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Role of Signal Transducer and Activator of Transcription 3 in Neuronal Survival and Regeneration

Suzan Dziennis[1] and **Nabil J. Alkayed**[1,2]

1*Department of Anesthesiology & Peri-Operative Medicine, University, Portland, OR, USA*

2*Physiology & Pharmacology, Oregon Health & Science University, Portland, OR, USA*

Synopsis

Signal Transducers and Activators of Transcription (STATs) comprise a family of transcription factors that mediate a wide variety of biological functions in the central and peripheral nervous systems. Injury to neural tissue induces STAT activation, and STATs are increasingly recognized for their role in neuronal survival. In this review, we discuss the role of STAT3 during neural development and following ischemic and traumatic injury in brain, spinal cord and peripheral nerves. We focus on STAT3 because of the expanding body of literature that investigates protective and regenerative effects of growth factors, hormones and cytokines that use STAT3 to mediate their effect, in part through transcriptional upregulation of neuroprotective and neurotrophic genes. Defining the endogenous molecular mechanisms that lead to neuroprotection by STAT3 after injury might identify novel therapeutic targets against acute neural tissue damage as well as chronic neurodegenerative disorders.

Keywords

STAT3; ischemia; neuroregeneration; neuroprotection; axotomy; spinal cord injury; neurodevelopment

Introduction

STATs are activated in the central nervous system (CNS) following injury in response to multiple signaling pathways (Fig. 1). The primary mechanism for STAT activation in brain and spinal cord is believed to be in response to the multiple cytokines and growth factors that are released after injury. However, STATs can also be activated by free radicals, excitatory neurotransmitters and other inflammatory mediators that are also produced in damaged neural tissue [3]. STATs comprise a family of seven transcription factors: STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b and STAT6 [119]. STATs are expressed during development in both neurons and glia. In adult brain, STATs are normally quiescent, but as summarized in Tables 1 and 2, several STAT family members, including STAT1, STAT3 and STAT5, have been shown to be activated after injury. Interestingly, however, different STATs seem to play different roles in injured tissue. For example, whereas STAT3 and STAT5 have been linked to neuroprotection by trophic factors and cytokines after ischemic brain or nerve injury, STAT1 activation has been associated with neuronal cell death. STAT3 and STAT5 promote neuronal survival by inducing neuroprotective genes, whereas STAT1 promotes neurodegeneration by inducing apoptotic and other cell death promoting genes. Finally, although several STAT

Reprint address: Nabil J. Alkayed, M.D., Ph.D., Department of Anesthesiology & Peri-Operative Medicine, Oregon Health and Science University, 3181 S.W. Sam Jackson Park Rd., UHS-2, Portland, Oregon 97239-3098, USA, e-mail: E-mail: alkayedn@ohsu.edu.

family members influence neuronal survival during development and in response to trophic factors and cytokines, STAT3 emerges as a key effector of neuronal survival after injury (Table 3).

Mechanisms of Stat Activation

The cellular and molecular mechanisms of STAT activation have been well characterized in the context of growth factor and cytokine signaling. Growth factors are signaling proteins that stimulate cellular proliferation and differentiation. They include cytokines and hormones that act on multiple cell types in the nervous system during development and following injury. Figure 1 demonstrates that several of those growth factors, which are protective against cell death and promote repair after injury, converge on STAT3.

As mentioned above, in the adult CNS, STATs reside in the cytosol in an inactivated state. The Janus kinase (JAK) family of tyrosine kinases, which are closely associated with the cognate receptors for multiple cytokines and growth factors, including interleukin-6 (IL-6), leukemia inhibitory factor (LIF), ciliary neurotrophic factor (CNTF), granulocyte-colony stimulating factor (G-CSF), erythropoietin (EPO) and insulin-like growth factor-I (IGF-I) [86,136,144] activate and work through STATs. Binding of these growth factors or cytokines to their cognate receptors activates JAK kinases, which phosphorylate tyrosine residues on the cytoplasmic portion of the receptor complex. These receptor-phosphorylated tyrosine residues then become docking sites for the STATs, which get phosphorylated by Jaks on a tyrosine residue into phosphorylated STAT (P-STAT). STATs can also be activated by growth factor receptors with intrinsic tyrosine kinase activity, such as the cognate receptors for epidermal growth factor (EGF) [5], platelet-derived growth factor (PDGF) [154], nerve growth factor (NGF) [34,95] and brain-derived neurotrophic factor (BDNF) [80]. In addition, STATs can also be activated by non-receptor associated tyrosine kinases belonging to the Src family of kinases.

Upon activation by tyrosine phosphorylation, members of the STAT family either heterodimerize or homodimerize and translocate to the nucleus to induce gene transcription (Fig. 1). STATs recognize and bind to a known consensus DNA sequence consisting of the 8-10 base pair (bp) inverted repeat 5′-TTn4-6AA-3′. In particular, consensus sites for STAT3 are evident in several neuroprotective gene promoters (see Fig. 1). The STAT binding consensus sequences are sometimes referred to as SIE (sis-inducible element), GAS (gamma activated sequence) or ISRE (interferon stimulated regulating element). STAT actions are terminated by deactivation in the nucleus by phosphatases [149] or by targeted degradation [65]. The unphosphorylated STATs are then recycled back to the cytosol [109]. There are further complexities of STAT deactivation as STAT activation can also be abrogated in the cytosol [116,161]. For example, activated STAT3 upregulates the suppressor of cytokine signaling 3 (SOCS3) gene, that inhibits STAT3 activation by suppressing JAK activity [70, 79].

