Mechanisms of Ageing and Development 183 (2019) 111147

Contents lists available at ScienceDirect

Mechanisms of Ageing and Development

journal homepage: www.elsevier.com/locate/mechagedev

Ischemic stroke in neonatal and adult astrocytes

Miren Revuelta[a,b,1,*], Amaia Elicegui[a,1], Leire Moreno-Cugnon[b], Christoph Bührer[a], Ander Matheu[b,c,d], Thomas Schmitz[a,**]

[a] Department for Neonatology, Charité University Medical Center, Chariteplatz 1, 10117, Berlin, Germany
[b] Cellular Oncology Group, Biodonostia Health Research Institute, Paseo Doctor Begiristain, 20014, San Sebastian, Spain
[c] IKERBASQUE, Basque Foundation for Science, María Díaz Haroko 3, 48013, Bilbao, Spain
[d] CIBERfes, Monforte de Lemos 3-5, 28029, Madrid, Spain

ARTICLE INFO

Keywords:
Astrocytes
Stroke
Neonatal
Ageing
Hypoxia ischemia

ABSTRACT

The objective of this paper is to review current information regarding astrocytes function after a stroke in neonatal and adult brain. Based on the current literature, there are some molecular differences related to blood brain barrier (BBB) homeostasis disruption, inflammation and reactive oxygen species (ROS) mediated injury between the immature and mature brain after an ischemic event. In particular, astrocytes, the main glial cells in brain, play a different role in neonatal and adult brain after stroke, as time course of glial activation is strongly age dependent. Moreover, the present review provides further insight into the therapeutic approaches of using neonatal and adult astrocytes after stroke. More research will be needed in order to translate them into an effective treatment against stroke, the second main cause of death and disability worldwide.

1. Ischemic-stroke in neonatal and aged humans

Ischemic stroke is the second cause of death and disability worldwide (Feigin and Norrving, 2014) and is the most common type of stroke (Wallace et al., 2016). It occurs after a blockage of blood flow in the neck or in the brain (Randolph, 2016). Since the brain is a metabolically very active organ that has very low or no oxygen reserve, occlusion of the cerebral circulation leads to brain cell death if perfusion is not restored in a short period of time (Ten and Starkov, 2012). While functionality of surviving neurons may at least partially compensate some of the brain damage after stroke, the capacity for effective repair is very limited in neurons of the human brain (Ding et al., 2013).

In infants, stroke occurs in 1 in 4000 live birth being the majority of them ischemic (Myers and Ment, 2012). Indeed, stroke is 17 times more common in neonates than in children and adolescents (Nelson, 2007), and despite the plasticity of the developing brain, neonatal brain injury turns out in a substantial morbidity in the pediatric population (Bernson-Leung and Rivkin, 2016). The most common cause of ischemic injury during the perinatal period is the intrauterine asphyxia leading to hypoxic ischemic (HI) encephalopathy (Fatemi et al., 2009; Nelson, 2007). After HI injury neonates show cortical sparing and deep gray matter injury that can lead to neurological and developmental difficulties including cognitive decline, such as cerebral palsy, epilepsy, learning impairment and visual and hearing disabilities (Chen et al., 2019).

In adults, stroke is the second main cause of death in the adulthood with a death impact of 5.7 million people per year and affecting approximately 16 million people annually (Sarikaya and Steinlin, 2018; Strong et al., 2007). Stroke can affect individuals of any age but the incidence increases with age, doubling the rates after 55 years both in men and women (Chen et al., 2010). Among all types of stroke, brain ischemic stroke represent 87% of all cases, and neurological deficits such as motor impairment and inability to read or even aphasia are common consequences (Chen et al., 2010). However, the type and the extent of the damage depend on the location and the severity of the ischemic insult. Age and individual risk factors, such as high blood pressure, smoking, diabetes, obesity or high cholesterol among others, directly correlate with the severity of the stroke (Comi and Johnston, 2009).

1.1. Ischemic stroke mechanism in adult vs. neonatal brain

During the last decades, most of experimental studies of stroke have been performed in young animals, but ageing has a distinct as well as

* Corresponding author at: Cellular Oncology Group, Biodonostia Health Research Institute, Paseo Doctor Begiristain, 20014 San Sebastian, Spain.
** Corresponding author at: Department for Neonatology, Charité University Medical Center, Chariteplatz 1, 10117 Berlin, Germany.
E-mail addresses: miren.revuelta@biodonostia.org (M. Revuelta), thomas.schmitz@charite.de (T. Schmitz).
[1] These authors have contributed equally and should be considered as co-first authors.

https://doi.org/10.1016/j.mad.2019.111147
Received 27 May 2019; Received in revised form 6 August 2019; Accepted 2 September 2019
Available online 04 September 2019
0047-6374/

critical role for the response of the brain to stroke (Badan et al., 2003).

The molecular mechanism of brain ischemic stroke is similar in neonate and adult animal models. Cerebral ischemic stroke triggers the release of glutamate neurotransmitters that open ion channels, provoking lethal intracellular events (Back et al., 2007; Lipton, 1993). Different animal models are commonly used to mimic this brain ischemic event. The most used one in neonates is the ligation of the common carotid artery followed by the hypoxic event (the HI model) (Rice et al., 1981). Meanwhile, the middle cerebral artery occlusion (MCAO) has been established as a stroke model in adult animals (Koizumi et al., 1986). In vitro, the model to mimic cerebral ischemia is the oxygen glucose deprivation model (OGD) (Sommer, 2017).

However, there are some molecular differences between the immature and mature brain after stroke. While apoptosis occurs within a week after the ischemic event in the neonate brain, this period is much shorter in adult brain, taking place immediately after the damage. There is a rapid activation of microglia after the injury in the neonatal brain, with an increase in the number of macrophages from 1 to 4 in a few weeks after HI, whereas it occurs slowly in the adult brain. The different patterns of superoxide accumulation in macrophages after stroke in adults and neonates may be the most important mechanism for this aged dependent glial activation (Fernandez-Lopez et al., 2014). The most important difference may however be that the neonatal brain is far more susceptible to oxidative stress that occurs after the stroke, also as a result of the higher oxygen demand and low concentration of antioxidants of the immature brain (Vexler et al., 2006) (Table 1).

1.1.1. Blood brain barrier integrity after stroke

Blood brain barrier (BBB) is a diffusion barrier that can prevent certain molecules from flowing into the brain parenchyma, but let oxygen, hormones and some chemokines and cytokines to penetrate. There are three cell types in BBB: endothelial cells, astrocytes and pericytes (Daneman and Prat, 2015). Its integrity is constituted by various elements including tight junctions (TJ), extracellular matrix components and adherent junctions which are responsible of maintaining paracellular permeability and maintenance of cell polarity (Bazzoni et al., 2000). After brain injury, BBB can be disrupted in the adult brain and the consequent extended permeability may cause a secondary damage including brain edema and inflammation (Arba et al., 2017). However, brain immaturity affects differently concerning inflammation, neuronal injury, appearance of reactive oxygen species (ROS) or even BBB integrity.

It has been demonstrated that adult brain BBB is disrupted after stroke, whereas the neonatal brain is able to maintain BBB integrity (Fernandez-Lopez et al., 2012). In that study, it is demonstrated how genes and proteins that take part in TJ, extracellular matrix composition and cell infiltration differ between neonatal and adult brain after stroke. Protein expression of Col-IV, which is the principal collagen type in basal lamina, and several TJ proteins such as claudin-5 and occludin were higher in injured neonates compared to adults. This alters not only matrix composition but also cell signaling via interaction with some receptors and adhesion molecules (Khoshnoodi et al., 2008). Besides, Fernández-López et al. (2012) showed that metalloproteinase-9 (MMP-9), which is one of the most studied metalloproteinases in BBB disruption, was remarkably increased in adult brain (up to 63.2 fold change) but not in the developing brain of neonates.

The infiltration of neutrophiles enhances BBB disruption after brain ischemia due to their contribution in ROS and proteolytic enzyme production. Fernández-López et al., in 2012 demonstrated the negligible presence of this cell type in injured neonatal brains, and overexpression of vascular permeability molecules such as vascular endothelial growth factor receptor-2 (VEGFR-2) in adult but not in neonates. These all together, confirms the lack of leukocyte infiltration protects neonatal brain from BBB integrity after ischemic injury.

Table 1
Main differences in the neurovascular units and inflammation after hypoxic ischemic event in the immature and mature brain. ↑, ↓, -,?, symbols mean increased, decreased, standard values or unknown, respectively.

