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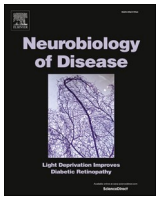

The NG2-glia is a potential target to maintain the integrity of neurovascular unit after acute ischemic stroke

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ABSTRACT

The neurovascular unit (NVU) plays a critical role in health and disease. In the current review, we discuss the critical role of a class of neural/glial antigen 2 (NG2)-expressing glial cells (NG2-glia) in regulating NVU after acute ischemic stroke (AIS). We first introduce the role of NG2-glia in the formation of NVU during development as well as aging-induced damage to NVU and accompanying NG2-glia change. We then discuss the reciprocal interactions between NG2-glia and the other component cells of NVU, emphasizing the factors that could influence NG2-glia. Damage to the NVU integrity is the pathological basis of edema and hemorrhagic transformation, the most dreaded complication after AIS. The role of NG2-glia in AIS-induced NVU damage and the effect of NG2-glia transplantation on AIS-induced NVU damage are summarized. We next discuss the role of NG2-glia and the effect of NG2-glia transplantation in oligodendrogenesis and white matter repair as well as angiogenesis which is associated with the outcome of the patients after AIS. Finally, we review the current strategies to promote NG2-glia proliferation and differentiation and propose to use the dental pulp stem cells (DPSC)-derived exosome as a promising strategy to reduce AIS-induced injury and promote repair through maintaining the integrity of NVU by regulating endogenous NG2-glia proliferation and differentiation.

Abbreviations: AIS, acute ischemic stroke; AKAP12, A kinase anchor protein 12; BBB, blood-brain barrier; BDNF, brain-derived neurotrophic factor; BM, basement membrane; bFGF, basic fibroblast growth factor; BMPs, bone morphogenetic protein; Cav-1, caveolin-1; CNTF, ciliary neurotrophic factor; COX, cyclooxygenase; CSPGs, chondroitin sulfate proteoglycans; CXCL12, chemokine ligand 12; CX3CR1, CX3C chemokine receptor 1; CXCR4, chemokine receptors 4; CXCR7, chemokine receptors 7; DCX, dual cortical proteins; DPSC, dental pulp stem cells; EAE, experimental autoimmune encephalomyelitis; EVs, extracellular vesicles; FGF, fibroblast growth factor; FGF-2, fibroblast growth factor 2; FSIs, fast-spiking interneurons; GABA, gamma-aminobutyric acid; GGF2, glial growth factor 2; HDACs, histone deacetylases; HSP90, heat shock protein 90; ID2, differentiation inhibitory factor; IFN-γ, interferon-gamma; IGF-1, insulin growth factor-1; IL33, interleukin 33; MAIs, myelin-associated inhibitory proteins; MAG, myelin-associated glycolin; MCAO, middle cerebral artery occlusion; MLKL, mixed lineage kinase domain-like protein; MRI, magnetic resonance imaging; MS, multiple sclerosis; MSCs, mesenchymal stem cells; NFSIs, non-fast-spiking interneurons; NgR1, Nogo receptor-1; NOS, NO synthase; Nrp1, neuropilin-1; NVU, Neurovascular unit; OPCs, oligodendrocyte precursor cells; OMgp, oligodendrocyte myelin glycoprotein; PDGF-BB, platelet-derived growth factor-BB; PDGFR-α, platelet-derived growth factor receptor alpha; PDGFR-β, platelet-derived growth factor receptor beta; PGE2, prostaglandin E2; PIPK, phosphatidylinositol phosphate kinase; S1P, sphingosine-1-phosphate; ST2, suppression of tumorigenicity 2 receptor; STAT1, signal transducer and activator of transcription 1; STAT6, signal transducer and activator of transcription 6; TGFBR2, TGF-β type II receptor; TGFα, transforming growth factor-α; TGF-β, transforming growth factor-beta; TGF-β2, transforming growth factor-β2; TNFR2, tumor necrosis factor receptor-2; VEGF, vascular endothelial-derived growth factor..

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1. Brief introduction of the neurovascular unit and NG2-glia

Stroke is the second leading cause of death worldwide and imposes a heavy economic and health burden on society, in particular, ischemic stroke is more common (Feske, 2021). With the failure of decades of large-scale clinical trials of neuroprotective drugs, the focus of stroke treatment has shifted from neuroprotection to neurovascular unit protection (Ayer et al., 2012; Lo et al., 2004), which is composed of neurons, astrocytes, endothelial cells, microglia cells, basal membrane and extracellular matrix (Schaeffer and Iadecola, 2021).

It is well known that oligodendrocytes (OLs) are the myelin sheath cells of the central nervous system (CNS), whereas the oligodendrocytes precursor cells (OPCs), also termed as polydendrocytes, refer to NG2-glia, a class of glial cells expressing neural/glial antigen 2 (NG2, a chondroitin sulfate proteoglycan). OPCs and NG2-glia are used interchangeably in the literature. NG2-glia have the same self-renewal ability as pluripotent stem cells (Song et al., 2017). It has been reported that NG2-glia serve as a reserve pool after injury and differentiate into OLs to promote the remyelination of axons after injury (Song et al., 2017). Owing to the ability to promote remyelination of axons after injury, NG2-glia play important roles in the restoration of demyelinating conditions (Akay et al., 2021) in diseases such as multiple sclerosis (Girolamo et al., 2019; Song et al., 2017), ischemic stroke (Bonfanti et al., 2017) and spinal cord injury (Levine, 2016). The role of NG2-glia in brain physiology and repair has been reviewed elsewhere (Akay et al., 2021; Nishiyama et al., 2014).

NG2-glia can differentiate to generate astrocytes in a variety of contexts. For example, the addition of fetal bovine serum to culture media of NG2-glia can generate type 2 astrocytes (Raff et al., 1985; Suzuki et al., 2017; Zhu et al., 2011). In response to bone morphogenetic protein (BMPs) and interferon-gamma (IFNγ) treatment, NG2-glia exit the cell cycle and differentiate to produce astrocytes (Tanner et al., 2011). When NG2-glia are exposed to fibrinogen which is activated by BMP signaling, NG2-glia differentiate into astrocytes and prevent myelin production (Petersen et al., 2017). It is of note that NG2-glia can also differentiate into neurons in the ventral forebrain, dorsal cerebral cortex, and hippocampal region in postnatal and adult animals (Guo et al., 2009; Guo et al., 2010; Rivers et al., 2008). NG2-glia can differentiate into NeuN-positive neurons in vitro (Belachew et al., 2003) and NG2-glia express low levels of neuronal precursor markers such as Sox2, Pax6, and dual cortical proteins (DCX), suggesting that the NG2-glia may have an intrinsic potential for neuronal differentiation (Dayer et al., 2005; Guo et al., 2010; Robins et al., 2013).

