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Review

Astrocytes in Spinal Cord Injury: Current Opportunities and Prospects for Directional Polarization

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Abstract

Activation of astrocytes during spinal cord injury (SCI) is accompanied by changes in their morphology and functional activity, possibly having severity-, localization-, and time-dependent features. The understanding of the role of reactive astrocytes has undergone significant changes over the last decades, and new data are still emerging to assess the diversity of functional manifestations of reactive cells. This review discusses the current understanding of astrocyte behavior, possible manifestations of their negative and positive roles in SCI, and the prospects for using various methods of directed polarization of astrocytes to improve post-traumatic outcomes. Despite the existing difficulties regarding the disclosure of the complex cascade of molecular changes of reactive astrocytes in different posttraumatic periods, researchers do not give up hope for the development of astrocyte-targeted methods that could reduce the severity of secondary injury by regulating the negative effects of these cells.

Keywords: astrocytes; phenotypes; polarization; spinal cord injury

1. Introduction

Spinal cord injury (SCI) results in the formation of a glial scar that contributes to maintaining the structural integrity of neural tissue during the acute period of injury. However, the importance of the formed physical barrier, mainly mediated by reactive astrocytes and perivascular/meningeal fibroblasts, is worth considering during the chronic period of SCI.

Astrocytes are the most abundant cell type in adult neural tissue and are indispensable for maintaining normal health and function of the central nervous system (CNS) [1,2]. In response to neurotrauma, neurodegeneration, or infection, astrocyte activation occurs, accompanied by changes in gene expression and consequent morphology and function of these cells [3,4]. Summarizing previously obtained data, it was found that reactive astrocytes are capable of both negative effects, such as increased neuroinflammation and inhibition of axon growth, and positive effects, including neuroprotection and participation in the restoration of the blood–brain barrier [5,6]. This finding confirms the heterogeneity of reactive astrocytes, which is associated with various specific environmental signals that affect them [7]. In this regard, similar to microglia, several polarization variants are distinguished for astrocytes, leading to the formation of A1 (proinflammatory) and A2 phenotypes (anti-inflammatory/neuroprotective). It should be noted that several researchers recommend avoiding binary terms when describing cell phenotypes because the classification of reactive astrocytes should take into account multiple criteria, including transcriptome, proteome, morphology, and specific cellular functions, as well as their impact on pathological characteristics [8]. It is also worth emphasizing the importance of using multivariate data to detect differences in astrocyte phenotypes since the A1 and A2 transcriptomes of astrocytes may represent only two variants among many [9]. However, the classification of astrocyte phenotypes into A1 and A2 is still used in scientific research, perhaps to facilitate understanding of the bidirectional functions of these cells after activation. We are aware of the heterogeneity of the astrocyte cell population, but when quoting other people's works, we allow the use of terms used by other authors with the most complete description of the molecular and morphological changes found in these cells.

Polarization of reactive astrocytes towards the A1 phenotype shows increased expression of many cascade genes of the complement system, such as *C1r*, *C1s*, *C3*, and *C4* [10], as well as the induction of pro-inflammatory factors IL-1β, TNF-α, and NO [11]. This subtype of reactive astrocytes has been found to be induced by classically activated microglia cells (M1 phenotype). Pro-inflammatory microglia, through the secretion of cytokines like IL-1α, IL-1β, TNF-α, and C1q, are involved in the induction of astrocyte polarization toward the A1 phenotype. A co-culture of astrocytes or reactive A1 astrocytes with retinal ganglion cells (RGCs) was performed, and their viability was subsequently assessed. As a result, RGCs were found to die

rapidly when grown in a medium containing higher concentrations of factors secreted by A1 astrocytes. These results indicate that A1 astrocytes begin to secrete a soluble neurotoxin that leads to the death of neurons and oligodendrocytes [12]. Not only do A1 astrocytes lose their function of maintaining synapses, but they also lose their ability to negatively impact them through the activation of complement cascade genes and the induction of NF-κB signaling [13,14]. Thus, the A1 phenotype of astrocytes is characterized as neurotoxic, capable of influencing the development of pathological processes in the CNS [15].

Modulation of astrocytes toward the A2 phenotype leads to the secretion of cardiotrophin-like cytokine factor (CLCF1), hypoxia-inducible factor (HIF), IL-6, IL-10, and thrombospondins by these cells, promoting neuronal survival and growth [10]. A2 reactive astrocytes are present during glial scar formation because the borders of the glial scar are formed by newly proliferating elongated astrocytes via STAT3 signaling. Specific to A2 astrocytes is Emp1, a high expression of which was observed in the acute phase of brain damage against the background of the destruction of the blood–brain barrier [16]. Some researchers are inclined to distinguish a separate population of synaptogenic astrocytes capable of forming more functional synapses when co-cultured with neurons. This population of astrocytes appears to be largely absent from the intact adult spinal cord [17]. However, it is not known whether the synaptogenic potential in other subpopulations of astrocytes can be enhanced against the background of neuropathology [18].

