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Glial cell line-derived neurotrophic factor in brain repair after focal ischemic stroke

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Cerebral focal ischemic stroke (FIS) is a leading brain disorder associated with human debilitation and death. It is induced by the formation of a thrombus in the arteries that supply blood to the central nervous system. FIS patients may suddenly experience paralysis, impairment of speech and loss of vision. Most patients develop permanent disabilities. In the past decades, enormous efforts have been taken to develop FIS treatment strategies, but unfortunately, there is still a lack of effective ones for this disease. Currently, tissue plasminogen activator is the only Food and Drug Administration approved drug for FIS treatment, however, this method has limited application since it is only effective within 3–5 hours after the onset of FIS. Because of this, the recovery from FIS largely depends on self-brain repair and rehabilitation (Campbell et al., 2019).

Astrocytes are the predominant glia cell type in the central nervous system. These cells fulfill many significant functions in central nervous system. After FIS, astrocytes, especially in the peri-infarct region, are activated and experience spatiotemporal changes in morphology and proliferation capacity (Li et al., 2014; Barreto et al., 2011). This process is known as reactive astrogliosis and the activated astrocytes are called reactive astrocytes (RAs). In the subacute phase, RAs become hypertrophic with large processes that can be characterized by significant upregulation of glial fibrillary acidic protein. Proliferating RAs may impact tissue preservation, repair, remodeling, and functional outcome. The molecular phenotype exhibiting rapid and transient induction of gene expression in RAs after ischemia supports the notion that RAs may offer beneficial or protective effects to neurons for brain repair (Zamanian et al., 2012). Upon prolonged time (in the chronic phase) following FIS, proliferation of RAs starts to decline and glial scars are formed and stabilized. Reactive astrogliosis and glial scar formation eventually cause substantial tissue remodeling and structural changes including tissue shrinking and scar maturation in the peri-infarct region.

Recent studies suggest that one important mechanism for brain protection and repair by RAs might be through the releases of growth factors (Poyhonen et al., 2019). These growth factors include: brain-derived neurotrophic factor, glial cell line-derived neurotrophic factor (GDNF), nerve growth factor, cerebral dopamine neurotrophic factor and ciliary neurotrophic factor. Among them, GDNF has been studied extensively in Parkinson's disease (PD). GDNF is a member of the transforming growth factor-β super family (Airaksinen and Saarma, 2002). It was originally isolated from the supernatant of a rat glioma cell-line and was thought to act as a trophic factor for midbrain dopaminergic neurons. GDNF is expressed in neurons, but little in astrocytes under normal condition. On the membrane of neurons, GDNF can bind with GFRα protein and form GDNF-GFRα complex (Airaksinen and Saarma, 2002) (**Figure 1A**). The complex combines with a RET receptor and leads to the phosphorylation of tyrosine residues in RET protein (Airaksinen and Saarma, 2002). The activated RET could serve as a binding site for various intracellular proteins within the neuron. In addition, activated RET can also activate phosphoinositide 3-kinases, mitogen-activated protein kinase and their downstream pathways. These pathways were thought important for neuronal survival, differentiation and neurogenesis. However, new evidence suggests RET is not the only GDNF receptor in neurons. Studies have revealed the NCAM receptor could bind with the GDNF-GFRα complex and lead to the activation of tyrosine-protein kinase Fyn (Airaksinen et al 2002). The activated Fyn protein can activate MET protein, initiating many downstream pathways for neuronal survival.