1.1. Role of NG2-glia in the development of neurovascular unit

NG2-glia are a heterogeneous, multifunctional population of cells that emerge during embryonic development and are resident in the adult brain parenchyma (Nishiyama et al., 1999). During the embryonic phase, NG2-glia regulate branching, connection and refinement of the blood vessels by secreting VEGF or regulating the expression of signaling molecular which are involved in angiogenesis and blood vessel maturation, such as angiopoietin1/Tie2 and Notch/Delta-like-4/JAG1 (Minocha et al., 2015). During the postnatal phase, NG2-glia migrate along vasculature and jump between blood vessel. Wnt-Cxcr4 (chemokine receptor 4) signaling plays an important role in regulating NG2-glia-endothelial interactions (Tsai et al., 2016). In addition, NG2-glia regulate endothelial cell proliferation and increase white matter angiogenesis through hypoxia-inducible factors 1/2 (HIF1/2) signaling (Yuen et al., 2014) (Fig. 1A). Meanwhile, NG2-glia are required for normal BBB development (Seo et al., 2014).

NG2-glia in the vicinity of blood vessels may be integrated with other cells in the NVU due to their developmental alignment and response to neuroinflammation and growth factors. The simultaneous activation of vascular NG2-glia and pericytes during BBB development and dysfunction suggests that NG2-glia are key regulator of vascular development (Girolamo et al., 2019).

1.2. Role of NG2-glia in aging-induced damage of the neurovascular unit

The cerebral blood flow has been shown to decrease with age (Mokhber et al., 2021). This decrease can cause hypertension, atherosclerosis, and cerebral small vessel disease (Nagata et al., 2016). During aging, neurovascular uncoupling and BBB damage occur most, which in turn leads to altered cerebral blood flow and local perfusion. Moreover, aging-induced NVU dysfunction allows the infiltration of toxic proteins or leukocytes from blood into the brain, leading to white matter (WM) damage, NVU disorders, and neuroinflammation, ultimately resulting in cognitive deficits (Li et al., 2019). In addition, studies have shown that a decrease in age-related OLs production may disrupt WM repair in mice (Miyamoto et al., 2013) and senile oligodendrocytes exhibit a swollen morphology that causes irreversible demyelination of neurons and damages the proliferative capacity of NG2-glia (Peters, 2009). In summary, aging causes severe impairment of the normal function of NG2-glia, and maintaining the normal function of NG2-glia after aging may represent as a potential therapeutic strategy for the treatment of aging-induced neurological diseases.

2. Interactions between NG2-glia and the neurovascular unit in health and disease

Interactions between NG2-glia and the cells that consist in the neurovascular unit have been summarized in Fig. 2A.

2.1. Interaction of NG2-glia with endothelial cells

When NG2-glia are co-cultured with endothelial cells, NG2-glia increase the barrier integrity of endothelial cells through platelet-derived growth factor-BB (PDGF-BB)/platelet-derived growth factor receptor α (PDGFRα) signaling pathway (Kimura et al., 2020). Oligodendrocytes have been reported to regulate endothelial cell barrier integrity through Ras (Mayes et al., 2013) and WNT/β-catenin signaling pathways (Zhang et al., 2014). NG2-glia-endothelial cell interactions can regulate neonatal WM vascular development in a Wnt-dependent manner (Chavali et al., 2020). In addition, endothelial cells can regulate the survival and proliferation of NG2-glia by secreting cytokines. For example, endothelial cells secrete trophic factors such as brain-derived neurotrophic factor (BDNF) and fibroblast growth factor (FGF), which activate the Src/Akt signaling pathway and promote the expression of platelet-derived growth factor receptor alpha (PDGFR-α) in NG2-glia to promote the survival and proliferation of NG2-glia (McKinnon et al., 1990). Endothelial progenitor cells (EPCs), as precursor cells of endothelial cells, interact with NG2-glia in the CNS. Overexpression of chemokine ligand 12 (CXCL12) by EPCs promoted the expression of PDGFR-α, basic fibroblast growth factor (bFGF), chemokine receptors 4 (CXCR4), and chemokine receptors 7 (CXCR7) in NG2-glia while promoting the proliferation and migration of NG2-glia. Blocking CXCR4 attenuated the beneficial effects of CXCL12 on the proliferation and migration of NG2-glia, whereas the knockdown of CXCR7 inhibited NG2-glia differentiation. It is shown that modification of EPCs by the *CXCL12* gene further promotes the ability of EPCs to enhance the remyelinating properties of NG2-glia, indicating that CXCL12-EPC has great potential in WM repair (Yuan et al., 2018).

During neonatal angiogenesis, NG2-glia interact with the tip cells of germinating vessels in the WM to promote angiogenesis. Meanwhile, the cell number of NG2-glia is positively correlated with the density of blood vessels in the WM (Chavali et al., 2020). NG2-glia are not only in close contact with the tip cells during angiogenesis, but are also critical for the formation of vascular networks during development and can affect other vascular-associated cells (Minocha et al., 2015). NG2-glia require the vasculature as a physical matrix and interact with the vascular endothelium for migration. The migration of NG2-glia was blocked when the