Recent studies have shown that astrocytes located in different areas of the brain and spinal cord have been found to respond to pathological processes and change their phenotype differently [19–24]. These results indicate the relevance of studies aimed at establishing qualitative and dynamic shifts in astrocyte populations, including in SCI, to reveal their role in disease progression, search for therapeutic targets, and determine the most favorable period for therapeutic action. Despite a long period of research into post-traumatic processes in the spinal cord and the discovery of most, but probably not all, mechanisms of astrocyte activation that mediate certain effects, severity- and time-dependent approaches have not been developed to modulate the protective potential of these cells. In our opinion, the development of gentle, step-by-step, targeted therapies, including those aimed at astrocytes, in SCI is becoming increasingly relevant compared to debates about the inconsistency of the binary system of cell phenotypes. Our review is aimed at improving the understanding of the role of reactive astrocytes in connection with the emergence of new data on the diversity of their functional manifestations in SCI, as well as revealing the possible prospects for using various methods of targeted polarization of these cells to improve post-traumatic outcomes.

2. Astrocytes in Spinal Cord Injury

The process of the development of posttraumatic reactions includes two phases: primary and secondary. Primary trauma is defined as mechanical damage to the spinal cord, most often caused by the consequences of the fracture or dislocation of vertebrae with their displacement towards the spinal canal. This period is directly related to the compression of the spinal cord and acute disruption of its blood supply, leading to changes such as cell death by necrosis and edema of nerve tissue, culminating in secondary ischemia. Secondary spinal cord injury occurs as a consequence of the primary and lasts for several weeks or months. This period is characterized by the development of inflammatory processes, activation of astrocytes and microglia, glial scar formation, and disruption of axonal connections [25].

One of the main pathological signs of spinal cord injury is reactive astrogliosis, characterized by significant morphological and functional changes in astrocytes after SCI. The severity of the injury, the progression period of the disease, and the distance from the site of injury are factors that determine the degree of astrocyte reactivity and possibly their polarization. It was noted earlier that astrogliosis can be categorized into several degrees, ranging from moderate changes in the morphology and phenotype of astrocytes to pronounced proliferation of these cells [26].

A chronology of the transformation of astrocytes forming the glial scar after SCI can be distinguished. Within a few hours after SCI, there is activation and hypertrophy of astrocytes, with increased expression of glial fibrillary acidic protein (GFAP). However, morphologically, most reactive astrocytes do not differ from normal ones. During the 3–5 day period following SCI, there is a rapid proliferation of astrocytes. This proliferation is characterized by the extension of elongated outgrowths perpendicular to the area of damage, corresponding to the transformation of these astrocytes into scar-forming cells. After two weeks, astrocyte proliferation ceases, and their phenotypic changes are likely complete. The outgrowths become more parallel and overlap with each other [27].

It is important to note that there are two types of astrocytes (protoplasmic and fibrous). Protoplasmic astrocytes are located in the gray matter and have short, strongly branching outgrowths that form a network around neurons and synapses, supporting their structure and function. On the other hand, fibrous astrocytes are located in the white matter, larger in size, and have long ramifications that accompany nerve fibers. The classification mentioned above does not overlap with the terminology of A1/A2 astrocytes. However, some studies have linked the reactivity of astrocytes in SCI with their morphology and localization.

Taking as a basis the basic signs of astrocyte activation, including complex three-dimensional morphometry of astrocytes, it was shown that the increased expression of A1 astrocyte markers (C3 and Serping1) and downregulation

of A2 astrocyte markers (SphK1 and tm4sf1) are accompanied by changes in cell volume, surface area, filament length, glial branches, and cell body density decreasing in terms of the astrocyte shell [28]. This has shown a correlation of morphologic parameters with markers A1 and A2. However, how these morphological changes in A1/A2 astrocytes ultimately affect their deleterious or beneficial functions remains unclear.

Many researchers have noted the dual role of the glial scar and the astrocytes that make up it. In terms of positive properties, it is important to note that the glial scar acts as a physical barrier to prevent the spread of inflammation and degenerative processes during the acute period of SCI. In addition, astrocytes absorb excess glutamate, the release of which into the extracellular environment is one of the early consequences of SCI, thereby reducing its excitotoxic effects on the microenvironment [29]. Reactive astrocytes have been shown to potentially promote tissue regeneration as they enhance the expression of FGF-2 and S100β in the injured spinal cord. In addition, there are studies suggesting that removal of the glial scar does not promote spinal cord regeneration [30]. Thus, to date, it is not excluded that astrocyte activation is necessary to minimize the severity of SCI [31].

Astrocytes have a key function in homeostatic regulation, which includes buffering ions and controlling neurotransmitters [32]. The disruption of this function in astrocytes plays a crucial role in the process of secondary injury in SCI. Due to their highly negative resting membrane potential and low membrane resistance, astrocytes are ideally suited to regulate K^+ levels in nervous tissue. Various potassium channels in astrocytes, such as TREK-1 and Kir4.1, have been demonstrated. Studies on TREK-1 knock-out mice have shown increased reactive astrogliosis and production of chondroitin sulfate proteoglycans (CSPGs), as well as decreased GLT-1 expression [33]. Hyperalgesia and altered firing patterns of neurons in the dorsal spinal cord were observed when Kir4.1 was knocked down in spinal astrocytes [34]. Kir4.1 channels are typically concentrated in perivascular endings and perisynaptic outgrowths of astrocytes at the cell level [35]. These astrocytes play a crucial role in maintaining the spatial isolation of synaptic transmission through glutamate transporters, thereby preserving the specificity of signal transduction. The $Ca(2^+)$-activated K^+ channel KCa3.1 plays an important role; blocking it pharmacologically or knocking it out leads to a decrease in reactive astrogliosis, which improves tissue protection and locomotor recovery after SCI [36,37].

