Chapter 1

Role of TGF-β Signaling in Neurogenic Regions After Brain Injury

Sonia Villapol, Trevor T. Logan and Aviva J. Symes

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/53941

1. Introduction

In 1928 Santiago Ramón y Cajal penned what became the accepted view about neurons in the central nervous system; *"everything may die, nothing can be regenerated"*. He later exhibited his wisdom by adding; *"It's the job of science to rewrite, if possible, this cruel phrase"* [1]. Up until 20 years ago, the scientific literature had emphasized that neurogenesis only occurs during development with no new neurons generated in the adult mammalian brain. However, since the discovery of adult neurogenesis, an extensive literature has emerged supporting the constant generation of new neurons in two neurogenic regions of the adult brain: the subventricular zone around the lateral ventricles (SVZ) and the subgranular zone (SGZ) of the hippocampal dentate gyrus (DG) [2].

The existence of adult neurogenesis gave hope for recovery and regeneration from the many different insults that can damage the brain. After stroke or traumatic brain injury (TBI), immediate massive necrosis occurs followed by a subsequent prolonged period of inflammation and further neuronal death [3]. Although brain injury induces massive cell loss, it also induces an increase in proliferation of NSCs residing in the neurogenic niches [4]. The environment of the neurogenic niche in adult animals is exquisitely regulated, with a finely-tuned balance of soluble and cell-intrinsic factors that regulate the many different processes that are critical to neurogenesis: cell survival, proliferation, differentiation, and migration [5]. Dramatic changes occur in this environment as a consequence of the injury. The careful regulation of neurogenesis is disrupted by the many different cellular, soluble and vascular signals detected by the different cell types in the SVZ and DG. This major environmental alteration leads to increased proliferation of progenitor cells for long periods after the acute injury, yet the ability of the neural progenitor cells to fully differentiate, migrate and integrate into the lesioned area is limited [6]. Understanding the signals that regulate adult neurogenesis

in the naïve and injured animals is key to ultimately being able to harness the potential of neuronal replacement and improve stem cell therapy.

There are many different factors important to regulation of neurogenesis, many of which are discussed in other chapters in this book. Here we will focus on the role of the transforming growth factor-β (TGF-β) superfamily and its associated signaling pathways in regulating neurogenesis after brain injury. Members of this family, including the bone morphogenetic proteins (BMPs), Activin, and TGF-β1, -β2 and -β3 have a profound influence on the neurogenic process in naïve animals [7]. Many of these cytokines are induced by injury and play critical roles in many kinds of brain damage related processes around the lesion [3]. We and others recently started to accumulate data on their induction in the neurogenic niches after different types of injury. Here we will focus on the relevance of their induction in these specific brain regions, and the mechanisms through which they may influence the neurogenic response to injury. As there are significant differences between the behavior of cells contributing to neurogenesis during development and in the adult, we will restrict our analysis to that observed in adult animals after injury. Delineation of the specific role of members of the TGF-β superfamily in injury-induced neurogenesis may provide specific therapeutic targets for enhancing neurogenesis after trauma.

2. The TGF-β superfamily; cytokines, receptors and signaling

The TGF-β cytokine superfamily is a large group of proteins comprising 33 different members that include: bone morphogenetic proteins (BMPs), growth differentiation factors (GDFs), activins, inhibins, nodal, lefty, mülllerian inhibiting substance (MIS) together with the TGF-β proteins [8, 9]. All members of this cytokine family mediate their effects in a broadly analogous manner, binding specific type I and II transmembrane serine threonine kinase receptors and transducing their signal through similar intracellular Smad proteins [10]. These cytokines are divided into two distinct groups: those of the TGF-β/Activin group which mainly signal through the type I receptors ALK4, -5 and -7 activating Smad2 and -3, and those of the BMP/GDF group [11, 12] which employ ALK1, -2, -3 and -6 to activate Smad1, -5 and -8 [13, 14]. The specificity of Smad activation is therefore mainly determined by the identity of the type I receptor used to transduce the cytokine signal [15] (Figure 1).

TGF-β1, -β2 and -β3 together with some GDFs are unique in that they are synthesized as a large precursor molecule that is cleaved but remains non-covalently linked to its latency associated peptides, in either a small or large complex [18]. The bioavailability of TGF-βs is tightly regulated by the release of active TGF-β from these complexes in the extracellular matrix, so synthesis of TGF-β does not necessarily provide a reliable indication of available cytokine to initiate signaling. Similarly, the bioavailability of BMPs is regulated by binding to secreted extracellular antagonists that prevent BMP (and sometimes Activin) from binding to their receptor [19]. Expression levels of endogenous antagonists, including noggin, chordin, follistatin, gremlin and cerberus, thereby regulate the availability, and therefore, active signaling by their associated ligands [20]. TGF-β signaling is the archetype for signaling by

