Function of Astrocytes in Neuroprotection and Repair after Ischemic Stroke

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Keywords

Astrocytes · Stroke · Blood-brain barrier · Gap junctions · Aquaporin-4

Abstract

Background: Astrocytes are the most numerous cell types within the central nervous system, and many efforts have been put into determining the exact role of astrocytes in neuroprotection and repair after ischemic stroke. Although numerous studies have been done in recent years, there is still no thorough understanding of the exact function of astrocytes in the whole course of the stroke. **Summary:** According to the recent literature, there are many structures and factors that play important roles in the process of ischemic stroke, among which blood-brain barrier, various growth factors, gap junctions, AQP4, and glial scars have been studied most comprehensively, and all these factors are closely related to astrocytes. The role of astrocytes in ischemic stroke, therefore, can be analyzed more comprehensively. **Key Message:** The present review mainly summarized the current knowledge about astrocytes and their potential roles after ischemic stroke.

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Introduction

Stroke is the second leading cause of death globally [1], and every 1 person in 6 will have an experience of stroke in his or her life of whom 10% will die [1]. Ischemic stroke is the most common kind of stroke [2] which is caused by an interruption in the flow of blood to the brain and is the most common cause of serious, perennial disabilities. A sizable percentage of stroke survivors require long-term, even permanent, assistance [1]. So, it is necessary to explore the development and treatment mechanism of ischemic stroke.

A mass of studies focused on the roles of astrocytes in ischemic stroke have been conducted currently, since astrocytes account for almost half of the brain cells [3], and they are the most abundant subtypes of glial cells [4]. Astrocytes play roles in many aspects of the central nervous system (CNS) both in health and disease, including maintaining normal brain function, forming the BBB, controlling and supporting neurons, recycling neurotransmitters, and communicating with other cells [5].

Neurovascular unit (NVU) is a structural and functional unit composed of microglia, neurons, the BBB,

Fig. 1. The neurovascular unit consists of neurons, neuroglia (astrocytes, microglia, and oligodendrocytes), vascular cells (pericytes, endothelial cells, and vascular smooth muscle cells), and the basal lamina matrix of brain vasculature. From [10].

and the extracellular matrix [6] (shown in Fig. 1). Its main function is to respond to physiological or pathological stimulation of the CNS and meet the dynamic metabolic needs of the brain by regulating cerebral blood flow [7]. Astrocytes are considered to be indispensable elements for NVU or BBB expansion [8], dynamically regulating the interaction between neurons and the cerebrovascular system, and play a key role in maintaining BBB and neurovascular coupling. In NVU, astrocyte dysfunction accompanies and may lead to BBB impairment and neurovascular dysregulation [9]. Under physiological conditions, astrocytes restrict the entry of peripheral immune cells across the BBB. Under pathological conditions, astrocytes are involved in innate immune responses and adaptive immune responses [8]. Therefore, in the development of ischemic stroke, with the destruction of the brain environment, the status of astrocytes determines its pivotal role. Its positive effect will play a role of repair, but its negative development will play a role of fueling the fire.

However, until now, the specific effects of astrocytes over stroke are not thoroughly understood [11]. This article describes our current understanding of the function of astrocytes after ischemic stroke.

Astrocytes in Normal Brain

As the most abundant glial cells in the brain and the most abundant cell type within the CNS, astrocytes have many housekeeping functions [4], including the maintenance of BBB homeostasis [12] by releasing trophic factors [1], support and protection of delicate neurons via regulating cerebral blood flow [13], recycle of neurotransmitters and antioxidants [5], and cerebral metabolism. Astrocytes maintain the extracellular environment by regulating ion balance [14], providing a pathway for glucose [15] and other metabolites to travel between the blood and neurons [14]. And, improvements in glucose metabolism can have positive effects on several nerve functions, which emphasizes the role of astrocytes.

Gap junctions catenate astrocytes, and the endfeet of astrocytes are rich in gap junction channels [12]. The astrocyte endfeet of the basilar processes cover almost the whole surface of capillaries in the brain [16] and participate in forming and maintaining the integrity of BBB, which helps prevent harmful molecules into the brain and segregates the CNS from the rest of the body [17]. Astrocytes release multiple soluble factors to control maturation of synapses [3] and have a great ability to regulate synaptic strength in response to changes in neuronal ac-

Fig. 2. Astrocytes can affect the generation, differentiation, and migration of de novo generated neurons by secreting pro-neurogenic factors, such as IGF, NGF, VEGF, and EPO. IGF, insulin growth factor; NGF, nerve growth factor; VEGF, vascular endothelial growth factor; EPO, erythropoietin.

tivity [4], which demonstrates that astrocytes are active in the establishment and reconstruction of synaptic connections.

