HHS Public Access

Author manuscript

Nat Neurosci. Author manuscript; available in PMC 2018 July 01.

Published in final edited form as:

Nat Neurosci. 2018 January ; 21(1): 9–15. doi:10.1038/s41593-017-0033-9.

The diversity and disparity of the glial scar

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Abstract

Injury or disease to the CNS results in multifaceted cellular and molecular responses. One such response, the glial scar, is a structural formation of reactive glia around an area of severe tissue damage. While traditionally viewed as a barrier to axon regeneration, beneficial functions of the glial scar have also been recently identified. In this Perspective, we discuss the divergent roles of the glial scar during CNS regeneration and explore the possibility that these disparities are due to functional heterogeneity within the cells of the glial scar—specifically, astrocytes, NG2 glia and microglia.

Regeneration of the damaged mammalian CNS continues to represent the holy grail of regenerative medicine. The CNS is a complex network of neuronal connections that are supported, refined and modified by a population of glial cells of increasingly appreciated diversity, including astrocytes, oligodendrocytes and microglia. CNS pathologies—including injury or trauma, infection and neurodegenerative and autoimmune diseases—have debilitating and costly effects on human life. Therefore, much effort has focused on understanding the molecular mechanisms underlying endogenous cellular responses to injury and disease in the mammalian CNS. One cellular response that has sparked wide debate over its conflicting and varied roles during CNS repair is the glial scar.

The glial scar has been widely studied in the context of spinal cord injury (SCI), but it also occurs after traumatic brain injury, after ischemic stroke and in many neurodegenerative diseases, including multiple sclerosis. Upon damage to the CNS, newly proliferated reactive astrocytes[1], NG2 glia and microglia form a compact border around an area of severe tissue damage, or lesion core. The lesion core contains a mixture of perivascular-derived fibroblasts, pericytes, ependymal cells and phagocytic macrophages[2]. Some debate over the glial scar is likely caused by the differing and ambiguous use of the term. While multiple previous studies have referred to the entire CNS lesion as the glial scar, this can be misleading because the lesion core contains very few glial cells. Furthermore, the lesion core (also referred to as the fibrotic or mesenchymal scar) contains a rich deposit of extracellular matrix proteins that largely inhibit axonal growth and remyelination. Therefore, we will

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Competing interests

The authors declare no competing financial interests.

instead use the term “glial scar” to refer only to the glial cell border that surrounds the non-neural lesion core (Fig. 1).

Traditionally, the glial scar has been viewed as a barrier to CNS regeneration. However, over the past decade, increasing evidence has suggested that the glial scar can also support CNS repair. Simultaneously, increased evidence of the complexity and heterogeneity of glial cell physiology implies that glial cells within the scar may be more heterogeneous than previously believed. In this Perspective, we discuss functional heterogeneity of reactive astrocytes, NG2 glia and microglia—the three primary cell types that make up the glial scar. We then examine the contrasting roles of the glial scar during CNS repair in view of this cellular heterogeneity. We argue that further understanding of the distinct roles played by different glial cell populations, both within and across different injuries and diseases, is critical for developing effective future therapies.

Inherent heterogeneity of the glial scar

Damage to the mammalian CNS results in widely varied cellular, molecular and structural changes in the lesion site and nearby affected regions. This is due to (i) the myriad of CNS diseases and injuries, (ii) the variability among individuals with a specific injury or disease, (iii) the location within the brain and severity of the insult, and (iv) the heterogeneous cell populations that respond differently to injury or disease. Increased understanding of CNS cellular diversity raises the question of whether glial scar heterogeneity is fundamentally shaped by functionally diverse glial populations that make up the scar. Furthermore, are the divergent functions of the glial scar due to distinct cellular responses that vary with anatomical location and time after injury?

Is glial cell diversity preserved in the injured CNS?

Originally believed to be nothing more than support cells for neurons, glial cells are now accepted to play critical roles in CNS development, homeostasis and repair. Because of these diverse functions, the perception that astrocytes, NG2 glia and microglia are homogenous cell populations in the healthy CNS has been largely rejected. Astrocytes display distinct regional identities and functional properties in both the mouse spinal cord[3,4] and adult brain[5–7]. A recent study by the Deneen laboratory identified five distinct astrocyte subpopulations that differentially support synaptogenesis[5]. Similarly, gray and white matter NG2 glia exhibit differences in proliferation and differentiation rates[8,9], as well as physiological properties[10]. Within a given region, NG2 glia also display differences in protein expression[11]. Lastly, microglia from different regions of the adult mouse brain display distinct gene expression profiles[12] and were recently found to differ significantly in morphology, membrane properties and lysosome content[13]. Overall, these studies indicate that both intrinsic factors and environmental cues are likely to direct neural-circuit-specialized or region-specific glial cells. Whether this diversity is preserved following injury or disease and whether it shapes distinct cellular responses in the damaged CNS remain critical questions to address.