http://dx.doi.org/10.5772/53941

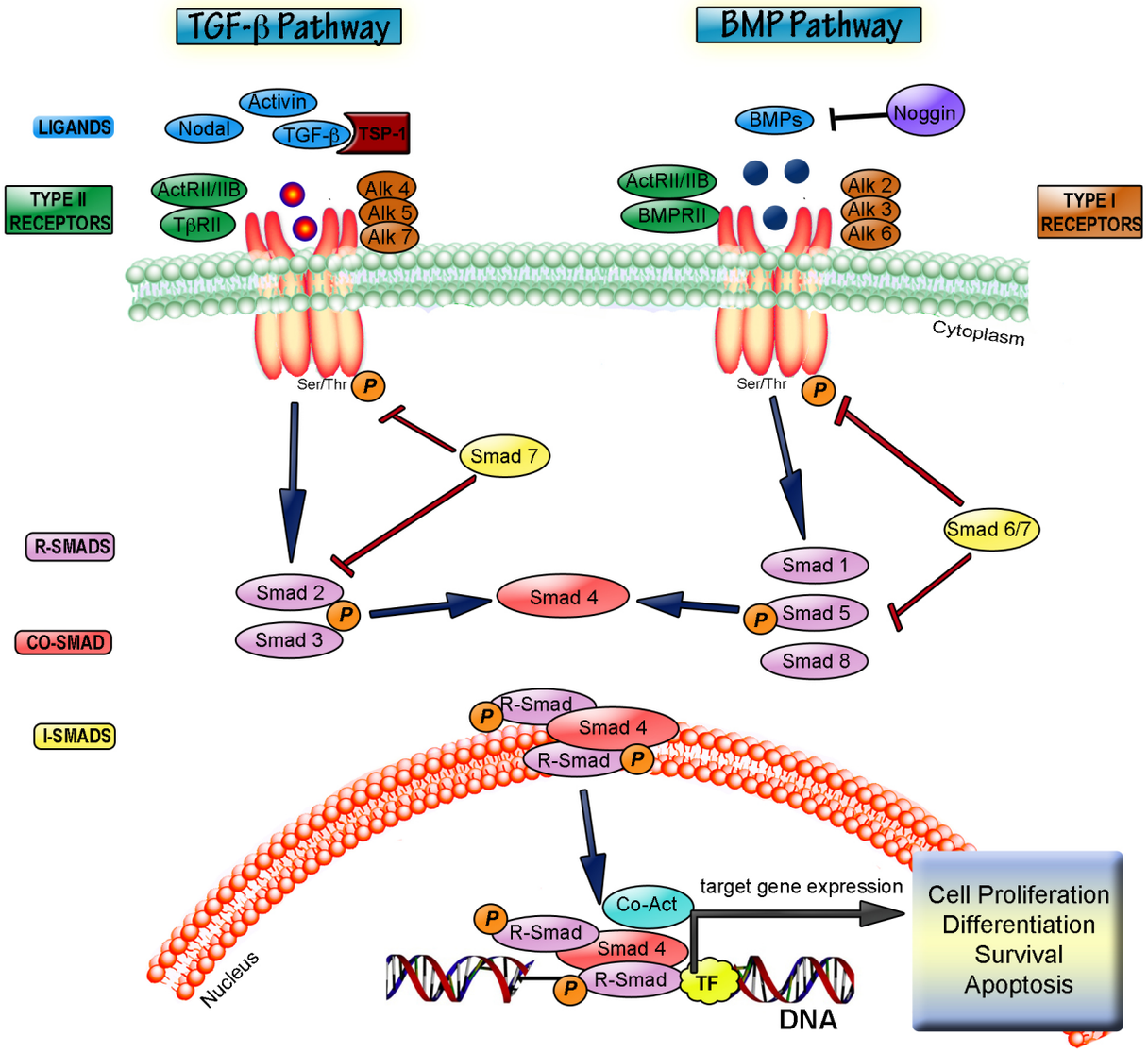

Figure 1. TGF-β superfamily signal transduction. TGF-β, nodal or activin ligands bind to Type II receptors, which then recruit Type I receptors leading to transphosphorylation of type 1 receptors. Activated type I receptors phosphorylate Smad 2/3 (*i.e.* R-Smads) which then complex with the co-Smad, Smad4 and translocate to the nucleus to bind DNA at specific DNA motifs. Smad proteins activate or repress transcription through association with various co-activator (Co-Act) or co-repressor proteins. This pathway is inhibited by Smad7. BMP signaling operates by a similar paradigm. BMP6 and BMP7 bind to their Type II receptor before the complex recruits the Type I receptors, Alk-3 or Alk-6. BMP2 and BMP4, however bind first to their type I receptor before recruiting the type II receptor BMPRII. BMP binding to either receptor can be inhibited by first binding to various extracellular inhibitor proteins, such as noggin. Activation of the receptor complex leads to phosphorylation of the receptors and subsequent phosphorylation of Smad1, Smad5, or Smad8, allowing them to form a complex with Smad4. This heteromeric complex translocates to the nucleus, to target BMP-regulated genes through interaction with co-activators or repressors. Smad 6 and Smad7 may act similarly to inhibit the BMP pathway through interactions with the receptor complex and thus inhibiting R-Smad activation. TGF-β and BMP pathways induce the expression of proteins involved in proliferation, differentiation, survival and apoptosis. The diagram is adapted from [16] and [17].

this cytokine family. TGF-β binds to the constitutively active TGF-β receptor II (TβRII) which can then recruit the type I receptor TGF-β receptor I (TβRI/ALK5). Activation of TβRI by transphosphorylation activates it, initiating downstream signaling [21]. Canonical signaling

by these cytokines is through the receptor regulated Smads (R-smads). As previously mentioned, TGF-β and activin signal through activation of Smad2 and Smad3, which are phosphorylated by the Type I receptor, and form a heteromeric complex with the common or co-Smad, Smad4 [22]. This Smad complex translocates to the nucleus where it regulates the transcription of numerous genes in cooperation with other transcription factors, coactivators and corepressors. Inhibitory Smads, or I-smads, are Smad-activated proteins that provide negative feedback to the Smad pathway through a variety of mechanisms [16, 23]. BMP signaling is similar in form to TGF-β signaling, although the specifics of individual receptors and R-Smads (1, 5, 8/9) involved vary according to the specific cytokine. For a full review of signaling and receptor nomenclature by this cytokine family please refer to some excellent reviews [14, 24]. The Smad pathway is by no means the only mechanism through which TGF-β cytokine signals are transduced from the receptor to the nucleus. Smad-independent pathways include activation of MAPKs, Ras/ERK, JNK, p38, PI3K-Akt, NF-kappaB, JAK/STAT, PP2A/S6 phosphatases and small Rho-related GTPases (16, 25). Some of the non-Smad kinases can influence Smad directed signaling by complexing with, or modifying the Smad proteins directly [16, 25]. Another level of control was found when it was shown that TGF-β/BMP signaling is both regulated by, and can regulate transcription of miRNAs [26]. Smads can also influence miRNA biogenesis by binding directly to the pri-miRNA to enhance Drosha processing of these molecules to pre-miRNA [27]. An intricate balance between Smad and non-Smad signaling superimposed on cell intrinsic and environmental conditions determines the specificity and the ultimate response of each cell to TGF-β signaling. Thus, there is a complexity to TGF-β superfamily signaling that befits cytokines that signal to multiple different cell types, in context dependent manners to influence many different physiologic processes [28].

Genetic evidence indicates that TGF-β family members regulate embryonic, perinatal or neonatal development of the mouse embryo. Most mice null for one TGF-β superfamily ligand, receptor, protein or signaling protein fail in either gastrulation or mesoderm differentiation. Table 1 lists known phenotypes of mice that are null for specific proteins in the TGF-β superfamily signaling pathways.

Conventional knockout mouse model of TGF-β proteins	**Phenotype**	**References**
TβRI	Failed angiogenesis, Embryonic lethality (E8)	[29]
TβRII	Embryonic lethality (E10.5)	[30]
TβRIII	Failed coronary vessel development accompanied by reduced epicardial cell invasion. Embryonic lethality (E14.5)	[31]
TGFβ-1	Loss of a critical regulator of immune function	[32, 33]
TGFβ-2	Perinatal lethal, craniofacial defects	[34]
TGFβ-3	Perinatal lethal, delayed lung development	[33]

Conventional knockout mouse model of TGF-β proteins	Phenotype	References
Smad1	Embryonic lethality (E10)	[35, 36]
Smad2	Embryonic lethality (E7.5–E12.5)	[37]
Smad3	Viable and fertile. Impaired immune function, including defective neutrophil chemotaxis, and impaired mucosal immunity	[38, 39]
Smad4	Increased number of Olig2-expressing progeny	[40]
Smad5	Embryonic lethality: defective vascular development	[41, 42]
Smad7	Significantly smaller than wild-type mice, died within a few days of birth	[43]
Smad8	Viable and fertile	[41, 44]
BMPRIA	Embryonic lethality (E9.5)	[45]
BMPRIB	Viable and exhibit defects in the appendicular skeleton	[46]
BMPRII	Embryonic lethality (E9.5), arrest at gastrulation	[47]
BMP2	Embryonic lethality (E7.5-10.5), defective cardiac development and have defects in cardiac development	[48]
BMP3	Increased bone density in adult	[49]
BMP4	Embryonic lethality (E6.5-E9.5), no mesoderm differentiation and show little or no mesodermal differentiation	[50]
BMP5	Viable, skeletal and cartilage abnormalities	[51]
BMP6	Viable and fertile; slight delay in ossification.	[52]
BMP7	Perinatal lethal because of poor kidney development, eye defects that appear to originate during lens induction.	[53-56]
BMP8A	Viable: male infertility due to germ cell degeneration	[57]
BMP8B	Viable: male infertility due to germ cell depletion	[58]
BMP15	Viable: female subfertility	[59]
Endoglin	Embryonic lethality (E11.5)	[60, 61]
Activin receptor IA (ALK2)	Embryonic lethality (E9.5)	[62]
Activin receptor IIB (ActR2B)	Perinatal lethal	[63]
Activin-βA	Neonatal lethal, craniofacial defects (cleft palate and loss of whiskers, upper incisors, lower incisors and molars)	[64]

Conventional knockout mouse model of TGF-β proteins	Phenotype	References
Activin-βB	Large litters but delayed parturition; nursing defects; Eye lid closure defects at birth	[65]
Noggin	Perinatal lethal, cartilage hyperplasia	[66]
Follistatin	Neonatal lethal, craniofacial defects, growth retardation and skin defects retardation and skin defects	[67]

Table 1. Phenotype of mice that do not express specific TGF-β ligands, receptors or signaling molecules.

3. TGF-*β* superfamily expression and function in normal adult brain: Role in neurogenesis

Adult neurogenesis involves proliferation of neural stem cells (NSCs), cell cycle exit, differentiation, maturation, and integration into the neural circuits, in a process that is involved in learning and memory in the normal adult brain [68]. The neurogenic niche of the adult forebrain subventricular zone (SVZ) is comprised of three major proliferative cell types; A, B and C. Multipotent, self-renewing type B cells occur earliest in the neurogenic lineage of the SVZ and give rise to the rapidly dividing type C cells, or transit amplifying progenitors. Type A cells or neuroblasts differentiate from Type C cells and are migratory neuronal progenitors with proliferative capacity, which migrate to the olfactory bulb where they differentiate into interneurons (reviewed in [69-71]. In the subgranular zone (SGZ) of the hippocampal dentate gyrus (DG), type 1 and type 2 slowly-dividing progenitors give rise to more rapidly dividing intermediate progenitor cells, and these in turn differentiate into immature neuroblasts, which migrate into the granule cell layer, then differentiate into mature neurons and integrate with the existing hippocampal circuitry [71].