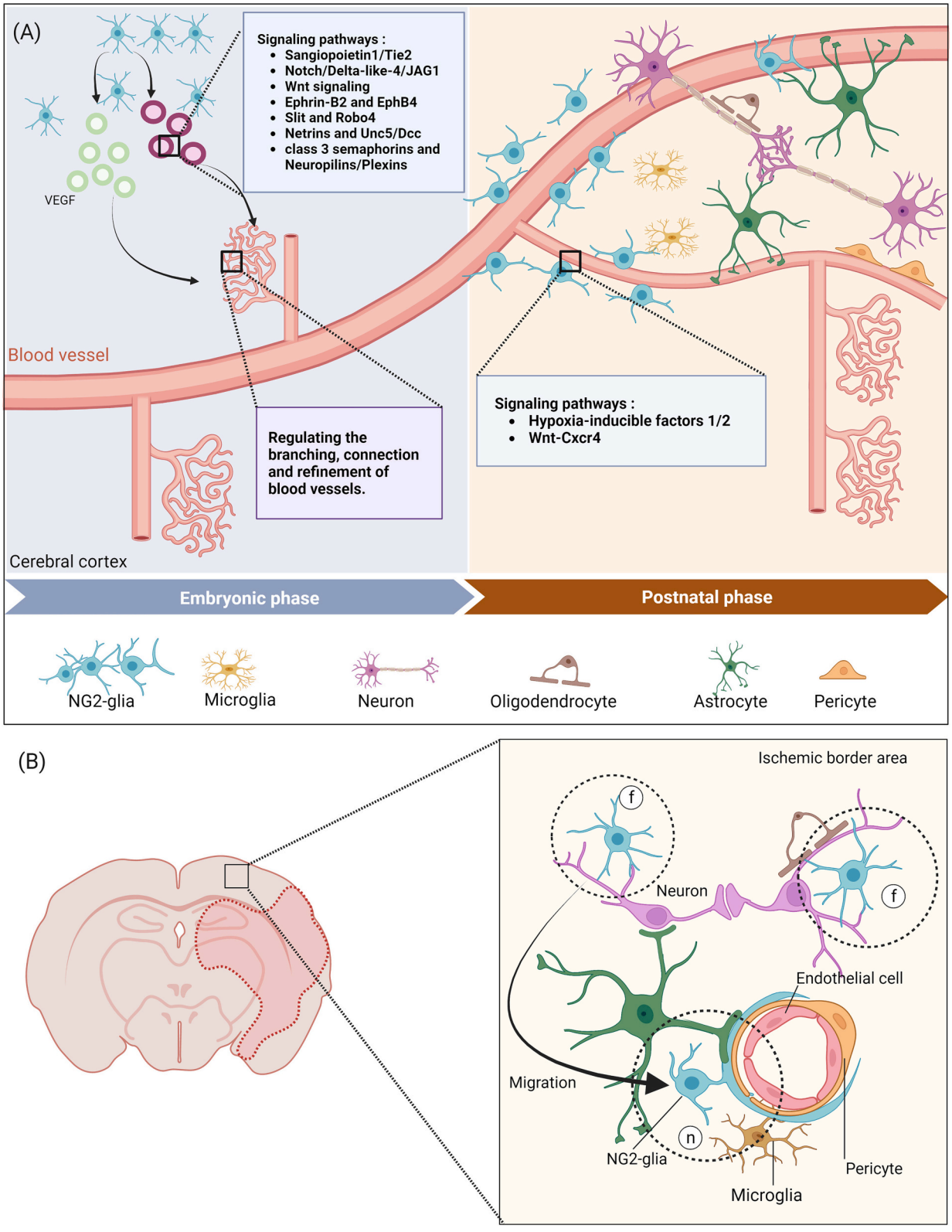

Fig. 1. NG2-glia regulate blood vessel during the embryonic and postnatal phase and NG2-glia subtype shifts to stimulate angiogenesis after ischemic stroke. A) During the embryonic phase, NG2-glia regulate branching, connection and refinement of the blood vessels by secreting VEGF or regulating the expression of signaling molecular (angiopoietin1/Tie2 and Notch/Delta-like-4/JAG1). During the postnatal phase, NG2-glia migrate and jump along vasculature. Wnt-Cxcr4 signaling plays an important role in regulating NG2-glia-endothelial interactions. In addition, NG2-glia regulate endothelial cell proliferation and increase white matter angiogenesis through HIF1/2 signaling. B) In the central nervous system, most of NG2-glia are parenchymal subtype (f) which interacts with other neural cells. Following ischemic stroke, the phenotype of NG2-glia in cortex shifts from the parenchymal subtype (f) to the perivascular subtype (n) which can stimulate angiogenesis. "n" means near, "f" means far.