Functional diversity of glial cells in the injured CNS

Reactive astrocytes

Reactive astrocytes have been traditionally identified by hypertrophy and high expression of glial fibrillary acidic protein (GFAP), but increasing evidence now indicates a more complex and heterogeneous nature. The endogenous astrocytic cellular response to CNS damage ranges from mild reactive astrogliosis following mild non-contusive trauma to formation of a compact astroglial scar, and includes a wide spectrum of changes in gene expression, proliferation, morphology and physiology[2]. Transcriptional profiling of reactive astrocytes isolated from ischemic stroke and neuroinflammation mouse models found that, despite a small core of shared genes, reactive astrocytes upregulate genes specific to the type of injury or disease[14]. Interestingly, the Barres laboratory recently characterized the functional properties of neuroinflammation-induced reactive astrocytes (termed A1 astrocytes) and found that they secrete a neurotoxin that promotes neuronal and oligodendrocyte cell death[15]. They identified A1 reactive astrocytes in tissue samples from patients with neurodegenerative diseases, suggesting that A1 astrocytes may represent a new common cellular target for therapies. In contrast to these results, reactive astrocytes induced by ischemia appear to acquire a more protective phenotype, increasing expression of neurotrophic factors and transferring mitochondria to injured neurons[14,16]. The mechanisms regulating these diverse functional properties remain unknown, but evidence suggests that environmental cues, especially microglia-derived signals[15,17], are important.

Distinct subtypes of reactive astrocytes are also found in individual animal models of CNS injury. The degree of astrogliosis is highly dependent on the distance of astrocytes from lesions, with mildly reactive astrocytes found distal to the lesion site[1]. However, distinct reactive astrocyte populations have also been observed within the same CNS region. For example, in spinal cord glial scars, reactive astrocytes have been found to express differing levels of GFAP, nestin and brain lipid-binding protein (BLBP)[18]. Furthermore, only subsets of astrocytes were found to react to a cortical stab injury, either by polarization toward lesion sites or by proliferation[19]. These proliferative astrocytes were largely localized to juxtavascular sites, indicating that niche-specific cues may direct functional properties of reactive astrocytes (see Box 1). Interestingly, this proliferative population of reactive astrocytes was also shown by clonal analysis to be derived from distinct progenitors[20], suggesting that one source of reactive astrocyte heterogeneity may be distinct cellular origins. In support of this, neural stem cell (NSC)-derived reactive astrocytes have been shown to contribute to glial scars in the brain[21], while a recent study found little contribution of NSC-derived reactive astrocytes to SCI-induced glial scars[22]. Therefore, reactive astrocyte heterogeneity is dependent on the site of injury. Whether NSC-derived reactive astrocytes are more permissive or inhibitory for regeneration remains unknown. However, studies have found that NSC-derived reactive astrocytes are more important in restricting inflammation[23] and can be converted to neurons in vivo[21], indicating that they may be a useful cellular target for promoting repair.

Taken together, these findings raise several important questions. Do specific subsets of astrocytes respond to different types of CNS damage? Is reactive astrocyte heterogeneity

primarily induced by extrinsic factors (that is, context-dependent cues and non-cell-autonomous mechanisms) or intrinsic factors (cell-autonomous mechanisms)? To begin answering these questions, reactive astrocytes from different CNS regions following the same injury must be compared. With recent advances in molecular tools (Fig. 2), the ability to identify disease- or injury-specific reactive astrocytes will have important implications for developing new therapies[24].

NG2 glia

NG2 glia constitute approximately 4–5% of the total cells in the postnatal and adult brain[25] and a large percentage of the proliferating cells in the glial scar[26]. Following injury and in many neurodegenerative diseases, NG2 glia in the glial scar share several characteristics with reactive astrocytes: cellular hypertrophy, increased proliferation and increased expression of proteoglycans[26]. However, their function remains controversial, partly because there are multiple cell types in the scar and lesion core that upregulate the proteoglycan NG2 after injury: oligodendrocyte progenitor cells, pericytes, Schwann cells and macrophages. Unfortunately, this makes it difficult to interpret studies where further qualifying characteristics were not used to specify the cell type being investigated (for example, coexpression of NG2 and Olig2 for oligodendrocyte progenitor cells). Therefore, we use the term NG2 glia to refer specifically to NG2-expressing cells that give rise to glia.