Neurovascular unit and inflammation	IMMATURE BRAIN	ADULT BRAIN
Blood-Brain Barrier (BBB)	↓permeability	↓permeability
MATRIX METALLOPROTEINASES (MMP) AND EXTRACELLULAR MATRIX		
Metallopeptidase 9 (MM-9)	↑	↑
Microglia	rapid activation	slow activation
Inflammatory citokyne	↑	↑
Metalloproteinases (MMPs)	↑	–
Astrocytes		
Glial fibrilary acidic protein (GFAP)	↑	↑
Apoptosis regulator BAX	↑	↑
CASPASE3	↑	↑
poli ADP ribose polimerase (PARP)	↑	↑
PROINFLAMMATORY CYTOKINES		
Interleukin 1 (IL-1)	↑	↑
Tumor necrosis factor (TNFα)	↑	↑
Interleukin 6 (IL-6)	↑	↑
Interleukin 18 (IL-18)	↑	↑
ANTI-INFLAMMATORY CYTOKINES		
Interleukin 10 (IL-10)	↑	↑
Transforming growth factor beta (TGF-1β)	↑ in microglia	↑ in microglia
	↓ in neurons	↓ in neurons
CHEMOKINES		
Monocyte-attracting chemokine (MCP)	↑	↑
Macrophage Inflammatory Protein 1-α (MIP-1α)	↑	↑
Interleukin 8 (IL-8)	↑	↑
REACTIVE OXYGEN SPECIES (ROS)		
H_2O_2	↑	↑
Cyclooxygenase (COX)	↑	↑
Myeloperoxidase (MPO)	↑	↑
Monoamine oxidase(MAO)	↑	↑
Antioxidants	↓	–
Antioxidants enzymes	Imbalanced	–
Nitric oxide synthases (NOS)	↑	↑
Inducible nitric oxide synthases (iNOS)	↑	↑
Mitogen activated protein kinase (MAPKs)		
Stress-activated protein kinase (SAPK)/ c-Jun NH2-terminal kinase (JNK)	↑	↑
P38MAPK	↑ (?)	↑
Extracellular signal regulated kinase (ERKs)	↑	↑
APOPTOSIS	7 days after the injury	Just after the damage

1.1.2. Inflammation after stroke

Ischemic injury triggers pro-inflammatory cytokine production and chemotactic molecule expression. This results in an increase of leukocyte trafficking, which depends on the activated cytokine pattern and adhesion molecule expression (Docagne et al., 2005) (Fig. 1).

Some of the most studied cytokines in brain stroke are interleukin-1 (IL-1), tumor necrosis factor (TNFα) and interleukin-8 (IL-8), among others. It is documented that early release of IL-1 potentiates brain injury in neonatal and adult brain after stroke (Fig. 1). Likewise, TNFα is upregulated in adult brain and enhances brain damage, whereas it is not proven any overexpression in neonates (Jin et al., 2010).

Besides, TNFα is a major activator of the nuclear factor kβ (NFkβ) pathway (Varfolomeev et al., 2008), which appears to be upregulated and to promote neuronal death in ischemia models (Schneider et al., 1999). Inhibition of the Iκβ kinase (IKK), which is the responsible of NFkβ translocation to the nucleus by the phosphorylation of Ikβ, demonstrated the decrease of the infarct size in mouse brains (Herrmann et al., 2005).

In adults, accumulation of microglia, the resident macrophages of the brain, starts 24 h after stroke (Fox et al., 2005). Activated microglia can amplify inflammation by secretion or expression of inflammatory

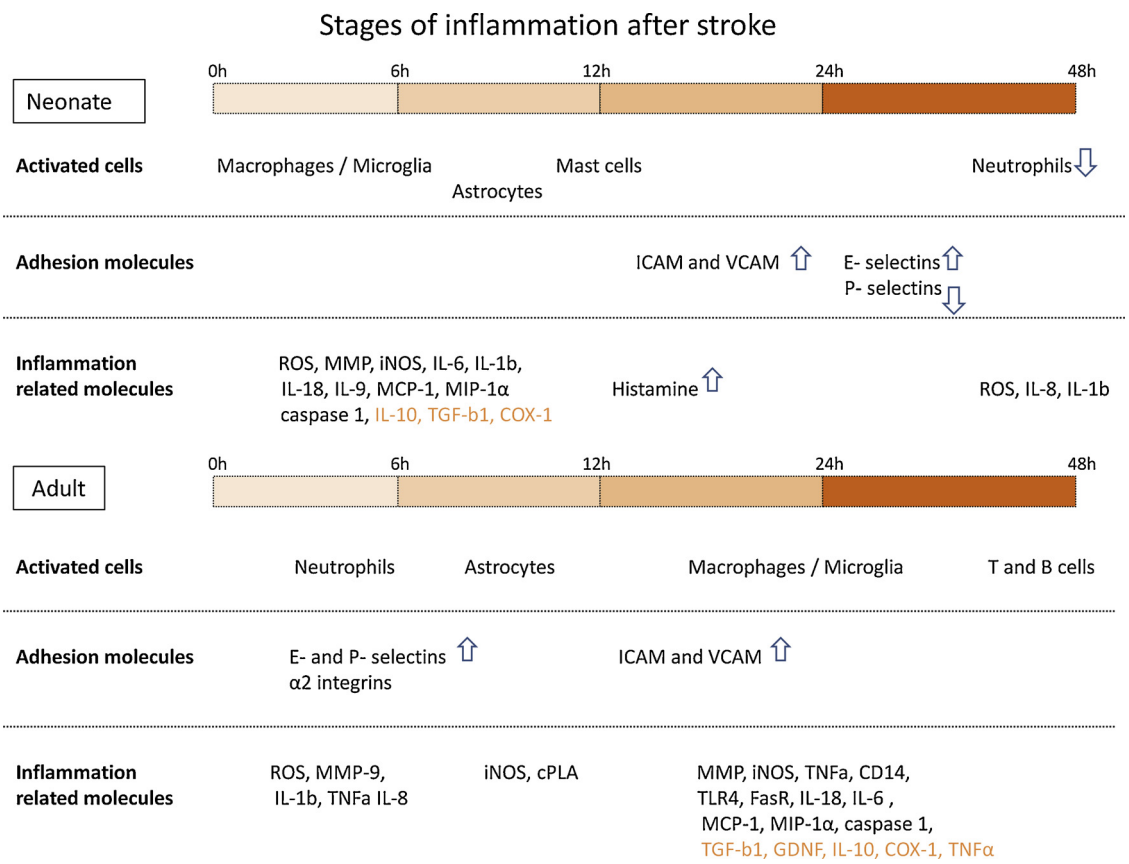

Fig. 1. Stage of inflammation after stroke during the first 48 h in the neonatal and adult brain representing the activated cells, adhesion molecules and inflammation related molecules during this timeline. ↑ and ↓ means increased and decreased, respectively. Molecules showed in orange are anti-inflammatory. Abbreviation: Intercellular Adhesion Molecule 1 (ICAM); vascular cell adhesion molecule (VCAM); cytosolic Phospholipase A (cPLA)).

cytokines, chemokines or inducible nitric oxide synthases (iNOS) (Fig. 1), but can also contribute in tissue debris phagocytosis.

Another signaling pathway that can be activated after ischemic stroke is the p38 mitogen-activated protein kinase (MAPK) pathway (Krupinski et al., 2003; Kyriakis and Avruch, 2001). p38 MAPK promotes the stabilization and enhanced translation of mRNAs encoding proinflammatory proteins, and p38 inhibitors seem to be effective reducing infarct volume (Barone et al., 2001). Some preclinical studies demonstrate that knockout of p38 reduced astrogliosis in ischemic mouse models. Consequently, p38 MAPK signaling pathway may play a critical role in reactive astrogliosis after ischemic stroke, and could be an interesting target for modulation (Roy Choudhury et al., 2014). P38 MAPK inhibitors have shown promising treatment effects in adult stroke models but the role of this kinase in neonatal ischemic injury remains to be elucidated (Vexler et al., 2006).

On the contrary, the overexpression of anti-inflammatory cytokines such as interleukin 10 (IL-10) is observed in both neonatal and adult ischemic brains, which appear to have neuroprotective effects and to diminish excitotoxic brain lesions (Mesples et al., 2003).

1.1.3. Reactive oxygen species after stroke

When inflammation occurs, brain generates ROS, as a defense mechanism, via some enzyme systems (cyclooxygenase (COX), NADPH, xantine dehydrogenase, myeloperoxidase (MPO) or monoamine oxidase (MAO) and nitric oxide synthase (NOS) among others) that lead to superoxide anions or H_2O_2 production contributing to the injury in the developing brain (Fig. 2) but also in the adult brain. Indeed, ROS-mediated injury is the main cause of injury in the neonatal brain as the antioxidative defense mechanism is imbalanced with high oxygen consumption and low concentration of antioxidants (Vexler et al., 2006).

Among the main important sources of ROS after stroke, the NADPH oxidase (NOX) is the main superoxide producers (Weston et al., 2013). Indeed, NADPH intensifies brain damage in the adult brain (Ma et al., 2017a). Besides, mice lacking a functional NOX in the central nervous system had smaller infarct volume (Fig. 2).

Another important origin of ROS in the neonatal and in the adult brain is the mitochondrial electron transport chain. Indeed, the ubiquinone-cytochrome b region of the electron transport chain has been proposed as the major site for ROS production during ischemia (Murphy, 2009). Xanthine oxidase (XO) has also been described being a factor for ROS production after ischemic stroke. The activity of XO is increased in rat brains after stroke and which seems to contribute to brain edema (Li and Yang, 2016). Moreover, the intracellular enzymes COXs, lipoxygenases (LOXs), and cytochrome P450 enzymes are also involved in the metabolism of free arachidonic acid, being this arachidonic acid one of the major source of superoxide generation during ischemic stroke (Li and Yang, 2016) (Fig. 2).

Indeed, reperfusion and reoxygenation that occur after ischemic stroke represent the main causes of the second peak or wave of ROS production. As a result, there is a necrotic neuronal and glial cell death in the ischemic region that is *continuum* in neonatal brain as is more susceptible to excitotoxic-damage while this occurs in a particular moment in adults (Kratzer et al., 2014; Titomanlio et al., 2015).