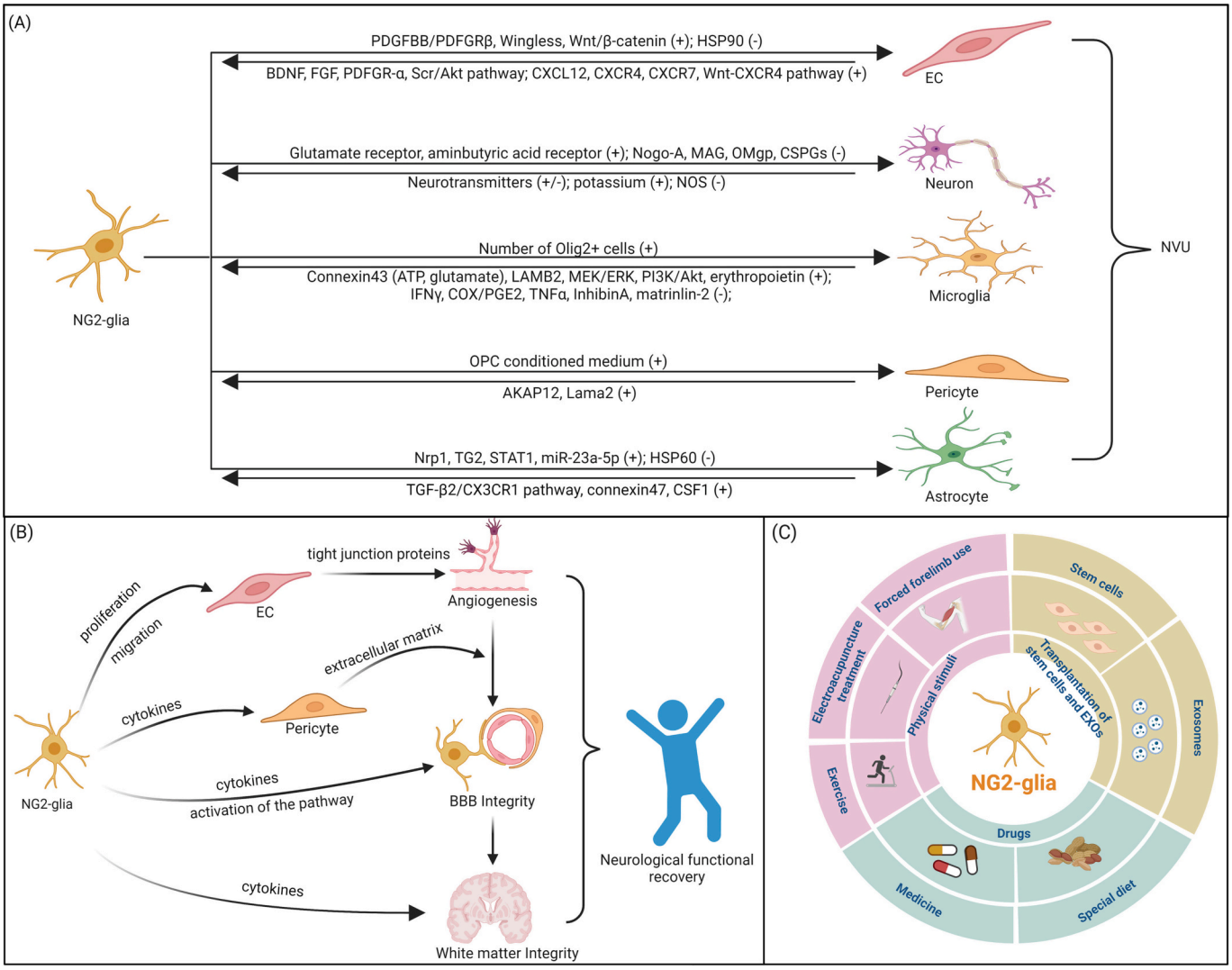

Fig. 2. NG2-glia interact with other cells in the NVU and the strategies to augment the function of NG2-glia. A) Under physiological and pathological conditions, NG2-glia interacts with ECs, neurons, astrocytes, pericytes, and microglia via multiple molecular or signaling pathways. "+" means a protective effect, and "-" means a detrimental effect. B) Function of NG2-glia in promoting neurological functional recovery by protecting BBB integrity, promoting angiogenesis and white matter integrity repair. C) Strategies to augment the function of NG2-glia by promoting NG2-glia proliferation, migration and maturation.

endothelial cell structure was disrupted in mice, but not when pericytes were absent (Tsai et al., 2016). The interaction between NG2-glia and endothelial cells was also found to be regulated by the Wnt-Cxcr4 signaling pathway, which regulates the migration and differentiation of NG2-glia (Tsai et al., 2016). It has also been shown that the crosstalk existing between ECs and NG2-glia/OLs is mediated by Wingless and Wnt/β-catenin pathway (Manukjan et al., 2020).

In mice with bilateral carotid artery stenosis, NG2-glia accumulated progressively on the damaged vessels and caveolin-1 (Cav-1) was lost in large amounts in endothelial cells, which secreted large amounts of heat shock protein 90 (HSP90) and aggravated vascular injury (Zhao et al., 2022). When Cav-1 was overexpressed or HSP90 was downregulated, the interaction between endothelial cells with NG2-glia was restored, promoting oligodendrocytes production and attenuating myelin injury (Zhao et al., 2022). In multiple sclerosis (MS), NG2-glia are unable to properly detach from blood vessels after migrating along them, allowing NG2-glia to accumulate around the blood vessels. Defective interaction of NG2-glia with blood vessels can interfere with the integrity of the TJPs between astrocytes and endothelial cells, leading to altered vascular permeability and CNS inflammation (Niu et al., 2019).

In rat WM infarction areas, brain microvascular endothelial cells (MVEC) and MVEC-derived exosome transplantation were found to promote the remyelination of demyelinated axons in the infarcted area (Kurachi et al., 2016). Studies have shown that fibronectin is abundant on the surface of extracellular vesicles (EVs) of MVECs. Fibronectin promotes the survival and proliferation of NG2-glia through the integrin signaling pathway (Osawa et al., 2017). However, some studies have reported that fibronectin on EVs of endothelial cells mediates their internalization into NG2-glia and that EVs promotes the survival and proliferation of NG2-glia independent of the integrin signaling pathway (Osawa et al., 2017; Zhou et al., 2021).

2.2. *Interaction of NG2-glia with neurons*

NG2-glia express ionotropic receptors for glutamate and amino-butyric acid and NG2-glia connect to synapses for rapid activation, quantification of responses, facilitation and inhibition, and presynaptic inhibition in the white and gray matter (Bergles et al., 2010; Ziskin et al., 2007). Electron microscopy results indicate that synapses of NG2-glia formed morphologically distinct connections at with hippocampal or callosal neuronal axons in mice, suggesting that NG2-glia can be targets for axonal projections (Bergles et al., 2010; Ziskin et al., 2007). NG2-glia

respond to neurotransmitters and potassium released during neuronal transmission while in contact with synapses. NG2-glia cells express a variety of neurotransmitter receptors and ion channels. Interestingly, glutamate signaling and potassium channels play prominent roles in NG2-glia differentiation (Sakry and Trotter, 2016). Electrophysiological recordings showed that stimulation of CA1 neurons by hippocampal NG2-glia produced excitatory postsynaptic currents (Bergles et al., 2000). In the corpus callosum, NG2-glia receive synaptic signal inputs from many functionally connected cortical and thalamic regions, suggesting that NG2-glia can integrate signals from whole-brain circuits (Mount et al., 2019). Besides excitatory synapses, inhibitory synapses are also formed between NG2-glia and gamma-aminobutyric acid (GABA) neurons, resulting in small inhibitory postsynaptic currents (Lin and Bergles, 2004). In addition, fast-spiking interneurons (FSIs) and non-fast-spiking interneurons (NFSIs) connect to NG2-glia, but FSIs connect more extensively to NG2-glia. Therefore, NG2-glia contribute to the coordination of cortical oscillations through their close relationship with FSIs, facilitating higher-order cognitive functions (Cardin et al., 2009; Sohal et al., 2009).

When the mouse corpus callosum receives ischemic insult, stress triggers the Ca^{2+}-dependent release of vesicular glutamate from axons, leading to damage to oligodendrocyte myelin. In contrast, pretreatment with NMDA receptor inhibitors effectively reduces WM damage, suggesting a new perspective on oligodendrocyte-neuron interactions under lesion conditions (Doyle et al., 2018). Moreover, following CNS damage, oligodendrocytes produce large amounts of endogenous molecules such as Nogo-A, myelin-associated glycolin (MAG), oligodendrocyte myelin glycoprotein (OMgp), and chondroitin sulfate proteoglycans (CSPGs), which inhibit axonal growth and associated neuroplasticity. These endogenous molecules bind to myelin-associated inhibitory proteins (MAIs) receptor complexes expressed by neuronal axons (e.g., Nogo receptor-1 (NgR1)) and inhibit axonal growth by suppressing Rho/ROCK signaling (Atwal et al., 2008; Kurihara et al., 2017).

Studies have shown that NG2-glia are all susceptible to lipopolysaccharide (LPS)-mediated activation and nitric oxide (NO)-induced cell damage. This types of neuronal NO synthase (nNOS)-mediated oligodendrocyte injury provides a mechanistic insight into demyelinating diseases, suggesting that NO-induced cell damage may underlie oligodendrocyte loss and demyelination under diseased conditions (Yao et al., 2010).

2.3. Interaction of NG2-glia with microglia cells

Microglia enter the CNS during embryonic development as phagocytes of the CNS (Barron, 1995; Xu et al., 2015). Microglial cells have been shown to interact with the NVU under physiological and pathological conditions (Zhao et al., 2018), and overactivated microglia exert important effects on the inflammatory response, neuron damage, and BBB breakdown after AIS (Chen et al., 2022).

