Tech Science Press

Systems Neuroprotective Mechanisms in Ischemic Stroke

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Abstract: Ischemic stroke, although causing brain infarction and neurological deficits, can activate innate neuroprotective mechanisms, including regional mechanisms within the ischemic brain and distant mechanisms from non-ischemic organs such as the liver, spleen, and pancreas, supporting neuronal survival, confining brain infarction, and alleviating neurological deficits. Both regional and distant mechanisms are defined as systems neuroprotective mechanisms. The regional neuroprotective mechanisms involve release and activation of neuroprotective factors such as adenosine and bradykinin, inflammatory responses, expression of growth factors such as nerve growth factors and neurotrophins, and activation and differentiation of resident neural stem cells to neurons and glial cells. The distant neuroprotective mechanisms are implemented by expression and release of endocrine neuroprotective factors such as fibroblast growth factor 21, resistin like molecule γ, and trefoil factor 3 from the liver; brain-derived neurotrophic factor and nerve growth factor from the spleen; and neurotrophin 3 and vascular endothelial growth factor C from the pancreas. Furthermore, ischemic stroke induces mobilization of bone marrow hematopoietic stem cells and endothelial progenitor cells into the circulatory system and brain, contributing to neuroprotection. The regional and distant mechanisms may act in coordination and synergy to protect the ischemic brain from injury and death. This paper addresses these mechanisms and associated signaling networks.

Keywords: Neuroprotection; cell survival signaling mechanisms; ischemic stroke

1 Introduction

Systems protective mechanisms are defined as natural biological processes that occur in not only an injured organ, but also in distant non-injured organs of an individual organism and can be activated to protect the injured organ from death in response to an environmental insult [1]. Such mechanisms are based on distinct regional and distant protective systems, involving a variety of molecules and cells, established in response to various environmental insults through evolution [1,2]. The ultimate goal of these protective mechanisms is to confine injury and support the survival of the organism. Although organisms are subjected to various environmental insults, including microbiological (viruses, bacteria), physical (temperature, radiations, mechanical injury), and chemical (toxins) insults, a common set of protective systems often develops. The same set of systems is exploited for protection against various environmental insults, a design conforming to the principle of energy minimization. The mammalian species have established the most sophisticated and most effective protective systems. Here, ischemic stroke is used as an example to demonstrate the concept of systems protective mechanisms.

Ischemic stroke, although causing brain infarction, can activate natural neuroprotective mechanisms that support neuronal survival, confine brain injury, and alleviate neurological deficits, thereby minimizing morbidity and mortality [3-5]. Two types of neuroprotective mechanism are activated in response to ischemic stroke-regional and distant protections, both defined as system neuroprotective mechanisms. The regional neuroprotective mechanisms occur in the ischemic brain, including release and actions of early

DOI: 10.32604/mcb.2019.06920

protective factors, expression of growth factor genes, inflammatory responses, and activation and differentiation of resident neural stem cells to neurons and glial cells [3-5]. The distant neuroprotective mechanisms occur in non-injured organs in response to ischemic stroke. Such organs include the liver [6], spleen [unpublished data], and pancreas [unpublished data], which express neuroprotective factors in ischemic stroke. The bone marrow can mobilize its hematopoietic stem cells and endothelial progenitor cells into the circulatory system and brain in response to ischemic stroke, contributing to neuroprotection [7,8]. Other organs may also be involved, but have not been experimentally tested. The activation of the distant organs requires messengers that transmit injury signals from the brain to the distant organs. Cytokines released from injured brain cells may serve as such messengers. Through the mediation of the cytokines, the regional and distant protective mechanisms act in coordination and synergy to minimize the level of brain injury. In the following sections, the regional and distant neuroprotective mechanisms are discussed based on data from experimental and clinical investigations.