2. Astrocytes: a central element in stroke

Astrocytes are the main glial cells in the brain that regulate ion balance, blood flow and present antioxidant functions in the brain (Sun et al., 2018). During brain injury there is a massive glial response or reactive gliosis, characterized by an increased expression of glial fibrillary acidic protein (GFAP) and extracellular matrix molecules

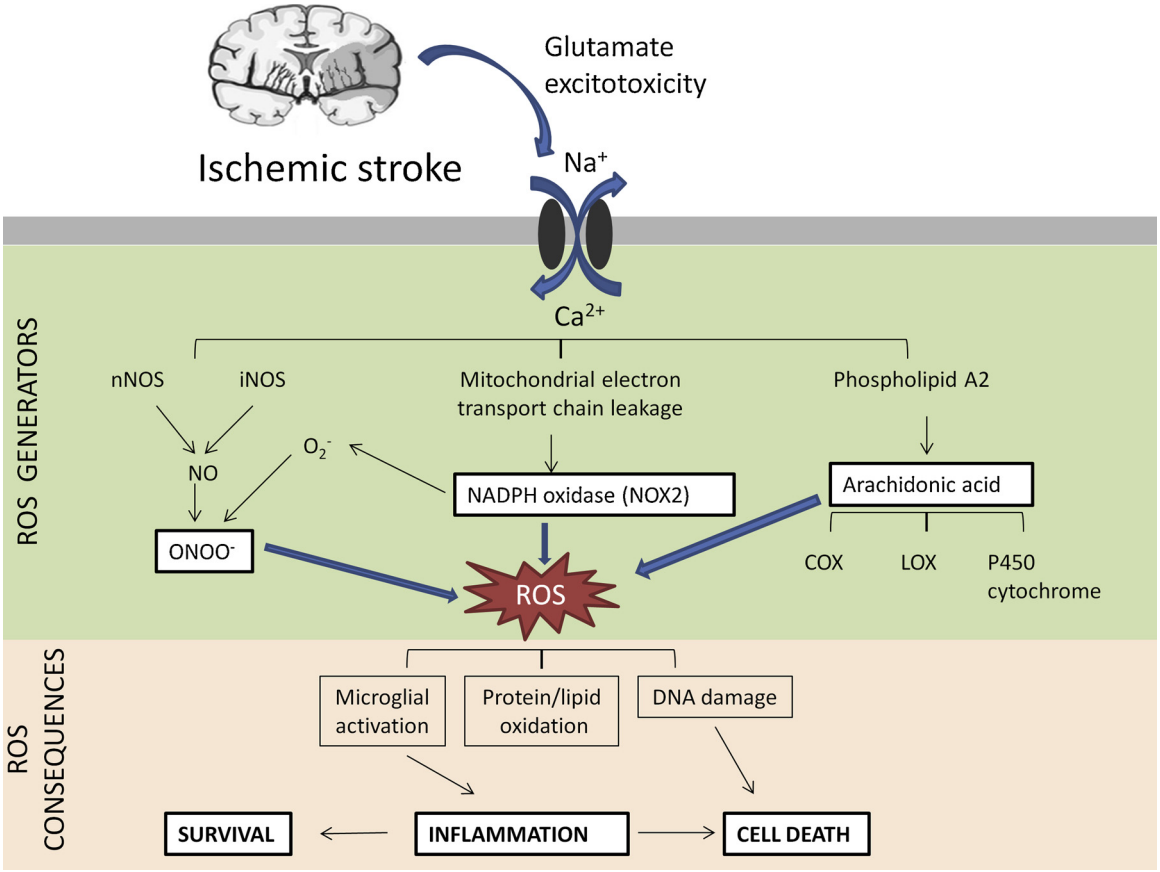

Fig. 2. Diagram of ROS production sources and ROS generation consequences after stroke. The increase of Ca2+ influx promote NOS production, mitochondrial membrane disruption and phospholipid A2 production leading to the activation of severe enzyme system that provoke ROS. This ROS production consequently activates glial cells, oxidates lipid and proteins and produces DNA damage that increases cell death and inflammation.

(ECM) such as chondroitin sulfate proteoglycans (CSPGs). Indeed, in severe cases, this massive glial response leads to the formation of a glial scar surrounding the injury site (Huang et al., 2014).

Glial scar, orchestrated by molecules responsible of astrocytic hypertrophy, proliferation, migration and gliogenesis, is considered a source of proinflammatory molecules, ROS and neurotoxicity. Besides, reactive astrocytes enhance production of inhibitory molecules that block axonal regeneration such as CSPGs (Gris et al., 2007). In the same way, it is seen that reactive astrocytes in the glial scar are necessary to demarcate the damaged tissue as they decrease the area of inflammation and do not let inflammation spread over (Faulkner et al., 2004; Liu and Chopp, 2016). Therefore, reactive astrocytes not only have detrimental functions, but may also enhance neuroprotection.

Furthermore, there are some anti-inflammatory cytokines that are upregulated after stroke in astrocytes, such as tumor growth factor β (TGF-β). It is demonstrated that its signaling is a molecular mechanism by which astrocytes control neuroinflammation and maintain brain function in the subacute period after stroke (Cekanaviciute et al., 2014). Astrocytes, together with microglia are able to release neurotrophic factors that protect neurons, enhance neuronal plasticity and improve functional outcome after stroke (Liu and Chopp, 2016). Some of this examples are nerve growth factor (NGF), basic fibroblast growth factor (bFGF), brain-derived neurotrophic factor (BDNF), glia-derived neurotrophic factor (GDNF), vascular endothelial growth factor (VEGF) and erythropoietin (EPO) (Bernaudin et al., 2000; Ho and Blum, 1997; Lee et al., 1998; Nicole et al., 2001; Tokumine et al., 2003; Wick et al., 2002; Yamagata et al., 2002).

Among the proinflammatory molecules, aquaporin-4 (AQP4), the most abundant water channel in the brain and localized in the endfeet of astrocytes (Ikeshima-Kataoka, 2016), has also an important role in brain edema, as it controls the water fluxes into and out of the brain parenchyma. Indeed, it has been showed that AQP4-deficient mice improved neurological outcome and had reduced brain edema (Li et al., 2011; Saadoun et al., 2005) There are some signal molecules or transcription factors of these activated astrocytes that have an important role in the mentioned glial scar formation. Among these transcription factors, signal transducer and activator of transcription 3 (STAT3) is increased in astrocytes by a number of cytokines implicated in injury responses such as interleukin 6 (IL-6), ciliary neurotrophic factor (CNTF) and leukemia inhibitory factor (LIF) (Wang et al., 2018).

2.1. Astrocyte function in neurogenesis after stroke

Astrocytes play contradictory roles in the interplay with neurons they may either have detrimental or beneficial effects, depending on the way of action and on the circumstances under which they are triggered. Thus, increase of astrocytic activity may e.g., activate catabolic processes via mediation of proteases, lipases and nucleases that contribute to neuronal inhibition and lead to cell death (Becerra-Calixto and Cardona-Gomez, 2017). However, astroglia can also exert beneficial properties leading to neuroprotective functions (Ma et al., 2017b); (Anderson et al., 2003) (Li et al., 2008). In acute ischemia, transgenic rats with double deletion of GFAP and vimentin ($GFAP^{-/-}Vim^{-/-}$) resulted in decreased glutamate uptake, increased susceptibility to oxidative stress and significantly increased infarct volumes (2–3 times compared to WT mice), suggesting that GFAP and Vimentin are crucial for nervous tissue recovery and possess protective functions. It is therefore reasonable to further investigate astroglial involvement in the recovery after stroke (Li et al., 2008).

The genesis of new neurons in the rodent brain is ongoing during

early developing and also adult ages, although it usually declines during adulthood. However, experimental data reveal that ischemic stroke strongly stimulates neurogenesis in the hippocampal dentate gyrus (DG) and subventricular zone (SVZ) of adult rodents (Ming and Song, 2005). Neural precursors in the hippocampal subgranular zone (SGZ) migrate to the dentate granular layer while the ones in SVZ migrate to the cortex, olfactory bulb and striatum, where they will differentiate into neurons (Yoneyama et al., 2011).

Astrocytes seem to acquire stem cell hallmarks in vitro properties after an ischemic event. In vitro assays have shown that some of the reactive astrocytes in the ischemic brain are able to gain neurosphere-forming capacity, multipotency and long-term self-renewal, while others remain within their astrocyte lineage (Gotz et al., 2015).

2.2. Stroke in neonatal astrocytes

Although neurons are primarily susceptible to injury by HI, the impairment of supporting glial cells such as astrocytes can also be induced, which contributes to secondary injury in neurons (Gouix et al., 2014). A proper astroglial functioning is essential for the development, proliferation, maturation and survival of neurons and oligodendroglia during brain development (Clemente et al., 2013; Wuestefeld et al., 2012) but also under pathological circumstances.

Supportive properties of astrocytes are reflected by the production of growth factors such as platelet-derived growth factor (PDGF) and insulin-like growth factor 1 (IGF-1) (Scheuer et al., 2015), clearance of radicals via superoxide dismutase activity (Xu et al., 2010), anti-oxidant defense with glutathione synthesis (Miyamoto et al., 2015; Sa Santos et al., 2011) and removal of glutamate from the synaptic cleft (Zhou and Zhang, 2014), among others (Fig. 3).

The mechanisms of ischemic brain damage are still not fully understood in the neonatal brain, but neurotoxic actions of glutamate and related excitatory amino acids have been implicated in its pathogenesis (Dang et al., 2017). Increased glutamate levels and release of inflammatory cytokines can induce production of ROS in the brain. Hence, regulation of glutamate homeostasis is one of the major functions of astrocytes in the brain. A function of glutamate transporter proteins in the membranes of astrocytes is to reduce extracellular glutamate levels by re-uptake into the cell to avoid excitotoxicity. The enzyme glutamine synthetase (GS) is needed to transform glutamate intracellularly into glutamine which is stored in vesicles (Dang et al., 2017).