Most studies have focused on mechanisms underlying the differentiation of NG2 glia into oligodendrocytes and their contribution to remyelination. Several developmental signals have been found to be upregulated in the glial scar that promote NG2 glial proliferation and migration to trauma sites: fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), Wnts[27]. However, many NG2 glia in the scar do not differentiate into myelinating oligodendrocytes. Interestingly, NG2 glia have been found to also generate astrocytes and Schwann cells following injury. In NG2-Cre estrogen receptor (CreER) mice, up to 25% of reporter-labeled cells express GFAP 1 week after SCI[28], while only 8% express GFAP 10 d after cortical stab injury[29]. Both of these numbers decrease to 10% or less at 1 month after injury. Conversely, very few (~3% of) platelet-derived growth factor receptor-α (PDGFRα)-CreER reporter-labeled cells express GFAP in the spinal cord after lysolecithin-induced demyelination[30]. Instead, ~20% of these reporter-labeled cells expressed the Schwann cell marker periaxin[30]. This is especially surprising because Schwann cells are neural crest–derived, whereas NG2 glia arise from the neural ectoderm. NG2-derived Schwann cells were also identified following contusion SCI[31], but not after cortical stab wound injury[29]. Together these results indicate that the differentiation potential of NG2 glia greatly differs depending on the type and location of CNS injury. Whether all NG2 glia acquire the ability to transdifferentiate or if certain subsets are restricted to specific lineages remains unknown. While NG2-derived Schwann cells likely contribute to remyelination, albeit at low levels, the role of NG2-derived reactive astrocytes remains unclear. Because NG2-derived reactive astrocytes are a transient population that do not upregulate proteins expressed highly by scar-forming astrocytes (nestin and vimentin), it is possible that they resemble more ‘helpful’ reactive astrocytes[32]. Overall, NG2 glia participate in the formation and resolution of glial scars beyond serving simply as a reservoir for generating oligodendrocytes.

Microglia

While microglia are widely known for their role as sentinels and effectors of the CNS immune response, evidence now shows that they display an array of functions: synaptic organization[33], phagocytosis of cellular debris[34], trophic support for neurons[35] and the regulation of neuronal excitability[36]. Microglia are among the first cells to respond to CNS injury or disease, proliferating and migrating to lesion sites. As with reactive astrocytes and NG2 glia, it remains uncertain to what extent microglia both promote and hinder CNS recovery and repair[37]. This is likely context-dependent, varying with regard to the type of injury or disease, environmental cues and phase of recovery. Recent evidence for brain-region-specific[12] and neurodegeneration-specific[38] microglial gene expression signatures highlights how the environment influences microglial phenotype. Interestingly, microglia from distinct regions of the adult mouse brain display differences in expression of immune regulation and activation genes[12], indicating that the microglial cellular response may vary depending on the site of CNS damage.

Microglia have been largely grouped into two types: a ramified or ‘resting’ state, critical for CNS homeostasis, and a reactive or amoeboid state, induced by CNS damage. Reactive microglia are sometimes further classified into M1 (classically activated, pro-inflammatory) and M2 (alternatively activated, anti-inflammatory) subtypes, but there is debate as to whether this classification is appropriate[39]. Evidence has shown that M1 microglia are the dominant phenotype following SCI[40], stroke[41] and traumatic brain injury[42]. Lipopolysaccharide-induced M1 microglia promoted a toxic reactive astrocyte phenotype via secretion of interleukin-1α, tumor necrosis factor and complement component C1q[15]. M1 microglia were also found to promote proliferation of oligodendrocyte progenitor cells following focal demyelination, while M2 microglia promoted oligodendrocyte differentiation via secretion of activin-A[43]. Therefore, stimulating microglial polarization toward an M2 phenotype has been promoted as a potential therapeutic tool.

This interpretation is likely an oversimplification of a spectrum of several different microglial activation states with different functions. Because reactive microglia are largely indistinguishable from infiltrating macrophages, it has been difficult to obtain direct evidence for functionally diverse microglial subtypes in the glial scar. Recent technical advances in purification protocols, single-cell sequencing and unique cell surface markers[44] will hopefully result in better insight into microglial heterogeneity and its effects on axon regeneration (Fig. 2). Determining how environmental cues differentially affect microglia and the subsequent cross-talk between glial cells and regenerating axons in the glial scar remains an important but daunting challenge for neuroscientists. Overall, the presence of functionally heterogeneous cell types in the glial scar is likely to strongly contribute to the contrasting roles of the glial scar during regeneration.

