are nonproliferative and hypertrophic, maintain their basic structure and individual domains, and interact functionally with neurons and synapses (ref. 20 and Figure 1, A and B). Emerging evidence suggests that these hypertrophic reactive astrocytes are critically involved in inducing local axon sprouting and regulating synapse plasticity and circuit reorganization (Figure 3A and discussed below).

NG2-OPCs are glial cells with progenitor potential and other functions (66, 67). After CNS injury, NG2-OPCs are present in two compartments, scar borders and spared reactive neural tissue (Figure 1A and Figure 3A). In scar borders, NG2-OPCs are hypertrophied and are reported to restrict axon dieback and axon regrowth (68–70). Other reports show that robust axon growth can

be stimulated through areas of dense NG2 in vivo (71, 72), NG2-OPCs robustly support axon regrowth (73), and deletion of *NG2* fails to increase spontaneous axon growth in vivo (74). Thus, with respect to axon regrowth after SCI, the roles of NG2-OPCs and of NG2 are not fully understood.

Pericytes and fibroblast-lineage cells are major components of non-neural lesion core (Figure 1A, Figure 3A, and refs. 8, 9). Molecules produced by these cells have axon-inhibitory properties in vitro, including certain collagens and chondroitin sulfate proteoglycans (CSPGs) (75). Nevertheless, failure of axon regrowth through lesion core may depend as much on the absence of required growth-stimulatory chemoattractive molecules as on the presence of putative inhibitors. For example, fibroblast cell grafts support robust axon regeneration after SCI but do so only when they produce specific axon-stimulatory growth factors (76). Growth factors required for sensory axon growth during development are absent from SCI lesion core, and delivery of those growth factors via biomaterial depots attracts substantive sensory axon regrowth into SCI lesion cores (19).

Immune and inflammatory cells also influence regrowth of injured CNS axons. Activated macrophages induce retraction and dieback of axons after SCI (77). In contrast, neutrophils around neuronal cell bodies can stimulate axon regeneration (78). More work is needed on understanding how inflammatory cells influence axon regrowth.

Environmental molecular regulators of axon regrowth. Developmental axon growth and guidance are regulated by four main types of environmental molecular cues: diffusible chemoattraction, contact chemoattraction, diffusible chemorepulsion, and contact chemorepulsion (79). Similar environmental cues influence axon regrowth after SCI (ref. 80 and Figure 3A). Notably, the effects of certain cues are context-dependent and can be modified by receptor-mediated signaling in growth cones that changes responses from repulsion to attraction or vice versa (81, 82). Both the presence and absence of molecular cues can influence axon regrowth capacity.