NG2-glia have been reported to inhibit microglia activation and maintain immune homeostasis in the brain through the transforming growth factor-β2 (TGF-β2)-TGF-β type II receptor (TGFBR2)-CX3C chemokine receptor 1 (CX3CR1) signaling pathway (Zhang et al., 2019a). A recent report showed that NG2-glia are required for maintaining the homeostatic state of microglia (Liu and Aguzzi, 2020). In the absence of oligodendrocyte gap junctions (connexin 47 deletion), the experimental autoimmune encephalomyelitis (EAE) mouse phenotype was pronounced, with marked microglia activation and severe myelin and axon loss. It also showed severe disruption of the blood-spinal cord barrier (BSCB) and severe infiltration of immune cells (Papaneophytou et al., 2018). The effect of NG2-glia on microglia is also considered to play an important role in nerve recovery by maintaining microglia's homeostatic state (Liu and Aguzzi, 2020).

Activation of colony-stimulating factor receptor (CSF1R) can regulate microglia development, survival, and proliferation (Dai et al., 2002). Oligodendrocytes express colony-stimulating factor 1 (CSF1). When CSF1 is knocked down in OLs, it results in WM microglia deletion (Badimon et al., 2020; Irfan et al., 2022). Microglia-specific deletion of type 1 integral membrane protein neuropilin-1 (Nrp1) inhibits the proliferation of NG2-glia in WM and also affects the migration and myelin repair of NG2-glia after acute demyelination (Sherafat et al., 2021). In addition, it has been shown that NG2-glia do not express cell death markers when microglia contact with or phagocytose NG2-glia, suggesting a novel mechanism that favors the proper NG2-glia/axon ratio (Irfan et al., 2022). Microglia-derived transglutaminase-2 (TG2) sends signals to the adhesion G protein-coupled receptor (ADGRG1) on the surface of NG2-glia and can promote NG2-glia proliferation when the extracellular matrix is intact (Giera et al., 2018). In addition, heat shock protein 60 (HSP60) secreted by microglia can bind to toll-like receptors on the surface of NG2-glia and promote NG2-glia apoptosis by activating the TLR4/NF-κB signaling pathway (Li et al., 2017), implying that HSP60 could be a potential target for microglia activation-induced myelin-related neurodegenerative diseases.

LPS-activated microglia can severely impair the development of OLs (Pang et al., 2010). The use of conditioned medium with LPS-activated microglia promotes the survival of mature OLs (Taylor et al., 2010). In the aged brain, activated microglia inhibit the differentiation of NG2-glia by releasing inflammatory factors (Ramarao et al., 2022), however, signal transducer and activator of transcription 1 (STAT1) can inhibit this effect (Luan et al., 2021).

In the early stages of demyelination and OLs loss, microglia phagocytose myelin debris and apoptotic cells, preparing for subsequent resheathing of demyelinated pavements. During the stage of myelin remodeling, microglia transform from M1 to M2 type, and microglia-conditioned medium promotes the generation of OLs in vitro (Miron et al., 2013). Activated microglia can induce the generation of NG2-glia in the subventricular zone (SVZ) of the corpus callosum after demyelination (Naruse et al., 2018). In addition, M2 microglia-derived EVs promote white matter repair after ischemic stroke by directly targeting Olig3 via miR-23a-5p (Li et al., 2022b). Meanwhile, intracerebral injection of microglia-derived EVs can limit senescence in the post-stroke phase, enhance the maturation of peri-lesion NG2-glia, and improve neurological function (Raffaele et al., 2021). Therefore, the mechanism of M1/M2 phenotype transition in microglia can be therapeutically important for WM remyelination and repair.

2.4. Interaction of NG2-glia with pericytes

Pericytes are located at the abluminal surface of endothelial cells of capillaries, arterioles, and venules (Fisher, 2009). They provide support to endothelial cells and other cells, which in turn play a critical role in the development and maintenance of BBB as well as in angiogenesis (Ozerdem and Stallcup, 2004). Pericytes are embedded within the basement membrane and play multiple roles in the perivascular niche in the brain. Recently, NG2-glia have also been reported to associate with cerebral endothelium. Pericytes and NG2-glia are in localized contact with each other in mouse WM on postnatal days. Electron microscopic analysis confirmed that pericytes are attached to NG2-glia via the basement membrane in the perivascular region. The proximity between these two cell types was also observed in postmortem human brains. When NG2-glia cultures were treated with pericyte-conditioned media, the NG2-glia number increased (De La Fuente et al., 2017). Similarly, the pericyte number increased when pericytes were maintained in NG2-glia-conditioned media (Maki et al., 2015).

Meanwhile, pericytes are important for maintaining WM function, and when they are absent, myelin thinning occurs, WM bundles become disorganized and vacuolated, and oligodendrocytes undergo apoptosis (Montagne et al., 2018). For example, pericytes are one of the major A kinase anchor protein 12 (AKAP12)-expressing cells. Conditioned culture media from pericytes promoted in-vitro NG2-glia maturation. However, conditioned media from AKAP12-deficient pericytes did not support the NG2-glia maturation, suggesting that AKAP12 signaling in

pericytes may be required for NG2-glia differentiation into oligodendrocyte as a renewal mechanism to maintain the WM homeostasis in the adult brain (Maki et al., 2018).

In addition, during remyelination, mature oligodendrocytes were found near pericytes. NG2-glia differentiation was delayed if pericytes numbers were reduced, although remyelination proceeded to completion. Pericytes-conditioned medium accelerated and enhanced NG2-glia differentiation in vitro and increased the rate of remyelination in an ex vivo cerebellar slice model of demyelination. Lama2 is a pericytes-derived factor that promotes NG2-glia differentiation. Thus, the functional role of pericytes is not restricted to vascular homeostasis but includes the modulation of adult CNS progenitor cells involved in regeneration (De La Fuente et al., 2017). NG2-glia differentiation and remyelination were significantly attenuated in pericyte-deficient mice (Shibahara et al., 2020). It is of note that stroke-induced cerebral ischemia-reperfusion leads to pathological changes in the pericytes. The close anatomical and functional interactions between pericytes and other NVU components play a key role in the progression of stroke pathology (Cai et al., 2017b).