Within the CNS, all three isoforms of TGF-β are produced by both glial and neuronal cells [72]. Immunohistochemical studies show widespread expression of TGF-β2 and -β3 in the developing CNS, and these proteins play a role in regulation of neuronal migration, glial proliferation and differentiation [73-76]. In adult brain, TGF-β receptors are found in all areas of the CNS including the cortex, hippocampus, striatum, brainstem and cerebellum [77, 78]. Immunoreactivity for TβRI and TβRII is detected on neurons, astrocytes and microglia and endothelial cells located in the cortical gray matter, suggesting that almost every cell type in the CNS is a potential target for TGF-β signaling [79].

The TGF-β superfamily and its downstream targets are capable of controlling proliferation, differentiation, maturation and survival of stem cells and precursors in the neurogenic niches of adult brain [18]. TβRI and TβRII are expressed by Nestin-positive type B and C cells in the SVZ [80, 81]. Our data show mRNA expression of TGF-β1, -β2, and -β3 in both the adult SVZ

and DG [82]. In the adult human brain, TGF-β1 protein expression has been reported in the hippocampus, and the protein levels significantly increased with the age of the individual [83]. As neurogenesis declines with age [84], it has been suggested that TGF-β is a possible regulator of this age-related decline [83]. Signaling by the Smad2/3 pathway is high in the hippocampus and specifically the dentate gyrus, indicating a role for TGF-β and/or activin in regulation of neurogenesis [85, 86]. When TGF-β protein is overexpressed or infused directly into the lateral ventricles of uninjured animals, hippocampal neurogenesis is dramatically inhibited [81, 87]. This may be due to a direct anti-proliferative effect of TGF-β on type 1 and 2 primary NSCs [17]. A direct effect of TGF-β on NSCs is supported by *in vitro* studies showing that TGF-β1 treatment of cultured adult NSCs induces the cyclin-dependent kinase inhibitor (p21) and leads to cell cycle termination, without altering the differentiation choices of the NSCs [81]. Additionally, overexpression studies lead to increased TGF-β signaling in many different cell types within the neurogenic niche, making the exact contribution of more restricted, endogenous TGF-β difficult to determine. Recent data have suggested that TGF-β signaling at later stages of neurogenesis is critical for newborn neuron survival and maturation in the DG. Conditional deletion of the TβRI (ALK5) gene specifically in immature and mature neurons, leads to decreased neurogenesis and reduced survival of newborn neurons [85]. Thus, TGF-β potentially has opposing roles at different stages of neurogenesis, providing an additional example of the contextual nature of TGF-β action.

Activin receptors are expressed throughout the brain, with strong expression in the neuronal layers of the hippocampus [88-90]. We have found that mRNA for activin-A and for activin's endogenous high affinity inhibitor, follistatin, are expressed in both the SVZ and DG of the adult mouse [82] and several recent reports have demonstrated that activin-A modulates adult neurogenesis [88, 91, 92]. Chronic overexpression of follistatin by neurons of the hippocampus almost entirely ablates adult DG neurogenesis, due to drastically lowered survival of adult-generated neurons [91], although short-term infusion of follistatin does not affect neurogenesis in uninjured animals [88]. Infusion of activin to the lateral ventricle of uninjured mice mildly increases the rate of NSC proliferation and neuron generation in the DG, indicating that activin might stimulate division of NSCs. This effect may be indirect as activin has a potent anti-inflammatory effect in the CNS, and may modulate local microglia to stimulate neurogenesis [88]. Smad3 knockout mice have decreased levels of cell proliferation in the SVZ and along the rostral migratory stream, and decreased levels of olfactory bulb neurogenesis [93]. As these mice have defective signaling by both TGF-β and activin, these data suggest that activin signaling in the SVZ may be the predominant Smad3-utilizing cytokine in defining basal levels of neurogenesis. In the DG pSmad2 is normally absent from Sox2-positive type 1 and 2 primary NSCs in the DG of adult mice [17]. However, Smad3 knockout mice also have reduced proliferation in the DG potentially pointing to a different role for Smad2 and Smad3 in the DG [93].

The BMP family of proteins regulates cell proliferation and fate commitment throughout development and within the adult neurogenic niches [19]. Expression of BMP2, -4 and -7 mRNAs have been reported in neurogenic regions of adult rodent brain [94], and the BMP receptors BMPRIA, -IB and -II are expressed abundantly in neurons, as well as in astrocytes

and ependymal cells [95]. All three of these receptors are expressed in type A cells of the SVZ, while type B and C cells express BMPRIA and BMPRII [96]. In the DG, radial stem cells of the SGZ marked with glial fibrillary acidic protein (GFAP) and Nestin or Sox2 primarily express BMPRIA but not BMPRIB, while mature neurons express only BMPRIB [97]. BMP ligands are also expressed in the adult rat brain [98, 99]. BMP2, -4, -6, and -7 are expressed by cells of the SVZ and DG [96, 97]. In the DG, the BMP signal transducer pSmad1 is strongly expressed in non-dividing primary NSCs and neuroblasts, but is absent in dividing primary NSCs [97], while in the SVZ, pSmad1/5/8 has been reported in primary NSCs and transit amplifying progenitors, but not in DCX-positive neuroblasts [40]. The soluble BMP inhibitor noggin is also expressed by ependymal cells of the SVZ [96] and by cells of the DG [100].

Changing the ratio of BMP to noggin alters the rates of NSC proliferation and neurogenesis in adult animals, indicating that these proteins are primary regulators of basal adult neurogenesis [96, 97, 100]. Administration of exogenous BMP4 or BMP7 potently inhibits the division of NSCs and generation of new neurons in vivo and in vitro [96, 97], as does inhibition of noggin expression [101]. Conversely, infusion of noggin or genetic deletion of the BMPRIA receptor causes an increase in NSC proliferation and generation of NeuN-expressing neurons in the DG [96, 97]. However this increase is transient, there is an eventual depletion of the primary NSC pool and a drastically reduced level of neurogenesis [97]. Decreased BMP signaling in the DG is thought to be responsible for increased neurogenesis driven by exercise [102]. It has been proposed that secretion of noggin from ependymal cells inhibits BMP signaling allowing a low level of basal neurogenesis to occur, while BMP signaling maintains the overall quiescence of the primary NSC pool [96, 97, 100]. Exogenous noggin infusion potentially has a different effect on SVZ NSCs, leaving their proliferation rate unaffected, but causing an increase in the generation of oligodendrocyte precursor cells from primary NSCs at the expense of immature neuroblasts [40]. This noggin infusion phenocopies the effect of conditionally deleting Smad4 in NSCs using GLAST-cre [40] and is in contrast to the pro-neurogenic effects of noggin described by Lim et al [96]. Thus, although there is still some controversy in the field it its clear that the balance between BMP and noggin is critical to proper maintenance of the adult NSC population.

4. Expression of TGF-β related cytokines in the adult rodent brain after injury

TGF-β family proteins are present in the brain immediately after injury as they are carried into the wound by the blood [103]. Additionally, extracellular TGF-β proteins are activated and released from their latent protein complexes in the brain parenchyma [104]. Local CNS expression of TGF-β, activin, and BMP proteins is increased after many different injuries [72, 105, 106]. Following acute brain injury, TGF-β1 levels are elevated in astrocytes, microglia, macrophages, neurons, ependymal cells and choroid plexus cells with peak expression around 3 days [107-110]. TGF-β2 and -β3 expression has also been found in astrocytes, microglia, endothelial cells and neurons after both ischemic and TBI [111, 112]. We have recently found TGF-β2 expression in oligodendrocytes in the lesioned cortex and corpus callosum [113]. Ischemic lesions as well as TBI show elevated activin-A mRNA as well as mRNA for the BMPRII receptor [90, 94,

114]. Smad proteins are also upregulated after injury and were mainly located in the cerebral cortex, typically in the nucleus and/or in the cytoplasm of astrocytes, oligodendrocytes or neurons [86, 108, 115, 116]. We have summarized many studies that have examined changes in the TGF-β superfamily of cytokines after central nervous system injury in Table 2.