Is the glial scar inhibitory or beneficial to regeneration?

The question of whether formation of the glial scar aids CNS regeneration and functional recovery has been discussed and debated for many years. There is widespread evidence supporting the notion that compact astroglial scars prevent axon regeneration. Evolutionarily, there is a stark contrast in regenerative abilities between mammals and lower

vertebrate classes (fish, reptiles and amphibians). Species such as salamanders maintain a surprisingly robust ability to regenerate the CNS throughout life and do so without formation of a glial scar[45]. There is also a large difference in regenerative capabilities between the mammalian CNS and peripheral nervous system (PNS). Unlike the CNS, peripheral nerves can regenerate over long distances, find their appropriate target cells and form functional synapses. This dissimilarity is believed to be due to differences in intrinsic properties of the neurons[46] and in the composition of the injured CNS and PNS environmental milieu. Most strikingly, PNS axons transplanted into the injured CNS fail to regenerate, while injured CNS neurons are able to project axons within bridges of peripheral nerve tissue[47]. Since these pioneering studies, the repressive nature of the glial scar has been largely attributed to a high concentration of inhibitory proteins, including chondroitin sulfate proteoglycans (CSPGs) and myelin proteins.

Inhibitory environment of the glial scar

The glial scar environmental milieu likely varies across different types of CNS injury and disease. For example, lipopolysaccharide injection or optic nerve crush both result in production of inflammatory factors from reactive M1 microglia that promote an A1 reactive astrocyte phenotype[15]. These A1 reactive astrocytes in turn secrete an unidentified neurotoxin that kills neurons and oligodendrocytes in vitro and in vivo[15]. Gene expression profiling of A1 reactive astrocytes also identified strong expression of several genes of the classical complement cascade that are known to be destructive to synapses[14]. Therefore, injuries or neurodegenerative diseases that induce A1 reactive gliosis presumably create a highly toxic environment for regenerating axons and NG2 glia. By contrast, ischemia-induced reactive astrocytes produce several neuroprotective factors and cytokines, such as cardiotrophin-like cytokine factor 1 (CLCF1), leukemia inhibitory factor (LIF) and thrombospondins[14]. Within the same injury, subtypes of reactive astrocytes may also express differing levels of inhibitory proteins. Following contusion SCI, scar-forming astrocytes upregulate several genes that distinguish them from milder reactive astrocytes, including CSPGs and the repulsive axon guidance protein Slit2[32].

CSPGs—which include the lecticans (aggrecan, versican, neurocan and brevican), phosphacan, and NG2—have been largely credited with axon regeneration failure in the CNS[48]. Following SCI, CSPGs are highly upregulated by both reactive astrocytes and other cells in the glial scar[49]. As in their developmental role, CSPGs have been shown to efficiently repel regenerating axons in vitro[50]. CSPGs also directly prevent oligodendrocyte maturation and remyelination in vitro[51] and in animal models of multiple sclerosis[52]. Degradation of CSPGs by treatment with chondroitinase ABC following SCI[53] and focal ischemia[54] has resulted in locomotor improvement due to sprouting of spared axons. A recent study found that modulation of the CSPG receptor protein tyrosine phosphatase-σ (PTPσ) following SCI restores serotonergic innervation to the injured mouse spinal cord, along with functional recovery of locomotor and urinary systems[55]. Overall, reducing CSPG signaling in the glial scar has been a major therapeutic focus, with promising but varying results. Targeted ablation of individual CSPGs from specific cell populations in the glial scar is needed to better understand the respective roles of CSPGs during axon regeneration.

In addition to axonal growth, the glial scar also presents an inhibitory environment for endogenous remyelination. Our laboratory recently characterized a protein secreted by reactive astrocytes, endothelin-1 (ET-1), as a negative regulator of NG2 glial differentiation and functional remyelination[56,57]. Blocking ET-1 signaling by either pharmacological or genetic approaches enhances maturation of NG2 glia into oligodendrocytes after focal demyelination of the corpus callosum. Notably, ET-1 signaling increases Jagged1 expression in reactive astrocytes, activating Notch signaling in neighboring NG2 glia and preventing their differentiation. Therefore, ET-1 modulates both the astrocytic and oligodendroglial responses to CNS damage. Other signaling proteins in the glial scar, such as bone morphogenetic proteins (BMPs), have been shown to play similar roles[58]. Intriguingly, gray matter tracts have been found to undergo more remyelination than white matter lesions in patients with multiple sclerosis[59]. This may be due to different environmental factors (for example, levels of ET-1 or differential accumulation of microglia) and/or the different proliferative states of resident NG2 glia in gray and white matter. Determining whether high ET-1 production is restricted to specific subtypes of reactive astrocytes remains an important issue to address.