2.2.1. Neonatal astrocyte function in neurogenesis after stroke

As aforementioned, reactive astrocytes are able to secrete neurogenic factors that will influence the differentiation and migration of newly generated neurons as well as protection of surviving neurons (Wagenaar et al., 2018). Overexpression of VEGF after neonatal cerebral ischemia is associated with neural stem cell (NSC) proliferation and differentiation, and its inhibition leads to worsened injury, increased cell death, and reduced endothelial cell proliferation in 10-day-old rats (Shimotake et al., 2010) (Fig. 3). Several in vitro studies show that BDNF overexpression caused by ischemic stroke reduces apoptosis of hypoxic hyperglycemic hippocampal neurons and stimulates neurite outgrowth of neonatal cortical neurons in the presence of astrocytes in neonates (Deumens et al., 2006; Huang et al., 2017). Besides, these neurotrophic factors promote neuronal plasticity and formation of synapses that will allow the integration of the newly generated neural cells. Therefore, neurotrophic factors can be crucial for regeneration of neurons after damage and could also be a tool for treatment after HI (Huang et al., 2017).

2.3. Stroke in aged astrocytes

Ageing alone increases astroglial reactivity with GFAP overexpression (Lively et al., 2011) but this glial response is exaggerated following ischemia-stroke in brain accelerating the glial scar formation (Badan et al., 2003). Indeed, Badan et al. (2003) confirmed that the time course of glial activation is strongly age dependent with an induction of proteins with very important biological effects, such as, TGF, β-amyloid precursor protein (β-APP), apolipoprotein E (APOE), and lipocortin (also called annexin) (Fig. 3).

Moreover the intensity of GFAP staining is different after stroke in young and old rats hemispheres, being more intense in the contralateral hemispheres of aged rats after ischemic stroke (Gordon et al., 1997).

2.3.1. Adult astrocyte function in neurogenesis after stroke

As mentioned, stroke increases neurogenesis in the SVZ and in the

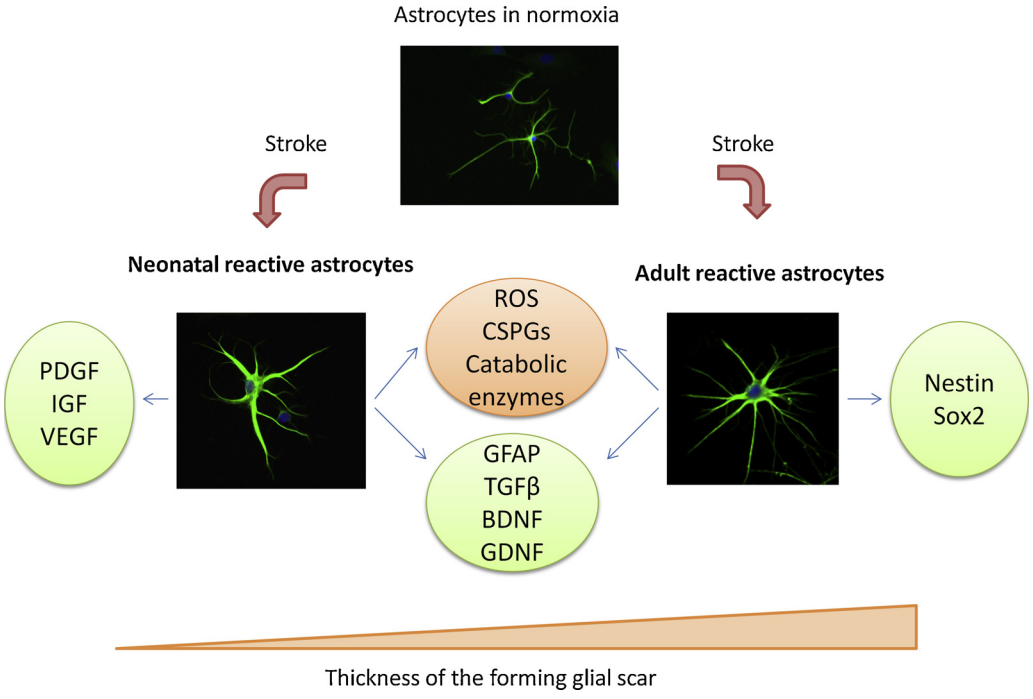

Fig. 3. Reactive astrocytosis after stroke. Reactive astrocytes form a glial scar around the infarct area and express genes and secret molecules that can promote inflammation (shown in orange) or neuroprotection (shown in green). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

DG of adult rodents and this neurogenesis may contribute to functional recovery following stroke. It has been demonstrated that astrocytes isolated from adult hippocampus may support cell proliferation as well as neurogenesis (Anderson et al., 2003). However, aged astrocytes show impairment in their ability to promote neuronal differentiation and this impairment is exacerbated after stroke (Lewis et al., 2012).

Moreover, aged astrocytes show less secretion of Wnt3, IGF-1, fibroblast growth factor receptor 2 (FGFR-2) and VEGF which has been associated with reduced ability for neurogenesis in the adult brain (Fig. 3).

3. Astrocytes as targets for stroke therapy

During the last decades, the treatment for ischemic stroke injury was focused on neurons as primary targets for neuroprotection, however, such a specific and yet exclusive approach may be insufficient to improve neurological outcomes after stroke. Due to the important role of astrocytes after ischemic stroke, broader therapeutic strategies have been proposed (Liu and Chopp, 2016). As astrocytes interact with neurons, regulate $Ca2^{+}$ signaling, neurotransmitter and immune response and also modulate axon regeneration among others (Sun et al., 2018), they might be an adjustable target for stroke recovery. Therefore, in this section different astrocyte-based therapeutic approaches will be described.

3.1. Astroglial MicroRNAs for protection after stroke

MicroRNAs (miRNAs) are small non-coding RNA molecules, expressed in a wide variety of organisms and very conserved across species (Pillai, 2005). miRNAs regulate gene expression and play an important role in cell development, differentiation and apoptosis. In the last years, the relevance of some miRNAs in astrocyte protection or injury after stroke has been addressed. Among these protective miRNAs, miR-424 overexpression, a member of the miR-16 family that play an important role in cell differentiation, inhibits astroglial activation by the repression of cell-cycle activators, such as CCND1, CDC25A, and CDK6 and consequently reduces ischemic brain injury (Zhao et al., 2013). miR-200c inhibition in astrocytes has been also associated to the reduction of brain injury as miR-200c induces FAS mediated apoptosis by repressing the expression of FAS associated protein-tyrosine phosphatase 1 (FAP-1) (Tan et al., 2011) as well as miR-29a that ameliorates ischemic injury targeting AQP4 (Zheng et al., 2019). Moreover it was found that miR-181a protects female mice from transient focal cerebral ischemia by targeting astrocyte estrogen receptor-α (ER-α) (Stary et al., 2017). However, other miRNAs have been associated to the opposite effect, such as miR-24, which induces Neuro2A (N2A) neuroblastoma cell proliferation after OGD (Zhou and Zhang, 2014) or miR-182, which aggravates cerebral ischemia injury by targeting inhibitory member of p53 family (Yi et al., 2017). Hence, a whole variety of miRNAs may be available as potential therapeutic targets in astrocytes.

3.2. Astrocytes as neuronal differentiation promoter

As mentioned previously, a subset of astrocytes are considered as cellular components of stem cell niches and named as niche astrocytes (NAs) (Luo et al., 2017). Indeed, these astrocytes that are isolated from neonatal brain or from adult hippocampal SGZs can enhance the capacity of NSCs to differentiate into new neurons under co-culture conditions in vitro (Liu et al., 2012). Moreover, it has been proposed to use transplantation of NSC together with astrocytes as a novel therapeutic tool to promote differentiation of newly generated neurons from the NSCs and finally reduce the effect of neuron injury after stroke (Cai et al., 2015). These results were corroborated with the transplantation of NSC in primary co-existence with NAs (Luo et al., 2017).

3.3. ROS and mitochondrial function modulation in astrocytes as therapeutic role

Stroke triggers increased levels of free radicals that cause tissue damage in the brain. Three different strategies have been proposed in order to mitigate the free radical effect after stroke via modulation by application of: 1) ROS scavengers, 2) ROS degradators, and 3) ROS reducers (Li and Yang, 2016).

Among ROS scavengers, the most frequently used antioxidants are represented by vitamin E, that reduces lesion volume and behavioral impairments in mice (Mishima et al., 2003), N-acetylcysteine (NAC), an antioxidant with a free thiol group that is able to react with ROS and lipoic acids (LA), such as docosahexaenoic acid (DHA), a fatty acid that can lead to long-lasting neuroprotective effects mitigating axonal changes (Arteaga et al., 2017).

There are two main enzymes that can degrade ROS levels, superoxide dismutase (SOD) and catalase (CAT). SOD reduces the superoxide to hydrogen peroxide, and CAT metabolizes hydrogen peroxide to water and oxygen, hence providing the first line “defense system” against ROS damage. Indeed, intravenous administration of polyethylene glycol-conjugated SOD (PEG-SOD) and CAT (PEG-CAT) reduced infarct volume in rats (Li and Yang, 2016).

However, preventing or attenuating excessive ROS production has been proposed as the most effective strategy for treatment of ischemic stroke. In a recent study, elastin-derived peptides have been proposed as scavengers of the free radicals. In particular, it was demonstrated that the Val-Gly-Val-Ala-Pro-Gly (VGVAPG) hexapeptide, known for its chemotactic activity and MMP upregulation properties, decreased nitric oxide (NO) release during ROS production in mouse astrocytes after OGD in vitro (Szychowski and Gminski, 2019). Silencing the galactosidase beta 1 (Glb1) gene reversed the effects caused by the VGVAPG peptide, decreasing ROS production.

