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Portrait of glial scar in neurological diseases

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Abstract

Fibrosis is formed after injury in most of the organs as a common and complex response that profoundly affects regeneration of damaged tissue. In central nervous system (CNS), glial scar grows as a major physical and chemical barrier against regeneration of neurons as it forms dense isolation and creates an inhibitory environment, resulting in limitation of optimal neural function and permanent deficits of human body. In neurological damages, glial scar is mainly attributed to the activation of resident astrocytes which surrounds the lesion core and walls off intact neurons. Glial cells induce the infiltration of immune cells, resulting in transient increase in extracellular matrix deposition and inflammatory factors which inhibit axonal regeneration, impede functional recovery, and may contribute to the occurrence of neurological complications. However, recent studies have underscored the importance of glial scar in neural protection and functional improvement depending on the specific insults which involves various pivotal molecules and signaling. Thus, to uncover the veil of scar formation in CNS may provide rewarding therapeutic targets to CNS diseases such as chronic neuroinflammation, brain stroke, spinal cord injury (SCI), traumatic brain injury (TBI), brain tumor, and epileptogenesis. In this article, we try to describe the new portrait of glial scar and trending of research in neurological diseases to readers.

Keywords

fibrosis, glial scar, inflammation, neurological diseases

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Introduction

Scar formation after injury or disease leaves permanent deficits for patients, especially in central nervous system (CNS) diseases. Since scar formation is the most common response in damaged tissue, most of the patients who have neurological disorders suffer from the loss of normal sensorimotor function which means losing their basic life guarantee and their fundamental life support abilities.[1] Furthermore, with the reduction in quality of life

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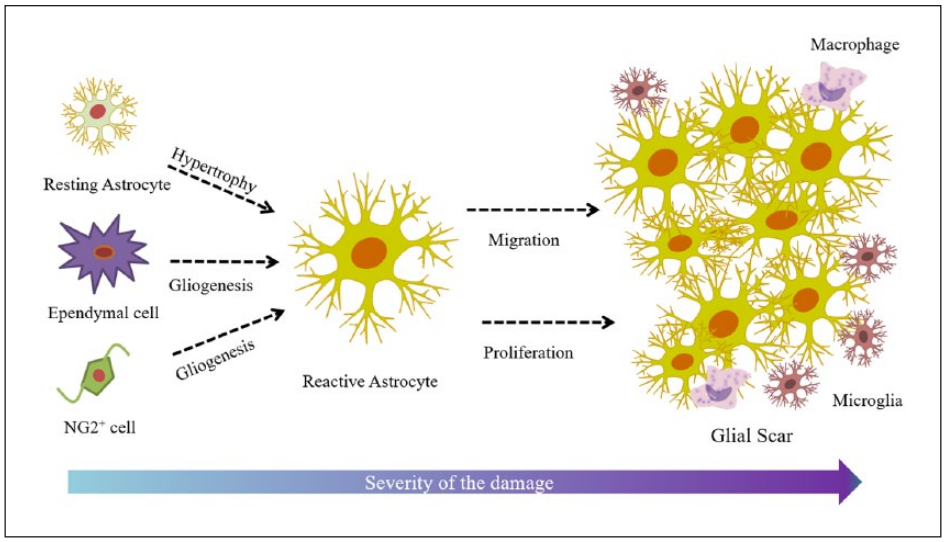

Figure 1. Reactive astrocytes in the formation of glial scar and where they form.
The astrocyte goes through tremendous changes including hypertrophy, migration, proliferation, gene expression, and functional alternations, depending on the distance from the lesion core and the severity of the damage in many CNS pathologies. Astrocytic proliferation, migration, and activation are involved in glial scar formation in a coordinating manner. As for induction of reactive astrocytes leading to glial scar formation, several studies showed that some neural progenitors, such as ependymal cells and NG2+ cells, are involved in gliogenesis in this process at the limited brain regions. It is reported that the forming of glial scar involves these phenotypic alternations which is regulated by many different signal mechanisms. The key molecules (including AQP, CX30, CX43, ET-1, TGF-β1, and MMP9) play pivotal roles in regulating the induction of glial scar formation.