Under physiological conditions, astrocytes exhibit both spontaneous and receptor-activated $Ca(2^+)$ signals [38]. This calcium signaling plays a crucial role in enabling bidirectional communication between neurons and astrocytes at the synapse [39]. It has been suggested that $Ca(2^+)$ has a damaging effect on white matter in SCI, which may be partially mediated by potential-dependent L- and N-type $Ca(2^+)$-channels on periaxonal astrocytes. These channels are involved in the posttraumatic impairment of axonal conduction [40]. A recent study demonstrated that upregulation of the non-selective cation channel TRPC6 inhibits astrocyte activation and proliferation after SCI in rats by suppressing AQP4 expression [40].

Currently, there is no direct evidence linking potential-dependent Na^+ channels to action potential initiation in astrocytes. However, it is believed that these channels play a crucial role in astrocyte function, particularly in maintaining ion homeostasis, regulating neurotransmitters, and reactive astrogliosis [35]. Na^+ accumulation-induced depolarization of the cell membrane can result in neuronal death by releasing glutamate into the presynaptic region. Research has demonstrated that after SCI, there is an occurrence of glutamate release at an excitotoxic level into the extracellular space [41]. The vast majority of functional uptake of extracellular glutamate after SCI is carried out by astrocytic glutamate transporters. Studies have shown that decreased expression of the astrocyte glutamate transporter (GLT-1) in heterozygous mice (GLT1+/−) leads to worsened functional outcomes, increased apoptosis, and neuronal loss following SCI [42]. However, overexpression of GLT-1 in astrocytes via virus mediation also increased lesion size, neuronal damage, and respiratory impairment after cervical SCI [43]. Therefore, it can be concluded that overactivation of glutamate receptors may have diffuse and nonspecific effects on nervous tissue.

As researchers note, along with its protective function, the glial scar also plays a negative role in the pathological processes of SCI. The glial scar and the reactive astrocytes that comprise it secrete inhibitory proteins that prevent functional recovery. Among the main factors mentioned are CSPGs, whose expression is increased by reactive astrocytes. In selectively isolated *in situ* using laser microdissection and immunohistochemistry, astrocytes also showed a high level of CSPG expression [4]. Some studies show that chronic SCI astrocytes characterized by increased expression of the *Sox9* gene exhibit lower expression of CSPG-related genes than scar-forming astrocytes [44]. Recently, evidence for enhanced expression of CSPG4 by protoplasmic astrocytes, including in the region of their perisynaptic outgrowths, has been reported during acute and chronic periods of rat SCI not only in the epicenter of the injury, but also in the area remote from the lesion epicenter [45]. However, there has been no conclusive evidence that enhancing astrocyte expression of CSPG4 has an inhibitory effect on axon growth. Therefore, whether the astrocytic component of the glial scar can be considered an inhibitory mechanism in SCI is still controversial.

Research on astrocyte behavior in SCI is still ongoing, with the hope that the knowledge gained could be applied to more effective therapies in the future. Since the glial scar plays a dual role, new therapies should attenuate

