

● PERSPECTIVES

## Reactive astrocytes promote axonal remodeling and neurological recovery after stroke

Stroke is a leading cause of death and disability in adults worldwide. For decades, the primary approach and goal of therapy for stroke has focused on neuroprotection, namely treating the injured tissue, with interventions designed to reduce the volume of cerebral infarction. Enormous effort in the laboratory has been devoted to the development of neuroprotective agents in an attempt to salvage ischemic neurons in the brain from irreversible injury; however, all these efforts have failed to demonstrate efficacy in clinical trials of stroke. In order to treat stroke, we have to re-conceptualize and redefine our therapeutic targets. Acute neuroprotective treatments for stroke fight a temporal battle of salvaging cerebral tissue before the onset of death, as well as a physiological impediment of delivery of therapy to tissue which has inadequate blood flow. Thus, a more promising therapeutic approach would be to promote remodeling of the central nervous system (CNS) *via* neurovascular plasticity, and thereby to foster neurological recovery. Stroke affects all cellular elements of the brain *i.e.*, vascular cells, neurons, astrocytes, oligodendrocytes, microglia and ependymocytes. Therefore, to accomplish this and to broaden treatment targets, we must consider therapeutic approaches that benefit multiple cell types, and in our view, particularly, astrocytes, which are in contact with and interact all parenchymal cells (Li et al., 2014).

Our knowledge of astrocytes in stroke is largely based on cortical injury in rodents, and might be even more important when we consider the increased complexity of astrocytes in the human brain as well as differences between regions of the CNS. As an integral part of the neuron-glia system, astrocytes provide many housekeeping functions, including structural support, neuronal metabolism, maintenance of the extracellular environment, regulation of cerebral blood flow, stabilization of cell-cell communications, neurotransmitter synthesis, and defense against oxidative stress. In the injured CNS, astrocytes undergo important morphological modifications, such as hyperplasia and hypertrophy, to form a glial scar, a physical and functional wall surrounding the damage area. These astrocytes, also referred to as, reactive gliotic, exhibit increased expression of the intermediate filament proteins including glial fibrillary acidic protein (GFAP), vimentin and nestin, and have altered expression of many other genes (Ridet et al., 1997).

The functional role of reactive astrocytes after stroke is controversial. In the glial scar, reactive astrocytes express a broad range of inhibitory molecules against axonal regeneration, such as chondroitin sulfate proteoglycans (CSPGs). However, the glial scar may also seclude the injury site from healthy tissue, preventing a cascading wave of uncontrolled tissue damage. In addition, reactive astrocytes take up excess glutamate, and produce neurotrophic factors, to protect the

neurons from ischemic lesion. Thus, the reactivity of astrocytes after stroke may potentially play both detrimental and beneficial roles under certain spatio-temporal conditions. In response to brain damage of stroke or trauma, reactive astrocytes up-regulate GFAP and vimentin and re-express nestin (Li and Chopp, 1999). In mice lacking both GFAP and vimentin (GFAP<sup>-/-</sup>Vim<sup>-/-</sup>), reactive gliosis and the glial scar are attenuated after neurotrauma (Pekny et al., 1999); however, at 7 days after induction of middle cerebral artery occlusion (MCAo), the infarct volume is 2.1–3.5 fold larger than in wild-type (WT) mice (Li et al., 2008). In addition, a recent *in vitro* study demonstrated that GFAP<sup>-/-</sup> Vim<sup>-/-</sup> astrocytes exposed to oxygen-glucose deprivation and reperfusion exhibit increased cell death and confer lower degree of protection to co-cultured neurons than WT astrocytes (de Pablo et al., 2013), suggesting that reactive astrocytes are protective during brain ischemia.

The astrocytic nanofilament system is a structural component of the cytoskeleton and serves as an important signaling platform in situations linked to cellular stress. In injured brains of GFAP<sup>-/-</sup>Vim<sup>-/-</sup> mice, astrocytes show similar abundance and access comparable volumes of brain tissue as astrocytes of wild-type mice, but do not exhibit the reactive phenotype with characteristic hypertrophic processes as astrocytes in wild-type mice (Wilhelmsson et al., 2004). Glial scar formation is attenuated in GFAP<sup>-/-</sup>Vim<sup>-/-</sup> mice, healing after trauma takes longer and post-traumatic synaptic loss is more prominent (Pekny et al., 1999; Wilhelmsson et al., 2004). Regenerative responses and functional recovery after spinal cord trauma is improved in GFAP<sup>-/-</sup>Vim<sup>-/-</sup> mice (Menet et al., 2003), which also show increased hippocampal neurogenesis (Larsson et al., 2004). Thus, at least in some disease contexts, the benefits of reactive gliosis at the acute injury phase seem to be counteracted by restricted regenerative potential at later stages.