Stat Structure-Function Relationship

STAT family members share several common domains. STATs are comprised of an amino terminal, coiled-coil, DNA binding domain (except STAT2), linker, SH2 and transcriptional activation domain (TAD). The amino terminal domain is required for STAT dimerization after activation [59]. The coiled-coil domain allows interaction with other transcription factors and regulatory proteins [19,155]. The DNA binding domain, which varies slightly among individual STATs, recognizes the consensus binding regions (SIEs) on gene promoters. STAT3 favors the canonical binding site 5′-TTn4AA-3′ [125], whereas STAT1 favors the sequence 5′-TTn3AA-3′ [120]. As mentioned above, STAT1 binding sites are present in the pro-apoptotic genes caspase-1, caspase-2 and caspase-3 [22,24,72], whereas STAT3 binding sites are present in the anti-apoptotic genes *bcl-2* and *bcl-xL* [137]. The linker region is involved in

transcriptional activation [124] and protein-protein interaction [84]. Adjacent to the linker region is the SH2 domain that recognizes phosphotyrosine residues and docks the STAT protein to tyrosine phosphorylated cytokine and growth factor receptors. The SH2 domain also contains the highly conserved tyrosine residue that is required for STAT activation. In close proximity to the tyrosine residue is a serine residue, located in the transcriptional activation domain (TAD). Serine phosphorylation at this site can either enhance or suppress gene transcription (P-Ser-STAT) [19,30,57,69]. This site is a putative mitogen-activated protein kinase (MAPK) phosphorylation motif, but the identity of the kinase(s) responsible for these modifications is not entirely elucidated [19]. Therefore other signaling pathways activated by ischemia, such as the MAPK and the AKT pathway [66], may directly or indirectly alter STAT function. While the other domains are highly conserved among STAT family members, the TADs are variable, which may contribute to their specificity of function. This may explain why STAT3, in addition to preferring known promoter sequences, is uniquely able to induce protective genes, in contrast to STAT1, which induces apoptotic genes (see the section below on STAT induction of neuroprotective genes). Although little is known about the structure-function relationship of the TAD in STAT3, STAT3 may partner with transcriptional co-activators and the combination of STAT3 with other transcription factors may increase the transcription of neuroprotective genes.

Stat Expression in the Developing and Mature Nervous System

In rodents, protein expression of STAT3 is detected in the cerebral cortex and striatum starting at embryonic day 14 (E14), and in the hippocampus at E18. STAT3 protein [29] and mRNA [171] are expressed continually from E14 to 3 months. During postnatal development, STAT3 expression increases from postnatal day 3 (P3) through P21 [40]. Immunohistochemistry has been employed to visualize activity, by localization in cytoplasm versus nucleus, and cell type specificity. STAT3 is localized to the nucleus in the developing rat brain at P3 with increasing intensity at P10 in the corpus callosum, cortex, cerebellar white matter, and stellate cells, suggesting that STAT3 is transcriptionally active during neurodevelopment. Total STAT3 is expressed in both neuronal and glial cell types throughout the rat brain during development [91] and in the adult [99]. Gautron *et al.* [40] found that during development, glial cells posses more nuclear reactivity than neurons, with the exception of robust immunoreactivity in brainstem cranial nerves (facial, hypoglossal) at P3 and P10. Of particular interest to nerve injury, brainstem cranial nerves have regenerative capabilities in the adult after nerve transection.

STAT3 is implicated in developmental processes that are later needed for support and repair of neurons in the adult after injury. STAT3 is required for vertebrate motor neuron pathfinding and guidance cues [16,26] and neurite outgrowth [50,55,162]. Glia provide structural and trophic support for neurons, and STAT3 is an important factor for the development of glial cell phenotype [17,107,148,153,168]. STAT3 induces the glia-specific protein glial fibrillary acidic protein (GFAP) [146] and is required for glial cell differentiation from cortical precursors cells *in vitro* [17]. *In vivo*, robust STAT3 activity precedes the onset of astrogliogenesis [49], suggesting that STAT3 is an integral component for differentiation of glial cells. Interestingly, neurons and glia arise from the same precursor cell population [9]. Although the precise mechanisms of neural development remain unclear, it has been proposed that failure of STAT3 to initiate glial gene expression in neuronal precursors is attributed to STAT-independent mechanisms, such as the requirement of additional transcription factors for neuronal development.

By P21, STAT3 activity declines in the brain with a few exceptions. One exception is in hypothalamic leptin-responsive neurons, where STAT3 phosphorylation is constitutive, suggesting that in addition to a developmental role, STAT3 is required for maintenance of the

hypothalamic neuroendocrine axis in the adult [40,138]. Another exception is that activated STAT3 (P-STAT3) is detected in a small population of cells in the adult brain that continually undergo self-renewal [33,38,83]. This ongoing postnatal neurogenesis occurs throughout life in the subventricular zone (SVZ) of the forebrain and in the subgranular zone (SGZ) of the hippocampus. STAT3 activation is ongoing in precursor cells within these regions throughout development and in the adult. Since endogenous neuronal precursor cells may be a source to replace dying cells after ischemic brain injury, STAT3 activation may contribute to the repair of neurons and glia after stroke.

The continual expression and nuclear localization of STAT3 during development suggests that STAT3 is an important mediator of the development and maintenance of the nervous system. These developmental processes involving STAT3 activation are important to consider because many of these developmental paradigms become reinstated after injury [49,51,71,81,82,87, 140,142,143]. Thus, STAT3 is poised to play a functional role in neuroprotection and repair after injury.

The importance of STAT3 in neurodevelopment and neuronal survival is highlighted by the fact that total STAT3 knockout animals are embryonic lethal [147]. These animals die prior to gastrulation (E6.5). In order to specifically determine the role of STAT3 in neuronal survival, several groups have circumvented embryonic lethality by generating tissue-, cell-type and time-specific knockout animals (Table 3). In particular, Cre-Lox technology has greatly improved our ability to study the effects of tissue specific and/or time specific gene deletion of STAT3 on neuronal survival (for review see [4]).

Alonzi *et al.* used the balancer strain (bal1) promoter to delete the STAT3 gene after E6.5 [8]. Bal1-cre is expressed in many tissues, including the brain, shortly after gastrulation (E6.5). The bal1-cre STAT3 knockout animals exhibit impaired survival of cranial sensory neurons from the nodose ganglia *in vivo* at El8. *In vitro*, cultured sensory neurons from bal1-STAT3 knock out animals show impaired responses to the neurotrophic cytokines CNTF and LIF. Interestingly, these knockout animals are deficient in their ability to activate signal transduction of another survival pathway, the PI3K/Akt pathway, in response to CNTF [8], suggesting a novel function of STAT3 in cytosolic signaling, apart from its role as a nuclear transcription factor. Bal1-Cre mediated STAT3 deletion, however, results in death within 5-8 hours after birth.

