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Review article

Astrocyte reactivity and astrogliosis after spinal cord injury

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ABSTRACT

After traumatic injuries of the central nervous system (CNS), including spinal cord injury (SCI), astrocytes surrounding the lesion become reactive and typically undergo hypertrophy and process extension. These reactive astrocytes migrate centripetally to the lesion epicenter and aid in the tissue repair process, however, they eventually become scar-forming astrocytes and form a glial scar which produces axonal growth inhibitors and prevents axonal regeneration. This sequential phenotypic change has long been considered to be unidirectional and irreversible; thus glial scarring is one of the main causes of the limited regenerative capability of the CNS. We recently demonstrated that the process of glial scar formation is regulated by environmental cues, such as fibrotic extracellular matrix material. In this review, we discuss the role and mechanism underlying glial scar formation after SCI as well as plasticity of astrogliosis, which helps to foster axonal regeneration and functional recovery after CNS injury.

Contents

1. Spinal cord injury and glial scar formation

There are approximately 5000 new cases of traumatic spinal cord injury (SCI) recorded each year in Japan, and 17,000 new cases in the USA; approximately 100,000 people in Japan and 1.2 million people in the USA are paralyzed due to SCI, mainly because of motor vehicle, sports, and work accidents (Lee-Liu et al., 2013; Ide-Okochi et al., 2013). The incidence of fall-related injuries in the elderly population has significantly increased with the aging of the population (Okada et al., 2009). Traumatic SCI often causes irreversible motor and sensory dysfunction as well as the loss of bladder, bowel and sexual function, resulting in a significant reduction in the quality of life (Adams and Hicks, 2005). Since there are no approved therapies to restore the spinal cord function, a better understanding of the mechanism for compromising functional recovery after SCI is significantly important.

Glial scarring, which impedes axonal regeneration and functional recovery has been considered to be the main cause of the limited regenerative capability in the mammalian CNS (Silver and Miller, 2004). The impeded axonal regeneration and functional

Abbreviations: CNS, central nervous system; SCI, spinal cord injury; USA, United States of America; GFAP, glial fibrillary acidic protein; STAT, signal transducer and activator of transcription; SOCS, Suppressor of cytokine signaling; LMD, laser microdissection; GFP, green fluorescent protein; FACS, fluorescence activated cell sorting; Col I, the type 1 collagen; NAs, naïve astrocytes; RAs, reactive astrocytes; SAs, scar-forming astrocytes.

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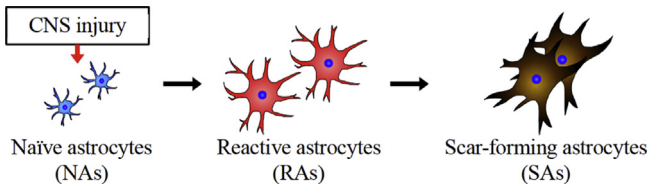

Fig. 1. The changes of astrocytes after CNS injury. Naïve astrocytes are activated by several types of injury (*i.e.*, inflammation, infection, ischemia, and traumatic injury) and they exhibit sequential phenotypic changes, including cellular hypertrophy and process extension; these are referred to as reactive astrocytes. Around the area of the lesion, reactive astrocytes express cell adhesion molecules, such as N-cadherin, and transform into scar-forming astrocytes. Scar-forming astrocytes adhere to each other and form the glial scar.

recovery have been shown to be irreversible and to permanently inhibit axonal regrowth in both rodents and humans with SCI (Lee-Liu et al., 2013). In fact, in some non-mammalian vertebrate animals (*i.e.*, urodele amphibians and lampreys) in which little glial scar formation is detected, the spinal cord has been shown to regenerate, substantially restoring the motor function (Lee-Liu et al., 2013; Diaz Quiroz and Echeverri, 2013). Chondroitin and keratin sulphate proteoglycans are among the main inhibitory extracellular matrix molecules to be produced by reactive astrocytes in glial scars, and they play a crucial part in regeneration failure (Silver, 2016). Due to the presence of these inhibitory molecules, severed axons cannot regenerate past the lesion. In fact, after SCI, treatment with chondroitinase results in the degradation of chondroitin sulphate proteoglycans at the lesion site and allows axonal regeneration and functional recovery (Bradbury et al., 2002).