Mitochondria also undergo functional impairment during and after brain ischemia, contributing to increased oxidative stress and tissue damage by ROS (George and Steinberg, 2015). Stimulation of astrocyte mitochondrial ATP production with triiodo-l-thyronine (T3) (Sayre et al., 2017) and the disruption of astrocytic Ca^{2+} signaling by deleting inositol trisphosphate receptor type 2 (IP3R2s) (Li et al., 2015) have been proposed as important modulators of this mitochondrial dysfunction.

3.4. BBB protection in astrocytes

Since cell infiltration contributes to BBB disruption after ischemic stroke, strategies have been designed to focus on BBB preservation. For example, pharmacological intervention has been proposed to precondition the brain via activation of the Nrf2 anti-oxidative defense pathway in the cerebral microvasculature to prevent BBB breakdown (Alfieri et al., 2013). Another strategy has been tested by using nafamostat mesilate (NM) in astrocytes, a synthetic serine protease inhibitor to preserve BBB integrity through the regulation of PKCα/RhoA/MLC2 pathway components (Wang et al., 2018). Moreover, Apelin-13 was applied as a peptide being involved in the regulation of cell homeostasis, against ischemic BBB injury across the effect of AQP4 in astrocytes (Chu et al., 2017).

A distinct pathway for BBB stabilization has been addressed by stimulating astrocytic synthesis of sonic hedgehog (Shh). Shh seems to upregulate tight junction proteins in capillary endothelial cells (He et al., 2013) and to promote cerebral angiogenesis after stroke. For example, Shh induced an upregulation of angiopoietin-1 in astrocytes, necessary for vessel maturation (He et al., 2013).

3.5. Hypotermia in astrocytes as therapeutic role

Therapeutic hypothermia, due to its multiple synergistic effects on ischemia and reperfusion, is one of the main neuroprotectants against

stroke at preclinical and clinical level (Kurisu and Yenari, 2018; Tahir and Pabaney, 2016) and seems to be a promising candidate to be a component of multimodal clinical strategies (Faridar et al., 2011). Indeed, it exerts strong neuroprotective effects modulating astrocytic function through decreasing metabolic rate, reducing glutamate release and ROS, regulating inflammatory and apoptotic factor expression and even preventing BBB destruction (Wang et al., 2014) so therapeutic hypothermia may be effective in improving patient outcome through the regulation of astrocytic functions.

3.6. Cannabinoids (CB) treatment as therapeutic role

The endocannabinoid system (ECS) (consist of cell-surface cannabinoid receptors CB1R, CB2R and endogenous ligands) has been shown to have neuroprotective roles against cerebral stroke (Kolb et al., 2019). CB are divided in three categories due to its affinity to the mentioned cannabinoid receptors or ligads: endocannabinoids, phytocannabinoids, and synthetic CBs. The best-studied endocannabinoids are anandamide and 2-arachidonoylglycerol (CB1/2R agonists). Meanwhile, phytocannabinoids, are the main source of 60 different compounds, such as Δ9-tetrahydrocannabinol (THC) (a partial agonist for CB receptors) and cannabidiol (CBD) (low affinity for CB receptors) (England et al., 2015).

CB demonstrate high anti-oxidant capacity and anti-inflammatory activity as well as reduce glutamate release, maintain mitochondrial membrane homeostasis and prevent NF-κB activation (Ceprian et al., 2017; Lafuente et al., 2011). Moreover concerning to astrocytes, CB regulates glial activation and toxicity reducing inflammatory mediators and increasing prosurvival factors (Fernandez-Ruiz et al., 2015; Kozela et al., 2017). This potential is promising for acute and chronic neurodegenerative pathological conditions such as stroke through the modulation of astrocytes reactivity.

3.7. Cerebral ischemic preconditioning

In the last decade, it has been proposed that cerebral ischemic preconditioning (IPC) in astrocytes can upregulate protective mechanisms to tolerate subsequent hypoxia or ischemia (Li et al., 2017). Among others, IPC promote the activation of c-Jun N-terminal kinase (JNK), extracellular signal-related kinase (ERK)-1/2, p38, and protein kinase B (Akt) signaling pathways (Pang et al., 2015). In astrocytes, this IPC induced tolerance is mediated by TLR3 signaling, so reprogramming this TLR3/TRIF/IRF3 signaling pathway may play an important role in the suppression of the inflammatory response after stroke (Pan et al., 2014).

3.8. Transcription factors regulation for glial scar formation

The inflammation response contributes to a secondary ischemic brain damage by multiple mechanisms such as activation of cell signaling pathways that promote the formation of glial scar in astrocytes (Wang et al., 2018).

One of these proinflammatory molecules is tumor necrosis factor like weak inducer of apoptosis (TWEAK), a member of the TNF family. TWEAK binds Fn14, a member of TNF receptor and this binding activates different transcription factors, such as, NF-κβ, ERK and JNK17 and contributing to glial scar formation. The intracerebroventricular injection of a soluble Fn14-Fc decoy receptor just after MCAO significantly reduced infarct volume (Yepes et al., 2005) (Fig. 4).

On the contrary, it has recently been suggested that tyrosol, a natural phenolic compound, represents a promising candidate for stroke treatment in astrocytes due to its inhibition of the inflammatory pathway. In cultured astrocytes exposed to OGD, tyrosol effectively reduced the released TNFα and IL-6 levels via decreasing STAT3 activation via JNK (Luo et al., 2018) (Fig. 4).

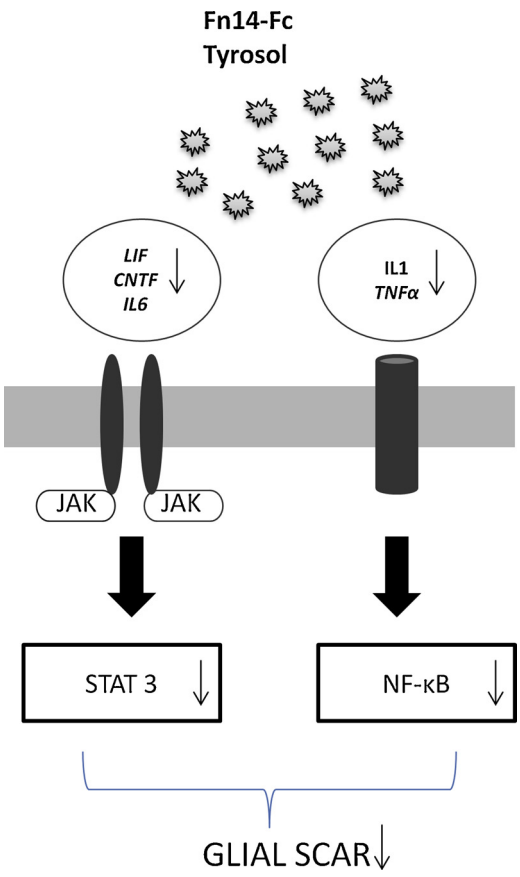

Fig. 4. Diagram of the mechanism of two different anti-inflammatory molecules (Fn14-FC and tyrosol) that reduce cytokines and interleukins responsible for the activation of different transcription factors (indicated with a thick arrow) that promote the formation of glial scar in astrocytes. ↓ means that when cytokines and interleukins are reduced due to Fn14-FC and tyrosol activation STAT 3 and NF-κB transcription factors are decreased and consequently glial scar formation is inhibited.

4. Conclusion

The present review examines various factors involved in the pathogenesis of astrocytes in the immature and mature brain after stroke. From what we have reviewed, it seems that during stroke, neonatal astrocytes reflect supportive properties by the production of growth factors. As the main neurotoxic action during the injury is due to the production of glutamate and other excitatory amino acids, regulation of glutamate homeostasis is one of the major functions of astrocytes in the developing brain. On the other side, since ageing increases astrocytic and microglial reactivity, glial scar formation is accelerated in an adult ischemic event as compared to the immature brain.

Moreover, astrocyte reactivity can play a protective or detrimental effect in neurogenesis, so reactive astrocytes could be an adjustable target for stroke recovery. Thus, it seems that new doors are opening for the treatment of stroke both, in the mature and in the immature brain, although more research will be needed in order to translate novel insights into an effective clinical therapy.

Funding

M.R is recipient of a postdoctoral fellowship from the Department of Education, University and Research of the Basque Government (ID: POS_2017_1_0095). T.S was supported by Förderverein für frühgeborene Kinder an der Charité e.V.

Declaration of Competing Interest

The authors have no other sponsorship or funding arrangements relating to their research, or conflicts of interest to disclose.

References

Alfieri, A., Srivastava, S., Siow, R.C.M., Cash, D., Modo, M., Duchen, M.R., Fraser, P.A., Williams, S.C.R., Mann, G.E., 2013. Sulforaphane preconditioning of the Nrf2/HO-1 defense pathway protects the cerebral vasculature against blood-brain barrier disruption and neurological deficits in stroke. Free Radic. Biol. Med. 65, 1012–1022.

Anderson, M.F., Blomstrand, F., Blomstrand, C., Eriksson, P.S., Nilsson, M., 2003. Astrocytes and stroke: networking for survival? Neurochem. Res. 28, 293–305.

Arba, F., Leigh, R., Inzitari, D., Warach, S.J., Luby, M., Lees, K.R., Collaboration, 2017. Blood-brain barrier leakage increases with small vessel disease in acute ischemic stroke. Neurology 89, 2143–2150.