Neuron-specific STAT3 knockout mice are viable. However, mice harboring neural tissue-specific deletion of STAT3 during early development are obese, infertile, diabetic and are unable to maintain body temperature when challenged with cold exposure [39]. This phenotype is consistent with the strong STAT3 expression in hypothalamic leptin-responsive neurons [40,138], and suggest a role for STAT3 in the development of neuronal circuitry in the neuroendocrine axis, which controls feeding, appetite, thermoregulation and reproduction.

In the aging rodent brain, STAT3 expression progressively decreases between 3 and 26 months. In contrast, STAT1, which plays a deleterious role in stroke [145], remains unchanged in the cortex, striatum and hippocampus [28]. It is enticing, therefore, to speculate that these changes may be linked to age-related susceptibility and risk of stroke, especially, as mentioned above, that the loss of STAT3 is associated with the development of stroke risk factors, such as weight gain and diabetes mellitus. Whether age-related decline in STAT3 correlates with obesity, diabetes mellitus and increased risk and sensitivity to stroke in reproductively senescent animals, however, remains to be determined.

STAT3 Contributes to Neuroprotection After Ischemic Brain Injury

STAT3 activation following ischemic injury

STAT3 is activated in a time- and cell type-specific manner in ischemic brain (Table 1). STAT3 phosphorylation is observed in neurons [36,60,112,140], astrocytes, microglia [25,60,101, 140] and endothelial cells [112,140] in cortex, striatum and hippocampus in multiple models of ischemic brain injury both *in vivo* and *in vitro* (Table 1). In general, STAT3 phosphorylation is present in neurons during the acute phase of neural tissue injury, approximately from 0.5-24 h, and in astrocytes, microglia and endothelial cells during the chronic phases of injury, starting from 24 h to several days.

In transient cerebral ischemia models, neuronal STAT3 activation is slightly induced as early as 30 min after reperfusion [140] in the peri-ischemic area of the cerebral cortex, and increases at approximately 3 hours of reperfusion [36,140]. Robust nuclear P-STAT3 immunoreactivity is observed at 24 h, suggesting that STAT3 is functionally active and engaged in inducing gene transcription [36]. The majority of the data show that onset of glial STAT3 activation is most prevalent after 24 h of injury onset [64,112]. In some cases, however, STAT3 activation is observed in astrocytes at an earlier time. For example, cytosolic to nuclear translocation of STAT3 is observed in astrocytes as early as 1 h following focal cerebral ischemia [60]. STAT3 activation in glia remains high after several days, which may be linked to the role of STAT3 in the delayed inflammatory response to cerebral ischemia [169]. STAT3 activation is also apparent in endothelial cells of blood vessels at 24 h [112] and 48 h after experimental stroke induced in the rat by middle cerebral artery occlusion (MCAO) [140].

Role in neuroprotection

STAT3 activation in neurons correlates with survival in animal models of transient focal ischemia (MCAO). P-STAT3 co-localizes with the neuronal marker MAP-2 [36,140] and the anti-apoptotic protein bcl-2 [36,68], but not with cleaved-caspase 3 [166], suggesting that STAT3 is correlated with neuronal survival. In contrast, using a permanent occlusion MCAO model, Wen *et al.* [159] observed that P-STAT3-immunoreactive neurons were TUNEL-positive, leading the authors to conclude that STAT3 activation is associated with ischemic cell death. However, as seen in Table 3, the majority of functional, rather than correlative studies, which used STAT3 inhibitors and gene deletion approaches, support a role of STAT3 as a mediator of neuronal survival (Table 3).

Neurons depend on glia to provide structural, trophic and metabolic support after ischemia [10,46,94]. Therefore, STAT3 activation in astrocytes may indirectly be linked to neuronal survival. In support of this idea, ischemic preconditioning increases STAT3 expression in reactive astrocytes in the hippocampus [64], and STAT3 phosphorylation correlates with increased expression of cIAP2, a member of the inhibitor of apoptosis protein family, in glial cells in the penumbra [134]. Interestingly, STAT3 phosphorylation correlates with decreased gliosis in the most severely damaged part of the brain, the striatal core of the infarct. In addition, Satriotomo and colleagues found that STAT3 knockdown by *si*RNA, rather than inhibition of STAT3 activity, reduced brain damage after MCAO in spontaneously hypertensive rats [112]. Furthermore, in that study, STAT3 phosphorylation was predominantly localized in microglia/macrophages, the main source of inflammatory cytokines in the ischemic brain. Because STAT3 is activated in both neurons and glia, it is possible that severe ischemic damage, such as observed in spontaneously hypertensive rats or in the core of the infarct, and in inflammatory cells [169], may override the neuronal protection.

Endothelial cell STAT3 is protective against ischemic injury in the heart [52,158] and will be an important avenue for further study in the brain. However, little is currently known about

the role of STAT3 in endothelial cells after ischemic brain injury. For example, it has not been determined whether STAT3 phosphorylation observed within blood vessels originates in endothelial cells or is also due to the presence of inflammatory cells within the vasculature.

Role of STAT3 in Regeneration After Spinal Cord and Peripheral Nerve Injury

STAT3 may act as an injury-induced signaling molecule and transcription factor that promotes regeneration, and may serve as a new target for therapeutic intervention after traumatic injury to the spinal column or peripheral nerves. Neuronal regeneration after spinal cord injury depends on the survival of axotomized neurons. Therefore, experimental studies have focused on investigating the effect of STAT3 activation/phosphorylation on regeneration after injury induced by nerve crush, axotomy or neurochemical lesion.

Spinal cord compression injury induces phosphorylation of STAT3 in neurons in a pattern that resembles that seen after ischemic damage (Table 2). P-STAT3 is induced immediately following compression, and activation is sustained for up to 7 days, the latest time point analyzed [167]. STAT3 phosphorylation is first observed in motor neurons around 12 h, and later in reactivate astrocytes/ microglia at approximately 48 h after injury. AG-490, an inhibitor of JAK/STAT signaling, decreases hind limb motor function recovery after spinal cord compression injury, suggesting that the JAK/STAT pathway mediates motor neuron recovery. However, in this study, JAK phosphorylation was inhibited to a greater extent than STAT3 phosphorylation, suggesting that additional signaling cascades contribute to STAT3 activation following injury [167].