In addition, astrocytes communicate with many capillaries and other cells. Astrocytes are not typical immune cells, but they can function as inflammatory cells and produce inflammatory factors [13] and produce and release various mediators, such as nitric oxide (NO), aminogluturic acid, prostaglandins, and arachidonic acid [12], that help regulate blood vessel diameter and blood flow [4]. In the meantime, astrocytes secrete insulin growth factors (IGF), nerve growth factors, vascular endothelial growth factors (VEGF), and erythropoietin [3] to stimulate neurogenesis (shown in Fig. 2).

Astrocytes in Ischemic Brain

Astrocytes respond to all forms of CNS insults through a process referred to as reactive astrogliosis, which has become a pathological hallmark of CNS structural lesions [4]. The outcome after ischemic stroke is mainly dependent on the response of astrocytes to injury. Reactive astrocytes have different effects in different stages. In the acute phase, reactive astrocytes play neuroprotective roles, but in the subacute and chronic phases, they have both positive and negative effects to the function of the brain [1]. For instance, in the acute phase of ischemic

stroke, the proliferation and hypertrophy of astrocytes in the peri-infarct area is conducive to sealing the site of injury, remodeling the tissue, and controlling the local immune response both spatially and temporally [18]. However, in the chronic phase, the formation of glial scarring by excessive astrocyte proliferation may limit recovery of central nervous function [19].

Astrocyte-related structures/proteins play different roles in the activation of astrocytes at different developmental stages of ischemic stroke, and understanding this alteration of astrocytes will be beneficial for finding therapeutic strategies for the disease. Therefore, we will elaborate the specific role of astrocytes in ischemic stroke from the 5 aspects mentioned above.

BBB Integrity Has Neuroprotective Properties

The BBB is formed before astrocyte formation and vascular coverage, so these cells do not play a role in initial BBB induction, but once astrocytes mature, they modulate and maintain the BBB stability. Many factors unique to NVU microcracks maintain and regulate the structural function of the BBB. Astrocytes-BBB-EC interact under physiological and pathological conditions [20].

The early manifestation of ischemic stroke is usually BBB destruction. BBB leakage occurs within the first hour after stroke and may lead to ischemic tissue damage, secondary neuroinflammation, vasogenic edema formation, and intracerebral hemorrhage [21]. After further injury,

Fig. 3. In brain disorders, astrocytes release various kinds of extracellular signaling molecules. **a** Vascular permeability factors: astrocyte-derived VEGFs, MMPs, NO, glutamate, and ETs cause endothelial apoptosis and downregulation of TJ-related proteins, resulting in BBB disruption. **b** Vascular protective factors: astrocyte-derived ANG-1, SHH, GDNF, RA, IGF-1, and APOE

protect endothelial cells from apoptosis and promote recovery of TJ function. From [22]. VEGF, vascular endothelial growth factor; MMPs, matrix metalloproteinases; ETs, endothelins; ANG-1, angiopoietin-1; SHH, sonic hedgehog; GDNF, glial-derived neurotrophic factor; RA, retinoic acid; IGF-1, insulin-like growth factor-1; APOE, apolipoprotein E.

astrocytes and some factors produced by them induce apoptosis of endothelial cells, reduce the expression of endothelial TJ-related proteins, and lead to further deterioration of BBB (shown in Fig. 3). In addition, some astrocyte-derived factors also control white blood cells crossing the BBB that causes further inflammation in the brain [22]. Reperfusion in time can restore BBB permeability. However, due to the production of ROS and the release of proteases, late reperfusion can aggravate BBB damage [21]. Therefore, protecting BBB and restoring it are crucial to slow the progression of brain damage.

In addition, astrocytes secrete angiogenin, promoting angiogenesis and inducing decreased endothelial permeability. However, when certain factors such as VEGF that can impair BBB function are coexpressed with angiogenin, the barrier integrity can be enhanced, and neuroprotective characteristics can be induced [20] (shown in Fig. 3). Astrocytes also produce angiotensin-converting enzyme 1, convert angiotensin I to angiotensin II, and act on the type 1 angiotensin receptor (AT1) expressed by BBB ECs to induce vasoconstriction, activate AT1, and inhibit the permeability of the BBB [20].