Arteaga, O., Alvarez, A., Revuelta, M., Santaolalla, F., Urtasun, A., Hilario, E., 2017. Role of antioxidants in neonatal hypoxic-ischemic brain injury: new therapeutic approaches. Int. J. Mol. Sci. 18.

Back, S.A., Craig, A., Kayton, R.J., Luo, N.L., Meshul, C.K., Allcock, N., Fern, R., 2007. Hypoxia-ischemia preferentially triggers glutamate depletion from oligodendroglia and axons in perinatal cerebral white matter. J. Cereb. Blood Flow Metab. 27, 334–347.

Badan, I., Buchhold, B., Hamm, A., Gratz, M., Walker, L.C., Platt, D., Kessler, C., Popa-Wagner, A., 2003. Accelerated glial reactivity to stroke in aged rats correlates with reduced functional recovery. J. Cereb. Blood Flow Metab. 23, 845–854.

Barone, F.C., Irving, E.A., Ray, A.M., Lee, J.C., Kassis, S., Kumar, S., Badger, A.M., Legos, J.J., Erhardt, J.A., Ohlstein, E.H., Hunter, A.J., Harrison, D.C., Philpott, K., Smith, B.R., Adams, J.L., Parsons, A.A., 2001. Inhibition of p38 mitogen-activated protein kinase provides neuroprotection in cerebral focal ischemia. Med. Res. Rev. 21, 129–145.

Bazzoni, G., Martinez-Estrada, O.M., Orsenigo, F., Cordenonsi, M., Citi, S., Dejana, E., 2000. Interaction of junctional adhesion molecule with the tight junction components ZO-1, cingulin, and occludin. J. Biol. Chem. 275, 20520–20526.

Becerra-Calixto, A., Cardona-Gomez, G.P., 2017. The role of astrocytes in neuroprotection after brain stroke: potential in cell therapy. Front. Mol. Neurosci. 10, 88.

Bernaudin, M., Bellail, A., Marti, H.H., Yvon, A., Vivien, D., Duchatelle, I., Mackenzie, E.T., Petit, E., 2000. Neurons and astrocytes express EPO mRNA: oxygen-sensing mechanisms that involve the redox-state of the brain. Glia 30, 271–278.

Bernson-Leung, M.E., Rivkin, M.J., 2016. Stroke in neonates and children. Pediatr. Rev. 37, 463–477.

Cai, Q., Chen, Z., Song, P., Wu, L., Wang, L., Deng, G., Liu, B., Chen, Q., 2015. Co-transplantation of hippocampal neural stem cells and astrocytes and microvascular endothelial cells improve the memory in ischemic stroke rat. Int. J. Clin. Exp. Med. 8, 13109–13117.

Cekanaviciute, E., Fathali, N., Doyle, K.P., Williams, A.M., Han, J., Buckwalter, M.S., 2014. Astrocytic transforming growth factor-beta signaling reduces subacute neuroinflammation after stroke in mice. Glia 62, 1227–1240.

Ceprian, M., Jimenez-Sanchez, L., Vargas, C., Barata, L., Hind, W., Martinez-Orgado, J., 2017. Cannabidiol reduces brain damage and improves functional recovery in a neonatal rat model of arterial ischemic stroke. Neuropharmacology 116, 151–159.

Chen, R.L., Balami, J.S., Esiri, M.M., Chen, L.K., Buchan, A.M., 2010. Ischemic stroke in the elderly: an overview of evidence. Nat. Rev. Neurol. 6, 256–265.

Chen, X., Nakada, S., Donahue, J.E., Chen, R.H., Tucker, R., Qiu, J., Lim, Y.P., Stopa, E.G., Stonestreet, B.S., 2019. Neuroprotective effects of inter-alpha inhibitor proteins after hypoxic-ischemic brain injury in neonatal rats. Exp. Neurol. 317, 244–259.

Chu, H., Yang, X., Huang, C., Gao, Z., Tang, Y., Dong, Q., 2017. Apelin-13 protects against ischemic blood-brain barrier damage through the effects of aquaporin-4. Cerebrovasc. Dis. 44, 10–25.

Clemente, D., Ortega, M.C., Melero-Jerez, C., de Castro, F., 2013. The effect of glia-glia interactions on oligodendrocyte precursor cell biology during development and in demyelinating diseases. Front. Cell. Neurosci. 7, 268.

Comi, A.M., Johnston, M.V., 2009. Stroke: neonates vs adult. In: Hof, P., Mobbs, C. (Eds.), Handbook of the Neuroscience of Aging. Elsevier and Academic Press, London, pp. 491–494.

Daneman, R., Prat, A., 2015. The blood-brain barrier. Cold Spring Harb. Perspect. Biol. 7, a020412.

Dang, Y.X., Shi, K.N., Wang, X.M., 2017. Early changes in glutamate metabolism and perfusion in basal ganglia following hypoxia-ischemia in neonatal piglets: a multi-sequence 3.0T MR study. Front. Physiol. 8, 237.

Deumens, R., Koopmans, G.C., Jaken, R.J., Morren, K., Comhair, T., Kosar, S., Steinbusch, H.W., Den Bakker, C.G., Joosten, E.A., 2006. Stimulation of neurite outgrowth on neonatal cerebral astrocytes is enhanced in the presence of BDNF. Neurosci. Lett. 407, 268–273.

Ding, D.C., Lin, C.H., Shyu, W.C., Lin, S.Z., 2013. Neural stem cells and stroke. Cell Transplant. 22, 619–630.

Docagne, F., Campbell, S.J., Bristow, A.F., Poole, S., Vigues, S., Guaza, C., Perry, V.H., Anthony, D.C., 2005. Differential regulation of type I and type II interleukin-1 receptors in focal brain inflammation. Eur. J. Neurosci. 21, 1205–1214.

England, T.J., Hind, W.H., Rasid, N.A., O'Sullivan, S.E., 2015. Cannabinoids in experimental stroke: a systematic review and meta-analysis. J. Cereb. Blood Flow Metab. 35, 348–358.

Faridar, A., Bershad, E.M., Emiru, T., Iaizzo, P.A., Suarez, J.I., Divani, A.A., 2011. Therapeutic hypothermia in stroke and traumatic brain injury. Front. Neurol. 2, 80.

Fatemi, A., Wilson, M.A., Johnston, M.V., 2009. Hypoxic-ischemic encephalopathy in the term infant. Clin. Perinatol. 36, 835–858 vii.

Faulkner, J.R., Herrmann, J.E., Woo, M.J., Tansey, K.E., Doan, N.B., Sofroniew, M.V., 2004. Reactive astrocytes protect tissue and preserve function after spinal cord injury. J. Neurosci. 24, 2143–2155.

Feigin, V.L., Norrving, B., 2014. A new paradigm for primary prevention strategy in people with elevated risk of stroke. Int. J. Stroke 9, 624–626.

Fernandez-Lopez, D., Faustino, J., Daneman, R., Zhou, L., Lee, S.Y., Derugin, N., Wendland, M.F., Vexler, Z.S., 2012. Blood-brain barrier permeability is increased after acute adult stroke but not neonatal stroke in the rat. J. Neurosci. 32, 9588–9600.

Fernandez-Lopez, D., Natarajan, N., Ashwal, S., Vexler, Z.S., 2014. Mechanisms of perinatal arterial ischemic stroke. J. Cereb. Blood Flow Metab. 34, 921–932.

Fernandez-Ruiz, J., Moro, M.A., Martinez-Orgado, J., 2015. Cannabinoids in neurodegenerative disorders and stroke/brain trauma: from preclinical models to clinical applications. Neurotherapeutics 12, 793–806.

Fox, C., Dingman, A., Derugin, N., Wendland, M.F., Manabat, C., Ji, S., Ferriero, D.M., Vexler, Z.S., 2005. Minocycline confers early but transient protection in the immature brain following focal cerebral ischemia-reperfusion. J. Cereb. Blood Flow Metab. 25, 1138–1149.

George, P.M., Steinberg, G.K., 2015. Novel stroke therapeutics: unraveling stroke pathophysiology and its impact on clinical treatments. Neuron 87, 297–309.

Gordon, M.N., Schreier, W.A., Ou, X., Holcomb, L.A., Morgan, D.G., 1997. Exaggerated astrocyte reactivity after nigrostriatal deafferentation in the aged rat. J. Comp. Neurol. 388, 106–119.

Gotz, M., Sirko, S., Beckers, J., Irmler, M., 2015. Reactive astrocytes as neural stem or progenitor cells: in vivo lineage, in vitro potential, and Genome-wide expression analysis. Glia 63, 1452–1468.

Gouix, E., Buisson, A., Nieoullon, A., Kerkerian-Le Goff, L., Tauskela, J.S., Blondeau, N., Had-Aissouni, L., 2014. Oxygen glucose deprivation-induced astrocyte dysfunction provokes neuronal death through oxidative stress. Pharmacol. Res. 87, 8–17.

Gris, P., Tighe, A., Levin, D., Sharma, R., Brown, A., 2007. Transcriptional regulation of scar gene expression in primary astrocytes. Glia 55, 1145–1155.

He, Q.W., Xia, Y.P., Chen, S.C., Wang, Y., Huang, M., Huang, Y., Li, J.Y., Li, Y.N., Gao, Y., Mao, L., Mei, Y.W., Hu, B., 2013. Astrocyte-derived sonic hedgehog contributes to angiogenesis in brain microvascular endothelial cells via RhoA/ROCK pathway after oxygen-glucose deprivation. Mol. Neurobiol. 47, 976–987.