Conditional ablation of STAT3 in astrocytes revealed a surprising dual role for STAT3 after spinal cord injury [98]. Reactive astrocytes are known to migrate to the site of injury and form a physical barrier against inflammatory cells and neurotrophic factors. This barrier is commonly referred to as the glial scar and is believed to inhibit neuronal repair and axon regeneration through the scar. Since STAT3 is important for glial development, and therefore may contribute to the glial scar, it seems plausible that STAT3 deletion in astrocytes would be protective. Conditional ablation of STAT3 in astrocytes decreased migration of reactive astrocytes, but was accompanied by increased infiltration of CD11b+ (monocytes, macrophages and neutrophils; monocytic lineage) inflammatory cells, demyelination and more severe functional motor deficits, after spinal cord injury. When the SOCS3 gene was conditionally ablated, leading to prolonged activation of STAT3, increased reactive astrocyte migration to the site of injury was observed, and mice showed improvement of motor function compared to wild-type littermates, suggesting that P-STAT3-dependent recruitment of reactive astrocytes is beneficial, and that STAT3 activation dampens the influx of inflammatory cells, which may otherwise be harmful to the insult.

In general, axotomy increases P-STAT3 in the sciatic nerve [77,105,126], and STAT3 promotes axon regeneration after injury to the peripheral nervous system. Sensory neurons can regenerate if transected close to the nerve (sciatic), but not close to the spinal column. Qui and colleagues observed a positive correlation between sensory neurons' ability to regenerate and STAT3 phosphorylation [105]. STAT3 phosphorylation is observed in the dorsal root ganglia (DRG) sensory neurons following peripheral nerve injury to the sciatic nerve, but not in the spinal cord, when injury is induced by dorsal column crush. Neurochemical lesion with capsaicin, which destroys sensory neuron fibers but leaves their cell bodies intact [53], increases STAT3 phosphorylation in the DRG at 30 min, and in regenerating sensory fibers within the sciatic nerve at 6 h [34]. These findings are in agreement with the observation that mechanical lesion to the sciatic nerve increases P-STAT3 in spinal motor neurons and DRG during the regeneration period [77], and that P-STAT3 immunoreactivity decreases when

regeneration is complete at 6 weeks [126], again implicating STAT3 in the regenerative process.

In vitro, STAT3 induces neurite outgrowth in the DRG. In an *in vitro* model of nerve injury-induced stress and overexcitation, application of Cortisol and kanaic acid to cultured DRG neurons induces STAT3 phosphorylation on Ser-727 and promotes neurite outgrowth [152], suggesting that STAT3 phosphorylation may be involved in neuroregeneration and repair after injury. STAT3 also correlates with the expression of an important protein for growth and repair of neurons, growth-associated protein 43 (GAP-43). GAP-43, which is used as a marker for nerve regeneration after nerve injury [97,150], is upregulated in STAT3 expressing cells [105] on growth cones. Furthermore, STAT3 induces GAP-43 transcriptional activity [162], and GAP-43 expression is inhibited with the JAK inhibitor AG-490, suggesting a role for the JAK/STAT3 pathway in GAP-43 expression and neurite outgrowth and regeneration.

STAT3 activation and correlation with regeneration has also been described in cranial motor neurons following axotomy. For example, axotomy induces STAT3 phosphorylation in hypoglossal motor neurons [122], and activated STAT3 is observed in astrocytes and sprouting septal neurons after endorhinal cortical lesion [163]. Schwaiger *et al.* found that STAT3 phosphorylation is increased in facial and hypoglossal neurons, which can regenerate, but not in neurons from the Clark's nucleus, which cannot regenerate [122]. In that study, increased STAT3 phosphorylation was observed acutely in neurons at 3 h with later activation in astrocytes at 24 h. Haas and colleagues [45] also observed STAT3 activation at 24 h and 5 d after facial motor neuron axotomy. These observations suggest that STAT3 plays an important role in neuronal repair after injury. In contrast, Luo *et al.* [85] found that STAT3 is activated following optic nerve transection and that inhibition of JAK/STAT promoted, rather than inhibited, retinal ganglion cell survival and axon regeneration. Ganglion cell survival was accompanied by inhibition of macrophage recruitment into the eye, suggesting that JAK/STAT recruits macrophages to the site of injury, which exacerbates retinal injury, and that inhibition of JAK/STAT may promote regeneration by inhibiting macrophage recruitment.

Although most nerve and spinal cord injury studies examined the role of STAT3 in the context of regeneration, Schweizer and colleagues [123] employed a tissue-specific gene ablation of STAT3 in motoneurons to study the role of neural STAT3 in neuronal survival following nerve injury in the adult. Cre recombinase was expressed under the neurofilament light chain promoter (NF-L), which is not expressed in neural tissue until E12. The animals survived into adulthood with no apparent gross phenotypic abnormalities. However, facial motor neuron survival was significantly reduced in these mice after nerve lesion in the adult. Moreover, the upregulation of two important survival genes, *Reg-2* and *bcl-xL*, was reduced in STAT3 conditional knockout animals. The results from the NF-L/Cre STAT3 knockout study suggest an important role for STAT3 in promoting neuronal survival after injury, in part though the upregulation of neuroprotective genes.

Interestingly, STAT3 has been shown to be activated at the site of injury within nerve terminals, and then translocates retrogradely to the nucleus. Using compartmentalized cultures, addition of the neuropoeitic cytokine LIF to neurites of cultured sympathetic neurons induces P-STAT3 activation and subsequent translocation to nucleus [96]. Furthermore, STAT3 has been shown to be involved in retrograde signaling of Trk receptors [95]. A similar process seems to take place after injury *in vivo*, since STAT3 is localized to postsynaptic density (PSD) in cortical neurons, and STAT3 is phosphorylated by endogenous PSD tyrosine kinases [91]. Finally, P-STAT3 is observed in axons prior to its appearance in nuclei of sensory and motor neurons after injury [77,126].

Role of STAT3 in Neuroprotection by Growth Factors, Hormones and Cytokines

STAT3 is activated in response to growth factors, cytokines and hormones that are known to play a protective role after cerebral ischemia and nerve injury (Table 4). The neuropoeitic cytokines IL-6, LIF and CNTF, which are known to be induced after ischemia and exert neuroprotective effects [51,81,82,87,142], share the signaling receptor gp130. Cytokine-binding to this receptor complex induces either heterodimerization or homodimerization of receptor subunits, with IL-6 using a gp130 homodimer and all other family members a gp130/LIF receptor heterodimer [11,27,41,56,135]. CNTF and IL-6 first bind to a non-signaling glycosylphosphatidylinositol-linked alpha receptor, CNTF-RA or IL6-RA, respectively, which then complexes with the LIFR and/or gp130 receptors to form a functional receptor signaling complex.