Diffusible chemoattraction was first implicated in regulation of CNS axon regeneration by Cajal and Tello, who showed that (a) transected CNS axons would regrow into peripheral nerve grafts, (b) killing cells in the grafts with chloroform prevented CNS axon regeneration into them, and (c) chemical extracts obtained from peripheral nerves and loaded into cellulose matrix attracted CNS axon regrowth in vivo (see pages 388–392 and 742–750 in ref. 83). Based on these observations, Cajal theorized that in contrast with developing CNS and injured peripheral nerves, the injured adult CNS lacked diffusible chemoattractants required to promote axon regrowth. Modern axon-tracing technologies confirmed both that CNS axons regrow into peripheral nerve grafts (84, 85), and that this regrowth is critically dependent on neurotrophic factors produced by live cells in grafts (86, 87). Freezing nerves and killing resident cells prevented CNS axon growth into them, and injection of nerve growth factor (NGF) into frozen grafts restored their ability to attract regeneration of NGF-sensitive CNS axons, showing that permissive matrix alone is not sufficient for regrowth (86, 87). Numerous subsequent studies show that delivery into SCI lesions of developmentally active neurotrophins such as brain-derived neurotrophic factor
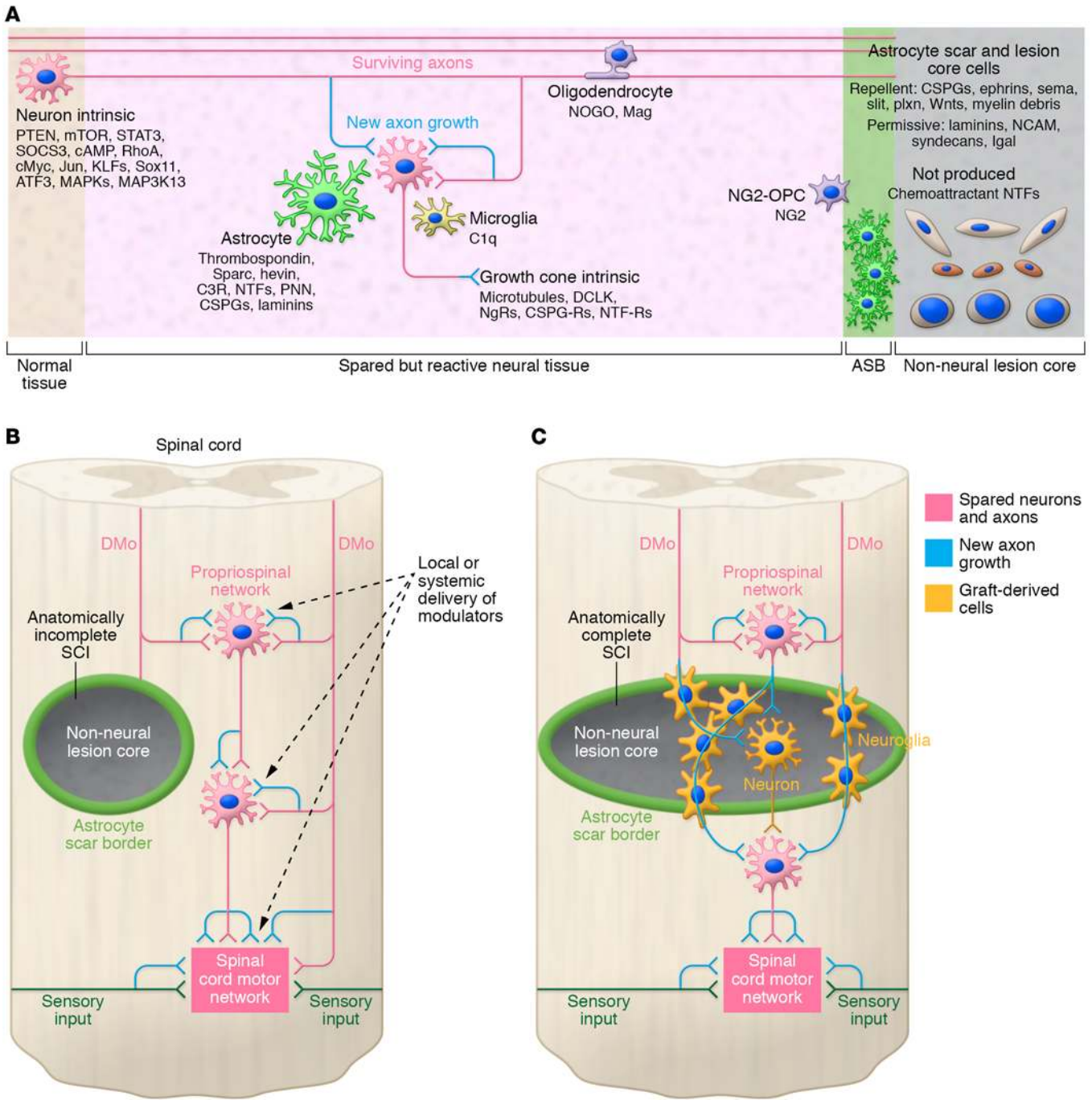
Figure 3. Growth potential of axons and synapses after SCI can be regulated by diverse cells and molecules in different lesion compartments. (A) Multiple cell types and molecules can influence and regulate the growth potential of axons and synapses in different lesion compartments. **(B)** Modulation of axon sprouting, synapse plasticity, or synapse function in spared but reactive and reorganizing neural tissue by delivery of molecules that modify peri-neuronal net, synapse formation, or synaptic transmission. **(C)** Transplantation to repopulate non-neural lesion core with neural cells that either provide new relay neurons, or provide neuroglia that support host axon regrowth, to restore circuit connectivity across the lesion. CSPG-Rs, CSPG receptors; KLFs, Krüppel-like factors; NgRs, NOGO-receptor; PNN, Peri-Neuronal-Net; NTFs, neurotrophic factors; NTF-Rs, neurotrophic factor receptors.

(BDNF), neurotrophin-3 (NT3), glia-derived neurotrophic factor (GDNF), and others, which are not detectably produced in the lesions, will promote injured axon regrowth in an axon-selective manner (19, 88, 89). Such findings highlight the importance of the absence of required chemoattractive molecules in the failure of spontaneous axon regeneration after CNS injury.