Beneficial functions of the glial scar

In face of the evidence above, a logical hypothesis is that blocking formation of the glial scar —the dense glial border surrounding the lesion core—should result in increased axonal growth and remyelination. However, a series of studies by the Sofroniew laboratory over the past decade has demonstrated that preventing formation of the astroglial scar following CNS injury does not result in increased regeneration[1,49,60]. Recently, this was further confirmed using two different genetic methods to ablate scar-forming astrocytes following severe crush SCI[49]. Selectively ablating proliferating astrocytes or deleting STAT3 signaling selectively from astrocytes each results in increased axonal dieback[49]. One explanation for the increased dieback is an altered inflammatory response. Previous studies have reported that reactive astrocytes are important in restricting the inflammatory response to the damaged CNS region, thereby protecting healthy CNS tissue. These protective influences include the sequestration of blood-derived macrophages and repair of the blood–brain barrier[1]. Ablation of scar-forming astrocytes has also been shown to exacerbate neuronal cell death and demyelination following injury, as a result of an influx of blood-derived macrophages and fibrotic cells[1,60]. Therefore, the glial scar is important in preserving tissue integrity and mitigating further inflammatory damage.

Unresolved discrepancies regarding the glial scar

One proposed explanation for the dual nature of the glial scar is that the scar has beneficial effects during the acute phase of injury, but prevents axon growth in chronic or later stages[61]. In support of this theory, a recent study by Hara et al. pharmacologically blocked integrin signaling 2 weeks after SCI, thereby attenuating astrocyte scar formation and improving locomotor performance[32]. However, Anderson et al. ablated reactive astrocytes in chronic glial scars 5 weeks after SCI and found that it did not promote axonal growth[49]. Whether this remains true for even more mature glial scars (months after injury) remains to be seen. Anderson et al. interpreted their results to signify that scar-forming astrocytes aid, rather than inhibit, axonal growth following injury[49]. This interpretation has been challenged

by others in the field[62], who claim that it ignores the deleterious effects of lesion-derived macrophages on regenerating axons. So what explains these differing outcomes? Anderson et al. ablated scar-forming astrocytes using genetically targeted diphtheria toxin receptor and low doses of diphtheria toxin[49], whereas Hara et al. administered an anti-β1-integrin antibody to the lesion epicenter, blocking the interaction of reactive astrocytes with collagen I[32]. It is possible that the latter approach preserved beneficial reactive astrocytes in the glial scar—perhaps akin to those in the ischemic glial scar. It is also likely that the anti-β1-integrin antibody affected other cellular interactions in the glial scar, in addition to reactive astrocytes. Unfortunately, neither study characterized the effects of scar ablation on other cells in the damaged CNS. It is therefore difficult to interpret whether the changes in axonal growth are due to the absence of astrocyte-derived cues or to altered cellular responses in microglia and/or NG2 glia.

In addition to reactive astrocytes, there are also conflicting reports on the effects of NG2 glia on axonal growth in the glial scar. NG2 glia express high levels of the CSPG NG2, which has been shown to inhibit neurite outgrowth in vitro[63]. Delivery of NG2 neutralizing antibody following SCI results in increased axonal growth and functional regeneration[64,65]. Furthermore, reducing proliferation of NG2 glia after optic nerve crush increases the number of axons crossing the proximal crush site[66]. Together, these findings suggest that NG2 glia inhibit axon regeneration. However, NG2 knockout mice display more axonal dieback from spinal cord lesions than wild-type controls[67,68]. NG2 null mice also exhibit less remyelination following lysolecithin-induced demyelination in the spinal cord[69], likely owing to a smaller pool of NG2 glial progenitors for oligodendrocyte generation. Intriguingly, regenerating axons have been observed closely associating with NG2-expressing cells in the glial scar, forming synapse-like connections[68]. These synaptic connections may mirror what is seen in the developing CNS, as NG2 glia have been found to receive direct synaptic inputs from excitatory and inhibitory neurons throughout the brain[70]. However, while these synaptic connections may be beneficial during early phases of glial scar formation, it is hypothesized that they ultimately trap regenerating axons in a dystrophic state[68]. Overall, these conflicting reports show that NG2 glia are likely to have both beneficial and inhibitory roles in the glial scar. Whether these diverse functions can be attributed to distinct subtypes of NG2 glia remains to be seen. It is also important to note that because NG2 glia, pericytes and infiltrating macrophages all express NG2, it is difficult to assess the individual roles of each cell type on axonal growth in NG2 null mice. Therefore, conditional ablation of NG2 from different cell populations in the glial scar is needed to better understand the effects of NG2 glia and the NG2 protein on axonal regeneration.