LIF is released after nerve injury [141,172] and activates STAT3 [108]. Exogenous LIF reduces infarct volume after stroke [143], which correlates with increased P-STAT3 and the presence of LIFR/gp130, suggesting that LIF attenuates ischemic brain injury by activating STAT3 downstream of the LIFR/gp130 receptor complex.

IL-6 has also been shown to be neuroprotective. Intraventricular injection of IL-6 decreases infarct size in animal models of cerebral ischemia [51,82]. *In vitro*, IL-6 improves neuronal survival and promotes axon outgrowth and neuronal differentiation [47,74]. Although IL-6, LIF and CNTF share receptor components, Yamashita and colleagues have shown that IL-6 is specifically neuroprotective in cerebral ischemia. Endogenous IL-6 is increased after cerebral ischemia [139,142], and blockade of IL-6 signaling with an IL-6RA antibody ïncreases infarct size, number of apoptotic cells in the peri-infarct and neurological deficits after MCAO [166]. STAT3 phosphorylation is also reduced in IL-6RA-blocked animals [166], suggesting that the mechanism of neuroprotection by endogenous IL-6 may be mediated via STAT3.

The hematopoeitic cytokines G-CSF and EPO were primarily known as factors that promote blood cell proliferation and differentiation [92,102,128] until their expression was discovered in brain. G-CSF is a well-characterized neuroprotectant [115,132], and is being evaluated for the treatment of stroke. G-CSF and G-CSFR are upregulated in neurons following ischemia [67,121], and recombinant human G-CSF is protective in both *in vitro* and *in vivo* models of cerebral ischemia [114]. The protection by G-CSF correlates with STAT3 phosphorylation, increased total STAT3 and increased anti-apoptotic proteins bcl-2 and Pim-1 in neurons [68, 134], and with increased cellular inhibitor of apoptosis protein 2 (cIAP2) in glia [134]. The promoters of *bcl-2*, *Pim-1* and *cIAP2* are known transcriptional targets of STAT3 [13,106, 127,137].

EPO and its receptor EPOR are also present in brain tissue [32,103,130], and EPO has been shown to be protective against hypoxia/ischemia [12,23,111], excitotoxicity [61,90], chemical toxicity [42] and oxidative stress [21]. EPO has also been shown to be protective against ischemia-induced apoptosis [37,133] and traumatic brain [18,43] and spinal cord [23,43] injury. Interestingly, EPO promotes regeneration of CNS neurons via STAT3 [71], and EPO also maintains bcl-2 expression, which correlates with cell survival after hypoxia/ischemia [73]. Like G-CSF, EPO is also being evaluated in clinical trials for the treatment of stroke-related brain damage [37,58,115].

EGF reduces infarct size [58], induces neurogenesis after cerebral ischemia [93], and activates STAT3 in vascular smooth muscle cell [76]. Since STAT3 activation is also associated with neuroprotection and stem cell proliferation, it seems likely that STAT3 activation would underlie the protective effects of EGF after cerebral ischemia. However, whether EGF elicits

neurogenesis and protects brain after stroke specifically via the STAT3 pathway remains to be studied.

IGF-I has also been shown to increase neuronal survival [62], and inhibition of the JAK/STAT pathway with AG-490 inhibits the ability of IGF-I to promote cell survival in primary cortical neurons. IGF-I increases neuritic length in cortical cultures [164], and concomitantly induces STAT3 phosphorylation, suggesting that STAT3 is involved in neurite outgrowth in response to IGF-I. One limitation of the use of the pharmacological inhibitor AG-490 is that it inhibits both JAK and STAT activities independently. Because JAKs activate other STAT family members in addition to STAT3, the effect of inhibiting other STATs cannot be separated in studies using AG-490.

Neurotrophins such as NGF and BDNF are critical for neuronal survival and neurodevelopment. *In vitro*, addition of NGF induces differentiation of neuronal phenotype in the rat adrenal pheochromocytoma cell line, which is a well-characterized model for studying NGF-induced neuronal differentiation. Recently, Ng *et al.* have shown that NGF activates STAT3 in these cells and results in STAT3 binding and upregulation of gene transcription [95], suggesting that STAT3 may play a role in neuronal differentiation in response to NGF. Interestingly, in this study STAT3 was activated by serine-727, rather than the classical tyrosine-705 phosphorylation. In the *in vivo* rodent model of capsaicin-induced sensory neuron lesion, regeneration was markedly enhanced with the addition of NGF [35,118]. Interestingly, addition of NGF maintains P-STAT3, when it would otherwise decrease during regeneration, suggesting that NGF may improve regeneration through P-STAT3 [34]. NGF also increases the STAT3 inducible GAP-43 gene in small sensory neurons after neurochemical lesion [117], further suggesting a potential role for STAT3 in regeneration by NGF after injury.

BDNF increases neurite outgrowth and arborization in hippocampal neurons *in vitro* [75] and *in vivo* [151]. Inhibition of STAT3 by *si*RNA abolishes BDNF-induced neurite extension in cultured hippocampal neurons, suggesting that BDNF promotes neurite outgrowth via STAT3. Lin *et al.* also demonstrated that BDNF promoted neurite extension through the JAK/STAT3 pathway in pelvic ganglia [80]. In one study, administration of BDNF after permanent MCAO reduced infarct volume at 24 h [165], whereas another study found no effect of BDNF on infarct volume, although neurological outcome was improved in that study at 6 weeks [113]. BDNF has not been linked to STAT3 in terms of its role in promoting functional recovery after stroke, and further studies are needed to determine whether BDNF plays a role in promoting axonal growth after either ischemic or nerve injury, and whether such an effect is mediated through STAT3.