2 Regional Neuroprotective Mechanisms

2.1 Paracrine Neuroprotective Factors

Regional neuroprotective mechanisms are activated in response to ischemic stroke and implemented by upregulation and/or release of paracrine factors from injured cells, including neurons and glial cells, and activated leukocytes [9-11]. Recognized paracrine neuroprotective factors include adenosine [12,13], opioids [14], interleukin 1α (IL1α)/IL1β [3,15], IL6 [16], erythropoietin [17], brain-derived neurotrophic factor (BDNF) [18], nerve growth factor (NGF) [19], vascular endothelial growth factor (VEGF) [20], and transforming growth factor β [21,22]. These paracrine factors are divided into two classes-early and later neuroprotective factors, based on the timing of release and activation. Early neuroprotective factors include adenosine and opioids. These factors are stored in cells and can be released within hours following cell injury in ischemic stroke. Selected cytokines, such as IL1α, IL1β, and IL6, are expressed in leukocytes and can be released rapidly in response to ischemic stimulation [23]. These cytokines may also be considered early factors, causing inflammatory responses, acting as protective factors, and activating the expression of additional protective factor genes. Late neuroprotective factors include brain-derived neurotrophic factor, nerve growth factor, vascular endothelial growth factor, and transforming growth factor β. These factors are expressed and released from injured neurons, glial cells, vascular endothelial cells, and smooth muscle cells in response to ischemic stroke. The expression of these protective factors requires de novo gene expression, a process occurring about 12 hrs to several days following ischemic stroke.

2.2 Paracrine Signaling Mechanisms

The action of neuroprotective factors involves complex signaling mechanisms. Neuroprotective factors can interact with cognate receptors, activate cell survival signaling networks, and suppress cell death signaling processes, thereby rescuing neurons from irreversible injury in the ischemic penumbra-areas peripheral to the core of ischemia. The early protective factor adenosine interacts with cognate G protein-coupled receptors of the neuron, activating cell survival signaling networks and alleviating neuronal death [12-14]. A major G protein-dependent cell survival signaling network involves phosphoinositide 3-kinase (PI3K) and serine/threonine protein kinase B (PKB or Akt) [24]. An increase in the concentration of adenosine enhances adenosine binding to its receptors. In response to adenosine binding, a trimeric G protein (composed of the α, β, and γ subunits) can separate the βγ subunits from the α subunit. The βγ subunits can activate and recruit PI3K to the cell membrane phospholipids [25,26]. Activated PI3K induces phosphorylation of phosphoinositides (PI), generating phosphatidylinositol (3,4)-bisphosphate (PIP2) and phosphatidylinositol (3,4,5)-trisphosphate (PIP3) [25]. PIP2 and PIP3 can interact with the serine/threonine protein kinase Akt, resulting in Akt recruitment to the cell membrane. PIP2 and PIP3 can also stimulate interaction of the protein kinase phosphoinositide-dependent protein kinase-1 (PDK1) with Akt, resulting in Akt phosphorylation and activation [27]. Activated Akt in turn phosphorylates the cell death-inducing protein Bcl-XL/Bcl-2-associated death promoter (BAD), reducing the activity of BAD and the rate of cell death [28]. BAD in tis dephosphorylated form is capable of

binding to and sequestering the anti-apoptotic proteins Bcl-2 and Bcl-XL, rendering the pro-apoptotic factors BCL2 associated X protein (BAX) and BCL2 antagonist/killer (BAK) relatively more active [29,30] BAX and BAK induce pore formation in the mitochondrial outer membrane, allowing cytochrome C to escape from the mitochondria to the cytoplasm. Cytochrome C in turn binds to apoptotic protease activating factor 1 (Apaf1), forming a complex with caspase 9. This complex can activate caspase-3, a proteinase capable of cleaving proteins and causing cell death. When phosphorylated, BAD dissociates from Bcl-2 and Bcl-XL. Free Bcl-2 and Bcl-XL become active and exert an inhibitory effect on the cell death-inducing factors BAX and BAK, thereby preventing cell death. Thus, adenosine-induced activation of the G protein-coupled receptor signaling network supports cell survival via a mechanism involving the phosphorylation of BAD and downregulation of its pro-apoptotic activities.