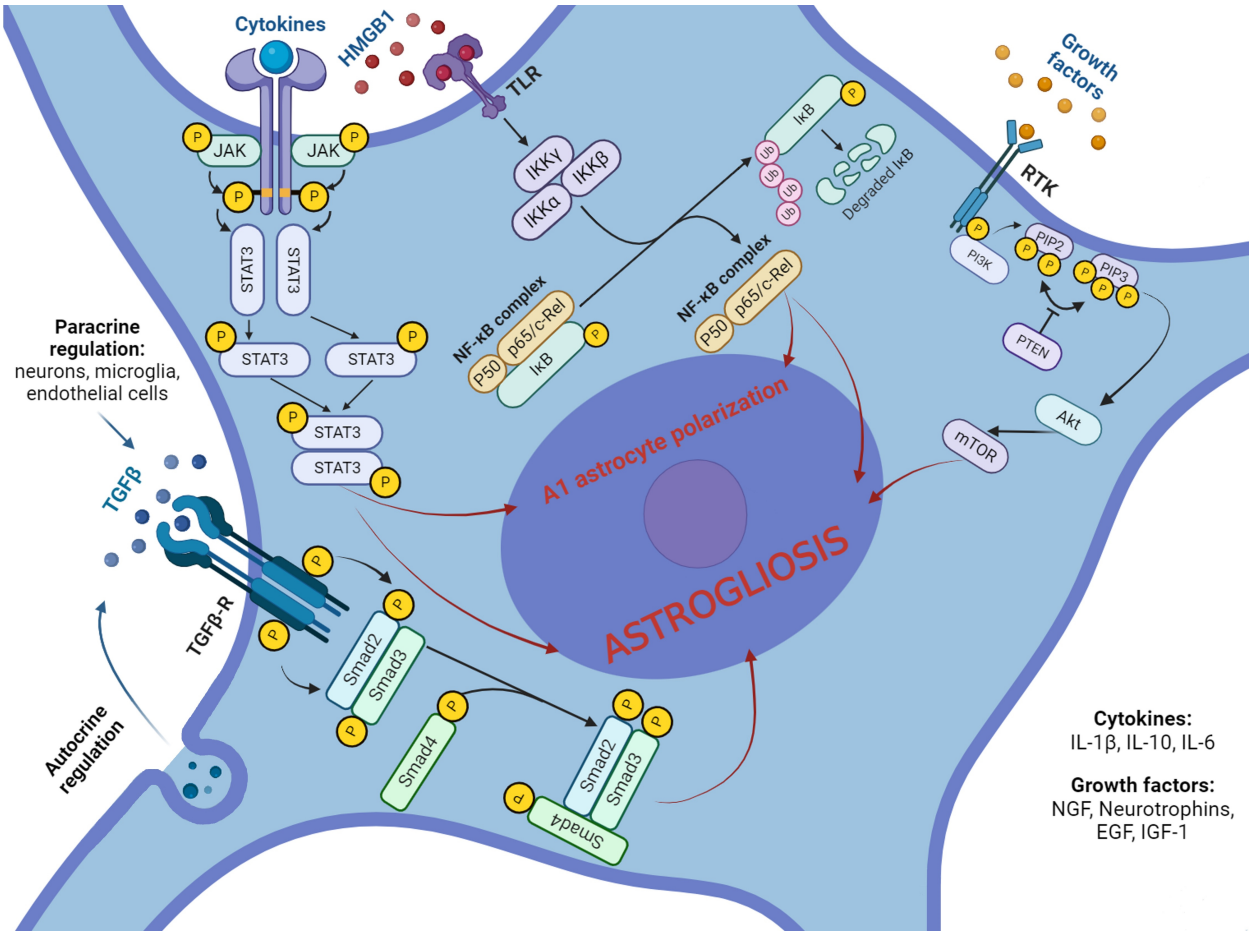

Fig. 1. Astrocyte signaling pathways determining reactivity and polarization of astrocytes. The most significant signaling pathways that regulate astrocyte polarization include JAK-STAT3, TGF-β/Smad, HMGB1/TLR4/NF-κB, and PI3K/Akt/mTOR. The TGF-β/Smad signaling pathway determines astrogliosis, while the other three pathways determine both astrogliosis and polarization towards A1 astrocytes. P indicates phosphorylation. TLR, toll-like receptor; RTK, receptor tyrosine kinase.

its inhibitory role while preserving its beneficial functions. To this end, novel approaches are being developed that target the various processes that regulate astrocyte function.

3. Signaling Pathways that Regulate Astrocyte Polarization

3.1 STAT3

Various signaling pathways have been implicated in the regulation of reactive astrogliosis and astrocyte polarization (Fig. 1), which will be discussed below. Activation of STAT3 in astrocytes through phosphorylation by Janus kinases has been demonstrated in various disease models associated with astrogliosis, including SCI [46,47]. STAT3 is considered a major component of the JAK-STAT3 signaling pathway, and its activation is a key prerequisite for astrocytic scar formation after SCI [48]. In mice with STAT3 knockout (GFAP-STAT3-CKO mice), no glial scar formation was observed after SCI: astrocytes near the lesion were dispersed without sealing off the damaged areas, which increased tissue infiltration by inflammatory cells [46].

STAT3 is also crucial in astrocyte differentiation and is essential for astrocyte maturation [49]. Recent studies show that STAT3 mediates the switch of astrocyte phenotypes upon activation. Lcn2/JAK2-STAT3 signaling was found to be involved in the activation of neurotoxic M1 microglia and A1 astrocytes during the acute and subacute periods after TCM [50]. STAT3 hyperphosphorylation has been reported to be induced in astrocytes with reduced IL-10 (IL-10tm1/tm1) by lipopolysaccharides (LPS), which was accompanied by an increased propensity for astrocyte polarization towards the A1 phenotype [51]. In the SCI model, activation of the Notch-STAT3 axis was also found to induce a phenotypic switch of astrocytes to type A1 and its neurotoxic effects [52]. However, some studies have shown that astrocytic STAT3 can also induce polarization toward the A2 phenotype of astrocytes. For example, STAT3-mediated (possibly A2) scar-forming reactive astrocytes were found to promote axon regeneration in an SCI model [53]. Ma *et al.* [54] showed that astrocytic STAT3 may be involved in the A2 astrocyte formation induced by prokineticin 2 in a model of subarachnoid hemorrhage. Su

et al. [55] demonstrated that miR-21 regulates the polarization of reactive astrocytes by targeting STAT3, as shown in a model of ischemic spinal cord injury. This experiment established that astrocytes, via STAT3-Gpc6 and STAT3-GNDF signaling, can promote synapse formation and nerve growth, and miR-21 may become a key SCI-targeting therapy molecule in the future.

3.2 TGF-β

In addition to STAT3, TGF-β signaling can also contribute to reactive astrogliosis. Astrocytes have TGF-β receptors and can synthesize TGF-β, which acts on these cells in both autocrine and paracrine ways. When the blood–brain barrier is disrupted, blood components such as fibrinogen, immunoglobulins, and other high molecular weight proteins can penetrate and accumulate in astrocytes, activating TGF-β signaling. Blood fibrinogen activates TGF-β/Smad signaling after SCI, leading to reactive astrogliosis and affecting the heterogeneity of astrocytes [56]. However, blocking TGF-β signaling has been shown to attenuate glial scar formation.