PDGF or CNTF enhance oligodendrocyte progenitor cells survival upon trophic factor withdrawal, and both factors maintain oligodendrocyte survival in cultured cells in the presence of the cytotoxic cytokine tumor necrosis factor-α (TNF-α) [31]. In oligodendrocyte progenitor cells, addition of either PDGF or CNTF rapidly activates and induced nuclear translocation of STAT3. However, not all oligodendrocytes responded with robust STAT activation, which may be due to differences in the developmental stage of oligodendrocyte progenitor cells *in vitro*. Interestingly, PDGF resulted in strong activation of both STAT1 and STAT3, suggesting that the cascades of survival and differentiation are complex and may involve multiple STATs.

The female sex hormone estradiol reduces lesion size and neuronal death after experimental cerebral ischemia when administered at physiological concentrations (for review see [54]). Young adult female rats sustain smaller infarcts after MCAO compared to age-matched males [1 10]. This sex difference in ischemic brain injury in young adult rats disappears after ovariectomy [6], and is absent in middle-aged, reproductively senescent female rats in which

ovarian function has naturally abated [7]. These observations suggest that ovarian hormones are protective against ischemic brain injury in young adult females.

Although ligand-bound estrogen receptor (ER) functions as a nuclear transcription factor, estrogen also induces rapid non-genomic cytosolic effects, such as changes in protein phosphorylation /78A Specifically, estradiol has been shown to rapidly activate STAT1, STAT3 and STAT5 in multiple cell types [14,15,63]. The role of STAT3 activation by estradiol has recently been linked to estrogen's neuroprotective effects against cerebral ischemia. We have recently reported that estradiol replacement increases STAT3 phosphorylation after transient focal ischemia relative to ovariectomized female rats. The protective effect of estradiol to reduce infarct volume appeared to be mediated via P-STAT3, because pharmacological inhibition of P-STAT3 abolished the protective effect of estradiol on infarct size [36]. Furthermore, we found that P-STAT3 was primarily activated in neurons, and that P-STAT3 co-localized in cells expressing the survival marker bcl-2. These findings suggest that STAT3 is an important player in mediating the protective effects of estradiol to reduce infarct size after MCAO in ovariectomized female rats.

Unlike the well-characterized activation of STAT3 by JAKs in response to growth factors and cytokines, the mechanism of ischemia-induced activation of STAT3 by estradiol is not known. However, recent studies suggest that a membrane-associated ER can interact with G-proteins [104] to activate Src [129], raising the possibility that estrogen may activate STAT3 after cerebral ischemia via Src.

STATs and Neuroprotective Genes

Several genes that promote survival and regeneration are activated in response to growth factors and cytokines, in part through STAT3 (Fig. 1). The bcl-2 family of proteins, including bcl-2 and bcl-xL, are critical in promoting neuronal survival after injury (reviewed in [88]). Bcl-2 is not expressed at high levels in mature neurons [1,89], but it is induced after ischemia. STAT3 expression directly induces *bcl-2* and *bcl-xL* gene expression in cell lines [137]. Conversely, STAT1 activity suppresses *bcl-2* and *bcl-xL* gene promoter activity [137] and induces pro-apoptotic genes such as *caspase-1, -2* and *-3* [24,72,145]. Interestingly, conditional knockout of the STAT3 gene in neurons led to decreased expression of survival genes *Reg-2* and *bcl-xL*, but had no effect on the expression of *bcl-2* [123], suggesting that additional factors may play a role in the expression of *bcl-2*, or that STAT3-induced expression of *bcl-2* may differ among various types of neurons.

STAT3 binds and activates the expression of members of the inhibitor of apoptosis protein family, including *survivin* [44] and *cIAP2* [48]. *Survivin* was identified as a potential STAT3-regulated gene by microarray analysis, which was confirmed by additional studies, including chromatin immunoprecipitation (ChIP), showing that STAT3 directly binds and regulates the *survivin* promoter in cancer cells. STAT3 is associated with the expression of GAP-43 during neurite outgrowth, indicating its importance in axonal repair [105]. Although STAT3 has been linked to the upregulation of multiple neuroprotective and neuro-repair genes after injury, few studies confirmed direct binding of STAT3 to these promoters. This may be due to technical difficulties in studying transcription factor/promoter interactions *in vivo*, since most studies characterizing STAT3 interaction with neuroprotective genes have been performed in cell lines. Whether STAT3 binds to and directly regulates *bcl-2, bcl-xL, survivin, cIAP2* or *GAP-43* expression *in vivo* after injury remains to be determined.

Summary

In this review we summarized accumulating evidence that STAT3 is an important player in neuroprotection and neuroregeneration, and a key factor for the development, differentiation,

maintenance and survival of neurons and glia in the central and peripheral nervous systems. STATs are activated following ischemic injury and axotomy in a time- and cell type-specific manner. Of all STAT family members, STAT3 seems to be specifically linked to neuronal survival during development and after injury. It co-localizes with survival markers in neurons after ischemic injury, and is associated with survival and regeneration of neurons after axotomy. Data from knockout animals suggest that loss of STAT3 leads to deficits in neuronal function and survival after injury. Multiple growth factors, hormones and cytokines, including IL-6, LIF, G-CSF, EPO, IGF-I, NGF and estrogen, utilize STAT3 to protect the brain against cell death by inducing pro-survival and repair genes, including *Reg-2, bcl-2, bcl-xL, survivin, Pirn-1, cIAP2* and *GAP-43.* The fact that signaling pathways utilized by growth factors, cytokines and hormones converge on STAT3 strongly suggests that STAT3 is a key and universal factor for promoting neuronal survival. Continuing to elucidate the molecular mechanisms of neuroprotection via the STAT pathway will, therefore, be crucial to our understanding of how the brain protects itself against insult, and may provide us with novel therapeutic approaches for neuroprotection in chronic neurodegenerative diseases as well as acute ischemic and traumatic brain and nerve injuries.