Contact chemoattraction or growth support is mediated by molecular families such as laminins, syndecans, and heparan sulfate proteoglycans (19, 90–93). During development, axon growth on substrates is determined by combinatorial mixtures of attractive and repellent molecules, and gradients of such mixtures guide growth directionality (79, 94). The degree of growth

support is determined by relative proportions of attractive and repellent molecules, such that increasing the concentration of one type of cue can overcome the effects of another (94, 95). Blockade of laminin-integrin interactions can prevent axon growth in vitro (90) and axon regeneration stimulated in vivo after SCI (19).

Diffusible chemorepulsion is mediated by Wnt signaling, semaphorins, netrins, and other molecules that create gradients during development (93, 96). Interestingly, semaphorins and netrins can be either repellent or attractive to different axons or even to the same axons depending on combinatorial signaling in growth cones (81, 82, 93). Roles of these molecules after SCI are being studied (93, 96, 97). Removal of semaphorins alone or in combination with removal of putative myelin inhibitors is insufficient for spontaneous regrowth of serotonin axons after SCI (98).

Contact chemorepulsion and growth inhibition of injured axons have been studied extensively, in particular as regards myelin-associated molecules (99) and CSPGs (54). The ability of these molecules to inhibit or repulse axon growth is clearly potent in vitro (95, 100). Nevertheless, their roles in vivo in the injured CNS with respect to different forms of injury-induced axon growth (Figure 2B) are less clearly defined and are being refined as experimental tests become more focused. Their effects on local synapse plasticity may be more relevant than effects on axon regrowth across lesions. For example, aggrecan, the prototypical CSPG used to demonstrate axon-inhibitory effects in vitro (95, 101), is not detectably present in SCI lesion core or astrocyte scar, but it is heavily present in perineuronal nets in spared and reorganizing neural tissue, where it may restrict synaptic plasticity (19, 102–104). Although NG2 (encoded by *Cspg4*) can inhibit axon growth in vitro, multiple in vivo studies show that robust axon regrowth occurs in the presence of NG2 and that deletion of *Cspg4* does not induce spontaneous axon regeneration (71–74). Digestion or blockade of CSPGs does not reproducibly enable spontaneous axon regrowth across anatomically complete SCI lesions with non-neural lesions cores. Deleting or blocking myelin is not sufficient to enable long-distance axon regrowth across severe SCI lesions and non-neural lesion cores (98, 105, 106). Thus, beneficial behavioral effects reported after blocking of either myelin-associated growth inhibitors or CSPG signaling are likely to be related to effects on synaptic plasticity and circuit reorganization after incomplete SCI (Figure 3B) rather than to enabling of axon regrowth across anatomically complete SCI (99, 102, 104, 107, 108). It also should be emphasized that molecular cues are not absolute, such that exposure to specific growth factors can reprogram growth cones to ignore inhibitory cues both during development and after injury (42, 43, 82). These observations highlight the importance of understanding the cell biology and molecular mechanisms regulating different forms of axon growth in different lesion compartments when interpreting behavioral observations and developing therapeutic strategies.

Multicellular and multimolecular regulation of synapse reorganization. As discussed above, trauma-induced synapse loss in spared but reactive CNS tissue spontaneously leads to formation of new synapses derived either from local surviving terminals or from more distant axons (Figure 1A, Figure 2, and Figure 3A). This spontaneous injury-induced synapse plasticity can be

associated with either adaptive or maladaptive effects, both of which represent important therapeutic targets. Understanding of the underlying cellular and molecular mechanisms is being facilitated by studies of synapse development that have identified critical roles for microglia and astrocytes, and for matrix molecules of the perineuronal net.

Microglia remove synapses from neurons responding to distant retrograde or anterograde injury (109). During development, microglia help sculpt postnatal circuits by pruning supernumerary synapses as regulated by classical complement signals, C1q, C3, and C3R (110–112), and the fractalkine receptor CX3CR1 (113). Related mechanisms regulate synapse turnover in adulthood, and under conditions of disease and trauma (112).