and the increase in the cost of care, patients who cannot live independently are more likely to experience depression after survival of neurological diseases.[1]

The consequence of scar formation in the CNS is usually considered more detrimental than that in the peripheral organs because of impaired neural function recovery.[2] Moreover, activated inflammatory cells can also induce secondary damage to intact cells and can impair post-injury synaptic regeneration. However, fibrotic scars provide perineuronal nets for both synaptic maturation and CNS plasticity by forming a dense extracellular matrix (ECM) to provide extracellular space and by inducing adult neurogenesis, respectively. Accumulating data suggest that the predominance of a detrimental or beneficial action of glial scar formation after neural diseases is considered to depend on the microenvironment at the injury site and the types of brain disorders and the severity.[3]

The activated form of astrocytes in pathologies

Astrocytic activation is the activated form of astrocytes in response to brain disorders. Reactive astrocytes are characterized by hypertrophy, high expression of intermediate filament (IF) proteins, and functional changes. Astrocytic activation is also accompanied by the production of various cytokines, chemokines, growth factors, and neurotrophic factors.

The astrocytes near the lesion undergo tremendous changes, including changes in morphology, gene expression, proliferation, and function, which are often collectively described as reactive astrogliosis. Many features of astrocytes are shared among various diseases, while astrocytic responses are not the same in all diseases due to the differences in specific diseases.

Signal molecules of activated astrocytes in glial scar formation

Signal molecules in coordination play crucial roles in astrocytes for glial scar formation after damage occurs (Figure 1). Some of the key molecules that are involved in astrocytic hypertrophy, proliferation, migration, and gliogenesis orchestrate the formation of glial scars.

The cytoskeletons of astrocytes are highly changeable; they have a strong potential to quickly remodel themselves to cooperate with the normal development of morphogenesis and tissue injury.[4] IF proteins are a part of the structure of cytoskeletal

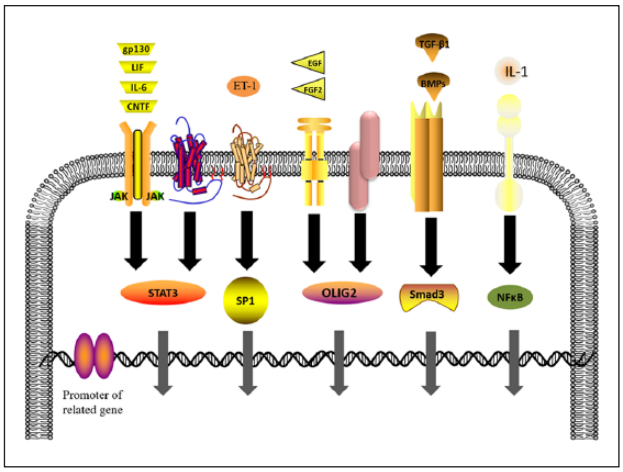

Figure 2. The key transcription factors regulating the glial scar formation in CNS pathologies.
The induction of glial scar formation is stimulated by a variety of signaling molecules (such as IL-1, IL-6, CNTF, ET-1, EGF, FGF2, TGF-β1, BMPs, and LIF) from the tissue near the lesion. These signaling molecules play crucial roles in activating the transcription factors that regulate the astrocytic hypertrophy, migration, proliferation, gliogenesis, and inflammation, which promotes the formation of glial scar. IL: interleukin; LIF: leukemia inhibitory factor; CNTF: ciliary neurotrophic factor; ET-1: endothelin-1; EGF: epidermal growth factor; FGF2: fibroblast growth factor-2; TGF-β1: transforming growth factor-b1; BMP: bone morphogenic protein; STAT3: signal transducer and activator of transcription 3; Sp1: specificity protein 1; OLIG2: oligodendrocyte transcription factor 2; SMAD: Sma- and Mad-related protein; NFκB: nuclear factor-kappa B.