Endogenous opioids are a group of peptides, including dynorphins, enkephalins, endorphins, endomorphins, and nociceptins. These peptides are encoded by the proopiomelanocortin (POMC) gene, generated by prohormone convertase-mediated cleavage of the POMC gene product, and collectively referred to as endorphins. These peptides are known to serve as analgesics, acting through interactions with G protein-coupled receptors [31,32]. There are four major types of opioid receptors-deferens (d or DOP), ketocyclazocine (k or KOP), morphine (m or MOP), and nociception (NOP) receptors. These receptors are primarily found in the central and peripheral sensory neurons [32]. Activation of the opioid receptors enhances tolerance to pain and also exerts a protective action against neuronal injury. Ischemic stroke can induce rapid release of opioids, which in turn act on cognate receptors to support neuronal survival. The G protein-mediated neuroprotective signaling mechanisms discussed above regulate the neuroprotective action of opioids.

The cytokines IL1α and IL1β are primarily expressed and released from leukocytes, which migrate to the injury sites in ischemic stroke. These cytokines can induce and regulate inflammatory responses and increase neuronal tolerance to ischemic injury [15]. Administration of recombinant IL1α or IL1β protects neurons from ischemic injury [3,15], whereas blockade of IL1 receptors by antagonists diminishes the neuroprotective effect of IL1 [15]. Both IL1α and IL1β can bind IL1 type I receptor (IL1R1). Interaction of IL1α or IL1β with IL1R1 induces conformational changes in the receptor, resulting in the recruitment of IL1 receptor accessory protein (IL1RAcP) and two adaptor proteins known as myeloid differentiation primary response gene 88 (MYD88) and IL1 receptor-activated protein kinase 4 (IRAK4) to the IL1/IL1R1 complex [33,34]. This activity causes autophosphorylation and activation of IRAK4. Activated IRAK4 phosphorylates and recruits IRAK1 and IRAK2 to the IL1/IL1R1 complex, followed by recruitment of tumor necrosis factor-associated factor 6 (TRAF6). IRAK1, IRAK2, and TRAF6 dissociate subsequently from IL1R1. TRAF6 serves as a ubiquitin E3 ligase and interacts with ubiquitin E2 ligase to cause attachment of the K63-linked polyubiquitin chain to several molecules, including IRAK1, TGFβ-activated protein kinase binding protein 2 (TAB2), TAB3, and TGFβ-activated kinase 1 (TAK1, a member of the mitogen-activated protein kinase kinase kinase family), inducing ubiquitination of these molecules. Ubiquitinated TAK1 can bind to TRAF6 and mitogen-activated protein kinase (MAPK)/extracellular regulated kinase (ERK) kinase kinase 3 (MEKK3), resulting in activation of MEKK3. MEKK3 in turn activates the transcription factors NFκB and AP-1, causing expression of mitogenic, inflammatory, and protective genes [35].

Interleukin 6 (IL6) is a cytokine expressed and released from activated leukocytes and vascular endothelial cells in response to almost all forms of injury. In patients with ischemic stroke, the serum level of IL6 elevates within 24 hrs [16]. IL6 exerts a profound protective effect on ischemic neurons [36]. IL6 interacts with its cognate receptor IL6Rα and activates the Janus kinase (JAK)-signal transducer and activator of transcription (STAT) signaling pathways, promoting cell survival and inflammatory responses. IL6-IL6Rα interaction causes recruitment of gp130 to IL6Rα, with two gp130 molecules to each IL6Rα, resulting in autophosphorylation of JAK1 and JAK3, which are constitutively associated with the IL6 receptors. These JAKs phosphorylate IL6 receptors on tyrosine residues, creating docking sites for SH2 domain-containing molecules, including STATs. One of the STAT family molecules, STAT3, is often recruited to the phospho-tyrosine sites of ILRα. JAKs can phosphorylate STAT3,

causing STAT3 detachment and dimerization. The STAT3 dimers can be translocated to the nucleus, inducing expression of inflammatory, protective, and proliferative genes.