TGF-β protein	Acute brain Insult (Animal model)	Expression in Brain	Expression in neurogenic niche	Cell types in which protein is expressed	mRNA and/or protein	References
TGF-β1	Ischemia	Cerebral cortex	_____	Microglia, neurons, oligodendrocytes, endothelial cells, astrocytes, macrophages, and ependymal cells	mRNA, protein	[107-110]
	Transient ischemia	Cerebellum, Cerebral cortex	Hippocampus, Subventricular zone	Microglia, T cells, neuroblasts and neurons	mRNA, protein	[117-120]
	Permanent ischemia	Cerebral cortex, Striatum	_____	Neurons, neuroblasts	mRNA, protein	[121-123]
	Bilateral cerebral ischemia	Cerebellum, Cerebral cortex	Dentate gyrus	Neurons, vessels	Protein	[124, 125]
	Hypoxic-ischemic	Cerebral cortex, Corpus callosum	_____	Astrocytes, Microglia and blood vessels	Protein	[126]
	Stab wound	Cerebral cortex	_____	Neurons	Protein	[116]
	Traumatic brain injury	Cerebral cortex	Hippocampus, Subventricular zone	Microglia, astrocytes and neurons	mRNA, protein	[82, 112, 127, 128]
	Excitotoxic lesion (NMDA)	Gray matter surrounding the lesion	_____	Astrocytes, neurons	Protein	[129]
	Triethyltin exposure	Cerebral cortex	Hippocampus	Neurons	mRNA, protein	[130, 131]
	Penetrating brain Injury	Cerebral cortex	_____	Activated glia, meningeal cells, choroid plexus	mRNA, protein	[132]
	Excitotoxic Injury	_____	Hippocampus	Neurons	Protein	[133]
	Irradiation	Cerebral cortex	_____	Macrophages and astrocytes	Protein	[134]

TGF-β protein	Acute brain Insult (Animal model)	Expression in Brain	Expression in neurogenic niche	Cell types in which protein is expressed	mRNA and/or protein	References
	Excitotoxicity with kainic acid	Cerebral cortex	Hippocampus	Microglia/macrophages, neurons and astrocytes	mRNA, protein	[86, 135-137]
	Stab wound	Cerebral cortex	_____	Astrocytes	Protein	[138]
TGF-β2	Ischemia	Cerebral cortex, cerebellum, striatum	Hippocampus	Neurons and endothelial cells, microglia and astrocytes	mRNA, protein	[108, 109, 111]
TGF-β3	Ischemia	Cerebral cortex	Dentate gyrus	Neurons	mRNA, protein	[111]
	Traumatic brain injury	Cerebral cortex	Hippocampus	Astrocytes	Protein	[112]
TβRI	Permanent ischemia	Cerebral cortex	_____	Astrocytes and neurons	mRNA, protein	[122]
TβRII	Ischemia	Cerebral cortex, midbrain, cerebellum, and brainstem	_____	Neurons, astrocytes, microglia, endothelial cells, and other non-neuronal cells found in the choroid plexus	mRNA, protein	[122, 139, 140]
	Traumatic brain injury	Cerebral Cortex	_____	Endothelial cells	Protein	[141]
Smad2	Excitotoxicity	Cerebral Cortex	Hippocampus	Neurons, astrocytes and microglia	Protein	[86]
pSmad2	Stroke	Cerebral Cortex	_____	Astrocytes, activated microglia	Protein	[108]
pSmad 1,5,8	Cuprizone-induced demyelination	_____	Subventricular zone	Oligodendrocytes	mRNA, protein	[115]
BMPRII	Traumatic brain injury	_____	Dentate gyrus	Neurons	mRNA, protein	[90]
BMPs and receptors	Ischemia	Cerebral Cortex, cerebellum	Hippocampus	Neurons	mRNA, protein	[124, 142, 143]
	Bilateral cerebral ischemia	Cerebral cortex, cerebellum	Subventricular zone, dentate gyrus	Neurons	mRNA, protein	[94]
	Traumatic brain injury	Cerebral cortex	Subventricular zone	Astrocytes	mRNA, protein	[144]

TGF-β protein	Acute brain Insult (Animal model)	Expression in Brain	Expression in neurogenic niche	Cell types in which protein is expressed	mRNA and/or protein	References
BMP4	Cuprizone-induced demyelination	_ _ _ _ _	Subventricular zone	Astrocytes and oligodendrocytes	mRNA, protein	[115]
BMP7	Traumatic brain injury	Cerebral cortex	_ _ _ _ _	Astrocytes	Protein	[144]
	Stroke	Cerebral cortex, corpus callosum	Subventricular zone	Progenitors cells and neurons	Protein	[145]
Noggin	Traumatic brain injury	Cerebral cortex	Subventricular zone	Astrocytes and progenitors cells	Protein	[144]
ActR-1A	Traumatic brain injury	_ _ _ _ _	Dentate gyrus	Neurons	mRNA, protein	[90]
Activin	Ischemia	Cerebral Cortex, striatum	Hippocampus	Neurons	mRNA, protein	[89, 146]
	Hypoxia-ischemia	Cerebral Cortex	Dentate gyrus	Microglia and blood vessels	mRNA, protein	[114]
	Excitotoxicity	Amygdala, Piriform cortex, and thalamus	Dentate gyrus	Neurons, blood vessels	mRNA, protein	[105, 146-148]

Table 2. TGF-β superfamily cytokine and signaling intermediate expression after different forms of injury.

Relatively few studies have examined changes in expression of the TGF-β superfamily of cytokines specifically within the neurogenic regions after brain injury. TGF-β1 expression increases in the SVZ [119] and DG [117, 118, 124] after ischemic injury. Its expression is also induced in neurons of the DG after a demyelinating lesion [131] or after local kainic acid injection [133]. Our group recently found that controlled cortical impact injury increased mRNA expression of many TGF-β cytokines, including TGF-β1 and -β2, activin-A, and BMPs -4, -5, -6, and -7 in the DG and SVZ, demonstrating that a distal injury can alter TGF-β signaling pathways in the neurogenic regions [82]. We have observed upregulation of TGF-β1 and -β3 in GFAP and Nestin positive progenitors in the SVZ and DG after TBI (Figure 2 and unpublished data). TβRII is expressed in these Nestin positive progenitors in the lateral SVZ (Figure 2d). Phospho-Smad3 (pSmad3) shows strong nuclear localization in these cells as well (Figure 2i and unpublished data) suggesting a role for TGF-β/activin signaling in the regulation of post-injury neurogenesis. In the DG, TβRII is expressed in GFAP-positive precursors with strong pSmad3 nuclear staining (Figure 2m, 2r) suggesting a similar role for TGF-β cytokines in this neurogenic niche.