networks that affect morphology and mortality.[4] Furthermore, these proteins are also tightly associated with reactive gliosis in glial scar formation. Compared to the quiescent state of astrocytes, the expression of glial fibrillary astrocytic protein (GFAP), which is the astrocyte-specific IF protein, as well as vimentin and nestin, is up-regulated in reactive astrocytes, causing hypertrophy via reorganizing the cytoskeleton of these cells. Although GFAP is one of the hallmarks of astrogliosis after injury, GFAP-null mice do not exhibit attenuation of glial scar formation or up-regulation of vimentin, but the new glial scar becomes softer. However, the formation of the glial scar in nerve injury is impaired when GFAP and vimentin are knocked out at the same time, which implies that the function of GFAP and vimentin are partly compensated. These indicate that the IF proteins, such as GFAP and vimentin, play important roles in nerve injury glial scar formation.

Astrocytic migration to the glial scar is suggested to be induced by matrix metalloproteinase (MMP) 9, which is produced by reactive astrocytes during injury and is released in extracellular space to degrade the protein in the ECM.[5] The production of MMP9 is stimulated by endothelin-1 (ET-1), transforming growth factor (TGF)-β1, and thrombin. When MMP9 is inhibited in mice with spinal cord injury, astrocytic migration and glial scar formation are inhibited. Aquaporin (APQ) water channels, which facilitate cell migration, are highly expressed in cell plasma membranes. AQP4 is expressed primarily in the astrocytic end-feet.[6] In AQP4-knockout mice, the migration of reactive astrocytes is slowed. This result suggests that AQP4 activities are important to astrocytic migration too.

Proliferation is another crucial factor for astrocytic accumulation in damaged parts of CNS that leads to glial scar formation. In neurological diseases, the neural stem cells and progenitors can transform into neurons and glial cells. Recent studies have proved that reactive astrocytes in the glial scar are derived from glial progenitors. NG2 cells can differentiate into proliferating reactive astrocytes in brain injuries, and ependymal cell precursors generate scar-forming astrocytes in spinal cord injury and stroke.[7]

Connexin 30 (CX30), a member of the connexin protein family, is involved in gap junctions regulating the expression of astrocytic IF proteins. The expression of CX30 is reduced in the activation of astrocytes. The expression of GFAP and the number of astrocytes are increased in CX30-knockout mice.[8] These suggest that CX30 may profoundly affect glial scar formation via impairing the hypertrophy and activation of astrocytes. Gap junctions are highly expressed in most astrocytes to serve their function in cellular communication among astrocytes, oligodendrocytes, and even some neurons. Connexins play important roles in astrocytic proliferation. Recent studies have successfully proven that the activation of astrocytes and astrocytic proliferation are enhanced when the expression of CX43 is inhibited by traumatic injury or tumor promoters.

Transcription factors of activated astrocytes in glial scar formation

STAT3 (signal transducer and activator of transcription 3), a transcription factor, is enhanced in astrocytes through stimulation of gp130, which activates Janus kinase (JAK) and phosphorylates STAT3 in CNS diseases (Figure 2).[9] In STAT3-knockout

mice, the proliferation and migration of reactive astrocytes are reduced; thus, the formation of glial scars is attenuated in CNS pathologies, but the functional recovery is impaired as well. Studies show that STAT3 is involved in the regulation of gene expression of reactive astrocytes including GFAP, CX43, and AQP4.[8] Furthermore, increase in leukemia inhibitory factor (LIF), interleukin-6 (IL-6), and ciliary neurotrophic factor (CNTF) in the damaged brain stimulates the astrocytic JAK/STAT3 signaling and enhances the glial scar formation. OLIG2 (oligodendrocyte transcription factor 2) is a transcription factor that regulates the conversion of neural precursors to neurons, oligodendrocytes, or astrocytes. In brain pathologies, OLIG2-positive NG2 cells differentiate into GFAP-positive astrocytes, which is the main source of reactive astrocytes for glial scar formation. The ablation of OLIG2 impairs the proliferation of GFAP-positive reactive astrocytes and glial scar formation. Brain injury increases the expression of fibroblast growth factor-2 (FGF2) and epidermal growth factor (EGF), which are involved in the stimulation of OLIG2 expression and astrocytic activation.