Glial scar formation after CNS insult is regulated by complex and combinatorial inter- and intracellular signal mechanisms. Under normal conditions, astrocytes are the predominant subtype of glial cells in the CNS; they maintain neurons as well as the blood-brain-barrier. They are activated in response to various types of injury, including inflammation, infection, ischemia, and traumatic injury, and play a crucial role in the pathophysiology of each through a phenotypic change known as reactive astrogliosis. After SCI, naïve astrocytes sequentially exhibit contrasting phenotypes, first as reactive astrocytes and then as scar-forming astrocytes (Fig. 1). Although the mechanism underlying reactive response of naïve astrocytes has not been fully elucidated, Shinozaki et al., recently have demonstrated that microglia transformed astrocytes phenotype *via* downregulation of the $P2Y_1$ purinergic receptor (Shinozaki et al., 2017). Reactive astrocytes are necessary in the acute wound healing and tissue remodeling processes; however, they eventually become scar-forming astrocytes and form a dense glial scar. Although these different astrocytic functions are multifaceted and phase-dependent, the term of astrogliosis has only been roughly defined, which makes the role of astrocytes unclear in the pathophysiology of SCI.

2. The beneficial role of reactive astrocytes

Although limited functional recovery occurs during the chronic phase of SCI, temporary functional recovery is often observed soon after incomplete SCI (Kobayakawa et al., 2014). The mechanism underlying this phenomenon has been unclear. We previously demonstrated that the astrocytic responses are crucial to tissue repair and functional recovery after SCI (Okada et al., 2006). We first created a contusive SCI (70 Kdyn) on the mouse 9th thoracic spinal cord using a commercially available SCI device (Infinite Horizons Impactor, Precision Systems Instrumentation, Lexington, KY), performed an immunohistological analysis, and evaluated the functional recovery. In this model of incomplete SCI, there was a substantial recovery in lower extremity movement until 2–3 weeks after injury. During this functional recovery phase, we found that reactive astrocytes emerged and eventually migrated centripetally to the area of the lesion and compacted the infiltrating inflammatory cells, resulting in the compaction of the area of the lesion. After the migration of reactive astrocytes and the completion of glial scar formation, the functional improvement also reached a plateau. Although reactive astrocytes were considered to be harmful for the pathophysiology of SCI due to their contribution to glial scar formation, this phenomenon indicates that the emergence and migration of reactive astrocytes had a prominent role in the repair of injured tissue and the restoration of the motor function before the completion of glial scar formation (Okada et al., 2006). In fact, selective ablation of dividing astrocytes using ganciclovir after SCI in the GFAP-TK transgenic mice resulted in severe leukocyte infiltration, tissue disruption, demyelination and neuronal death (Faulkner et al., 2004), which also suggested the beneficial role of reactive astrocytes after SCI. Since we found that phosphorylation and nuclear translocation of signal transducer and activator of transcription-3 (STAT3) were mainly observed in reactive astrocytes, we examined the role of STAT3 in reactive astrocytes in the mouse model of SCI (Okada et al., 2006). In the genetically modified mice in which STAT3 was selectively depleted in reactive astrocytes under the control of Nestin gene promoter/enhancer, reactive astrocytes showed limited migration to the lesion epicenter, which resulted in a wide area of injury, impaired contraction of infiltrating inflammatory cells, and limited functional recovery (Fig. 2). Similar results were observed after SCI in the STAT3-depleted mice in GFAP-expressing astrocytes, indicating that STAT3 is a critical regulator of astrogliosis (Herrmann et al., 2008). In contrast, in the genetically modified mice in which the protein suppressor of cytokine signaling-3 (SOCS3, negative feedback molecule of STAT3) was selectively depleted in reactive astrocytes, the rapid migration of reactive astrocytes to secluded inflammatory cells, enhanced contraction of the area of the lesion, and a dramatic improvement in functional recovery were observed after SCI. Similarly, in transgenic mice that showed the expression of astrocyte-targeted interleukin-6 (IL-6, one of the upstream effectors of STAT3 pathway) the prompt migration of reactive astrocytes and compaction of infiltrating inflammatory cells were observed after CNS injury (Penkowa et al., 2003). These result suggest that STAT3 signaling, which is associated with the migration of reactive astrocytes, is a key regulator in the healing process after SCI. In contrast, in the chronic phase of injury, reactive astrocytes form glial scars, which function as a physical and chemical barrier, impairing axonal regeneration.

3. The plasticity of astrocytic changes

Although astrocytic scars have been studied for more than half a century, the cellular and molecular mechanisms of this process remain unclear. One factor that limits basic research is the absence of specific markers of differentiation and reactivity. For example, the characterization of astrocytes is usually performed using intermediate filament GFAP; however, all phenotypes of astrocytes including reactive astrocytes and scar-forming astrocytes are strongly expressed with GFAP, even though these cell types show distinct morphological characteristics. These cells are conventionally distinguished from each other based on a histological analysis. Reactive astrocytes are characterized by cellular hypertrophy, process extension, and the increased expression of intermediate filaments such as GFAP and Nestin (Frisén et al., 1995). However, this immunohistological discrimination is neither objective nor quantitative. To investigate the regulatory mechanism underlying astrogliosis, clear definitions for naïve, reactive, and scar-forming astrocytes are necessary; we therefore developed

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