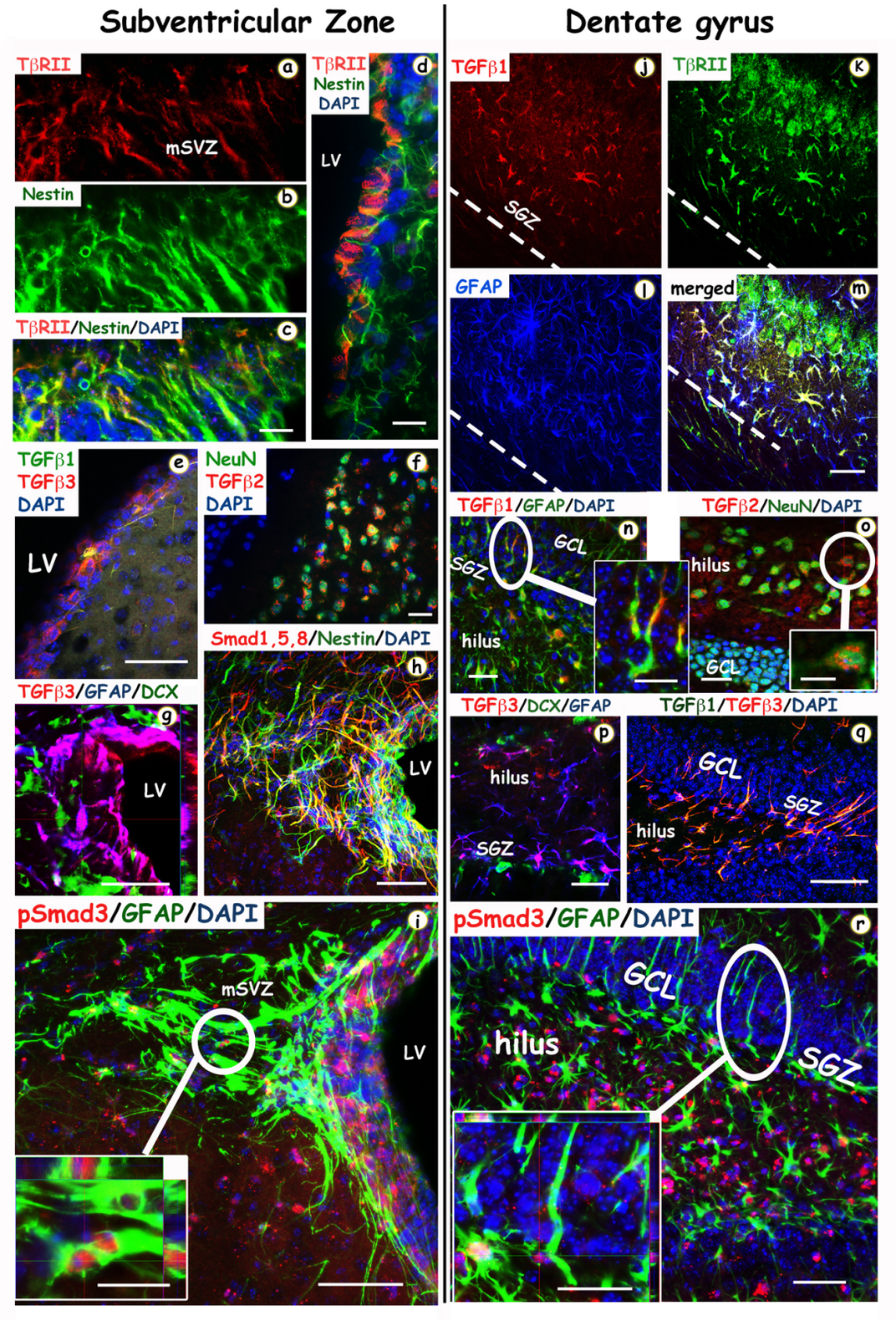

Figure 2. Confocal images of the TGF-β ligands, receptors and signaling proteins in the SVZ and DG in the injured adult mice brain. Double and triple labelled inmmunofluorescence staining for TGF-β proteins and receptors, with the following cell-type specific markers: Nestin (for undifferentiated neuronal precursors), NeuN (for mature neurons), GFAP (for progenitor and astroglial cells), DCX (for neuroblasts). The left column shows coronal sections within

the subventricular zone (SVZ) at 3 (a-g) and 7 (h and i) days after traumatic brain injury (TBI). TβRII (a, red) is expressed in Nestin positive (b, green) neural stem cells (NSCs) in the SVZ, and also in ependymal cells (d), lining the walls of the lateral ventricle (LV). Light TGFβ–1 (green) and predominant TGFβ–3 (red) expression is also found in the walls of the LV where the adult NSCs reside (e). (f) Neurons (NeuN, green) are co-localized with TGFβ–2 (red) in the damaged striatum. (h) The majority of Smad 1,5,8 proteins (red) are co-expressed with Nestin (green). (i) pSmad3 (red) colocalizes with GFAP (green) in the dorsolateral corner of the SVZ. The right column shows coronal sections within the dentate gyrus (DG) of the hippocampus at 3 (j-q) and 7 (r) days after TBI. (j-m) TGFβ–1 (red, j) and TβRII (green) are colocalized in astrocytes (GFAP, blue) in the hilus and GCL (granule cell layer) of the hippocampus (n) TGFβ–1 (red) is co-localized with astrocytes (GFAP positive cells) located in the subgranular zone (SGZ) of the hippocampus. In (o) TGFβ–2 (red) is co-localized with NeuN (green) positive neurons in the hilus of the dentate gyrus. (p) TGFβ–3 (red) is co-localized with GFAP positive (blue) immature progenitors in the SGZ but not with DCX (green) positive neuroblasts. (q) Immunostaining with TGFβ–1 (green) and TGFβ–3 (red) show they are almost entirely colocalized in the SGZ. (r) pSmad3 staining in the nuclei of GFAP positive progenitor cells in the SGZ and hilus of the hippocampus. Scale bars: (c, d, f, (inset in i), m, (inset in n), o, (inset in o), p, (inset in r)) 20 μm; (e, g, h, i, q, r) 50 μm.

Local injury to the hippocampus via saline injection produces a strong induction of activin-βA mRNA in the DG, which can be blocked by inhibiting NMDA receptors [114]. Activin expression in the DG is potently induced by seizures, local excitotoxic lesions, hypoxia/ischemia, TBI or permanent MCAO [89, 114, 146, 148, 149]. Cortical weight drop injury also elevates the expression of the activin receptor ActR-I and the BMP receptor BMPRII in the DG [90]. BMPRII expression is also elevated in the DG after global cerebral ischemia [94], and BMP4 levels increase in the SVZ after a demyelinating lesion [115].

The limited studies available indicate that TGF-β, BMP, and activin signaling may all be active in the neurogenic regions after injury. However, it is currently unclear the manner in which they affect the behavior of neural stem cells. Given that these cytokines clearly regulate adult neurogenesis in the uninjured adult, more research in this area is necessary to fully elucidate the effect of brain injury on these signaling pathways, and the mechanisms through which these changes alter post-injury neurogenesis.

5. Injury-induced neurogenesis and its regulation by TGF-β family proteins

We have described the role of TGF-β proteins in the regulation of neurogenesis under basal conditions. In response to various injuries, the rate of neurogenesis is increased and the fate and migration of the neural progenitors is changed. Cerebral ischemia, excitotoxicity and TBI can all promote neurogenesis in the adult DG and SVZ [88, 150-153]. After injury, the altered environment changes the basic processes of proliferation, differentiation, migration and integration. TGF-β related cytokines have the potential to regulate many of these processes. Alteration in the destination of progenitor cells means that many of the neuroblasts change their usual trajectory and migrate towards and into the lesion [154]. The cell fate of progenitor cells can be altered by the changed environment of the injured brain, in both the neurogenic niche and at the lesion site to which the progenitor cells migrate. The environment around the lesion is now very different than the normal location of these progenitors and thus further differentiation and integration occurs in an entirely unique environment [155]. Additionally, the actions of TGF-β cytokines are highly context dependent, and they can have very different effects in the injured as compared to the uninjured brain.

A major component of the brain post-injury in comparison to the uninjured brain is the inflammatory response, both of local CNS cells and invading macrophages. While the majority of studies have indicated that inflammation is detrimental to neurogenesis, it is now appreciated that the effect of inflammation on neurogenesis is multifaceted [156]. Of particular importance is the response of local microglia and astrocytes in the neurogenic regions. Microglia are potent regulators of neurogenesis, and in certain contexts can powerfully inhibit the process [157]. However microglia have also been shown to promote neurogenesis [158, 159], and studies have described differential action of acute vs. chronically activated microglia on NSC division and neurogenesis, as well as for microglia activated by different mechanisms or by different cytokines [160, 161]. As TGF-β proteins are prominent anti-inflammatory molecules [162], their actions after brain injury can regulate neurogenesis by acting directly on NSCs as well as indirectly through their effects on the glial inflammatory response [163].

Due to their pleiotropic actions, TGF-β superfamily proteins have been investigated as potential treatments for a variety of CNS injuries, and several studies have demonstrated potential uses for these cytokines as therapeutic molecules (see Table 3). They have also provided insights into the action of these molecules as regulators of neural stem/progenitor cell (NSPC) proliferation and differentiation, with respect to both endogenous and transplanted stem cell populations.

TGF-β related protein	Animal Model	Mode of administration	Effect on cell proliferation and neurogenesis	Behavioral Outcome	Reference
TGFβ-1	Transient ischemia	Intranasal aerosol spray	Decreased NSC proliferation and induce the number of DCX expressing neuronal precursors	Reduced Neurological Severity Score deficits	[164]
	Adrenalectomy	Intraventricular infusion	Decreased the percentage of dividing cells which co-express PSA-NCAM in the DG	None measured	[163]
	Adrenalectomy	Adenoviral overexpression	Increased NSC proliferation and neurogenesis in the SVZ	None measured	[165]
	Prenatal LPS inflammation	Adult adenoviral overexpression	Inhibited chronic microglial activation and restored neurogenesis	None measured	[166]
	Naïve animals	Injected into the cerebrospinal fluid	Number of proliferating cells in the hippocampus and in the lateral ventricle wall is substantially reduced, fewer neuronal precursor cells	None measured	[81]