Erythropoietin (Epo) is a cytokine known to regulate the development and maintenance of erythrocytes [37,38]. Erythropoietin and its receptor are expressed in developing and adult neural cells, including neurons and astrocytes [37]. During the embryonic stage, erythropoietin participates in the regulation of neural development and morphogenesis [37]. Erythropoietin-deficient mice exhibit unclosed neural tube and reduced brain size [37]. In the adult brain, erythropoietin supports the maintenance of the blood brain barrier [37]. Erythropoietin is upregulated in astrocytes and neurons in response to hypoxia and ischemic stroke [37]. Exposure of astrocytes to hypoxia can induce a ~100-fold increase in the level of erythropoietin expression [37]. Upregulated erythropoietin enhances neuronal tolerance to ischemia and hypoxia, protects neurons from injury and death, and reduces the size of brain infarcts in experimental stroke [37]. The neuroprotective action of erythropoietin is mediated by signaling networks involving erythropoietin receptor (EpoR), Janus kinase 2 (JAK2), and signal transducer and activator of transcription 5 (STAT5). EpoR is a type I cytokine receptor characterized by the presence of immunoglobulin-like domains and the signature motif of WSXWS in the extracellular domain. Binding of Epo to EpoR causes EpoR dimerization, activating JAK2, a protein tyrosine kinase constitutively associated with EpoR. JAK2 phosphorylates EpoR on tyrosine residues of the intracellular domain, establishing docking sites for recruiting STAT5. The recruited STAT5 is phosphorylated by JAK2, followed by several processes including STAT5 detachment, dimerization, and translocation to the nucleus. STAT5 dimers serve as transcription factors, causing expression of genes encoding proliferative and protective factors [39].

Brain-derived neurotrophic factor (BDNF) is a growth factor expressed in neurons and glial cells and is involved in the regulation of neuronal development and brain morphogenesis during the embryonic stage [40]. In the adult brain, this factor is responsible for controlling neuroplasticity, learning, and memory acquisition [41]. BDNF is upregulated in response to ischemic stroke to support neuronal survival, protect neurons from injury, alleviate brain infarction, and stimulate neuronal regeneration [42,43]. Administration of recombinant BDNF in experimental ischemic stroke reduces brain infarction and facilitates recovery from neurological deficits [42,43]. BDNF exerts a neuroprotective effect via interaction with its receptor known as tyrosine receptor kinase B (TrkB), a member of the Trk family that consists of TrkA (receptor for NGF), TrkB, and TrkC (receptor for neurotrophin 3) [44]. BDNF interaction with its receptor causes receptor dimerization and autophosphorylation on tyrosine residues within the receptor cytoplasmic domain. These processes lead to activation of signaling pathways involving phosphatidylinositol-3 kinase (PI3K) and mitogen-activated protein kinases (MAPKs), common cell survival and proliferative signaling molecules [41]. PI3K can be recruited to the phospho-tyrosine residues of the receptor, resulting in PI3K activation. Activated PI3K can phosphorylate cell membrane phospholipids, inducing activation of the Akt signaling network and suppression of cell death as discussed above. MAPK activation involves the Ras-MAPK kinase kinase signaling networks. The phospho-tyrosine residues of TrkB, established through receptor autophosphorylation in response to BDNF binding, serve as docking sites for recruiting the adaptor protein growth factor receptor-bound protein 2 (Grb2) [45,46]. Grb2 is coupled to a guanine nucleotide exchange factor known as son of sevenless homolog (Sos). Docking of Grb2 to the receptor phospho-tyrosine residues causes activation of Sos. Sos can activate signaling cascades involving Ras, Raf (a MAPK kinase kinase), MAPK/extracellular regulated kinase (ERK) kinases (MEKs), and MAPKs. MAPKs in turn activate the transcription factors cFos and cJun, both of which form homo- or hetero-dimers, known as activator protein 1 (AP1). These transcription factors can be translocated to the nucleus, acting on target gene promoters to cause expression of genes encoding mitogenic and protective factors [47].