Herrmann, O., Baumann, B., de Lorenzi, R., Muhammad, S., Zhang, W., Kleesiek, J., Malfertheiner, M., Kohrmann, M., Potrovita, I., Maegele, I., Beyer, C., Burke, J.R., Hasan, M.T., Bujard, H., Wirth, T., Pasparakis, M., Schwaninger, M., 2005. IKK mediates ischemia-induced neuronal death. Nat. Med. 11, 1322–1329.

Ho, A., Blum, M., 1997. Regulation of astroglial-derived dopaminergic neurotrophic factors by interleukin-1 beta in the striatum of young and middle-aged mice. Exp. Neurol. 148, 348–359.

Huang, L., Wu, Z.B., Zhuge, Q., Zheng, W., Shao, B., Wang, B., Sun, F., Jin, K., 2014. Glial scar formation occurs in the human brain after ischemic stroke. Int. J. Med. Sci. 11, 344–348.

Huang, W., Meng, F., Cao, J., Liu, X., Zhang, J., Li, M., 2017. Neuroprotective role of exogenous brain-derived neurotrophic factor in hypoxia-hypoglycemia-induced hippocampal neuron injury via regulating Trkb/MiR134 signaling. J. Mol. Neurosci. 62, 35–42.

Ikeshima-Kataoka, H., 2016. Neuroimmunological implications of AQP4 in astrocytes. Int. J. Mol. Sci. 17.

Jin, R., Yang, G., Li, G., 2010. Inflammatory mechanisms in ischemic stroke: role of inflammatory cells. J. Leukoc. Biol. 87, 779–789.

Khoshnoodi, J., Pedchenko, V., Hudson, B.G., 2008. Mammalian collagen IV. Microsc. Res. Tech. 71, 357–370.

Kolb, B., Saber, H., Fadel, H., Rajah, G., 2019. The endocannabinoid system and stroke: a focused review. Brain Circ. 5, 1–7.

Kozela, E., Juknat, A., Vogel, Z., 2017. Modulation of astrocyte activity by cannabidiol, a nonpsychoactive cannabinoid. Int. J. Mol. Sci. 18.

Kratzer, I., Chip, S., Vexler, Z.S., 2014. Barrier mechanisms in neonatal stroke. Front. Neurosci. 8, 359.

Krupinski, J., Slevin, M., Marti, E., Catena, E., Rubio, F., Gaffney, J., 2003. Time-course phosphorylation of the mitogen activated protein (MAP) kinase group of signalling proteins and related molecules following middle cerebral artery occlusion (MCAO) in rats. Neuropathol. Appl. Neurobiol. 29, 144–158.

Kurisu, K., Yenari, M.A., 2018. Therapeutic hypothermia for ischemic stroke; pathophysiology and future promise. Neuropharmacology 134, 302–309.

Kyriakis, J.M., Avruch, J., 2001. Mammalian mitogen-activated protein kinase signal transduction pathways activated by stress and inflammation. Physiol. Rev. 81, 807–869.

Lafuente, H., Alvarez, F.J., Pazos, M.R., Alvarez, A., Rey-Santano, M.C., Mielgo, V., Murgia-Esteve, X., Hilario, E., Martinez-Orgado, J., 2011. Cannabidiol reduces brain damage and improves functional recovery after acute hypoxia-ischemia in newborn pigs. Pediatr. Res. 70, 272–277.

Lee, T.H., Kato, H., Chen, S.T., Kogure, K., Itoyama, Y., 1998. Expression of nerve growth factor and trkA after transient focal cerebral ischemia in rats. Stroke 29, 1687–1696 discussion 1697.

Lewis, D.K., Thomas, K.T., Selvamani, A., Sohrabji, F., 2012. Age-related severity of focal ischemia in female rats is associated with impaired astrocyte function. Neurobiol. Aging 33 (1123), e1121–1116.

Li, H., Xie, Y., Zhang, N., Yu, Y., Zhang, Q., Ding, S., 2015. Disruption of IP(3)R2-mediated Ca(2)(+) signaling pathway in astrocytes ameliorates neuronal death and brain damage while reducing behavioral deficits after focal ischemic stroke. Cell Calcium 58, 565–576.