Conclusions and future directions

The CNS is a complex and structured organ system, and damage or disease to this system results in equally multifaceted cellular and molecular responses. As our knowledge of cellular diversity in the normal and pathological CNS continues to increase, it becomes even more important to compare cellular responses both within and across injury and preclinical disease models. Advanced molecular and imaging tools now make these experiments possible. Over the past decade, significant advancements have been made toward

understanding signaling processes that direct reactive astrogliosis. However, the full range of reactive astrocyte diversity remains to be determined. Furthermore, more attention must be directed toward molecular and physiological characterization of NG2 glia and microglia across different injury and disease models. Recognizing how different subtypes of reactive astrocytes, NG2 glia and microglia shape the environmental milieu of the glial scar is critical for correct interpretation of the glial scar's many roles during injury and repair. More studies are needed that characterize the roles of single molecules in specific cell types using state-of-the-art genetically targeted loss-of-function techniques.

Historically, treatments for CNS damage have been largely classified according to the inducing damage or injury (for example, SCI, demyelination, stroke or Alzheimer's disease) and the corresponding symptoms. However, with the arrival of high throughput sequencing methods, we are now close to classifying CNS pathologies according to their molecular profiles. An intriguing and perhaps more realistic possibility is classifying pathologies according to their cellular profiles—specifically, what subtypes of reactive astrocytes, NG2 glia and microglia are present in the damaged tissue. Each class of cells can be molecularly characterized and compared in different types of injury that lead to glial scar formation. Developing targeted therapies that repress or promote expression of specific gene pathways in distinct glial cell populations may provide the best approach for promoting maximal functional recovery across a broader range of CNS injury and disease.

Acknowledgments

The authors would like to kindly thank J. Triplett, E. Goldstein and T. Forbes for critically reading the manuscript. This work was supported by R01NS090383 from NINDS (V.G.), U54HD090257 from NICHD (District of Columbia Intellectual and Developmental Disabilities Research Center) (V.G.) and F32NS098647 from NINDS (K.L.A.). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

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Box 1

Reactive astrocytes as neural stem cells

During development and in the mature CNS, a restricted number of cells maintain the ability to self-renew and differentiate into multiple lineages. These radial glia or neural stem cells (NSCs) are defined by their ability to form neurospheres and differentiate into multiple lineages (neurons, astrocytes and oligodendrocytes) in vitro, but rarely do so in vivo[78]. Interestingly, reactive astrocytes have been described as sharing characteristics with NSCs. Fate-mapping studies have shown that a subset of reactive astrocytes resume proliferation in vivo following traumatic or ischemic brain injury[79–81], but usually undergo only one round of cell division[19]. In vitro, about 5% of all reactive astrocytes are able to form neurospheres with higher self-renewal capacity[79]. Additionally, both NSCs and reactive astrocytes display limited lineage potential in vivo but enhanced multipotency in vitro, generating both neurons and glia[80,81]. Lastly, transcriptomic analysis identified a group of genes activated in common between reactive astrocytes and NSCs, including genes involved in proliferation and neurogenesis[78]. Future studies using single-cell RNA sequencing will hopefully further explain why subtypes of reactive astrocytes respond differently to CNS damage. The ability to manipulate extrinsic signaling cues in regions of the glial scar to direct the behavior and lineage potential of reactive astrocytes would present valuable options for treating CNS injury and disease.