Nerve growth factor (NGF) is a secreted protein expressed in neurons and, to a lesser extent, in glial cells and is responsible for regulating neuronal differentiation and proliferation during the embryonic stage and supporting neuronal survival and function during the adult stage [44]. NGF is upregulated in the penumbral or peripheral region of brain infarcts from 3 to 14 days following experimental stroke [19] and

plays a role in protection against neuronal injury and recovery from brain infarction and neurological deficits [44]. NGF can interact with tyrosine receptor kinase A (TrkA) expressed in various neuronal types in the central and peripheral nerve systems [44]. NGF binding to TrkA activates the Ras- mitogen-activated protein kinase (MAPK) signaling networks described above.

Vascular endothelial growth factors (VEGFs) are a family of growth factors, including VEGF-A, VEGF-B, VEGF-C, VEGF-D and placental growth factor (PlGF). These growth factors are primarily expressed in endothelial cells, responsible for regulating endothelial cell development, proliferation, and angiogenesis. Selected VEGFs are expressed in neurons and glial cells, supporting neuronal development and survival [48]. These growth factors can be upregulated from 6 hours to 3 days following experimental brain ischemia, participating in neuroprotective processes [49]. VEGF suppression by anti-VEGF antibody or VEGF-specific antisense oligonucleotides results in intensification of brain infarction, whereas administration of recombinant VEGF alleviates brain infarction [49]. The neuroprotective effect of VEGFs is mediated by the VEGF receptor-PI3 kinase (PI3K) signaling network [50] as discussed above. VEGFs are also known as an angiogenesis-stimulating factor and are involved in regulating endothelial cell proliferation and blood vessel formation in the ischemic brain [48]. In addition, VEGFs stimulate neural stem cell proliferation and differentiation in ischemic injury [48]. These activities are regulated trough the VEGF receptor-Ras-MAPK signaling networks described above.

Transforming growth factor β (TGFβ) is a cytokine known to regulate extracellular matrix production from fibroblasts and smooth muscle cells [51]. In ischemic stroke, TGFβ is upregulated in primarily the penumbral region peripheral to the core of brain ischemia within 1 to 7 days in nonhuman primates [52]. Microglia and macrophages are the primary cell types that express TGFβ in the ischemic brain [53]. TGFβ contributes to protection against ischemic neuronal injury and wound healing by promoting extracellular matrix production and fibrosis [53]. Furthermore, TGFβ promotes neural stem cell proliferation and differentiation in ischemic brain injury, contributing to neuronal regeneration [54]. These activities are regulated by the TGFβ receptor (TGFβR) signaling networks. Two types of TGFβR, including type I and type II, are involved in TGFβ signaling. These receptors are present in the dimeric form in unstimulated cells. TGFβ binding causes the formation of a heterotetrameric complex composed of a type I TGFβR dimer and a type II TGFβR dimer. The type II receptors, possessing constitutively active serine/threonine kinases, can phosphorylate the type I receptors on selected serine and threonine residues within the intracellular domain. The phosphorylated receptors enable recruitment of the intracellular proteins SMADs (homologs of both the Caenorhabditis elegans protein SMA and the Drosophila protein mothers against decapentaplegic homolog or MAD) [55,56], including SMAD1, SMAD2, SMAD3, SMAD5, and/or SMAD8, to the receptor complex. The type I receptor serine/threonine kinase induces phosphorylation on 2 serine residues of each SMAD at the C-terminus. The phosphorylated SMADs dissociate from the receptor complex and combine with SMAD4, forming SMAD complexes. These SMAD complexes, serving as transcription factors, can be translocated to the cell nucleus, causing chromatin histone modifications, promoting SMAD binding to target genes, and inducing expression of genes encoding cell proliferative and regenerative factors as well as extracellular matrix proteins [57,58]. While extracellular matrix generation and fibrosis contribute to repairing processes, fibrotic structures serve as physical barriers to neural stem cell migration from their resident sites to brain injury regions, retarding neuronal regeneration. Thus, depending on the stage of injury, inhibition of extracellular matrix formation through repressing TGFβ expression may represent a treatment strategy for facilitating resident neural stem cell migration.