SMAD (Sma- and Mad-related protein) family of transcription factors regulate the differentiation of astrocytes via the stimulation of TGF-β1 and BMPs (bone morphogenic proteins) both in the developing CNS and in brain pathological conditions. The increase in TGF-β1 and BMPs after brain injury stimulates the conversion of neural progenitors to astrocytes and the induction of ECM deposition through the SMAD family. TGF-β stimulates SMAD in astrocytes to produce chondroitin sulfate proteoglycans (CSPGs), which are the main components of the glial scar ECM deposition that inhibit axonal regrowth and improvement of motor function.[10] After inhibiting TGF-β signaling, glial activation and CSPG expression are blocked.

Neuroinflammation in glial scar

Neuroinflammation involves multiple important inflammatory cytokines that play vital roles in triggering astrogliosis. Multiple inflammatory cytokines, such as interferon-γ, IL-1, IL-2, IL-6, tumor necrosis factor (TNF)-α, and macrophage colony stimulating factors, exhibit a dramatic influence in the formation of intense local inflammation after CNS injury that leads to progressive cavitation of the damaged area and significant stimulation of astrocytic activity. IL-6, a pro-inflammatory cytokine associated with secondary injury in CNS, can promote selective differentiation of endogenous neural stem/progenitor cells (NSPCs) into astrocytes via the JAK/STAT pathway. In addition, it has been reported that IL-1 can promote the expression of GFAP and astrocyte hypertrophy in rats. Furthermore, activated macrophages have the ability to up-regulate the expression of CSPGs, thus inhibiting regeneration after CNS injury. In contrast, type I interferon, an anti-inflammatory cytokine, has been found to have the power to decrease the activation of astrocytes through deactivating the MEK/ERK pathway. Collectively, these studies emphasize the role of neuroinflammation in regulating astrogliosis and glial scar formation.

Glial scar in CNS diseases

Reactive astrogliosis is the most characteristic feature of glial scar after stroke. It has been shown that the proliferation of astrocytes begins close to the tissue of infarct within 200 μm in 3–5 days after the onset of stroke, and half of the astrocytes reenter the cell cycle up to a week later. It has also been observed that, although most reactive astrocytes in scar development are reproduced by cells already residing immediately next to the infarct tissue, there is still a portion of proliferating and nonproliferating astrocytes close to the site of infarct derived from neural stem cells that migrate from the subventricular zone a few days after the stroke.[11] Astrocytes, microglia, and macrophages make up a large population of proliferating cells in the peri-infarct tissue. In addition, a recent study found that a subpopulation of perivascular pericytes or related stromal cells are involved in the proliferation in stroke as well. They release ECM components such as fibronectin and collagen type 1 in the core forming the fibrotic scar, which is part of the developing glial scar. Within weeks after stroke, a subset of these cells could survive and become a part of the mature glial scar.

The response of the glial scar to CNS injury, traumatic brain injury and spinal cord injury, involves a cascade of cellular and molecular changes that influence the local microenvironment and the recovery of neuronal function. Typically,

the glial scar from traumatic injury in the CNS contains two distinct regions: (1) the lesion core, which primarily consists of NG2 glia, fibroblasts/pericytes, and macrophages and (2) the penumbra, which contains mostly reactive astrocytes and activated microglia. Microglia/macrophages and NG2 cells accumulate at the injured white matter within the first week and occupy the core of the lesion. The number of astrocytes nearly doubles because of the proliferation of astrocytes within 500 μm from the damage in this region. The astrocytes adjacent to the lesion of contused injury in the CNS elongate their morphologies with overlapping processes, forming mesh-like structures as a part of glial scar formation.[12] More than half of the astrocytes undergo proliferation in spinal cord crush injury, while only up to 20% of the astrocytes mostly adjacent to capillaries in the peri-lesion tissue induce proliferation in traumatic brain injury.[12] Recent studies show that the response of astrocytes coincides with the severity of the insult as are the outcomes of regeneration and recovery. Severe insults stimulate the up-regulation of GFAP and the expression of related genes and promote the overlapping processes of astrocytes adjacent to the injury site, whereas reactive astrocytes suffering mild insults or being far from the damaged tissue only express GFAP with neither proliferation nor overlapping neighboring astrocytes. These data suggest that the roles of astrocytes and their response after glial scar formation are quite different.