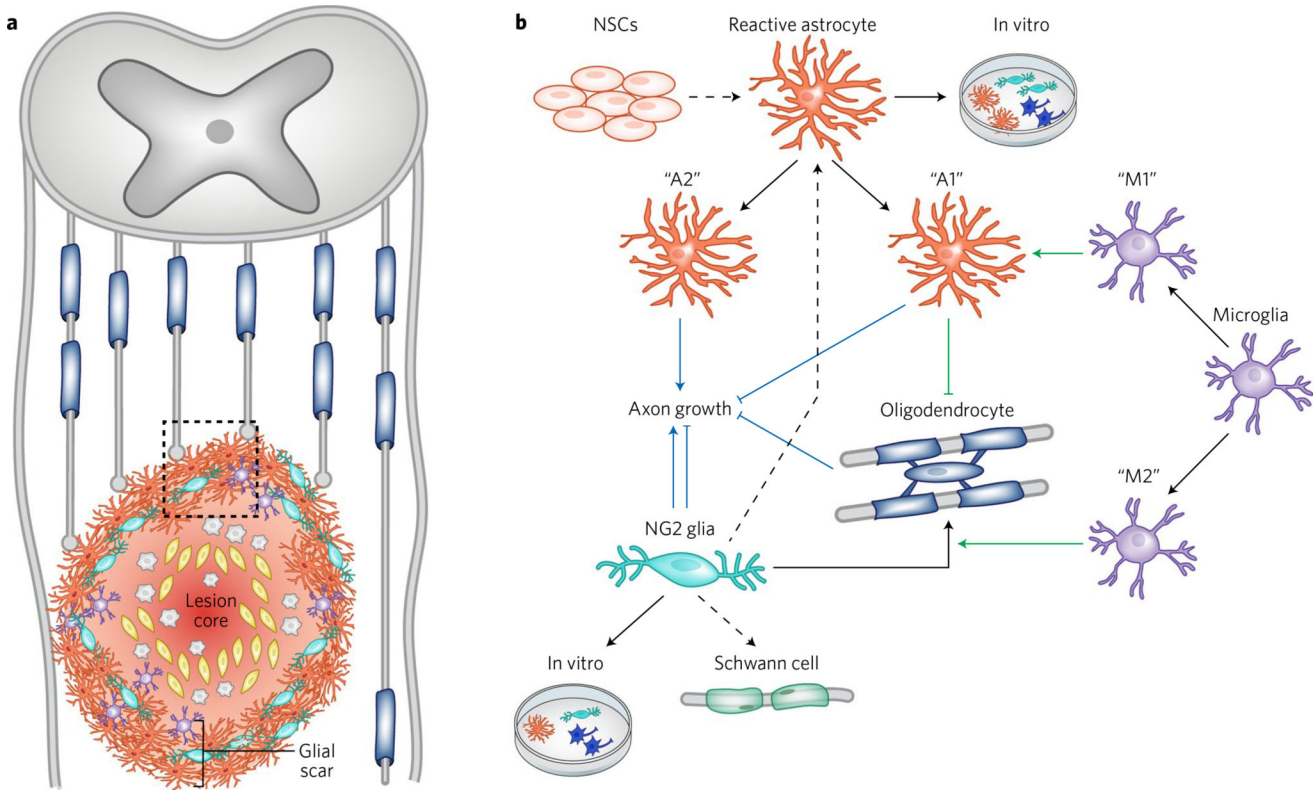

Fig. 1. Cellular interactions in the glial scar

a, Diagram of the glial scar after spinal cord injury. The glial scar is made up of reactive astrocytes (orange), NG2 glia (teal) and microglia (purple) that form a tight barrier around the lesion core, or area of severe tissue damage. The lesion core contains blood-borne macrophages (gray) and stromal cells (yellow). Injured axons (gray lines) fail to grow through the glial scar. **b**, The cellular interactions and developmental potential of heterogeneous glial cells within the glial scar (boxed region in **a**). Black arrows indicate the in vivo and in vitro lineage potential of each glial cell type, with black dashed arrows representing less common cell fates (that is, NG2 glial differentiation into Schwann cells or reactive astrocytes). Green lines depict cellular interactions among glial cells in the scar. Specifically, M1 microglia promote an A1 reactive astrocyte phenotype, while M2 microglia have been shown to promote differentiation of NG2 glia to oligodendrocytes. A1 reactive astrocytes secrete a toxin that kills oligodendrocytes. Blue lines depict the effect of each cell type on axonal growth (blue arrow indicates promotion of axon growth while blunt end indicates inhibition). The A1 and A2 astrocyte subtypes are based on Liddelow et al.[15] while the M1 and M2 microglial subtypes are based on Miron et al.[43]. NSCs, neural stem cells.