GDNF was first used to treat PD. Later, more evidence showed that GDNF not only protected dopaminergic neurons but many other neuronal subpopulations. Recent studies have pointed out that GDNF has many other functions, such as promoting neural progenitor differentiation and synapse formation (Poyhonen et al., 2019). Consequently, the investigation of GDNF is not limited in the field of PD but extends to many other neurological disorders, including FIS. In fact, treatments using recombinant GDNF and molecular genetic methods have demonstrated that GDNF could offer brain protection and promote neuronal survival after FIS (Wang et al., 1997; Duarte et al., 2012). In ischemic stroke, substantial interest has focused on the potential protective effects of GDNF owning to its upregulated production as well as expression of its receptors RET and GFRα-1. Based on treatment using recombinant GDNF and molecular genetic methods, multiple mechanisms for GDNF to protect neurons and brain after stroke have been reported. Those mechanisms include the reductions of excitotoxicity, apoptosis, synaptic loss and oxidative stress (**Figure 1B**) (Duarte et al., 2012; Zhang et al., 2021). Recombinant GDNF could activate p-AKT pathway and upregulate anti-apoptotic factors to inhibit apoptosis (Hoxhaj G et al., 2020). GDNF was also shown to protect against neuronal loss through attenuating the NMDA-induced cell death and calcium influx (Nicole et al., 2001). GDNF could reduce oxidative stress and protect neurons via transcriptional regulation of glutathione synthesis (Iwata-Ichikawa et al., 1999).

Recent studies indicate that GDNF is highly upregulated in RAs after FIS (Arvidsson et al., 2001; Zhang et al., 2020), raising the possibility that RAs-released GDNF might play a role in brain repair. To study the effects of RA-derived GDNF on brain protection and recovery, astrocyte-specific, inducible and conditional GDNF knockout (cKO) mice, i.e., GLAST-GDNF$^{-/-}$ cKO mice, by crossing floxed GDNF (GDNF$^{f/f}$) mice with GLAST-CreERT2 driver line were generated (Zhang et al., 2020). This study provided several novel findings using photothrombosis-induced FIS model: (1) The GLAST-GDNF$^{-/-}$ cKO exhibited more neuronal death and larger brain infarction than the wild type mice. (2) The GLAST-GDNF$^{-/-}$ cKO mice exhibited a more significant reduction in proliferation rate of RAs in subacute phase based on Brdu^{+} and Ki67^{+} and glial fibrillary acidic protein staining. These results indicate that the RAs-derived GDNF can affect the dynamics of reactive astrogliosis through a cell autonomous manner, and that reduced reactive astrogliosis can lead to worsening motor function recovery in the cKO mice. (3) GLAST-GDNF$^{-/-}$ cKO mice showed reduced adult neurogenesis in the hippocampus in normal brain. (4) Deletion of GDNF in astrocytes decreased glucose-6-phosphate dehydrogenase (G6PD) and increased reactive oxygen species production. Oxidative stress is an important contribution to neuronal death after FIS. Reduced glutathione serves as the primary antioxidant to remove reactive oxygen species to combat against oxidative stress-induced injury. Glutathione production is controlled by NADPH which is mainly generated by G6PD, a key enzyme in the pentose phosphate pathway. G6PD converts glucose-6-phosphate (G6P) to 6-p-gluconolactone and generates NADPH from NADP^{+}. Although the mechanism by which G6PD in RAs is regulated by GDNF is warranted to further study, the results indicate that astrocytic GDNF plays an important role in promoting anti-oxidative mechanisms. The activated GDNF receptors can trigger many intracellular signaling pathways. Among