Furthermore, astrocytes express FABP7, which has a high affinity for docosahexaenoic acid, and is essential in neurogenesis. Docosahexaenoic acid can prevent cerebral

edema after focal cerebral ischemia, suggesting that FABP7 plays a role in regulating the integrity of BBB [23].

The Multiple Functions of Growth Factors Released by Astrocytes

VEGF, also known as a vascular permeability factor [24], is a multifunctional protein that is involved in angiogenesis, inflammation, cancer, and wound healing and repair [25]. It is mainly secreted by astrocytes and widely expressed in brain tissues [25] and has a strong effect on increased vascular permeability.

Some studies have demonstrated that transcriptional activation of HIF-1 and HIF-2 induces VEGF expression in ischemic and anoxic brains. Within 6 h after ischemic injury, VEGF expression increased, especially in the ischemic limbic or penumbra regions [26]. Although the angiogenic functions of VEGF and HIF-1 promote the formation of edema in the acute phase, they have a protective effect in the long-term response to hypoxia injury [25].

Furthermore, VEGF expression is upregulated by activating the NF κ B/MMP-9 pathway under high salt conditions and destroys tight junctions in endothelial [24]. In addition to VEGF, astrocytes emit IGF-I signals in coordination with SCF secreted by astrocytes to protect neurons from oxidative stress [27].

Apart from VEGF and IGF, activated astrocytes can persistently release growth factors such as transforming growth factor- β , brain-derived neurotrophic factor, glial cell line-derived neurotrophic factor, basic fibroblast growth factor, ciliary neurotrophic factor, platelet-derived growth factor, nerve growth factor, and erythropoietin [28] through different pathways to engage in the survival and protection of neurons during the chronic phase [29], which is crucial to the maintenance of the integrity of BBB and remodeling of the NVU after ischemic stroke. The secretion of these factors can also influence the differentiation and migration of new neurons, control neuroinflammation, and participate in glia-mediated angiogenesis [12].

All of these mentioned above will improve functional outcome after stroke. Reactive astrocytes also secrete the neuroblast-attracting chemokines such as stromal cell-derived factor-1 after brain ischemic injury [3], which contributes to guiding migrating neuroblasts to the infarcted brain area. Astrocytes also play potentially helpful roles in neuroprotection and neurorestoration [30], by limiting lesion extension, reducing excitotoxicity [17], and releasing neurotrophins, which contribute to angiogenesis, neurogenesis, synaptogenesis, and axonal remodeling [4] after ischemic stroke.

Gap Junctions between Astrocytes Help Maintain Homeostasis during Ischemic Stroke

Astrocytes communicate with neighboring cells via gap junctions which may open up possibilities to communication over large distances [14]. Astrocyte gap junctions are axially arranged hexamers of connexin, which are the main structures of electrical transport, metabolism, and ion coupling between adjacent cells. Gap junctions are always open under physiological conditions, facilitating communication and information exchange between metabolites. At the same time, the astrocytic gap junction channels also carry chemical signals and metabolites (glucose and lactic acid) between glial cells, promoting the function of neurons, glia, and vascular tissue [31]. The endfeet are a part of astrocytes with plenty gap junction channels. The astrocyte endfeet of the basilar processes cover almost the whole surface of capillaries in brain [16] and participate in forming and maintaining the integrity of BBB, which helps prevent harmful molecules into the brain and segregates the CNS from the rest of the body [17].

Astrocytes surround neural circuits throughout the brain and maintain homeostasis in the interstitial environment, which is essential for neuronal signal and infor-

mation processing [32]. In CNS, abundant gap junctions between adjacent astrocytes (A/A junctions) and oligodendrocytes (O/A junctions) [33] make the movement of small molecules within and between glia cells relatively unrestricted [34], and the function of BBB will decrease with the ablation of gap junctions [12]. Astrocytes function coupling with adjacent astrocytes and oligodendrocytes through gap junctions, forming “glial syncytium” and maintaining the homeostasis of glial cells and neurons [35].

In ischemic stroke, occlusion of the middle cerebral artery results in rapid loss of the infarct core neurons, and the peri-infarct, also known as the penumbra, is an unstable and recoverable area. Around infarct, reactive astrocytes expressing high levels of connexin 43 (Cx43) in gap junctions have been identified [36].