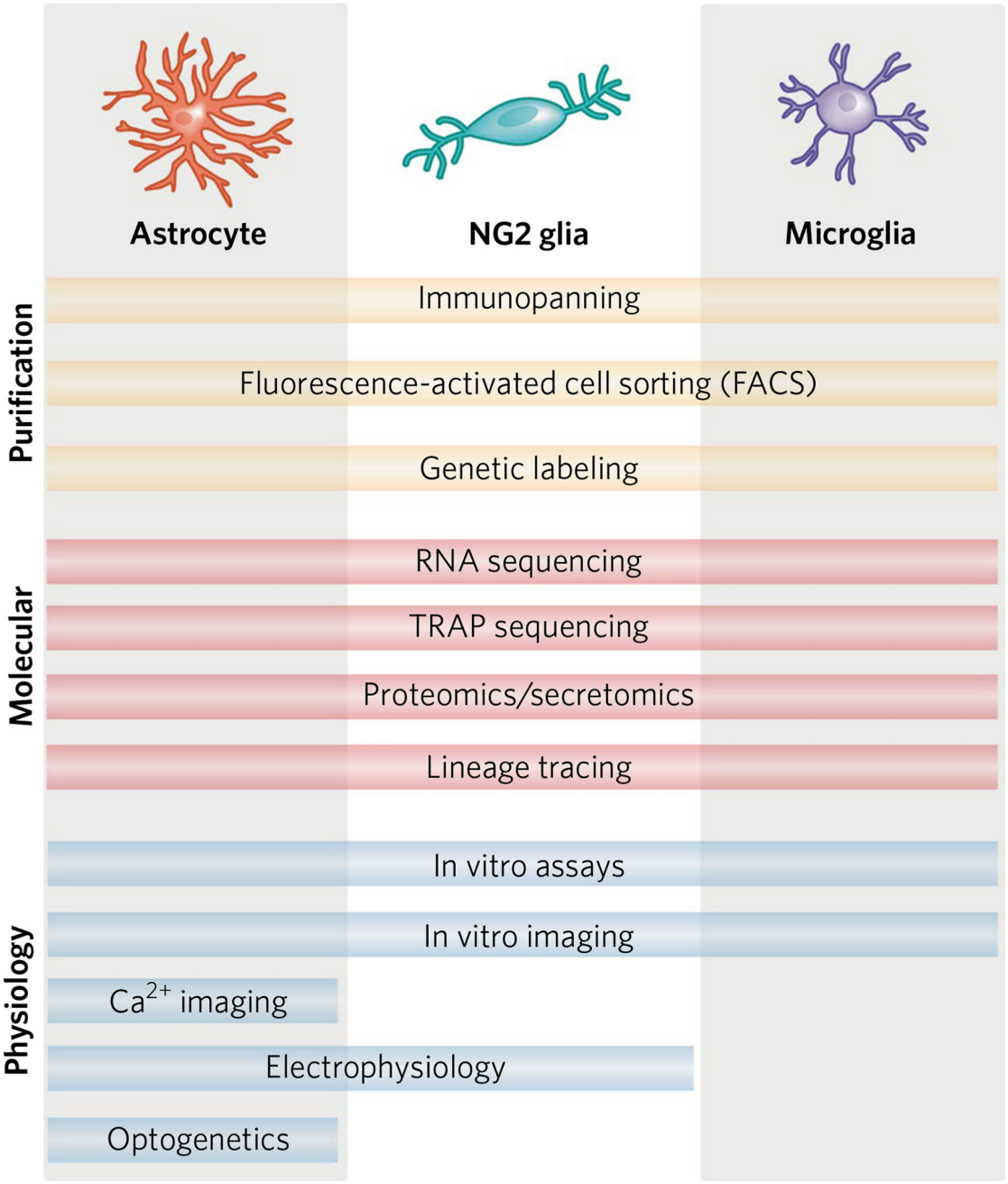

Fig. 2. Tools for assessing functional cellular diversity in glia

Elucidating cellular diversity requires robust purification protocols that effectively isolate astrocytes, NG2 glia or microglia from surrounding CNS tissue. Once cells are purified, they can be characterized using a range of different molecular tools, including new techniques such as single-cell RNA sequencing and translating ribosome affinity purification (TRAP) sequencing. These techniques result in molecular profiles that can be used to identify new molecular markers for glial subtypes, potential physiological differences among cellular subtypes and potential therapeutic targets for promoting functional repair following CNS damage. Assessing cellular physiology is critical for understanding functional heterogeneity

of astrocytes, NG2 glia and microglia. While in vitro assays (for example, cellular proliferation and synapse modulation) and in vivo imaging techniques have been used to characterize all three glial populations, there is a lack of sophisticated tools for analyzing microglial physiology. Refs. for purification protocols: Zhang et al.[71], Lin et al.[5], Bennett et al.[44]. Refs. for molecular tools: Doyle et al.[72], Kim et al.[73]. Refs. for physiology: Nimerjahn et al.[74], Perea et al.[75], Larson et al.[76], Gee et al.[77].