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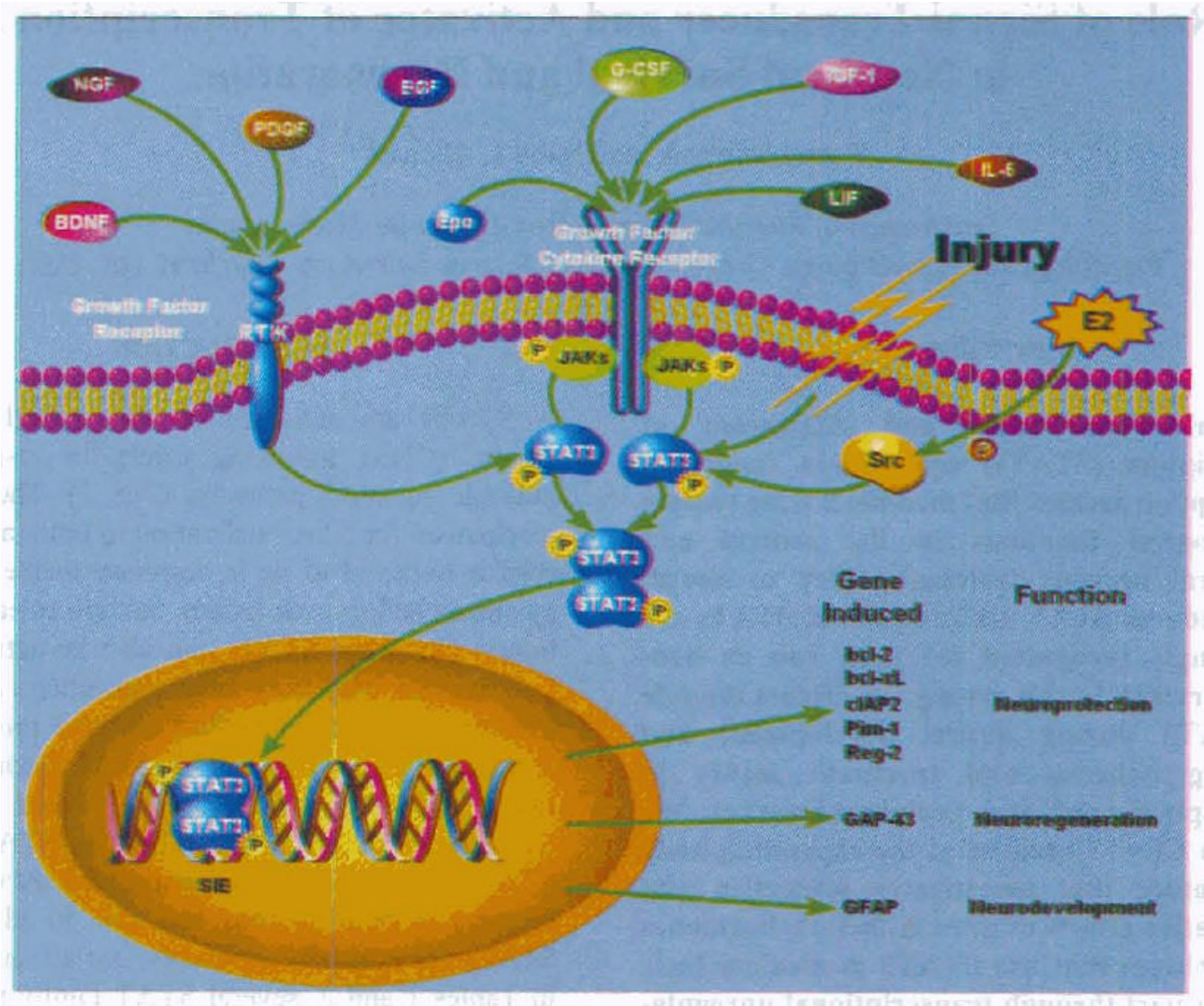

Fig. 1.

Schematic diagram summarizing mechanisms of STAT3 neuroprotection. Cytokines, growth factors and the hormone estradiol (E2) activate STAT3 by phosphorylation after ischemic or traumatic injury. STAT3 activation is linked to the neuroprotective role of these factors after injury. STAT3 activation results in the upregulation of genes that promote neuroprotection, neuroregeneration or neurodevelopment. RTK = receptor tyrosine kinase; P = phosphorylation; SIE = sis inducible element. See text for other abbreviations.

TABLE 1

Temporal activation and cell type specificity of STATs following ischemic injury

STAT	Type of ischemic injury	Species	Cell type	Time after reperfusion	Region	Reference
STAT1	MCAO	Rat	Not determined	6-24 h 4 d, 7 d, 15 d	Cerebral cortex - decrease Cerebral cortex - increase	[100]
STAT1	MCAO	Mouse	Neuron at 24 h	.5 h, 2 h, 24 h	Lateral striatum	[145]
P-STAT1	MCAO	Rat	Not determined	0 h, 3 h	Lesioned core and periphery	[160]
STAT3	MCAO	Rat	Microglia	4 d, 7 d, 15 d	Areas undergoing cell death Cortex and striatum	[101]
STAT3	MCAO	Rat	Neuron Neuron Astrocyte	First hours 12 h 1-12 h	Nuclear expression in cortex and striatum Boundaries of ischemic core of cortex and striatum Increased nuclear translocation	[60]
P-STAT3	MCAO	Rat	Neuron Neuron Endothelial Neuron Microglia-monocyte/macrophage	3.5 h-168 h Peak 3.5 h, decreased at 24 h 48 h 3.5 h, 24 h, 48 h .5 h-168 h	Peri-ischemic area-cortex Ischemic core-cortex Ischemic core-cortex and striatum Contralateral cortex and striatum No phosphorylation detected in any region	[140]
P-STAT3	pMCAO	Mouse	Neuron	6-24 h	Periphery of ischemic area	[159]
STAT3	Ischemic preconditioning	Rat	Astrocyte	3 d, 7 d	Hippocampus-dendritic CA1 molecular layer	[64]
P-STAT3	Bilateral carotid global forebrain ischemia	Rat	Astrocyte	4 h, 1 d, 3 d	Hippocampus-dentate hilar	[25]
P-STAT3	MCAO	Rat	Activated monocyte/ macrophage predominant cell type, neuron, astrocyte	24 h, 72 h	Core striatum and cortex Peri-infarct striatum and cortex No phosphorylation in contralateral side	[112]
P-STAT3	MCAO	Female rat	Undetermined Neuron	3 h 22 h	Cortex Peri-infarct region of cortex	[36]
P-STAT5	Hypoxic-ischemic neonatal model	Rat-PND7	Not determined	1 d, 3 d	Cortex	[131]
P-STAT5	Transient global ischemia	Rat	Not determined	1 h, 3 h	Hippocampus-CA1	[170]

MCAO = middle cerebral artery occlusion; pMCAO = permanent MCAO; PND = postnatal day.