It is known that glial scar formation depends on the interactions between CNS cells and non-CNS cells including hematogenous macrophages and fibroblasts. Traumatic injury causes direct large-scale death of neurons and glia around the site of the injury, shearing of ascending and descending axons and damage to the vasculature. Traumatic injury leads to hemorrhage at the lesion and release of factors associated with glial scar formation and immune response. Astrocytes and microglia quickly begin to accumulate around the lesion and increase the expression of pro-inflammatory cytokines and chemokines that inhibit axonal regeneration. Increased levels of pro-inflammatory cytokines, myelin debris, and CSPGs in the glial scar contribute to secondary damage to neurons, oligodendrocytes, and dystrophic endings of axonal dieback and inhibit the recovery. Perivascular fibroblasts are attracted by hematogenous macrophages, which infiltrate the lesion, and the perivascular fibroblasts form the fibrotic part of the scar. This part of the scar shows increased density in a week, and the scar starts to mature during the second week by forming tight borders between fibrotic components with glial components after spinal cord injury.[13]

ECM in glial scar

The components of ECM serve both causal and the modulatory roles in the pathogenesis of response to various CNS disorders. The components of ECM can be observed in the early stage of glial scar formation to limit the spread of damage. After months from its maturation, the scar shrinks and squeezes excess water. The size of the glial scar containing tightly intertwined astrocytic processes decreases, but the GFAP content persistently increases. ECM always serves as the extracellular scaffold and the barrier for the diffusion of soluble and membrane-associated molecules in the CNS. Therefore, it is pivotal to understand the important and key components of the ECM in CNS disorders.

CSPGs are the most abundant types of glycanated protein in the nervous system and are the major component of ECM. CSPGs are up-regulated in different types of CNS lesions. It was first shown that the dorsal root ganglia (DRG) and cerebellar granule neurons avoided CSPG-rich regions in vitro and cease growing when they encounter the CSPG-rich tissue of glial scars in vivo in adult CNS micro-transplanted DRG neurons. The level of CSPG expression in the ECM was greatly up-regulated and lasted for several months after CNS injury in glial scars. Furthermore, it has been demonstrated that there are four major groups of CSPGs with different functions. A member of the group predominantly secreted in the brain ECM is lecticans (a family that includes versican, aggrecan, neurocan, and brevican), which is secreted by reactive astrocytes and oligodendrocyte precursor cells (OPCs).

Discussion

In brain pathologies, multiple diseases can activate astrocytes through various pathways such as STAT3, OLIG2, SMAD, and sp1, which modulate the activities of reactivated astrocytes discussed above. In response to insults, the increased levels of immune and inflammatory factors stimulate astrocytes, microglia, and macrophages in glial scar formation to induce pathophysiological responses. Glial scars

in different pathological conditions such as CNS infection, brain stroke, and traumatic injuries exhibit their own patterns to reorganize the nerve tissue and to repair neuronal damages. Moreover, the ECM of the glial scar influences the prognosis and the onset of neurological complications. Therefore, in the past decades, glial scars have been thought to exacerbate brain damage. However, the neuroprotective benefits of glial scars have been discovered, which include not only sealing the injured tissue and preventing the spread of damage but also controlling the cerebral blood flow, modulating immunomodulation, and stimulating neurogenesis.[14] The exact role of glial scar formation in neurological diseases is complex and depends on the specific type of neurologic disease and its severity. Glial scars cannot be simply defined as beneficial or detrimental to CNS repair because each function of glial scars in neuropathology is determined by diverse key molecules and individual signal mechanisms.[15] Therefore, modulation of these molecules and signal mechanisms may improve the neuronal functional recovery and may reduce the inhibitory environment in CNS injuries, and they may be the next therapeutic targets to receive more and more attention.

Authors' Note

H.W. and G.S. contributed equally to this work. The manuscript was initially conceived by L.Z. and drafted by H.W. and G.S. A.A., Y.Y., and L.Z. further manipulated the language polishing and modification and managed the integral design of the manuscript.

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