Scientific data increasingly support the participation of TGF-β in regulating processes such as apoptosis, inflammation, and scarring after SCI [57–59]. Restricting TGF-β expression by astrocytes after SCI promotes axonal regeneration and neurological recovery [60]. During the subacute phase of SCI, microRNA-21 regulates reactive astrogliosis through TGF-β signaling. MicroRNA-21 and TGF-β are important regulators of astrogliosis. MicroRNA-21 can affect astrocyte release, proliferation, and apoptosis, promoting recovery from injury both *in vivo* and *in vitro* [61].

3.3 NF-κB

Nuclear transcription factor-κB (NF-κB) plays a central role in most inflammatory responses and can be activated by stimuli associated with damage, subsequently increasing the expression of pro-inflammatory cytokines [62,63]. In a study by Brambilla *et al*. [64], it was shown that reducing the inflammatory response induced by NF-κB activation can be achieved by inhibiting this signaling in astrocytes, resulting in improved functional recovery after SCI. This improvement is mediated by enhanced axon preservation, sprouting, and stimulation of spinal cord regeneration. In addition to reducing inflammation, inhibition of NF-κB activation in astrocytes significantly decreases the presence of CSPGs at the epicenter of injury and in the white matter near SCI [65]. In a recent study, astaxanthin, a fat-soluble carotenoid with anti-inflammatory effects, was shown to inhibit signaling pathways such as HMGB1/TLR4/NF-κB, reducing spinal cord edema and astrocyte activation [66]. The administration of the TLR9 antagonist (oligodeoxynucleotide 2088) via intrathecal injection resulted in a decrease in the number of proliferating astrocytes both rostral and caudal to the lesion border. This effect is believed to be due to the prevention of activation of the Erk/MAPK signaling pathway in these cells [67].

A recent study demonstrated that the LPS-induced proinflammatory response is attenuated in mouse astrocytes by the addition of non-metabolizable 2-deoxyglucose in culture [68]. This suggests that astrocytic glycolysis is a target for limiting NF-κB-mediated inflammation. In transgenic mice with astrocytes deficient in IκB kinase 2, inactivation of astrocytic NF-κB inhibited the increase induced by exposure to MnCl2 and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in A1 astrocytes expressing C3 [69]. These results suggest that astrocytic NF-κB activation can contribute to the acquisition of an A1 phenotype by astrocytes and play a critical role in astrocyte-mediated neuroinflammation.

Heat shock factor 1 (HSF1)-mediated inactivation of NF-κB was found to be involved in suppressing the expression of C3, a protein characteristic of the A1 phenotype of astrocytes. As an important transcription factor, HSF1, together with its inducible protein HSP70, has been shown to exert anti-inflammatory effects, regulating NF-κB activity either by reducing the degradation of the IκB protein [70] or by inhibiting the nuclear binding activity of NF-κB [63]. IGF-I signaling has also been found to contribute to the inhibition of NF-κB signaling in reactive astrocytes by recruiting calcineurin and PPARγ (peroxisome proliferator-activated receptor-γ) to activate IκB and prevent Foxo3 activation [71]. The possibility of inhibiting C3 production in A1 astrocytes was confirmed later in a study by Xu *et al*. [72], where injections of the NF-κB inhibitor pyrrolidine dithiocarbamate were made.

3.4 PI3K/Akt/mTOR

Evidence suggests that the activation of the PI3K/Akt/mTOR signaling pathway is involved in glial scar formation after SCI. Given that PTEN negatively regulates the PI3K/Akt/mTOR pathway, it was hypothesized that increasing PTEN expression in the spinal cord would contribute to a positive effect after SCI [73]. Another study demonstrated the role of miR-17 in regulating the PI3K/Akt/mTOR signaling pathway by affecting PTEN. It has been shown that the reduction of glial scar formation after SCI can be achieved by either inhibiting miR-17 or increasing PTEN expression [74].

4. Different Approaches to Astrocyte Modulation

4.1 Photobiomodulation

Photobiomodulation (PBM) is a treatment that employs low-intensity laser irradiation to activate positive biological processes in cells and tissues. In an *in vitro* experiment using primary astrocytes stimulated with conditioned medium from macrophages, researchers found that photobiomodulation inhibited astrocyte proliferation and the expression of genes related to their activation and pSTAT3 signaling [75] (Table 1, Ref. [12,75–88]).

Table 1. Different approaches to modulation of astrocytes *in vitro* and *in vivo*.