2.5. Interaction of NG2-glia with astrocytes

Astrocytes play a critical role in stroke-induced memory impairment through regulating the integrity of NVU (Zhang et al., 2019b). In terms of spatial location, astrocytes are connected to oligodendrocytes by gap junctions. Astrocytes supply small molecules such as adenosine triphosphate (ATP) and glutamate to oligodendrocytes through connexin 43 channels (Li et al., 2015; Rouach et al., 2002). Dicer deletion in astrocytes inhibits oligodendroglial differentiation and myelination (Liu et al., 2021). When NG2-glia are reduced, it affects the development of early astrocytes (Wang et al., 2021). Similarly, in the demyelination model, the myelination of NG2-glia is hampered by the lack of astrocytes (Lohrberg et al., 2020). This regulation may be achieved by altering the number of $Olig2^{+}$ cells (Madadi et al., 2019). In addition, astrocyte IFNγ overexpression inhibits the differentiation of NG2-glia (Kirby et al., 2019).

NG2-glia are known to migrate along the perivascular area. The astrocyte endfeet will become a terminator in the migration of NG2-glia (Su et al., 2022). Astrocytes can also protect NG2-glia through a variety of substances or molecular pathways, for example, laminin β2 in astrocyte-derived exosomes (Cheng et al., 2022), MEK/ERK and PI3K/Akt signaling pathways (Arai and Lo, 2010), erythropoietin (Kato et al., 2011) and tumor necrosis factor receptor-2 (TNFR-2) (Patel et al., 2012). On the other hand, activated astrocytes can inhibit the survival and differentiation of NG2-glia through cyclooxygenase (COX)/prostaglandin E2 (PGE2) and TNF-α production (Shiow et al., 2017; Su et al., 2011).

Under ischemic conditions, astrocytes release glutamate which acts on NMDA receptors in the NG2-glia and inhibits the differentiation of NG2-glia (Baltan et al., 2008). In stroke-induced WM injury, astrocyte secretion of Inhibin A and downregulation of Matrilin-2 in NG2-glia inhibit NG2-glia differentiation, tissue repair, and neurological recovery after stroke (Sozmen et al., 2019).

2.6. Regulation of BBB permeability by NG2-glia

During the aging process or in pathological states, at the capillary level, changes occur in the brain endothelium, which forms the BBB, including reduced cytoplasm, reduced mitochondria numbers, loss of TJPs, and thickening and collagenization of the basement membrane (BM) (Kalaria and Hase, 2019). In addition, a damaged BBB allows a large number of blood-derived proteins (such as fibrinogen) to enter the brain parenchyma (Cortes-Canteli et al., 2015). The massive accumulation of these proteins induces adaptive immune responses in the brain, inhibiting NG2-glia and suppressing demyelination (Ryu et al., 2015).

NG2-glia are required for BBB development (Seo et al., 2014), and when NG2-glia were ablated in embryonic mice, vascular network density and branching were reduced (Minocha et al., 2015). In addition, NG2-glia promote BBB integrity through TGF-β signaling, and inhibition of TGF-β secretion by NG2-glia leads to massive cerebral vascular rupture and hemorrhage. NG2-glia can promote the expression of tight junction proteins (TJPs) occludin and claudins through the activation of the TGF-β signaling pathway, which in turn promotes BBB integrity (Seo et al., 2014). When NG2-glia were ectopicly introduced, the BBB was disrupted (Morris et al., 2017). In MS, Wnt signaling dysfunction leads to blocked migration of NG2-glia along the vasculature, while NG2-glia accumulate around the vasculature, physically expelling astrocyte endfeet from the vascular surface, disrupting TJPs between endothelial cells, and increasing BBB permeability (Niu et al., 2019). Moreover, the proliferation of NG2-glia further exacerbates BBB injury in a diabetic model (Li et al., 2022a) and NG2-glia secrete matrix metalloproteinase 9 (MMP-9) to disrupt BBB integrity in the early stages of WM injury (Hu et al., 2022).

3. Roles of NG2-glia in regulating neurovascular unit via angiogenesis after ischemic stroke (Fig. 2B)

In addition to affecting BBB function, oligodendrocyte-spectrum cells may also be involved in angiogenic responses. Wnt7a and Wnt7b secreted by NG2-glia under hypoxic conditions promote angiogenesis in vivo and directly stimulate endothelial cell proliferation in vitro (Yuen et al., 2014). After birth, NG2-glia can promote WM angiogenesis via hypoxia-induced factor (HIF) and Wnt signaling (Chavali et al., 2020; Yuen et al., 2014).

Spontaneous repair of brain tissue is known to occur after AIS, but this repair is very limited (Li et al., 2020). After AIS, angiogenesis, and revascularization occur around the injured brain area (Yang and Torbey, 2020). Angiogenesis after stroke not only provides substances and energy for nerve regeneration, secretes neurotrophic factors and chemokines to support the survival of neonatal neurons, but also serves as a scaffold for the migration of neural precursor cells to the brain injury area, thereby promoting the structural repair and functional remodeling of the brain (Liu et al., 2014). The amount of neovascularization around the infarct area determines the prognosis and survival rate of patients, and angiogenesis after AIS is crucial for the recovery of neurological function (Liu et al., 2014). Notably, the restoration of neurological function after stroke is closely related to the reconstruction of WM integrity (Li et al., 2020), and the regeneration of OLs and maturity of NG2-glia in each brain region depend on the degree of local angiogenesis in the brain region (Jiang et al., 2011), and microvascular dysfunction and loss of WM integrity predict a poor prognosis in patients with AIS (Rost et al., 2018).

In the central nervous system, most of the NG2-glia are parenchymal subtype which interacts with other neural cells. Following ischemic stroke, the phenotype of NG2-glia in the cerebral cortex shifts from the parenchymal subtype to the perivascular subtype which can stimulate angiogenesis by secreting angiogenic factors (see Fig. 1B) (Kishida et al., 2019). Meanwhile, hypoxia-treated NG2-glia promote the expression of more pro-angiogenic factors (e.g., angiopoietin-1) and increase endothelial cell viability and tube formation (Kishida et al., 2019). In an in vivo study, treatment with conditioned medium from hypoxia-pretreated NG2-glia for 5 consecutive days starting two days after MCAO promoted post-stroke angiogenesis, reduced infarct volume, and improved functional impairment (Kishida et al., 2019). In addition, transplantation of NG2-glia after MCAO promotes angiogenesis and revascularization via the Wnt/β-catenin pathway (Wang et al., 2022b).

4. Role of NG2-glia in regulating neurovascular unit via white matter repair and BBB after ischemic stroke (Fig. 2B)

Arai and Lo proposed the concept of “wiring and plumbing “ to remodel the white matter and vascular, which provides the rationale for

strategies to promote post-stroke neurological recovery in patients (Arai and Lo, 2021).