2.3 Inflammatory Responses

In addition to the neuroprotective mechanisms discussed above, inflammation contributes to neuroprotection [59]. Indeed, the expression, release, and activation of the neuroprotective cytokines are an integral part of inflammation. In response to brain ischemia, inflammation evolves through several processes-elevation of endothelial permeability, edema in the interstitial space, upregulation and release of cytokines and growth factors from injured cells, extravasation of leukocytes, endothelial cell

proliferation and angiogenesis, glial cell proliferation, and overproduction of extracellular matrix, ultimately leading to fibrosis [59-61]. These are protective mechanisms to deploy neuroprotective and angiogenic factors to the injury sites, clean dead cell debris, prevent microorganism infections, enhance angiogenesis, and promote recovery from injury. Elevation of endothelial permeability is induced in response to histamine released from injured endothelial cells, mast cells, and basophils. Histamine can cause opening of the endothelial cell tight junctions, thereby increasing endothelial cell permeability, causing fluid leakage, and facilitating leukocyte extravasation. Injured neural cells and activated leukocytes can express and release cytokines, which can attract leukocytes to the injury sites to clean dead cell debris, release additional cytokines to boost inflammation, and prevent microorganism infection. Upregulated growth factors exert protective actions against neuronal injury. Selected growth factors, such as vascular endothelial growth factors, can cause endothelial cell proliferation, enhancing angiogenesis. Growth factors can also stimulate glial cell proliferation and generation of collagen matrix, resulting in fibrosis for repairing the injured brain. Thus, inflammation is a class of protective mechanisms in ischemic stroke. However, inflammation is often over-activated, exerting adverse impacts on the brain function-edema causes brain compression and secondary neuronal injury, and fibrotic tissue hinders resident stem cell migration and neuronal regeneration. Understanding the advantages and disadvantages of inflammatory responses is critical to developing neuroprotective engineering strategies for the treatment of ischemic stroke.

3 Distant Neuroprotective Mechanisms

3.1 Endocrine Neuroprotective Factors

Distant neuroprotective mechanisms from non-ischemic organs, such as the liver, spleen, and pancreas, are activated in response to ischemic stroke concurrently with the regional neuroprotective mechanisms discussed above. The liver can upregulate and release several proteins, including defensin β1 (DEFB1), fibroblast growth factor 21 (FGF21), kisspeptin 1 (KISS1), resistin-like molecule γ (RELMγ), and trefoil factor 3 (TFF3). Among these proteins, FGF21, RELMγ, and TFF3 have been shown to alleviate ischemic brain infarction and improve neuromuscular function following experimental stroke [6, unpublished data]. The neuroprotective action of TFF3 has been characterized in detail by using an experimental model of ischemic stroke [6]. TFF3 is a secreted protein originally found in the intestinal epithelial cells and involved in the maintenance of the intestinal epithelial function and protection against environmental insults [62,63]. In ischemic stroke, TFF3 is upregulated in the liver (Fig. 1) and released into the circulatory system to support neuronal survival, alleviate brain infarction, and improve neuromuscular function [6]. TFF3-deficient mice exhibit intensified brain infarction compared with wild-type mice. Administration of recombinant TFF3 to TFF3-deficient mice reduces brain infarction and alleviates neuromuscular deficits (Fig. 2) [6]. These investigations show that TFF3 is a potent neuroprotective factor and can be potentially used to treat ischemic stroke. However, the mechanisms of TFF3 action remain to be investigated.

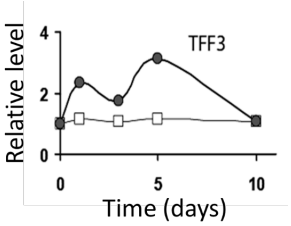

Figure 1: Time course of the relative expression of the trefoil factor 3 mRNA in hepatic cells in response to experimental ischemic stroke by differential gene expression profiling. Solid circles: TFF3 mRNA. Open squares: β actin mRNA. From [6]