TGF-β related protein	Animal Model	Mode of administration	Effect on cell proliferation and neurogenesis	Behavioral Outcome	Reference
	Naïve animals	Transgenic astrocytic overexpression	Decreased DG cell proliferation and generation of neuroblasts and neurons	None measured	[87]
Noggin	Permanent MCAO	Transgenic neuronal overexpression	Increased immature oligodendrocyte generation	Reduced motor deficits	[167]
	Naïve animals	Intraventricular infusion	Promoted neuronal differentiation of SVZ precursor cells transplanted to the striatum	None measured	[96]
	Cuprizone-induced corpus callosum demyelination	Intraventricular infusion	Decreased astrocyte and increased oligodendrocyte generation from the SVZ	None measured	[115]
	Spinal cord injury	Overexpression by transplanted NPCs.	Increased neuronal and oligodendroglial differentiation of transplanted NPCs	Improved motor recovery	[168]
BMP7	Transient ischemia	Intraventricular infusion	Increased SVZ proliferation and neurogenesis	Reduced motor deficits	[145]
	Naïve animals	Intraventricular infusion	Inhibited SVZ proliferation	None measured	[96]
Chordin	Lysolectithin-induced corpus callosum demyelination	Intraventricular infusion	Increased NPCs migrating to lesion, and increased oligodendrocyte differentiation	None measured	[169]
Activin-A	Excitotoxic hippocampal lesion	Continuous intraventricular infusion	Decreased astrocyte and microglial inflammation, and increases neurogenesis	None measured	[88]
	Naïve mice	Transgenic overexpression	Increases new neuron survival	Reduced anxiety-like behavior	[91]
Activin-A or Activin-B	Naïve mice	ICV injection	Not examined	Reduced depression-like behavior	[170]
Follistatin	Excitotoxic hippocampal lesion	Continuous intraventricular infusion	Increased NSC proliferation and neurogenesis.	None measured	[88]
	Naïve mice	Transgenic overexpression	Potently inhibited neurogenesis	Increased anxiety-like behavior	[91]

Table 3. Therapeutic application of TGF-β proteins in the normal and injured brain that affect neurogenesis.

5.1. TGF-β1

TGF-β1 treatment improves the outcome in several models of injury as it is strongly neuroprotective [76, 133, 171, 172] and in certain circumstances can promote neurogenesis after injury. After middle cerebral artery occlusion (MCAO) in mice, intranasal treatment with TGF-β1 increases the number of proliferative DCX-positive neural progenitors and the number of new neurons in the SVZ and striatum, while decreasing the fraction of proliferative cells that express GFAP [119]. After adrenalectomy, TGF-β also stimulates neurogenesis. TGF-β1 expression is upregulated and is necessary for the increased rates of neurogenesis in the SVZ and DG caused by adrenalectomy [163]. In this model TGF-β mediated downregulation of microglial activation and proliferation may be partially responsible for the increased neurogenesis [163, 165]. TGF-β1 can also inhibit chronic microglial activation induced by prenatal LPS exposure, and ameliorate the LPS-mediated decrease in neurogenesis [166] suggesting that the anti-inflammatory action of TGF-β participates in its pro-neurogenic effects. Conversely, in naïve animals intracerebroventricular infusion of TGF-β1 lowered the number of DCX-positive neuronal precursors in the neurogenic niches. This reduced level of proliferation in the TGF-β1 infused brains was strongly correlated with an increased accumulation of pSmad2 in Sox2/GFAP expressing cells of the SGZ [81]. Transgenic overexpression of TGF-β1 in naïve mice also leads to reduce neurogenesis [87]. The opposite effects of TGF-β1 in injured as compared to naïve animals illustrate the difficulty in assigning one specific role to TGF-β1 due to its context-dependent effects. Chronic inflammation, either after lesion or in neurodegenerative disease, provides a different environment for the consequences of TGF-β signaling. The anti-inflammatory actions of TGF-β can have an important role in influencing neurogenic processes, independent of direct effects on neural progenitor cells. Dysregulation of TGF-β signaling is being acknowledged as a potential source for chronic inflammation. Indeed, aberrant TGF-β signaling and consequent accumulation of activated microglia in the neurogenic regions may play an important role in the progression of Alzheimer's disease [171, 173].

5.2. Activin

Recent studies have demonstrated a critical role for activin signaling as a modulator of adult neurogenesis [91] in addition to its well-established role as a neuroprotective molecule [174, 175]. After local excitotoxic injury to the hippocampus, ablating activin signaling by infusion of the activin inhibitor follistatin potently inhibits post-injury neurogenesis and exacerbates the inflammatory response of astrocytes and microglia. Conversely, infusion of activin-A facilitates neurogenesis and represses gliosis [88]. Perhaps related to its effects on neurogenesis, activin can also regulate anxiety and depression-like behavior in rodents, and the activin pathway may be a useful therapeutic target for treating depression. Hippocampal infusion of activin-A or activin-B reduces measures of depression in a forced swim test, with a similar efficacy to that of the antidepressant fluoxetine [170]. Further, transgenic mice which overexpress activin-A, have decreased anxiety measures in spontaneous place preference tests, while mice which overexpress follistatin, display the reverse [91].

5.3. BMPs

In the naïve rodent, BMPs usually act to suppress neurogenesis in the SVZ and DG whereas the BMP inhibitor noggin promotes it [96]. In contrast, inhibition of BMP signaling by upregulation of the BMP inhibitor chordin after lysolecithin-induced demyelination of the corpus callosum, led to redirection of SVZ precursors away from a neuronal lineage towards that of oligodendrocytes [169]. This change in differentiation potential was accompanied by a change in the migration pattern of the SVZ precursors, away from the rostral migratory stream, and towards the corpus callosum. Injury-induced changes in expression of regulatory factors often alter the normal pattern of cell differentiation and migration [176, 177]. In a different model of demyelination, cuprizone-induced upregulation of BMP-4 resulted in more SVZ precursors becoming astrocytes, with a concomitant reduction in the number of mature oligodendrocytes [115]. Intraventricular infusion of noggin in this model increased the generation of oligodendrocytes from the SVZ [115] illustrating that inhibition of BMP signaling has the potential to promote remyelination in models of multiple sclerosis. The astrogliogenic potential of BMP has been demonstrated in multiple studies, where various precursors are pushed towards the astrocytic lineage [168, 178]. This is also true with transplanted neural stem cells or mesenchymal stem cells, where BMPs around the implantation site push the transplanted cells towards astrocytes [179]. If these cells are being used to enhance repair after spinal cord or TBI, inhibition of BMP becomes an attractive option to promote neuronal or oligodendrocyte differentiation rather than that of astrocytes. In contrast to all these studies, one group has shown that BMP-7 has neuroprotective properties which may enhance the survival of immature neurons [142, 180]. In one study, infusion of BMP-7 into the lateral ventricles of rats 24 hours after transient MCAO led to increased numbers of proliferating NSCs and more mature neurons generated in the SVZ while also facilitating behavioral recovery [145]. However, a different group has shown that transgenic expression of the BMP-inhibitor noggin in neurons after permanent MCAO in the mouse enhances functional recovery [167]. These conflicting data illustrate the sometimes confusing nature of the literature whereby BMP effects, similar to those of TGF-β are extremely contextual and are dependent on the exact model used. Overall, although some BMPs may have neuroprotective properties, the vast majority of the literature supports the view that BMP induction after injury is not beneficial for recovery, and that inhibition of BMP signaling may have therapeutic potential.

6. Future therapeutic strategies

In spite of extensive research in the field of brain injury or stroke, there is little effective treatment for these injuries [182]. Many of the neuroprotective treatments that have been successful in rodents have failed in clinical trials [183]. Harnessing the regenerative capacity of the adult brain is one strategy for repairing and replacing injured tissue, together with enhancing neurotrophic support of existing neurons to promote survival [184, 185]. A complementary strategy also under development is transplantation of neural stem cells or committed progenitors into the lesion. However, when multipotent NSCs were implanted