Astrocytes express 3 main gap junctional and hemichannel proteins, connexin 26 (Cx26), connexin 30 (Cx30), and Cx43 [34], among which Cx43 is the main astrocyte gap junctional protein [33]. Cx43 exists in the perivascular endfeet of astrocytes and is expressed less in white matter than in gray matter [33]. The potential effect of interstitial junctions is to “clamp” adjacent astrocytes to the same resting membrane potential, which is necessary to maintain consistent extracellular ion concentrations [32], and the system maintains a K^+ gradient, where there is more K^+ in the cell than there is outside the cell [34], which is essential to keep the normal physiological function.

In the case of ischemia and hypoxia, the gap junctions of astrocytes release glutamate, leading to excitotoxicity [37] that causes the increase of extracellular K^+ which enhances the resting potential of the neuronal membrane, making subsequent discharge activity more likely to occur [34]. In cerebral ischemia, Cx43 is phosphorylated at Ser368, Tyr247, and Tyr265 and subsequently phosphorylated at pUb (S65) which has been phosphorylated by PINK1. This modified Cx43 can recruit autophagy receptors OPTN and NDP52 for autophagy degradation. Inhibition of Cx43 autophagic degradation is conducive to the transformation of astrocytes from pro-inflammatory state to anti-inflammatory state, so as to prevent anti-inflammatory reactions during ischemia. In addition, under ischemic stress, depletion of Cx43 or acceleration of its degradation in astrocytes can fully protect cells from apoptosis [38]. Furthermore, ischemic preconditioning can effectively block the gap between astrocytes, reduce extracellular glutamate, reduce the release of glutamate and reactive oxygen species ROS in astrocytes, and reduce neuronal injury [37]. Also, GLP-

Fig. 4. During astrocyte swelling, Na^+/K^+ -ATPase (red channels) is involved in regulating the influx of ions and osmotic active molecules (red arrows). The swelling involves the entry of K^+ ions into the cell through CX43 gap junctions and hemichannels, Kir4.1, and Na^+/K^+ -ATPase. At the same time, glutamate enters the astrocytes via a transporter, increasing the osmotic gradient and causing water to enter the swelling astrocytes through the AQP4 channel. From [34].

1R signaling, especially at the hypothalamus level, is necessary to maintain the mitochondrial integrity and function of astrocytes [15].

Deficiency of Astrocyte-Related AQP4 Improves Neurological Function

In the brain, water is present in cerebrospinal fluid, blood, parenchymal cells, and interstitial components. The flow of fluid through the vascular, ventricular, and parenchymal compartments is essential for normal physiological function [39]. The neurovascular compartment, composed of blood vessels, neurons, and astrocytes, is the key to controlling the flow of water [40]. In ischemic stroke, edema causes increased intracranial pressure, which compresses the nerve tissue, making the situation worse.

Aquaporins (AQP) are nonselective bidirectional water channels that allow passive diffusion of water across cell membranes. There are 3 AQPs expressed in the CNS: AQP1, AQP4, and AQP9 [34], among which AQP4 is the most richly expressed [41]. It is specifically located in the endfeet of astrocytes and directly corresponds to CNS vessels. There are 6 subtypes of AQP4, a–f, but only AQP4c and AQP4e show water permeability [34]. AQP4

has diverse distribution in the whole brain, including the cerebral cortex, corpus callosum, retina, cerebellum, and large nucleus and brainstem of the hypothalamus. The tetrametric structure of AQP4 allows gas and ions to permeate through the central pore [41] helping maintain osmotic pressure equilibrium. AQP4 is involved in nerve excitation by making neurons release isotonic K^+ [41] and works together with gap junctions to keep normal function. In the normal CNS, AQP4 is expressed only at the endfeet of astrocytes but is widely expressed in the cytoplasm of activated astrocytes [22].

The role of AQP4 in ischemia was first observed in the combined model of cytotoxic and vasogenic brain edema (ischemic stroke model) and cytotoxic brain edema (acute water intoxication model). The presence of AQP4 has been found to aggravate postischemic cytotoxic edema in ischemic stroke models, while AQP4-KO mice showed the opposite effect and improved neurological function [42].