TABLE 2

Temporal activation and cell type specificity of STATs following nerve injury

STAT	Type of injury	Species	Cell type	Time after reperfusion	Comments	Reference
P-STAT3	Axotomy Right facial nerve	Rat	Motoneuron	24 h 5 d	Right facial nerve Facial nuclei-no change in total protein levels of STAT3	[45]
P-STAT3	Axotomy Facial and hypoglossal nerve	Rat	Neuron Astrocyte	3 h-42 d 1 d-133 d	P-STAT3 was not found in non-regenerating Clarks nucleus following axotomy	[122]
STAT3	N-Methyl-D-aspartate induced excitotoxic cell death	Rat PND9	Astrocyte, endothelial cells Microglia/macrophages Endothelial cells Neurons	0 h, 2 h, 4 h, 10 h, 24 h, 3 d, 7d 10 h 0 h, 2 h, 4 h, 10 h, 24 h, 3 d, 7d Not detected	Present solely in the nucleus of astrocytes at 2 h, 4 h, 10 h All other times present both in nucleus and cytosol Neocortex neurons showed STAT3 immunoreactivity prior to injury but not after	[2]
P-STAT3	Axotomy sciatic nerve	Rat	Neuron	15 min, 1 h, 6 h, 24 h, 1 wk, 6 wk	In the DRG nuclei and axons at 6 h and 24 h In motorneurons nuclei and axons at 24 h-1 wk, but gone at 6 wk	[126]
P-STAT3	Endorhinal cortex lesion	Rat	Reactive astrocytes Neuron (GABAergic)	24 h 24 h strong, 3 d reduced 7 d undetectable	Fascia dentate Medial septum	[163]
P-STAT3	Axotomy Sciatic nerve	Rat	Sensory neuron	6 h	P-STAT3 observed in axons before nuclei	[77]
P-STAT3	Neurochemical lesion with capsaicin	Rat	Sciatic nerve DRG	6 h 30 min-6 h	Peak at 6 h then decreased to baseline Peak 3-6 h then decreased to below baseline	[34]
P-STAT3	Axotomy Sciatic nerve Dorsal column crush	Rat	Neuron	6 h peak, present at 2 d, 7 d and 30 d	Observed in the DRG after sciatic nerve axotomy but not after dorsal column crush	[105]
P-STAT3	Spinal cord compression model	Female mouse	Neuron Reactive astrocyte/microglia Not determined	12 h strong, 48 h weak, 12 h weak, 48 h strong Peak 12 h, decreasing but present at 168 h	Observed in the anterior horn	[167]
P-STAT3	Spinal cord injury	Female rat	Not determined	4 h	P-Ser 727 observed, but not P-Tyr 705	[152]

PND = postnatal day; DRG = dorsal root ganglia.

TABLE 3

Effect of STAT inhibition/deletion on neuronal survival

STAT	Injury	Type of inhibition	Species	Phenotype	Reference
STAT1	MCAO	Gene deletion STAT1	Mouse	Deletion of STAT1 decreased infarct volume	[145]
STAT1	—	Gene deletion STAT1 bred with GFAP-IFNα	Mouse	Earlier onset and exacerbation of neurodegeneration	[157]
STAT2	—	Gene deletion STAT2 bred with GFAP-IFNα	Mouse	Cerebellar dysplasia and medulloblastoma	[156]
STAT3	—	Ball-Cre –STAT3 gene Deletion sensory neurons	Mouse	Enhanced neuronal apoptosis	[8]
STAT3	Axotomy of motoneuron	Neuron specific -STAT3 gene deletion (NFL-CRE)	Mouse	STAT3 deletion decreased motoneuron survival	[123]
STAT3	Contusive spinal cord injury	Conditional STAT3 gene deletion (Nestin-CRE)	Mouse	STAT3 inhibition decreased reactive astrocyte migration to injury site, accompanied by decreased motor function	[98]
STAT3	MCAO	si-RNA	Spontaneously hypertensive rat	STAT3 inhibition decreased infarct size	[112]
STAT3	MCAO	Pharmacological inhibition of P-STAT3	Wistar rat	STAT3 inhibition increased infarct size in estradiol-replaced female rats	[36]

MCAO = middle cerebral artery occlusion; GFAP = glial fibrillary acidic protein; IFNα = interferon-alpha.

TABLE 4

Protective factors utilizing STAT family members after injury

STAT	Factor	Method of admin.	Species	Injury model	Comments	Reference
STAT1	Theaflavin	Intravenous	Rat	Transient focal ischemia	Reduced infarct volume and reduced phosphorylation of STAT1	[20]
STAT3	G-CSF	Injection of recombinant G-CSF	Rat	Transient focal ischemia	Decreased infarct size	[114]
STAT3	G-CSF	Addition to culture media	Rat	Glutamate-induced excitotoxicity	Protection	[114]
STAT3	G-CSF	Injection of recombinant G-CSF	Mouse	Transient focal ischemia	Decreased infarct size	[68]
STAT3	NGF	Injection of recombinant NGF	Rat	Neurochemical lesion	Maintained P-STAT3 and increased regeneration	[34]
STAT3	IL-6	Blockade of IL-6RA	Mouse	Transient focal ischemia	Increased cerebral damage associated with loss of P-STAT3	[166]
STAT3	IGF-I	Addition to culture medium	Rat	Baseline survival	IGF-I promotes survival of primary cortical neurons and activation of STAT3	[164]
STAT3	EPO	Addition to culture medium	Rat	Optic nerve lesion	Regeneration of CNS neurons associated with P-STAT3	[71]
STAT3	Estradiol	Subcutaneous pellet	Female rat	MCAO	Increased P-STAT3 after MCAO	[36]
STAT5	EPO	Single IP injection	Rat PND7	MCAO	Neuroprotective	[131]
STAT5	EPO	ICV infusion of EPO	Rat	Transient global ischemia	Pro-survival of hippocampal neurons	[170]

MCAO = middle cerebral artery occlusion; PND = postnatal day; G-CSF = granulocyte-colony stimulating factor: NGF = nerve growth factor; IL-6RA = interleukin-6 receptor alpha; IGF-I = insulin-like growth factor-I; EPO = erythropoietin.