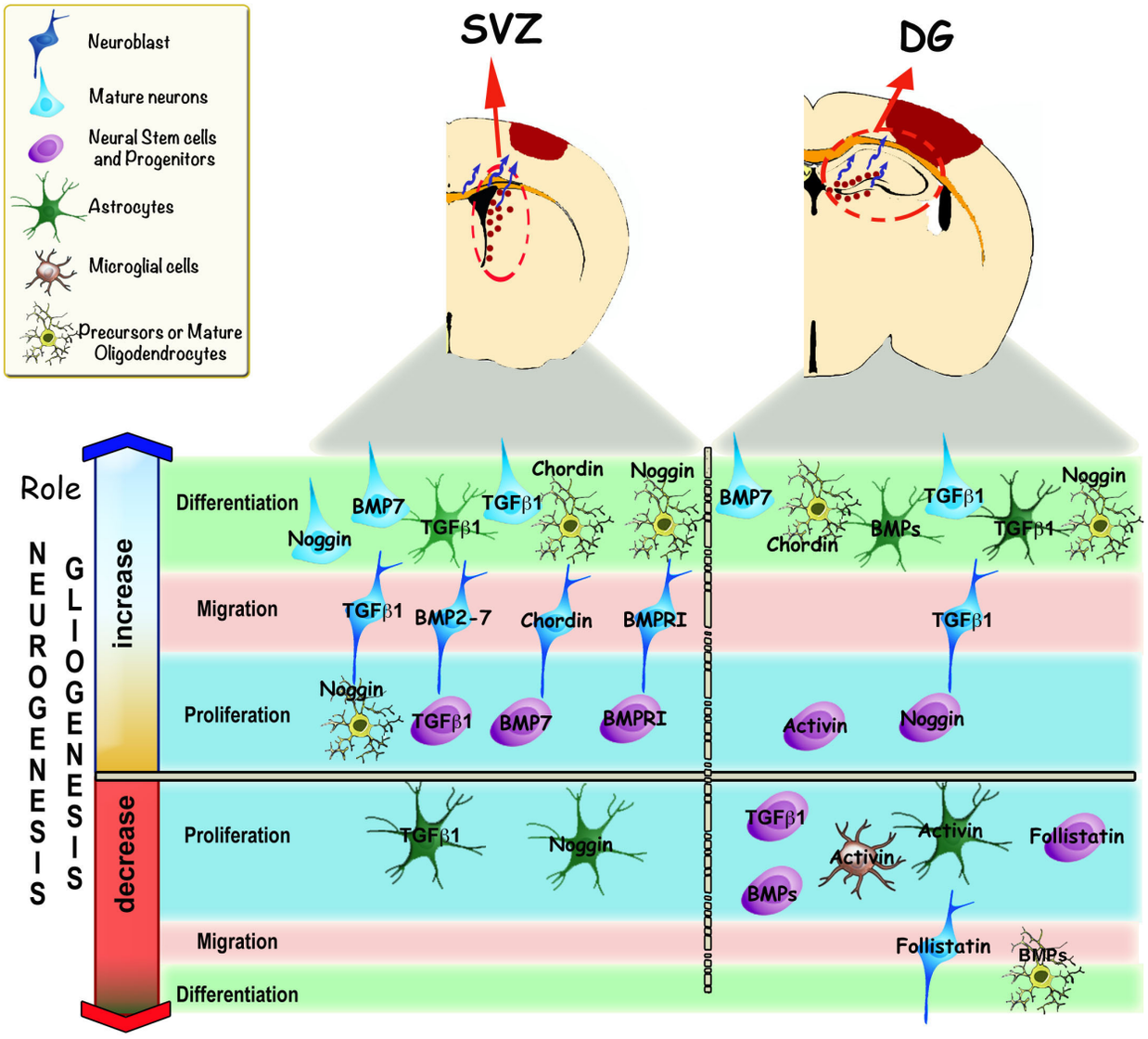

Figure 3. Modulation of neurogenesis and gliogenesis after adult brain injury by members of the TGF-β cytokine superfamily. In the top panel, the dentate gyrus (DG) in the hippocampus and subventricular zone (SVZ) of the lateral ventricles are shown after damage to the cerebral cortex. Note the proliferation and migration of cells from the SVZ and DG towards the infarcted area (blue arrows). Red dots represent proliferating and migrating neural stem cells and progenitors cells (NSPCs) located in these neurogenic regions. In the bottom panel, the role of TGF-β proteins at different stages of neurogenesis or gliogenesis after adult brain injury is illustrated. Proliferation, migration or differentiation are induced or inhibited by growth factors, such as: TGF-β, BMPs proteins, Activin, Follistatin or Noggin. After injury to the brain, TGF-β1 can increase proliferation of NSPCs and induce the differentiation of neuroblasts into neurons within the SVZ, [119]. BMP7 can induce neural stem cell proliferation, neuronal migration and differentiation [145]; other BMPs proteins (BMP2-7) also can stimulate neuronal migration [94]. The BMP inhibitor proteins noggin and chordin promote NSPC migration and oligodendrocyte proliferation and differentiation, while decreasing astrocyte proliferation [115, 169]. After injury to the brain, within the DG TGF-β1 can reduce the proliferation of immature neurons while increasing neuronal migration and differentiation [165, 166]. BMP7 can enhance NSPC proliferation and neuronal differentiation [96, 145]. Noggin can also increase NSPC proliferation [169]. Generally, BMPs can increase astroglial differentiation and inhibit oligodendrocyte generation, and the BMP inhibitors Chordin and Noggin can facilitate oligodendrocyte differentiation and proliferation [181]. Activin can induce NSPCs proliferation, and decrease microglial and astroglial proliferation. The activin antagonist, follistatin, reduces proliferating NSPCs and migrating neuroblasts [88]. In summary, the proliferation, migration and differentiation of cells in the SVZ and the DG may be influenced by the spatial and temporal expression profile of these TGF-β proteins after brain injury.

directly into non-neurogenic regions in the injured brain, such as in the cortex or striatum, they failed to generate neurons but instead generated glial cells [186, 187]. Endogenous neural progenitors also are limited in their differentiation potential, presumably because the post-lesion environment is one that supports glial differentiation in preference to that of neurons [188]. As TGF-β family members can promote astrogliogenesis [189, 190], it would seem that in some circumstances, inhibition of specific cytokine signals would increase neuronal differentiation. A further consideration for repair and neuronal survival is promotion of oligodendrocyte survival and differentiation, since remyelination is critical to continued survival and function of many neurons. Inhibition of BMP action through infusion of noggin can promote oligodendrocyte differentiation after demyelination [115]. Inflammation after injury is yet one more factor that alters the environment for regeneration. Although often thought of as a short-lived phenomenon, there can be longer lasting inflammatory changes that persist months after injury [191]. One of the major problems with development of members of the TGF-β superfamily or their inhibitors for therapeutic use are the pleiotropic nature of their effects. Thus TGF-β1 itself is neuroprotective and anti-inflammatory, which should promote recovery, but it inhibits proliferation of precursors, and also promotes development of the glial scar through upregulation of many extracellular matrix molecules, and through enhancing the migration of astrocytes [128, 192].

These cytokines act in a context dependent and concentration dependent manner, which adds an additional layer of complexity. To develop better therapeutic strategies we need a deeper understanding of the mechanisms through which the many actions of each cytokine are mediated. We may then be able to target specific molecules in the downstream signaling pathways, to avoid the pleiotropic effects that are emblematic of the activity of this cytokine family.

Acknowledgements

This study was supported by grant from the Center for Neuroscience and Regenerative Medicine (CNRM). SV is supported by a CNRM postdoctoral fellowship. The opinions and assertions contained herein are the private opinions of the authors and are not to be construed as reflecting the views of the Uniformed Services University of the Health Sciences or the US Department of Defense.

Author details

Sonia Villapol, Trevor T. Logan and Aviva J. Symes

Department of Pharmacology and Center for Neuroscience and Regenerative Medicine, Uniformed Services University of the Health Sciences, Bethesda, MD, USA

References

[1] Berciano J, Lafarga M, Berciano M: Santiago Ramon y Cajal. *Neurologia* 2001, 16(3): 118-121.

[2] Alvarez-Buylla A: Mechanism of neurogenesis in adult avian brain. *Experientia* 1990, 46(9):948-955.

[3] Parent JM: Injury-induced neurogenesis in the adult mammalian brain. *Neuroscientist* 2003, 9(4):261-272.

[4] Whitney NP, Eidem TM, Peng H, Huang Y, Zheng JC: Inflammation mediates varying effects in neurogenesis: relevance to the pathogenesis of brain injury and neurodegenerative disorders. *J Neurochem* 2009, 108(6):1343-1359.

[5] Hagg T: Molecular regulation of adult CNS neurogenesis: an integrated view. *Trends Neurosci* 2005, 28(11):589-595.

[6] Kronenberg G, Reuter K, Steiner B, Brandt MD, Jessberger S, Yamaguchi M, Kempermann G: Subpopulations of proliferating cells of the adult hippocampus respond differently to physiologic neurogenic stimuli. *J Comp Neurol* 2003, 467(4):455-463.

[7] Mackowiak M, Chocyk A, Markowicz-Kula K, Wedzony K: Neurogenesis in the adult brain. *Pol J Pharmacol* 2004, 56(6):673-687.

[8] Massague J, Cheifetz S, Ignotz RA, Boyd FT: Multiple type-beta transforming growth factors and their receptors. *J Cell Physiol Suppl* 1987, Suppl 5:43-47.