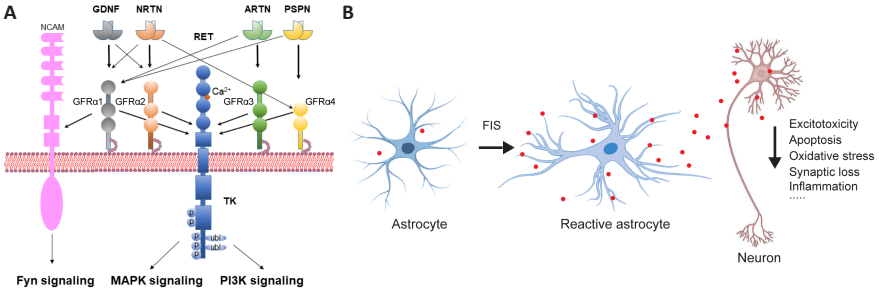

Figure 1 | GDNF family ligands and their receptors and potential mechanisms of RAs-derived GDNF on neuronal protection after stroke.
(A) GDNF family ligands and their receptors. The four members of GDNF family ligands include GDNF, neurturin, artemin, and persephin. They are homodimers and bind with high affinity to one of the four members of the GDNF receptor α family 1–4. These receptor-ligand complexes can interact with and activate the canonical GDNF receptor 'rearranged during transfection' (RET), which is a receptor tyrosine kinase (TK). GDNF can also activate alternative GDNF receptors, such as the neuronal cell adhesion molecule (NCAM). The intracellular domain of RET can be phosphorylated and ubiquitinylated and activate downstream pathways including MAPK, PI3K and Fyn signaling. (B) Potential mechanisms of RAs-derived GDNF on neuronal recovery after stroke. After FIS, quiescent astrocytes are activated and become RAs. RAs upregulate the expression and release of GDNF. GDNF can inhibit excitotoxicity, apoptosis, oxidative stress and promote neurogenesis and synaptogenesis, thereby facilitating neuronal survival and improving long-term FIS outcomes. The red dots represent GDNF released from reactive astrocytes. ARTN: Artemin; FIS: focal ischemic stroke; GDNF: glial cell line-derived neurotrophic factor; GFR: glial cell line-derived neurotrophic factor receptor; MAPK: Mitogen-activated protein kinase; NCAM: neuronal cell adhesion molecule; NRTN: Neurturin; PI3K: phosphoinositide 3-kinase; PSPN: Persephin; RET: rearranged during transfection.

them, the AKT/phosphoinositide 3-kinases pathway has attracted the most attention since it can regulate glycolysis and pentose phosphate pathway (Hoxhaj and Manning, 2020). (5) GLAST-GDNF$^{-/-}$ cKO mice exhibited higher motor function deficits than the wild type mice after photothrombosis, suggesting that RA-derived GDNF may play an important role in intrinsic brain repair and recovery after FIS. Taken together, these results further demonstrate that astrocytes- and/or RAs-derived GDNF is beneficial to protect against neuronal death and brain damage after FIS, stimulate reactive astrogliosis, promote anti-oxidative stress mechanism, and improve long-term stroke outcomes, and the endogenous GDNF in astrocytes/RAs is an important factor in brain repair processes.

RAs-derived GDNF may promote neuronal survival through inhibition of inflammation due to microglia activation. It was reported that the normal astrocyte conditioned media, not GDNF silenced astrocyte conditioned media was capable of modulating microglial activation, including reducing phagocytic activity and production of reactive oxygen species (Rocha et al., 2012). These results indicate that astrocyte derived GDNF can play an important role in the control of microglial activation in stroke, and that GDNF can protect against neurodegeneration through inhibition of neuroinflammation.

Evidence from clinical studies indicates that the brain is "primed" to recovery particularly during the subacute phase of ischemic stroke (Ronning and Guldvog, 1998; Poulin et al., 2016). Therefore, understanding the underlying mechanism will help to identify potential strategies to facilitate brain recovery and improve stroke outcomes. In this respect, GDNF released from RAs may offer multiple functions to mediate recovery of damaged neurons after FIS. With recent advance in molecular genetic tools, such as availability of astrocyte specific transgenic mouse lines and viral vectors, it is possible to look at the exclusive roles of astrocytes/RAs in neuronal circuitry during neuronal degeneration/death. Further studies on the downstream signaling pathways activated by GDNF in RAs may help to better understand the intrinsic brain repair mechanisms through the non-cell and cell autonomous effects, and in the context of neuron-glia interactions. In summary, targeting GDNF and related signaling pathways in RAs can be a potentially important strategy in stroke restorative therapy.

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