In recent years, studies have shown that WM injury accounts for about half of the infarct volume after AIS, which is an important pathological basis of neurological injury, and promoting WM integrity repair is of great significance to improve neurological function (Li et al., 2020). There is increasing evidence that the number of mature OLs that form myelin after WM injury is insufficient, possibly due to the acute mortality of immature OLs and impaired differentiation of NG2-glia (Li et al., 2020). In addition, animal and human studies have shown that adult brain tissue has a certain ability to repair itself after stroke, but it is very limited, one of the important reasons is the disordered NG2-glia differentiation into mature OLs (Goldman and Osorio, 2014). AIS-induced destruction of WM integrity is caused by OLs injury, so to enhance the differentiation of NG2-glia into mature OLs can be a strategy to promote WM integrity repair (Li et al., 2020).

OLs are myelocytic cells of the CNS and are responsible for ensuring axon conduction and neuronal support. Their numbers are tightly regulated and depend on the differentiation, survival, and proliferation of NG2-glia (Li et al., 2020). NG2-glia play an important role in the recovery of nerve function after stroke (Li et al., 2020). Studies have reported that after focal ischemia in rats, the number of NG2-glia in the core area of cerebral infarction is significantly reduced, while the number of NG2-glia in the area around the infarct is significantly increased (Li et al., 2020).

The interaction between oligodendrocytes and neurons during white matter repair also plays a role in neurological plasticity. However, such studies have been limited to human magnetic resonance imaging (MRI) studies. Studies have shown that a link between neuronal function and WM plasticity exists. The extensive instrumental practice was significantly associated with fiber tract organization (Bengtsson et al., 2005). In addition, myelin formation and plasticity in WM are controlled to some extent by the activation of neurons and their axons (Hamanaka et al., 2018). After WM injury, MMP-9 secreted by oligodendrocyte-spectrum cells contributes to vascular remodeling (Pham et al., 2012). Notably, under certain conditions, NG2-glia-derived MMP-9 has a damaging effect on blood vessels (Miyamoto et al., 2014).

Microglia/macrophages improve WM integrity and restore long-term neurological function after stroke via the interleukin 33 (IL-33)/ suppression of tumorigenicity 2 receptor (ST2)/signal transducer and activator of transcription 6 (STAT6) signaling pathway (Xie et al., 2021). Microglia-derived exosome vesicles improve recovery after AIS by inhibiting immune cell senescence and OLs production (Raffaele et al., 2021). Meanwhile, CD147 promotes the number of proliferating NG2-glia and mature oligodendrocytes after AIS through the CXCL12/CXCR4 signaling pathway. These may prevent neuronal and oligodendrocyte death in the acute phase of AIS, protect WM integrity in the late phase of AIS, reduce brain atrophy and tissue loss, and improve sensorimotor and cognitive function for at least 28 days after stroke (Liu et al., 2019). Transforming growth factor-α (TGF-α) protects oligodendrocyte spectrum cells and maintains WM integrity after AIS (Dai et al., 2020). In addition, G-protein coupled receptor 17 (GPR17), a receptor transiently expressed on early NG2-glia, has emerged as a therapeutic target for neurological function repair after AIS by promoting the maturation of NG2-glia (Bonfanti et al., 2017).

After AIS, neurological functions are impaired and excitability or coherent conduction of neurons as well as myelinated axons that innervate stimulated neurons is disrupted. Neurotransmitter release is accompanied by elevated Ca^{2+} levels in the WM, further triggering axonal damage. At this time, myelin-producing oligodendrocytes are located near neuronal axons and support axonal function in both direct and indirect ways. In particular, oligodendrocytes support axonal function through glucose delivery in a hypoglycemic state (Huang and Rasband, 2018). In turn, neurons secrete neuromodulators that promote the formation of myelin from oligodendrocytes, which play a neuroprotective role during CNS injury (Dammann et al., 2008; Li et al., 2007). Transplantation of NG2-glia promotes myelination, improves behavioral recovery, and reduces brain atrophy volume in animal models of MCAO (Zhang et al., 2013). Transplantation of NG2-glia promotes recovery after AIS by promoting endogenous oligodendrocyte generation, neurite growth, and synaptogenesis (Li et al., 2021). The latter two are mediated by the Netrin-1 and its receptor deleted in colorectal cancer (DCC) (Meyer et al., 2018).

Disruption of BBB integrity is a key factor in pathological damage of the WM (Yang et al., 2018). The degree of WM damage in patients with AIS is closely related to increased BBB permeability and damage to WM integrity. Microvascular dysfunction and loss of WM integrity after AIS predict a poor prognosis (Jiang et al., 2017; Rost et al., 2018). Some studies have shown that *ABCA1* deletion causes BBB leakage and WM and axonal damage, leading to severe neurological deficits after AIS (Cui et al., 2015). In contrast, omega-3 polyunsaturated fatty acids and 2-methoxyaminomethyl ketone improved BBB integrity and neurological function in aged mice (Cai et al., 2017a; Chern et al., 2014).

After the cerebrovascular injury, delayed cerebral edema and hemorrhagic transformation (HT) are the most important complications of stroke, leading to deterioration and death (Yang and Torbey, 2020), and destruction of the integrity of the BBB is the main pathological basis for the occurrence of vascular cerebral edema and HT (Hu et al., 2022), and is also one of the key factors leading to pathological damage of WM (Li et al., 2020). After transient MCAO, inhibition of NG2-glia improves BBB leakage via Wnt/β-catenin via increased TJPs expression, thereby reducing brain edema (Wang et al., 2020). Meanwhile, the expression level of MMP-9 in NG2-glia plays an important role in regulating BBB integrity (Zhao et al., 2006). Stroke causes differences in the distribution of different histone deacetylases (HDACs) in WM NG2-glia and OLs. It is implied that HDACs play an important role in the regulation of the proliferation and differentiation of NG2-glia during the repair process after stroke (Kassis et al., 2014).

5. Strategy to regulate NG2-glia (Fig. 2C)

As mentioned earlier, NG2-glia, as precursor cells for OLs, hold promise as effective new targets for post-stroke treatment (Song et al., 2017). Promoting the differentiation of NG2-glia and the production of OLs is a potential strategy to improve WM integrity and neurological function after AIS (Zhang et al., 2013), and the effect of drugs on NG2-glia has been summarized in Table 1.

After stroke, NG2-glia are activated to proliferate and differentiate into mature OLs. For example, both necrostatin-1 and melatonin can protect NG2-glia and promote neurological recovery after AIS (Chen et al., 2018; Chern et al., 2012), and BMP type I receptor blockers promote the recovery of myelinated oligodendrocytes in MS (Petersen et al., 2021). In addition, FTY720, a sphingosine-1-phosphate (S1P) receptor agonist, can modulate the survival of NG2-glia in MS, implying that the S1P signaling pathway plays a role in the regulation of NG2-glia (Miron et al., 2008). Moreover, minocycline promotes WM repair by reducing BBB damage, promoting TJPs expression and angiogenesis, and ultimately protecting OLs (Yang et al., 2018).