In ischemic stroke, AQP4 expression was rapidly up-regulated in perivascular endfeet of astrocytes and peaked 1 h after stroke [43] and is widely expressed from the normally polarized position at the end of astrocytes to the entire plasma membrane [34]. Upregulation of AQP4 in

astrocyte endfeet can reduce hemispheric enlargement in the early stage of middle cerebral artery ischemia [43]. In addition, there is evidence that compared with astrocytes expressing AQP4, astrocytes lacking AQP4 have a higher degree of interstitial connection, resulting in reduced water permeability of astrocytes [44], thus improving the spatial buffer of K^+ [34]. In the early stages of stroke, AQP4 loss leads to swelling of astrocytes and reduced water accumulation in the brain, thereby reducing BBB destruction, inflammation, and neuronal death [41] (shown in Fig. 4). AQP4 deficiency has good long-term efficacy and improves behavioral prognosis 14 days after stroke onset [43]. It suggests that AQP4 deficiency can improve neurological function and has a neuroprotective effect on cerebral ischemia. In addition, reactive astrocytes migrate in various brain injuries, isolate damaged tissues, and form glial scars, but the glial scars are reduced when AQP4 is in deficiency [44], which may promote the spread of inflammation and injury.

Glial Scar Composed of Astrocytes Inhibits the Spread of Injury

Glial scar impedes axon regeneration and functional recovery, which is the main reason for the limited regeneration of CNS. Glial scar formation after CNS injury is regulated by a complex combination of intercellular and intracellular signaling mechanisms. Under normal circumstances, astrocytes are the main subtype of glial cells in CNS, maintaining neurons and BBB. They are activated in different types of injury responses, including inflammation, infection, ischemia, and traumatic injury, and play a key role in the pathophysiology of each injury through a phenotypic change known as reactive astrogliosis [45].

In the late stage of stroke, a subgroup of astrocytes in the penumbra proliferates immediately while some migrate to the border of the infarct area and company to form a glial scar by secreting extracellular matrix molecules [1]. While the scar is developing, it contains astrocyte-derived chondroitin sulfate proteoglycans [28] and NG2-positive oligodendrocyte precursor cells [14]. Still, the astrocytes adjacent to the infarct are the main component of the core of the mature scar [4]. The formation of a perivascular scar is necessary to separate the damage tissue in the infarct from the healthy brain surrounded which helps restrict the damage area and prevent inflammation from spreading over [46]. In the early period of the glial scar development, proteoglycans deposit extracellularly which may be the reason to limit the spread of damage. As the characteristic of reactive astrogliosis, as-

trocytes far from scar development areas show morphological changes and a significant increased expression in glial fibrillary acidic protein [14], the iconic protein of astrogliosis and glial scar [47]. However, excessive astrocyte hyperplasia and glial scarring hinder neurite growth and neurogenesis [48].

In addition, age plays an important role in scar development. The proliferation and duration of reactive astrocytes after brain injury in the elderly resulted in accelerated glial scar formation, and compared with adult astrocytes, reactive astrocytes in the elderly showed upregulation of genes related to inflammation and scar formation [49].

Conclusion

Recombinant tissue-type plasminogen activator (tPA), which has been approved in the USA for the treatment of acute ischemic stroke since 1996 [50], is the only FDA-approved agent for the treatment of acute ischemic stroke, and its use in selected patients improves the overall outcome of ischemic stroke. However, tPA is effective 3–4.5 h after stroke, and the vast majority of stroke patients do not receive tPA therapy. So, a comprehensive understanding of the role of astrocytes can help to find better methods.

Astrocytes account for the largest proportion of cell subtype in the CNS. They are structurally and functionally involved in normal brain physiology and ischemic pathology. The astrocyte response to ischemic stroke is extremely complex and incompletely understood. After stroke, inflammation of astrocytes in the acute phase aggravates ischemic lesions, and glial scarring in the perinfarct area hinders axonal regeneration, thereby reducing functional outcomes after the acute phase. However, on the other hand, astrocytes also play a beneficial neuroprotective role, limiting lesion expansion. Astrocytes play an important role in nerve recovery after stroke by promoting angiogenesis and neurogenesis. Thus, the positive and negative effects of astrocytes on functional and neurological recovery after stroke make them a promising therapeutic target for pharmacological and cell-based therapies, which will be an important complement to cerebral ischemia treatment strategies.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Shufen Zhang and Han Shi carried out the search and selection of the studies. Writing was done by Shufen Zhang. Deshu Shang provided insightful comments and suggestions for manuscript improvement during revision. Weiyu Teng provided critical comments on the manuscript.

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