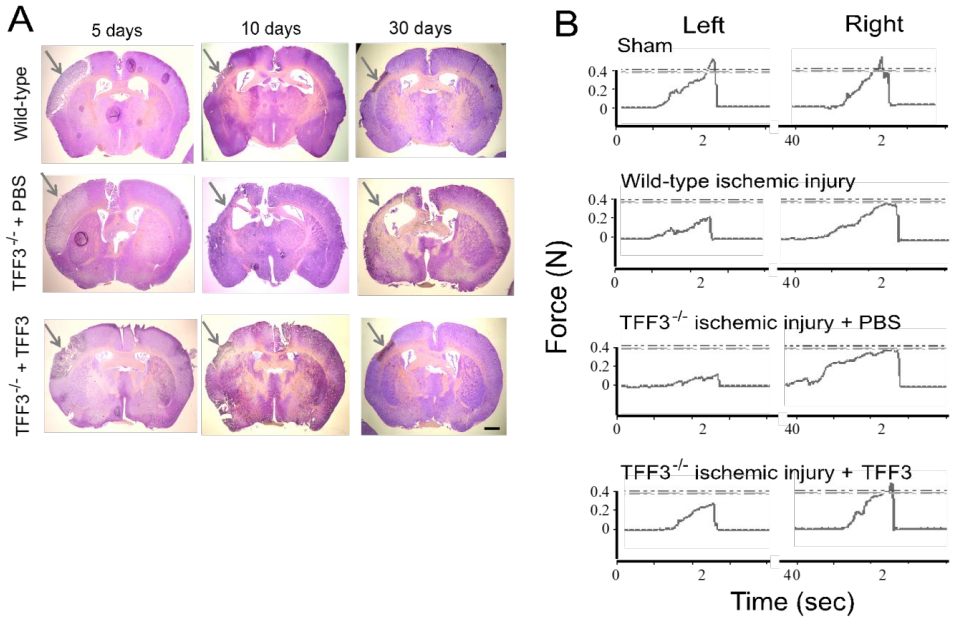

Figure 2: Neuroprotective action of trefoil factor 3 (TFF3) in experimental ischemic stroke. A. Brain slices from wild-type and TFF3-/- mice with ischemic stroke induced by 90-min ligation of the right cerebral artery and both common carotid arteries, showing the neuroprotective impact of TFF3. TFF3: Recombinant TFF3 administration. Cresyl violet staining. Arrows: Brain infarcts. Scale: 1 mm for all images. B. Gripping strengths (forces) of the left and right forelimbs of mice with sham brain operation and right-side ischemic stroke at 1 day. PBS: PBS administration. TFF3: Recombinant TFF3 administration. Note that the right forelimb gripping strength was not significantly changed, whereas the left forelimb gripping strength was reduced in right-side ischemic stroke. TFF3 deficiency causes a larger degree of increase in brain infarction and more decrease in left-forelimb gripping strength compared with wild-type controls, whereas TFF3 administration reduces brain infarction and improves the forelimb gripping strength. From [6]

In addition to the liver, the spleen and pancreas can respond to experimental ischemic stroke to upregulate numbers of genes encoding secreted proteins. The spleen can express and release adiponectin, bone morphogenetic protein 8 (BMP8), brain-derived neurotrophic factor (BDNF), growth differentiation factor 6 (GDF6), inhibin subunit βA (INHBA), interleukin 6 (IL6), left-right determination factor 1 (LEFTY1), and nerve growth factor (NGF) [unpublished data]. The pancreas can express and release adrenomedullin (ADM), BMP2, BMP6, fibroblast growth factor 9 (FGF9), FGF10, GDF11, leukemia inhibitory factor (LIF), macrophage migration inhibitory factor (MIF), netrin 1 (NTN1), neurotrophin 3 (NTF3), NTF5, vascular endothelial growth factor C (VEGFC), Wnt family member 2 (WNT2), and WNT10B [unpublished data]. The spleen- and pancreas-derived proteins can be released into the circulatory system to reach the ischemic brain, when the permeability of the blood brain barrier is increased under ischemia-induced inflammatory conditions. Although these secreted proteins have not been tested for protective actions, they may include neuroprotective factors. Indeed, several proteins from these populations, including BDNF, IL6, NGF, NTFs, and VEGF, have been shown to support neuronal survival and alleviate neuronal death in ischemic stroke as discussed above. These observations suggest the presence of distant neuroprotective mechanisms involving multiple remote non-ischemic organs in ischemic stroke. These mechanisms may act with the regional neuroprotective mechanisms in coordination and synergy to facilitate recovery from ischemic brain injury. The significance of the discovery of these distant neuroprotective mechanisms is that the distant protective genes can be manipulated to augment their neuroprotective impact by engineering approaches, such as gene transfer and editing, avoiding direct interventions of the ischemic brain.