Astrocytes extend fine processes that interact with all synapses and exert activities critical for normal synapse and circuit function (20, 114) and in circuit development (114, 115), where they secrete thrombospondins, glypicans, and hevin to promote synapse formation and activity (116–120); release the diffusible axon guidance cue semaphorin-3a, to regulate axon targeting (121); express phagocytic machinery for synapse pruning (122); and deposit extracellular matrix proteins, including tenascins and sulfated proteoglycans, which contribute to plasticity-restricting perineuronal nets (104). Increasing evidence links equivalent mechanisms to synapse plasticity and circuit reorganization after SCI, brain injury, and stroke. Hypertrophic astrocytes closely intermingle with viable neurons in spared but reactive tissue (refs. 5, 13, 123; Figure 1, A and B; and Figure 3A), where they may influence axon sprouting and synapse plasticity by modulating expression of perineuronal net–associated proteins such as neurocan and tenascin-C (124, 125) or by producing thrombospondin (126).

The perineuronal net, composed of matrix molecules including multiple CSPGs and tenascins, constrains synaptic plasticity and circuit reorganization (102, 104). During postnatal development, the perineuronal net is involved in closing critical periods of synaptic plasticity, such that enzymatic digestion of the perineuronal net by chondroitinase can reopen that plasticity (104, 127). Signaling related to NOGO (also known as reticulon 4 [RTN4]) and its receptors is also implicated in restricting synaptic plasticity via perineuronal net molecules during development and after injury (99, 102). Increasing evidence suggests that beneficial effects of blocking CSPGs or NOGO receptors after incomplete SCI are related to effects on perineuronal nets and synaptic plasticity in spared but reactive neural tissue (99, 102, 108) rather than axon regeneration across lesions.

Repair strategies

Developing reliable repair strategies will require understanding how potential interventions influence the cell biology of different SCI lesion compartments. The requirements to beneficially modulate synaptic plasticity in spared but reactive neural tissue (Figure 3B) will differ substantively from the requirements to bridge new axon growth across non-neural lesion cores of anatomically complete lesions (Figure 3C). Different cellular and molecular targets are now under investigation to improve outcome after SCI by providing tissue protection, modulating circuit reorganization, or regulating neural bridging connectivity across lesions.

Neuroprotection and control of inflammation. Damage caused by initial physical forces and anoxia may be beyond protective intervention. However, subacute phases after SCI may be amenable to delayed protective interventions that target and control peripheral infections and other peripheral sources of circulating inflammatory stimuli. Epidemiological studies show that comorbid infections such as pneumonia worsen functional outcome after human SCI (128–130). Potential cellular mechanisms may involve prolonged inflammation at the lesion site that results in increased tissue damage. Circulating microbial signaling molecules such as LPS can influence CNS glia and markedly alter their transcriptional profiles toward neurotoxic phenotypes (131, 132). After SCI, peripheral infections or other sources of inflammation may give rise to blood-borne molecular signals such as LPS or cytokines, or to activated immune cells that prolong inflammation within SCI lesions and exacerbate tissue damage. Early inflammation after traumatic injury is beneficial and should not be inhibited (133), but prolonged inflammation has neurodegenerative potential (134). The several-week time frame after SCI during which multiple cell types interact and organize into mature lesions with discrete compartments (5, 13) presents a window for potential protective interventions to maximize the sparing of functional tissue.

Repair strategies to restore neural connectivity and functions. Neuroprotective interventions alone will not be sufficient after SCI. Repair strategies will be required to improve or restore lost functions. Approaches to SCI repair include modulation of circuit reorganization after incomplete SCI (Figure 3B) and bridging of axons across complete SCI (Figure 3C). Both approaches rely conceptually on observations that after SCI, descending supraspinal axons in corticospinal and reticulospinal tracts can sprout and make new functional contacts (refs. 22–24, 30, 32, 135–138; Figure 2; and Figure 3, B and C). Functional studies show that relay circuits formed between supraspinal pathways and descending propriospinal neurons can relay supraspinal commands that control voluntary locomotion in rodents (31), and can mediate fine finger movements in nonhuman primates (138–140). Such observations provide a rationale for repair strategies that seek either to augment spontaneous relay circuit formation and efficacy after incomplete SCI (Figure 3B), or to restore short-distance neural connectivity across anatomically complete SCI lesions (Figure 3C).