Natural fish oil interacts with the BBB to promote the differentiation and maturation of NG2-glia to OLs in vitro (Piatek et al., 2022). In addition, dietary supplementation with omega-3 polyunsaturated fatty acids may promote OLs formation (Zhang et al., 2015). The peroxisome proliferator-activated receptor gamma (PPAR-γ) agonist rosiglitazone, a drug used for the treatment of diabetes, promotes the production of OLs (Han et al., 2015). Meanwhile, oral administration of the racemic DL-3-n-butylphthalide (dl-NBP) has been reported to promote the differentiation and maturation of NG2-glia in the WM (Cheng et al., 2019), and the cannabinoid receptor agonist WIN55,212-2 promoted the proliferation of NG2-glia in the peri-infarction zone after permanent focal ischemic stroke in rats, partially mediated by cannabinoid receptor type 1 (CB1) (Sun et al., 2013). Additionally, *N*-acetylglucosamine drives myelination by triggering NG2-glia differentiation (Sy et al., 2020). D-

Table 1
Mechanism underlying the effect of drugs on NG2-glia.

Drug	Mechanism	Proliferation	Migration	Differentiation
Necrostatin-1	Inhibited the activation of RIPK1, RIPK3, MLKL, and P-MLKL	√		
Melatonin	Reduces free radical production and cellular infiltration by interacting with MT2 receptors	√	√	√
BMP type I receptor blocker	Blocking fibrinogen deposition			√
FTY720	S1P receptor	√		√
Minocycline	Promotes VEGF-A expression and tight junction protein formation	√		
Natural fish oil	Increased expression of IGF-1, CNTF and BDNF and inhibition of FGF-2 synthesis			√
Omega-3	Promotes angiogenesis, neurogenesis and oligodendrocyte production	√		√
Rosiglitazone	PPAR-γ agonist; promotes microglia M2 polarization	√		√
Dl-NBP	Promotes BDNF expression and inhibits NogoA expression			√
WIN55,212-2	Activation cannabinoid receptor type 1	√		
N-acetylglucosamine	Inhibition of endocytosis of PDGFR-α cells			√
D-4F	Increased the expression of MBP and IGF-1	√		
VEGF	VEGF-A induces migration, VEGF-C promotes the proliferation	√	√	
GGF2	Inhibiting ID2	√		√

4F, an apolipoprotein A-I mimetic peptide, increased the number of NG2-glia in the striatum on the ischemic side and also increased the expression of myelin basic protein (MBP) and insulin-like growth factor-1 (IGF-1), thus promoting WM integrity and neurological function recovery after stroke in mice (Cui et al., 2016).

Vascular endothelial-derived growth factor (VEGF), as a pro-angiogenic factor, has different effects on different cells in the CNS. For NG2-glia, VEGF-A induces migration, while VEGF-C promotes the proliferation of NG2-glia after optic nerve and medulla oblongata demyelination (Hiratsuka et al., 2019). Administration of exogenous glial growth factor 2 (GGF2) after AIS promotes the proliferation, differentiation, and neurological recovery of NG2-glia by inhibiting the differentiation inhibitory factor (ID2) (Li et al., 2020).

Studies have shown that the activity and number of NG2-glia can be influenced by a variety of physical stimuli. For example. Studies have shown that electroacupuncture treatment alleviates AIS-induced motor deficits while increasing the number of NG2-glia by activating BDNF signaling pathways (Lee et al., 2022). In addition, forced forelimb use improves functional recovery in experimental ischemic stroke rats by altering Nogo-A expression and promoting the differentiation of NG2-glia (Zhao et al., 2020). Moreover, treadmill exercise increases the number of NG2-glia in WM in a prolonged brain hypoperfusion mouse model (Ohtomo et al., 2020).

The role and mechanisms of stem cells and their exosome transplantation in promoting the proliferation and differentiation of NG2-glia after AIS have received much attention in recent years. As an important component of mesenchymal stem cells (MSCs), dental pulp-derived stem cells (DPSCs) originate in the neural crest and have significant neuroectoderm features and the potential to repair damaged tissues (Liu et al., 2015) and express NG2 (Raza et al., 2018). Due to the ease of isolation, low ethical controversy, and low immunogenicity, DPSCs transplantation has neuroprotective effects and promotes neurological function recovery after AIS (Wang et al., 2022a). For example, the administration of DPSCs that overexpress hepatocyte growth factor (HGF) during the acute phase of AIS can regulate inflammation and BBB permeability and improve brain injury (Sowa et al., 2018). In addition, intracerebral transplantation of DPSCs significantly improves the sensorimotor function of the forelimbs in the fourth week after focal AIS in mice (Leong et al., 2012). It is of note that DPSCs are readily available from discarded third molars, do not require surgical intervention, and are not ethically bound. Compared with other sources, MSCs offer greater proliferative and immunosuppressive properties, higher expression of neuroprotective growth factors, and strong adaptability to hypoxia/ischemic conditions, making them an ideal source for MSCs to promote neurological function recovery after AIS (Pagella et al., 2020). Notably, evidence from preclinical studies suggests that the therapeutic effects of DPSCs are likely mediated by paracrine mechanisms (Mattei et al., 2021) and, less so by nerve cell replacement mechanisms (Leong et al., 2012). Exosomes (EXO) are small in size to cross the BBB, easy to store and transport, and do not form thrombus, with minimal proliferative capacity, and a low tumorigenicity risk (Maumus et al., 2020), making the use of DPSCs-derived EXO a new therapeutic strategy to promote neurological function recovery after AIS (Fig. 2).

6. Concluding remarks

With novel tools and investigative methodologies becoming increasingly available, more and more novel roles of NG2-glia are expected to be revealed in CNS development and diseases- (Galichet et al., 2021). Current accumulated evidence showing a critical role for NG2-glia in the integrity of the NVU, supports the notion that NG2-glia is a key therapeutic target to reduce AIS-induced injury and promote repair. Therefore, we propose to use DPSC-derived exposomes as a promising strategy to reduce AIS-induced BBB damage through stimulating endogenous NG2-glia proliferation and differentiation, and to promote outcome and functional recovery following AIS through stimulating oligogenesis and angiogenesis.

Authors' contribution

X Hu, P Geng, X Zhao, Q Wang, and C Liu collect the article and draft the manuscript. X Hu makes the figure and tables. C Guo edits the manuscript. W Dong, X Jin edit and finalize the manuscript.

Declaration of Competing Interest

The authors declare that they have no conflicts of interest.

Data availability

No data was used for the research described in the article.

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