Protocol details	Source of astrocytes (*in vitro* study)	Modulator, type of injection	Polarization/activation	Other reported effects	Reference
			Photobiomodulation		
Rats, clip-compression SCI	Brain of neonatal rats	PBM, 810 nm diode laser beam	Attenuation of A1 astrocyte activation; Promotion A2 astrocyte activation	*In vivo* ↓TNF-α, IL-6, IL-1β, iNOS, LCN2 ↑bFGF, TGF-β	Wang *et al.* [76], 2021
Female BALB/c mice, clip-compression SCI	Cerebral cortex of 1–3-day-old juvenile BALB/c mice	PBM, 810 nm diode laser beam	Reduces the activation of astrocyte; Inhibits the astrocyte proliferation	*In vivo* ↓the secretion of CSPG in the para-epicenter area, number of M1; recovery of motor function *In vitro* ↓gfap, nestin, ctnnb1, mmp2, axin2; pSTAT3	Sun *et al.* [75], 2020
			Cytokines		
N/A	Brain of rats or mice	Il-1α (3 ng mL^{-1}), TNF (30 ng mL^{-1}), C1q (400 ng mL^{-1})	Induce A1 astrocytes		Liddelow *et al.* [12], 2017
N/A	Cerebral cortices of newborn mice	Recombinant mouse IL-18 (100 ng/mL and 500 ng/mL, R&D Systems, USA)	Astrocyte conversion to the A1 phenotype	↑C3d, H2-T23, Fkbp5 и ligp1 ↓Emp1, S100a10 и Cd109	Hou *et al.* [77], 2020
			Gene therapy		
C57BL/6J mice, clip-compression SCI	Brains of neonatal C57BL/6J mice	AAV-L1 or AAV-GFP (3×10^7 transducing units in 1 µL)	Reduced proliferation of astrocytes	*In vivo* ↓GFAP, NG2 ↑Numb *In vitro* ↓migration of astrocytes	Chen *et al.* [79], 2007
Rat, contusion SCI	Spinal cord of 3-day-old rat	LV-p27kip1 or LV-GFP (3×10^7 transducing units in 1 µL)	Attenuation of reactive astrogliosis, inhibition of astrocyte proliferation	*In vivo* ↓CDK4, cyclin D1, GFAP, NG2 *In vitro* ↓migration of astrocytes	Chen *et al.* [80], 2016
WT mice, cerebral cortex	N/A	NeuroD1 (1.5 µL)	Attenuation of A1 astrocyte activation	↓GFAP, Lcn2, Gbp2, Serping1 ↓TNFα, IL-1β ↓neuroinflammation	Zhang *et al.* [78], 2020

Table 1. Continued.

Protocol details	Source of astrocytes (*in vitro* study)	Modulator, type of injection	Polarization/activation	Other reported effects	Reference
Other approaches					
Mice, clip-compression SCI	Spinal cord of 13-day-old C57BL/6J mouse embryos	NG (six 0.250 µL/0.025 mg/mL injections), NG-Cy5 loaded with Rolipram (nanogel 0.025 mg/mL), or free Rolipram (0.7 mg/mL)	Neuronal preservation and the reduced activation of astrocytes	*In vitro* ↓Lcn2 и iNOS	Vismara *et al*. [81], 2020
Rat, contusion SCI	Spinal cord of newborn Sprague–Dawley rats	Intraperitoneal injection of 100 µL of 20 mg/kg 17-AAG	To reduce the number of A1 astrocytes	*In vivo* ↓number of reactive astrocytes ↓expression of complement component C3	Li *et al*. [82], 2021
Mice, contusion SCI	Brains of neonatal mice	miR-NCOE-Exos, miR-124-3pOE-Exos, miR-NCKD-Exos, miR-124-3pKD-Exos (200 µg of exosome protein precipitated in 200 µL of PBS)	Inhibition of astrocyte A1 activation	Inhibition of microglia M1 activation	Jiang *et al*. [83], 2020
Rat, contusion SCI	Brains of 1–3-day-old neonatal Sprague–Dawley rats	Injection of 10 mg/mL ginsenoside Rg1 (10 mL/kg body weight)	Transdifferentiation of reactive astrocytes into neuron-like cells	*In vivo* ↓C3, GFAP; ↑MAP2, NeuN, NEUROD1, Myt1l, TUJ1, SYN1; ↓lesion cavity volume; repair of motor function	Shen *et al*. [84], 2023
Mice, clip-compression SCI	The BV2 microglial cell line	Intraperitoneally injected with parthenolide (2 mg/kg)	Decrease in A1 astrocyte number	*In vivo* ↓CSPG, reduction in the number of microglia and macrophages *In vitro* ↑anti-inflammatory cytokines (TGF-β, IL-10, and IL-13), ↓pro-inflammatory cytokines (TNF-α, IL-1β, and IL-6)	Gaojian *et al*. [85], 2020
Genetically modified mice, dorsal hemi-crush model SCI	N/A	Tamoxifen (75 mg/kg)	LZK overexpression enhances astrogliosis and scar formation	Activation of STAT3 and upregulation of Sox9	Chen *et al*. [86], 2018
Mice, contusion SCI	Mice, contusion SCI	Nec-1 (7.8 mg/kg)	Attenuates astrocyte death, rescues the neurotrophic function of astrocytes	*In vivo* ↓RIP3, MLKL, HMGB1	Fan *et al*. [87], 2016
Rat, contusion SCI	N/A	Nec-1	Reduces ERS in neuron, astrocyte, and microglia	↓CHOP, GRP78, XBP1	Wang *et al*. [88], 2017

PBM, photobiomodulation; N/A, not available; WT, wild type; AAV, adeno-associated viruses; LV, lentiviral vectors; NG, nanogel; LZK, leucine zipper-bearing kinase; ERS, endoplasmic reticulum stress; Nec-1, necrostatin-1; CDK, cyclin-dependent kinase. ↓: decrease; ↑: increase.