3.2 Bone Marrow Cell Mobilization

Another distant neuroprotective mechanism involves cell mobilization from the bone marrow in response to ischemic stroke [64-66]. The bone marrow contains hematopoietic stem cells and endothelial progenitor cells. These cells can be mobilized to the circulatory system and brain in response to ischemic stroke, contributing to cerebral angiogenesis and wound healing [64-66]. Several cytokines, including granulocyte colony-stimulating factor (G-CSF) and stem cell factor (SCF), are upregulated to stimulate bone marrow cell mobilization and homing to the ischemic brain [64,65]. Within the ischemic brain, the bone marrow-derived cells can upregulate and release growth and angiogenic factors, promoting neuronal regeneration and angiogenesis [64,65]. The bone marrow-derived endothelial progenitor cells may transform to vascular endothelial cells [67,68]. Thus, stimulating bone marrow mobilization and homing to the brain represents a potential treatment strategy for ischemic stroke.

4 Naturally Occurring Neuronal Regeneration

The adult brain contains resident neural stem cells capable of repopulating and differentiating into the neurons and glial cells in response to injury [69,70]. This is a concept different from the traditional view that the adult brain cannot regenerate in injury and disease. The resident neural stem cells are primarily neuroepithelial cells in nature and are scattered throughout the brain, ranging from the cortex, subventricular zone, to the olfactory bulb [71]. These cells can be activated in response to ischemic injury to migrate to the injury sites and differentiate into neurons and glial cells [71]. The neural stem cells can also express cell survival and proliferative factors, including brain-derived neurotrophic factor (BDNF), transforming growth factor β (TGFβ), and vascular endothelial growth factor (VEGF), which promote neuronal survival, wound healing, and angiogenesis [71]. However, the regeneration capacity of the resident neural stem cells is limited. The infarcted brain tissue is mostly replaced by glial cells and fibrotic tissue in spite of the regenerative activity of the neural stem cells. Even though neurons are regenerated from resident neural stem cells, they may not be able to integrate into the native neuronal circuits to exert neurological functions. Nonetheless, the discovery of the resident neural stem cells in the brain has changed the traditional view of brain regeneration and has suggested the possibility of regenerating injured brain tissue by engineering modulations of the neural stem cells. Critical tasks are to understand the mechanisms of resident neural stem cell activation and differentiation, increase the capacity of neural stem cell differentiation, and integrate the regenerated neurons into the preexisting neuronal circuits.

5 Concluding Remarks

Ischemic stroke is a serious disorder causing cognitive and neuromuscular deficits. The presence and activation of the innate neuroprotective mechanisms is critical to the survival of the ischemic brain. However, the innate neuroprotective mechanisms are often not promptly activated and do not reach the maximum level of effectiveness in ischemic stroke. The early regional neuroprotective factors such as adenosine and opioids are released within hrs and the late regional and distant neuroprotective factors such as nerve growth factor and trefoil factor 3 are introduced to the ischemic brain within 12 hrs to several days, leaving a wide time window without effective neuroprotection following ischemic stroke, during which neuronal injury and death occur. These observations suggest the necessity of developing neuroprotective engineering strategies for correcting the deficiencies of the naturally occurring neuroprotective mechanisms. The systems neuroprotective mechanisms are the foundation for designing such engineering strategies.

Acknowledgement: This work is supported by the National Science Foundation.

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