Beneficially modulating circuit reorganization in spared neural tissue. After SCI, substantial spontaneous synapse and circuit reorganization occurs in spared but reactive neural tissue (Figure 2 and Figure 3, A and B). Perineuronal nets constrain local synaptic plasticity and represent potential targets for therapeutic intervention. Enzymatic digestion of perineuronal net molecules or blockade of their neuronal receptors can augment task-specific rehabilitation after incomplete SCI (99, 102, 108). Perineuronal reactive astrocytes promote re-formation of excitatory synaptic inputs after injury via a thrombospondin- and STAT3-dependent mechanism (126). Sprouting of descending supraspinal axons to form new relay connections after incomplete SCI can be augmented by local delivery of growth factors (141), by chronic electrical stimulation (142), or by the increasing of intrinsic neuron growth capacity by deletion of PTEN and SOCS3 (143). Strategies that combine chemical modulation of neural activity with epidural stimulation of lumbar locomotor centers have shown effi-

cacy in restoring voluntary locomotor activity after incomplete SCI in rodents and humans (144–147). Such approaches have also shown benefit in regulating breathing (148) or autonomic functions such as bladder control (149). On the other hand, spontaneous synapse reorganization in spared but reactive neural tissue can lead to maladaptive consequences such as muscle spasticity (25), autonomic dysreflexia (26), or neuropathic pain (27, 28). More work is needed to understand mechanisms that drive maladaptive or adaptive synapse formation after SCI and how they can be beneficially modulated.

Reestablishing neural connections across anatomically complete SCI lesions. Anatomically complete lesions pose the biggest challenge to biological repair after SCI. Emerging repair strategies include facilitating the formation of new relay circuits either by endogenous axons or by grafted neurons that bridge lesions and connect into the distal propriospinal network (Figure 3C). Current evidence suggests that neural connections could be reestablished by combination of local delivery of axon chemoattractive factors and a growth supportive matrix with activation of intrinsic neuronal growth programs. Various means of reactivating neuron-intrinsic growth programs are emerging, including modulating specific genetic pathways (Figure 3A and discussed above), providing neuronal cell bodies with specific growth factors (150) or inflammatory factors (51, 55, 151), or stimulating neuronal activity (152). There is a growing list of chemoattractive growth factors that stimulate and guide regrowth of specific axons after SCI, including BDNF and NT3 for sensory axons (19, 88), GDNF for propriospinal axons (89), and IGF1 for corticospinal axons (153), as well as pleotropic growth factors such as FGF and EGF that act in beneficial but undefined ways (154–156). Modulating axonal cytoskeleton may improve growth cone formation and axon regeneration (38, 157, 158). Biomaterials are being explored to deliver specific molecules and bridge neural connectivity across SCI lesions, as reviewed elsewhere (19, 159, 160).

Cell grafts to bridge severe SCI lesions. Cell grafting after SCI has a long history comprehensively reviewed elsewhere (161, 162). In humans, anatomically complete lesions are dominated by large non-neural lesion cores (Figure 1C and ref. 6). Here, we briefly mention cell grafting strategies to repopulate non-neural lesion core with neuroglia that bridge host axons across the lesion, or to provide propriospinal relay neurons that receive host afferent input and relay information across the lesion to spared host neurons (Figure 3C). Repopulating non-neural lesion core with neuroglia may be required not only to provide axon regrowth–permissive substrates, but also to provide long-term support of regenerated axons or grafted neurons. Without glia, neuronal elements are unlikely to persist. Neuroglial cell grafts that can support host axon regrowth include Schwann cells (89, 161, 163) and astroglia (59–61). Coaxing of axons to regrow past grafts into spared host tissue and form connections can be facilitated by chemoattractive growth factors (88). Regarding neuronal grafts, there is now elegant proof-of-principle evidence that transplanted embryonic neurons can integrate into and function in adult neocortical circuits (164). Embryonic neurons and neural stem cell–derived neurons and glia transplanted after SCI can repopulate non-neural lesion core and receive host inputs, and send extensive connections into host, some of which form functional connections (165–168). Host

axons exhibit functionally appropriate preferences when forming contacts with grafted neurons (169). Injectable biomaterial carriers can improve graft survival by providing physically and/or chemically based protection (61, 159, 160). Biomaterial carriers can also deliver molecules to guide neural stem cell maturation and integration with host neurons, and may facilitate translation of neural cell transplantation strategies (160).

Concluding remarks

Substantial advances have been made in identifying multicellular interactions and molecular mechanisms that shape the response to SCI. Mature SCI lesions exhibit three main tissue compartments: (a) central non-neural lesion cores, often referred to as fibrotic or mesenchymal scar, (b) narrow astroglial scar borders that intimately surround lesion cores, and (c) large surrounding zones of spared neural tissue that is functional but reactive. Each

of these tissue compartments exhibits a unique cell biology, and deepening our understanding of their markedly different cellular and molecular interactions will be fundamental to developing rationally targeted repair strategies.

Acknowledgments

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