Similar results were obtained *in vivo*, showing that photobiomodulation reduces astrocyte activation and chondroitin sulfate proteoglycan (CSPG) secretion in the para-epicenter area after SCI, promoting the recovery of motor function [75]. Other authors demonstrated that the regulation of astrocyte activation through photobiomodulation may be due to a decrease in the number of A1 astrocytes in the region of the epicenter of injury and their polarization towards a neuroprotective A2 phenotype. The mechanism mediating these changes, as suggested by the authors, may be related to the regulation of NF-κB, Notch, JAK2-STAT3, and PI3K-Akt signaling pathways, which are crucial for astrocyte activation, and the increased expression level of basic fibroblast growth factor (bFGF) and TGF-β by these cells [76].

4.2 Cytokines

The phenotypic transformation of astrocytes is associated with changes in the microenvironment, in which some cytokines predominantly secreted by activated microglia are key regulators of the above process [7,12]. IL-1a, TNF, and C1q are secreted predominantly by activated microglia and can induce astrocyte polarization towards the A1 phenotype *in vitro*. Using single, double, or triple knockout mice for IL-1a, TNF, or C1qa, researchers found that A1 astrocyte reactivity was reduced in single (IL-1a$-/-$, TNF$-/-$, or C1qa$-/-$) and double (IL-1a$-/-$/TNF$-/-$) knockout mice, while triple knockout mice did not exhibit the indicated astrocyte reactivity in the background of systemic LPS injection. The authors also observed similar effects of IL-1a, TNF, and C1q on A1 transformation during normal aging [23]. A recent study showed that primary mouse astrocytes treated with exogenous IL-18 in culture showed increased expression of A1 astrocyte markers (C3d, H2-T23, Fkbp5, and ligp1) and decreased expression of A2 astrocyte markers (Emp1, S100a10, and Cd109) [77]. Therefore, the authors suggested that IL-18 may act as a novel inducer of A1-responsive astrocytes in addition to IL-1a, TNF, and C1q.

Although the pro-inflammatory cytokine IL-1β plays a significant role in neuroinflammation, [12] reported that IL-1β from a conditioned medium with LPS-activated microglia was unable to induce the expression of astrocyte A1 phenotype transcripts. Similarly, Hou *et al*. [77] also found that the level of astrocyte A1 marker C3d did not increase when IL-1β was added to astrocyte culture. However, during the same period, Zhang *et al*. [51] showed that IL-1β had the ability to increase A1- and A2-specific transcripts in astrocyte culture. In an *in vivo* model of IL-1β-induced white matter damage in newborn mice, astrocytes had increased A2 reactivity [89]. These results are controversial, and further studies are needed to determine the role of IL-1β, including dose-dependent effects, in the polarization in different experimental models.

IL-10 is a pivotal cytokine predominantly secreted by astrocytes and microglia, playing a crucial role in suppressing excessive inflammatory responses and promoting neuroprotection against various CNS injuries [90–93]. In both normal and pathological conditions, IL-10 receptor (IL-10R) expression has been detected in microglia, astrocytes, oligodendrocytes, and even neurons. Studies have demonstrated increased astrocyte immunoreactivity in the SCI area of IL-10 knockout mice, leading to enhanced secondary inflammatory processes [94]. Conversely, IL-10 administration has been observed to decrease the production of anti-inflammatory cytokines, suppress microglia and astrocyte activation, and reduce leukocyte infiltration [95]. IL-10 stimulates activated astrocytes to produce TGF-β, resulting in decreased microglia activation and reduced production of proinflammatory cytokines [96].

Given the above, it is essential to note that cytokines serve as potential tools for multidirectional modulation of functional rearrangements in reactive astrocytes following SCI. However, planned studies should carefully consider possible side effects and systemic reactions to cytokine administration, aiming to create targeted drugs that focus on signaling pathways regulating astrocyte activity.

4.3 Genetic Approaches

The application of genetic engineering approaches to modulate the phenotype of astrocytes appears to be highly relevant and promising. In a recent study, the neuronal transcription factor NeuroD1 was ectopically expressed in reactive astrocytes in the area of cortical damage in wild-type mice using adeno-associated viruses of serotype 9. It was observed that against the background of transduction in reactive astrocytes, the level of expression of markers characteristic of the A1 phenotype, namely GFAP, Lcn2, Gbp2, and Serping1, was reduced. Additionally, the release of toxic cytokines such as TNFα and IL-1β, contributing to neuroinflammation, was decreased. Genetic engineering-mediated differentiation of astrocytes into neurons showed the possibility of restoring the proportion of these cells in the area of injury, thereby improving the glial landscape [78].

There is evidence that by employing an AAV vector to express the cell adhesion molecule L1 in the injured spinal cord of mice, researchers observed a specific reduction in NG2 and GFAP expression in astrocytes, accompanied by improved motor function and enhanced 5-HT axon reinnervation [79]. The lentiviral vector-mediated expression of the cyclin-dependent kinase (CDK) inhibitor p27kip1 was used to reduce astrocytic reactivity and improve the local microenvironment in the injured rat spinal cord [80]. Another study revealed that direct gene therapy using AdV-GDNF left GFAP expression unaffected, indicating a potentially positive outcome. Additionally, S100B expression increased in the dorsal root entry zone (DREZ) due

to S100B+ Schwann cell migration, and AQP4 expression rose in the ventral funiculus (VF), suggesting specific phenotypic characteristics in white matter [97].