Li, L., Lundkvist, A., Andersson, D., Wilhelmsson, U., Nagai, N., Pardo, A.C., Nodin, C., Stahlberg, A., Aprico, K., Larsson, K., Yabe, T., Moons, L., Fotheringham, A., Davies, I., Carmeliet, P., Schwartz, J.P., Pekna, M., Kubista, M., Blomstrand, F., Maragakis, N., Nilsson, M., Pekny, M., 2008. Protective role of reactive astrocytes in brain ischemia. J. Cereb. Blood Flow Metab. 28, 468–481.
Li, L., Zhang, H., Varrin-Doyer, M., Zamvil, S.S., Verkman, A.S., 2011. Proinflammatory role of aquaporin-4 in autoimmune neuroinflammation. FASEB J. 25, 1556–1566.
Li, S., Hafeez, A., Noorulla, F., Geng, X., Shao, G., Ren, C., Lu, G., Zhao, H., Ding, Y., Ji, X., 2017. Preconditioning in neuroprotection: from hypoxia to ischemia. Prog. Neurobiol. 157, 79–91.
Li, W., Yang, S., 2016. Targeting oxidative stress for the treatment of ischemic stroke: upstream and downstream therapeutic strategies. Brain Circ. 2, 153–163.
Lipton, S.A., 1993. Molecular mechanisms of trauma-induced neuronal degeneration. Curr. Opin. Neurol. Neurosurg. 6, 588–596.
Liu, Y., Wang, L., Long, Z., Zeng, L., Wu, Y., 2012. Protoplasmic astrocytes enhance the ability of neural stem cells to differentiate into neurons in vitro. PLoS One 7, e38243.
Liu, Z., Chopp, M., 2016. Astrocytes, therapeutic targets for neuroprotection and neurorestoration in ischemic stroke. Prog. Neurobiol. 144, 103–120.
Lively, S., Moxon-Emre, I., Schlichter, L.C., 2011. SC1/hevin and reactive gliosis after transient ischemic stroke in young and aged rats. J. Neuropathol. Exp. Neurol. 70, 913–929.
Luo, G., Huang, Y., Mo, D., Ma, N., Gao, F., Song, L., Sun, X., Xu, X., Liu, L., Huo, X., Wang, B., Li, X., Jia, B., Deng, Y., Zhang, X., Fernandez-Escobar, A., Peng, G., Miao, Z., 2018. Tyrosol attenuates pro-inflammatory cytokines from cultured astrocytes and NF-kappaB activation in in vitro oxygen glucose deprivation. Neurochem. Int. 121, 140–145.
Luo, L., Shang, F.F., Long, H., Jiang, L., Zhu, R., Zhao, Q., Gu, H., Kong, J., Xu, W., Zhao, Y., Cen, X., 2017. Role of nicotinamide N-methyltransferase in dorsal striatum in cocaine place preference. Neuropsychopharmacology 42, 2333–2343.
Ma, M.W., Wang, J., Dhandapani, K.M., Brann, D.W., 2017a. NADPH oxidase 2 regulates NLRP3 inflammasome activation in the brain after traumatic brain injury. Oxid. Med. Cell. Longev. 2017, 6057609.
Ma, Y., Wang, J., Wang, Y., Yang, G.Y., 2017b. The biphasic function of microglia in ischemic stroke. Prog. Neurobiol. 157, 247–272.
Mesples, B., Plaisant, F., Gressens, P., 2003. Effects of interleukin-10 on neonatal excitotoxic brain lesions in mice. Brain Res. Dev. Brain Res. 141, 25–32.
Ming, G.L., Song, H., 2005. Adult neurogenesis in the mammalian central nervous system. Annu. Rev. Neurosci. 28, 223–250.
Mishima, K., Tanaka, T., Pu, F., Egashira, N., Iwasaki, K., Hidaka, R., Matsunaga, K., Takata, J., Karube, Y., Fujiwara, M., 2003. Vitamin E isoforms alpha-tocotrienol and gamma-tocopherol prevent cerebral infarction in mice. Neurosci. Lett. 337, 56–60.
Miyamoto, N., Maki, T., Shindo, A., Liang, A.C., Maeda, M., Egawa, N., Itoh, K., Lo, E.K., Lok, J., Ihara, M., Arai, K., 2015. Astrocytes promote oligodendrogenesis after white matter damage via brain-derived neurotrophic factor. J. Neurosci. 35, 14002–14008.
Murphy, M.P., 2009. How mitochondria produce reactive oxygen species. Biochem. J. 417, 1–13.
Myers, E.H., Ment, L.R., 2012. In: Perlman, J.M. (Ed.), Perinatal Stroke. Neurology: Neonatology Questions and Controversies. Elsevier Saunders, Philadelphia, pp. 91–107.
Nelson, K.B., 2007. Perinatal ischemic stroke. Stroke 38, 742–745.
Nicole, O., Ali, C., Docagne, F., Plawinski, L., MacKenzie, E.T., Vivien, D., Buisson, A., 2001. Neuroprotection mediated by glial cell line-derived neurotrophic factor: involvement of a reduction of NMDA-induced calcium influx by the mitogen-activated protein kinase pathway. J. Neurosci. 21, 3024–3033.
Pan, L.N., Zhu, W., Li, Y., Xu, X.L., Guo, L.J., Lu, Q., Wang, J., 2014. Astrocytic Toll-like receptor 3 is associated with ischemic preconditioning-induced protection against brain ischemia in rodents. PLoS One 9, e99526.
Pang, Y., Chai, C.R., Gao, K., Jia, X.H., Kong, J.G., Chen, X.Q., Vatcher, G., Chen, J.G., Yu, A.C., 2015. Ischemia preconditioning protects astrocytes from ischemic injury through 14-3-3gamma. J. Neurosci. Res. 93, 1507–1518.
Pillai, R.S., 2005. MicroRNA function: multiple mechanisms for a tiny RNA? RNA 11, 1753–1761.
Randolph, S.A., 2016. Ischemic stroke. Workplace Health Saf. 64, 444.
Rice 3rd, J.E., Vannucci, R.C., Brierley, J.B., 1981. The influence of immaturity on hypoxic-ischemic brain damage in the rat. Ann. Neurol. 9, 131–141.
Roy Choudhury, G., Ryou, M.G., Poteet, E., Wen, Y., He, R., Sun, F., Yuan, F., Jin, K., Yang, S.H., 2014. Involvement of p38 MAPK in reactive astrogliosis induced by ischemic stroke. Brain Res. 1551, 45–58.
Sa Santos, S., Sonnewald, U., Carrondo, M.J., Alves, P.M., 2011. The role of glia in neuronal recovery following anoxia: in vitro evidence of neuronal adaptation. Neurochem. Int. 58, 665–675.
Saadoun, S., Papadopoulos, M.C., Watanabe, H., Yan, D., Manley, G.T., Verkman, A.S., 2005. Involvement of aquaporin-4 in astroglial cell migration and glial scar formation. J. Cell. Sci. 118, 5691–5698.
Sarikaya, H., Steinlin, M., 2018. Cerebellar stroke in adults and children. Handb. Clin. Neurol. 155, 301–312.
Sayre, N.L., Sifuentes, M., Holstein, D., Cheng, S.Y., Zhu, X., Lechleiter, J.D., 2017. Stimulation of astrocyte fatty acid oxidation by thyroid hormone is protective against ischemic stroke-induced damage. J. Cereb. Blood Flow Metab. 37, 514–527.
Scheuer, T., Brockmoller, V., Blanco Knowlton, M., Weitkamp, J.H., Ruhwedel, T., Mueller, S., Endesfelder, S., Buhrer, C., Schmitz, T., 2015. Oligodendroglial maldevelopment in the cerebellum after postnatal hyperoxia and its prevention by minocycline. Glia 63, 1825–1839.
Schneider, A., Martin-Villalba, A., Weih, F., Vogel, J., Wirth, T., Schwaninger, M., 1999. NF-kappaB is activated and promotes cell death in focal cerebral ischemia. Nat. Med. 5, 554–559.
Shimotake, J., Derugin, N., Wendland, M., Vexler, Z.S., Ferriero, D.M., 2010. Vascular endothelial growth factor receptor-2 inhibition promotes cell death and limits endothelial cell proliferation in a neonatal rodent model of stroke. Stroke 41, 343–349.
Sommer, C.J., 2017. Ischemic stroke: experimental models and reality. Acta Neuropathol. 133, 245–261.
Stary, C.M., Xu, L., Li, L., Sun, X., Ouyang, Y.B., Xiong, X., Zhao, J., Giffard, R.G., 2017. Inhibition of miR-181a protects female mice from transient focal cerebral ischemia by targeting astrocyte estrogen receptor-alpha. Mol. Cell. Neurosci. 82, 118–125.
Strong, K., Mathers, C., Bonita, R., 2007. Preventing stroke: saving lives around the world. Lancet Neurol. 6, 182–187.
Sun, L., Zhang, Y., Liu, E., Ma, Q., Anatol, M., Han, H., Yan, J., 2018. The roles of astrocyte in the brain pathologies following ischemic stroke. Brain Inj. 1–5.
Szychowski, K.A., Gminski, J., 2019. The VGVAPG peptide regulates the production of nitric oxide synthases and reactive oxygen species in mouse astrocyte cells in vitro. Neurochem. Res.
Tahir, R.A., Pabaney, A.H., 2016. Therapeutic hypothermia and ischemic stroke: a literature review. Surg. Neurol. Int. 7, S381–386.
Tan, J.R., Koo, Y.X., Kaur, P., Liu, F., Armugam, A., Wong, P.T., Jeyaseelan, K., 2011. microRNAs in stroke pathogenesis. Curr. Mol. Med. 11, 76–92.
Ten, V.S., Starkov, A., 2012. Hypoxic-ischemic injury in the developing brain: the role of reactive oxygen species originating in mitochondria. Neurol. Res. Int. 2012, 542976.
Titomanlio, L., Fernandez-Lopez, D., Manganozzi, L., Moretti, R., Vexler, Z.S., Gressens, P., 2015. Pathophysiology and neuroprotection of global and focal perinatal brain injury: lessons from animal models. Pediatr. Neurol. 52, 566–584.
Tokumine, J., Kakinohana, O., Cizkova, D., Smith, D.W., Marsala, M., 2003. Changes in spinal GDNF, BDNF, and NT-3 expression after transient spinal cord ischemia in the rat. J. Neurosci. Res. 74, 552–561.
Varfolomeev, E., Goncharov, T., Fedorova, A.V., Dynek, J.N., Zobel, K., Deshayes, K., Fairbrother, W.J., Vucic, D., 2008. c-IAP1 and c-IAP2 are critical mediators of tumor necrosis factor alpha (TNFalpha)-induced NF-kappaB activation. J. Biol. Chem. 283, 24295–24299.
Vexler, Z.S., Tang, X.N., Yenari, M.A., 2006. Inflammation in adult and neonatal stroke. Clin. Neurosci. Res. 6, 293–313.
Wagenaar, N., Martinez-Biarge, M., van der Aa, N.E., van Haastert, I.C., Groenendaal, F., Benders, M., Cowan, F.M., de Vries, L.S., 2018. Neurodevelopment after perinatal arterial ischemic stroke. Pediatrics 142.
Wallace, A.N., Kansagra, A.P., McEachern, J., Moran, C.J., Cross 3rd, D.T., Derdeyn, C.P., 2016. Evolution of endovascular stroke therapies and devices. Expert Rev. Med. Devices 13, 263–270.
Wang, H., Song, G., Chuang, H., Chiu, C., Abdelmaksoud, A., Ye, Y., Zhao, L., 2018. Portrait of glial scar in neurological diseases. Int. J. Immunopathol. Pharmacol. 31, 2058738418801406.
Wang, L., Jiang, F., Li, Q., He, X., Ma, J., 2014. Mild hypothermia combined with neural stem cell transplantation for hypoxic-ischemic encephalopathy: neuroprotective effects of combined therapy. Neural Regen. Res. 9, 1745–1752.
Weston, R.M., Lin, B., Dusting, G.J., Roulston, C.L., 2013. Targeting oxidative stress injury after ischemic stroke in conscious rats: limited benefits with apocynin highlight the need to incorporate long term recovery. Stroke Res. Treat. 2013, 648061.
Wick, A., Wick, W., Waltenberger, J., Weller, M., Dichgans, J., Schulz, J.B., 2002. Neuroprotection by hypoxic preconditioning requires sequential activation of vascular endothelial growth factor receptor and Akt. J. Neurosci. 22, 6401–6407.
Wuestefeld, R., Chen, J., Meller, K., Brand-Saberi, B., Theiss, C., 2012. Impact of vegf on astrocytes: analysis of gap junctional intercellular communication, proliferation, and motility. Glia 60, 936–947.
Xu, L., Emery, J.F., Ouyang, Y.B., Voloboueva, L.A., Giffard, R.G., 2010. Astrocyte targeted overexpression of Hsp72 or SOD2 reduces neuronal vulnerability to forebrain ischemia. Glia 58, 1042–1049.
Yamagata, K., Tagami, M., Ikeda, K., Tsumagari, S., Yamori, Y., Nara, Y., 2002. Differential regulation of glial cell line-derived neurotrophic factor (GDNF) mRNA expression during hypoxia and reoxygenation in astrocytes isolated from stroke-prone spontaneously hypertensive rats. Glia 37, 1–7.
Yepes, M., Brown, S.A., Moore, E.G., Smith, E.P., Lawrence, D.A., Winkles, J.A., 2005. A soluble Fn14-Fc decoy receptor reduces infarct volume in a murine model of cerebral ischemia. Am. J. Pathol. 166, 511–520.
Yi, H., Huang, Y., Yang, F., Liu, W., He, S., Hu, X., 2017. MicroRNA-182 aggravates cerebral ischemia injury by targeting inhibitory member of the ASPP family (iASPP). Arch. Biochem. Biophys. 620, 52–58.
Yoneyama, M., Shiba, T., Hasebe, S., Ogita, K., 2011. Adult neurogenesis is regulated by endogenous factors produced during neurodegeneration. J. Pharmacol. Sci. 115, 425–432.
Zhao, H., Wang, J., Gao, L., Wang, R., Liu, X., Gao, Z., Tao, Z., Xu, C., Song, J., Ji, X., Luo, Y., 2013. MiRNA-424 protects against permanent focal cerebral ischemia injury in mice involving suppressing microglia activation. Stroke 44, 1706–1713.
Zheng, Y., Pan, C., Chen, M., Pei, A., Xie, L., Zhu, S., 2019. miR29a ameliorates ischemic injury of astrocytes in vitro by targeting the water channel protein aquaporin 4. Oncol. Rep. 41, 1707–1717.
Zhou, J., Zhang, J., 2014. Identification of miRNA-21 and miRNA-24 in plasma as potential early stage markers of acute cerebral infarction. Mol. Med. Rep. 10, 971–976.