Considering the multifaceted effects of reactive astrocytes as inhibiting axonal growth and supporting neuronal survival, it is interesting to address the question of how reactive astrocytes affect neurological recovery post stroke? We therefore examined whether the absence of the two major astrocytic intermediate filament proteins, GFAP and Vimentin, would impact functional recovery and axonal remodeling after stroke in GFAP<sup>-/-</sup>Vim<sup>-/-</sup> mice (Liu et al., 2014). In this study, we performed a unilateral photothrombosis to the forelimb motor area to generate a consistent focal cortical ischemia of equivalent size in both WT and GFAP<sup>-/-</sup>Vim<sup>-/-</sup> mice. We found that motor functional recovery of the stroke-impaired forelimb measured by single pellet reaching test and foot fault test, and corticospinal tract (CST) axonal outgrowth originating from the contralesional cortex in the denervated side of the cervical gray matter were significantly reduced, while CSPG expression was significantly increased in the lesion remote areas in both hemispheres, but decreased in the ischemic lesion boundary zone in GFAP<sup>-/-</sup>Vim<sup>-/-</sup> mice, compared to WT mice.

Consistent with previous studies (Pekny et al., 1999; Menet et al., 2003; Wilhelmsson et al., 2004), our results indicated that mice deficient in both GFAP and vimentin genes exhibit attenuated astrocytic reactivity after cortical stroke.

Unexpectedly, our behavioral functional data showed that the attenuated glial scar did not lead to improved functional recovery after stroke, as in the increased functional restoration observed in GFAP<sup>-/-</sup>Vim<sup>-/-</sup> mice subjected to spinal cord injury (Menet et al., 2003). This indicated that unlike spinal cord injury, the glial scar formation in the infarct proximal boundary region may not be a major barrier factor for neurological recovery after cerebral stroke. After spinal cord injury, extension of axons to bridge damage, may enhance functional recovery. Thus, reduced scar formation and reduced CSPG at the site of the damage may foster neurite growth and functional recovery. In contrast, after induction of stroke, reduction of the adjacent glial scar and reduction of CSPG, likely has no beneficial effect, since there is no neural cell survival in the ischemic infarct core area, thus, no necessity or benefit of promoting neurite extension and growth crossing the glial scar into the lesion boundary zone. To the contrary, our data suggest that the glial scar may have restorative effects, and restricts the ischemic lesion, and thereby promotes neurological recovery post stroke.

Astrocytic glial scar formation isolates the injury site to protect spared tissue from further damage, and may also restrict diffusible factors secreted from the lesion region into remote area. After injury, CSPG expression is rapidly upregulated by reactive astrocytes, forming an inhibitory gradient that is highest at the centre of the lesion and diminishes gradually into the penumbra (McKeon et al., 1991). Our results of increased CSPG expression in the areas remote from the lesion including the contralesional cerebral hemisphere and the cortical area outer lesion boundary zone in the ipsilesional hemisphere in GFAP<sup>-/-</sup>Vim<sup>-/-</sup> mice, suggest that subpopulations of astrocytes lacking GFAP and vimentin respond to injury to upregulate CSPG expression. Thus, the reduced CST axonal remodeling and neurological recovery in GFAP<sup>-/-</sup>Vim<sup>-/-</sup> mice after stroke may be attributed to upregulated CSPG expression in the remote areas surrounding a subpopulation of astrocytes and neurons, although the specific types of these astrocytes and neurons remain to be further characterized.

Neurite extension may depend on a balance of growth-promoting and growth-inhibiting molecules in the extracellular matrix after injury. The CNS response to stroke is a multicellular process that changes continually over time and is regulated by a multitude of extracellular and intracellular molecular signaling events. Depending on the timing and local environments after stroke, reactive astrocytes may be beneficial or detrimental. Astrocytes, together with microglia, also release trophic factors such as basic fibroblast growth factor, nerve growth factor, ciliary neurotrophic factor, glial cell line-derived growth factor and brain-derived neurotrophic factor, thus promoting neuronal plasticity, synaptic formation, and rebuilding of the nervous system to improve functional outcome after injury. On the other hand, reactive astrocytes may release proteolytic molecules, such as matrix metalloproteinases, to degrade CSPGs (Zuo et al., 1998), as observed in the present study that attenuated astrocytic activation induced increased CSPG expression in GFAP<sup>-/-</sup>Vim<sup>-/-</sup> mice.

Although reactive astrocytes form an inhibitory glial scar following stroke, they also perform functions important in neural repair. Our findings suggest that the involvement of

astrocytes in axonal remodeling and functional recovery after stroke may represent a possible therapeutic target for neurorestorative strategies. Further studies focused on preserving reactive astrocytes, to augment their protective functions, and/or reduce their detrimental effects, may lead to novel approaches to improve neurological recovery after stroke.

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