In general, these results confirm the possibility of using genetic engineering to modulate the phenotype of astrocytes and improve neurological functions. However, we should not forget about the heterogeneity of astrocytes and the different efficiency of their transduction in separate brain regions. Additionally, the possibility of transduction of various cell types other than astrocytes should be taken into account when using viral vectors. In this regard, the search for specific promoters for genetically targeted astrocytes in different regions of the CNS seems most promising [98–100]. However, if we are not talking about astrocyte transdifferentiation, the question remains open as to how significant a change in the expression of one or two genes can lead to the modulation of astrocyte functions.

4.4 Other Approaches

Polymeric nanoparticles have a wide range of applications and significant advantages in drug delivery. Rolipram, an anti-inflammatory drug that acts on the NF-κB signaling pathway in astrocytes, has been encapsulated in a nanogel. This formulation has been found to limit the expression of pro-inflammatory molecules, including Lcn2 and iNOS, in astrocytes of the A1 phenotype, thereby reducing the inflammatory response [81].

Heat shock transcription factor 1 (HSF1) also plays a crucial role in controlling the inflammatory response. Tissue damage or environmental stressors, such as elevated temperature or oxidative reactions, can lead to an increase in HSF1 expression. This increase inhibits the activity of MAPKs and NF-κB, which regulate the expression of C3, a key marker of the neurotoxic A1 phenotype of astrocytes [82]. In a recent study, scientists discovered that neuron-derived exosomes can block the activation of A1 astrocytes, resulting in a decrease in C3 and GFAP expression. This effect is achieved by regulating the PI3K/AKT/NF-κB signaling cascade, promoting functional recovery after SCI, both *in vitro* and *in vivo* [83].

In a recent study, it was found that the use of Rg1 ginsenoside can directly modulate reactive astrocytes into neuron-like cells *in vitro*. The mechanism of this process may proceed by blocking the Notch/Stat3 signaling pathway. In this sense, the application of Rg1 ginsenoside during SCI promoted the recovery of motor function in rats and reduced the area of damage in *in vivo* experiments [84]. Parthenolide treatment following SCI significantly reduced cavity volume, disrupted astrocytic scar formation marked by GFAP staining, and lowered total chondroitin sulfate proteoglycan (CSPG) levels. Notably, parthenolide administration significantly decreased the A1 astrocyte number at the lesion area, indicating its potential to modulate astrocyte phenotypes after SCI [85].

In another study, SCI was found to induce the expression of leucine kinase (LZK) in astrocytes. Scientists deleted the *Lzk* gene in the astrocytes of a group of injured mice, which resulted in decreased reactivity of these cells and increased the area of spinal cord injury. However, overexpression of the *Lzk* gene in astrocytes of another group of injured mice led to the activation of these cells against the background of damage and reduction of glial scarring. Overexpression of the *Lzk* gene in astrocytes of uninjured mice also promoted the activation of these cells. The above-mentioned confirms that LZK is an important positive regulator of astrocyte reactivity and glial scar formation after SCI [86].

It was found that after SCI, reactive astrocytes undergo RIP3/MLKL-mediated necroptosis. Astrocyte necroptosis can also be induced by microglia cells through TLR/MyD88 signaling. A study by Fan *et al*. [87] showed that inhibiting the necroptosis process via necrostatin-1 (Nec-1) in astrocytes may be useful in preventing secondary damage during SCI. The use of Nec-1 after SCI in mice not only prevented astrocyte death but also promoted the preservation of their neurotrophic function, which, in turn, rescued the survival of neighboring neurons. Another study also demonstrated that Nec-1 has the ability to reduce endoplasmic reticulum stress (ERS) in astrocytes, neurons, and microglia. Nec-1 use in SCI was found to reduce ultrastructural damage to the endoplasmic reticulum and mitochondria, as well as suppress the expression of genes (*CHOP*, *GRP78*, *XBP1*) and proteins encoded by them [88].

Thus, to modulate astrocyte reactivity and enhance their neuroprotective potential, various approaches aimed at different aspects of cell viability (proinflammatory signaling pathways, programmed cell death, etc.) are used. However, more in-depth studies in this area are required to confirm the possibility of translating these approaches into clinical conditions, taking into account the period and severity of neurological disease.

5. Conclusions

Recently, new data about astrocyte heterogeneity both across and within CNS regions, including healthy and affected nervous tissue, have increasingly appeared [101, 102]. The aforesaid seems to be one of the breakthroughs in modern neuroscience, but it complicates the process of interpreting the obtained data and the search for effective modulators for astrocyte directional polarization into significantly enhanced protective properties. There is still no answer to the question of what set of molecules expressed by astrocytes in different post-traumatic periods could reduce the severity of secondary damage, contributing to the restoration of lost functions. It should be taken into account that the set of molecules may simultaneously include cytokines that canonically have multidirectional action, including pro- and anti-inflammatory effects. Thus, we still have a long stage of accumulating new data obtained in lab-

oratories in different parts of the world to create a unified picture that can help in the development of effective therapeutic approaches targeting astrocytes.

Author Contributions

AB and OT contributed to the investigation and writing of the original draft. AR contributed to the formal analysis, reviewing, and editing of the manuscript. AB visualization. YM performed the conceptualization, methodology, supervision and funding acquisition. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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