[9] Massague J: Receptors for the TGF-beta family. *Cell* 1992, 69(7):1067-1070.

[10] Wrighton KH, Feng XH: To (TGF)beta or not to (TGF)beta: fine-tuning of Smad signaling via post-translational modifications. *Cell Signal* 2008, 20(9):1579-1591.

[11] Umulis D, O'Connor MB, Blair SS: The extracellular regulation of bone morphogenetic protein signaling. *Development* 2009, 136(22):3715-3728.

[12] Miyazono K, Maeda S, Imamura T: BMP receptor signaling: transcriptional targets, regulation of signals, and signaling cross-talk. *Cytokine Growth Factor Rev* 2005, 16(3): 251-263.

[13] Ross S, Hill CS: How the Smads regulate transcription. *Int J Biochem Cell Biol* 2008, 40(3):383-408.

[14] Heldin CH, Moustakas A: Role of Smads in TGFbeta signaling. *Cell Tissue Res* 2012, 347(1):21-36.

[15] Derynck R, Feng XH: TGF-beta receptor signaling. *Biochim Biophys Acta* 1997, 1333(2):F105-150.

[16] Derynck R, Zhang YE: Smad-dependent and Smad-independent pathways in TGF-beta family signalling. *Nature* 2003, 425(6958):577-584.

[17] Kandasamy M, Reilmann R, Winkler J, Bogdahn U, Aigner L: Transforming Growth Factor-Beta Signaling in the Neural Stem Cell Niche: A Therapeutic Target for Huntington's Disease. *Neurol Res Int* 2011, 2011:124256.

[18] Moustakas A, Heldin CH: The regulation of TGFbeta signal transduction. *Development* 2009, 136(22):3699-3714.

[19] Mehler MF, Mabie PC, Zhang D, Kessler JA: Bone morphogenetic proteins in the nervous system. *Trends Neurosci* 1997, 20(7):309-317.

[20] Walsh DW, Godson C, Brazil DP, Martin F: Extracellular BMP-antagonist regulation in development and disease: tied up in knots. *Trends Cell Biol* 2010, 20(5):244-256.

[21] Kang JS, Liu C, Derynck R: New regulatory mechanisms of TGF-beta receptor function. *Trends Cell Biol* 2009, 19(8):385-394.

[22] Kretzschmar M, Massague J: SMADs: mediators and regulators of TGF-beta signaling. *Curr Opin Genet Dev* 1998, 8(1):103-111.

[23] Vogt J, Traynor R, Sapkota GP: The specificities of small molecule inhibitors of the TGFss and BMP pathways. *Cell Signal* 2011, 23(11):1831-1842.

[24] de Caestecker M: The transforming growth factor-beta superfamily of receptors. *Cytokine Growth Factor Rev* 2004, 15(1):1-11.

[25] Zhang YE: Non-Smad pathways in TGF-beta signaling. *Cell Res* 2009, 19(1):128-139.

[26] Saj A, Lai EC: Control of microRNA biogenesis and transcription by cell signaling pathways. *Curr Opin Genet Dev* 2011, 21(4):504-510.

[27] Davis BN, Hilyard AC, Nguyen PH, Lagna G, Hata A: Smad proteins bind a conserved RNA sequence to promote microRNA maturation by Drosha. *Mol Cell* 2010, 39(3):373-384.

[28] Wharton K, Derynck R: TGFbeta family signaling: novel insights in development and disease. *Development* 2009, 136(22):3691-3697.

[29] Larsson J, Goumans MJ, Sjostrand LJ, van Rooijen MA, Ward D, Leveen P, Xu X, ten Dijke P, Mummery CL, Karlsson S: Abnormal angiogenesis but intact hematopoietic potential in TGF-beta type I receptor-deficient mice. *Embo J* 2001, 20(7):1663-1673.

[30] Oshima M, Oshima H, Taketo MM: TGF-beta receptor type II deficiency results in defects of yolk sac hematopoiesis and vasculogenesis. *Dev Biol* 1996, 179(1):297-302.

[31] Hill CR, Sanchez NS, Love JD, Arrieta JA, Hong CC, Brown CB, Austin AF, Barnett JV: BMP2 signals loss of epithelial character in epicardial cells but requires the Type III TGFbeta receptor to promote invasion. *Cell Signal* 2012, 24(5):1012-1022.

[32] Shull MM, Ormsby I, Kier AB, Pawlowski S, Diebold RJ, Yin M, Allen R, Sidman C, Proetzel G, Calvin D *et al*: Targeted disruption of the mouse transforming growth factor-beta 1 gene results in multifocal inflammatory disease. *Nature* 1992, 359(6397): 693-699.

[33] Kulkarni AB, Huh CG, Becker D, Geiser A, Lyght M, Flanders KC, Roberts AB, Sporn MB, Ward JM, Karlsson S: Transforming growth factor beta 1 null mutation in mice causes excessive inflammatory response and early death. *Proc Natl Acad Sci U S A* 1993, 90(2):770-774.

[34] Sanford LP, Ormsby I, Gittenberger-de Groot AC, Sariola H, Friedman R, Boivin GP, Cardell EL, Doetschman T: TGFbeta2 knockout mice have multiple developmental defects that are non-overlapping with other TGFbeta knockout phenotypes. *Development* 1997, 124(13):2659-2670.

[35] Huang S, Tang B, Usoskin D, Lechleider RJ, Jamin SP, Li C, Anzano MA, Ebendal T, Deng C, Roberts AB: Conditional knockout of the Smad1 gene. *Genesis* 2002, 32(2): 76-79.

[36] Tremblay KD, Dunn NR, Robertson EJ: Mouse embryos lacking Smad1 signals display defects in extra-embryonic tissues and germ cell formation. *Development* 2001, 128(18):3609-3621.

[37] Heyer J, Escalante-Alcalde D, Lia M, Boettinger E, Edelmann W, Stewart CL, Kucherlapati R: Postgastrulation Smad2-deficient embryos show defects in embryo turning and anterior morphogenesis. *Proc Natl Acad Sci U S A* 1999, 96(22):12595-12600.

[38] Datto MB, Frederick JP, Pan L, Borton AJ, Zhuang Y, Wang XF: Targeted disruption of Smad3 reveals an essential role in transforming growth factor beta-mediated signal transduction. *Mol Cell Biol* 1999, 19(4):2495-2504.

[39] Yang X, Letterio JJ, Lechleider RJ, Chen L, Hayman R, Gu H, Roberts AB, Deng C: Targeted disruption of SMAD3 results in impaired mucosal immunity and diminished T cell responsiveness to TGF-beta. *Embo J* 1999, 18(5):1280-1291.

[40] Colak D, Mori T, Brill MS, Pfeifer A, Falk S, Deng C, Monteiro R, Mummery C, Sommer L, Gotz M: Adult neurogenesis requires Smad4-mediated bone morphogenic protein signaling in stem cells. *J Neurosci* 2008, 28(2):434-446.

[41] Arnold SJ, Maretto S, Islam A, Bikoff EK, Robertson EJ: Dose-dependent Smad1, Smad5 and Smad8 signaling in the early mouse embryo. *Dev Biol* 2006, 296(1): 104-118.

[42] Chang H, Brown CW, Matzuk MM: Genetic analysis of the mammalian transforming growth factor-beta superfamily. *Endocr Rev* 2002, 23(6):787-823.

[43] Tojo M, Takebe A, Takahashi S, Tanaka K, Imamura T, Miyazono K, Chiba T: Smad7-deficient mice show growth retardation with reduced viability. *J Biochem* 2012, 151(6): 621-631.

[44] Huang Z, Wang D, Ihida-Stansbury K, Jones PL, Martin JF: Defective pulmonary vascular remodeling in Smad8 mutant mice. *Hum Mol Genet* 2009, 18(15):2791-2801.

[45] Mishina Y, Suzuki A, Ueno N, Behringer RR: Bmpr encodes a type I bone morphogenetic protein receptor that is essential for gastrulation during mouse embryogenesis. *Genes Dev* 1995, 9(24):3027-3037.

[46] Yi SE, Daluiski A, Pederson R, Rosen V, Lyons KM: The type I BMP receptor BMPRIB is required for chondrogenesis in the mouse limb. *Development* 2000, 127(3): 621-630.

[47] Beppu H, Kawabata M, Hamamoto T, Chytil A, Minowa O, Noda T, Miyazono K: BMP type II receptor is required for gastrulation and early development of mouse embryos. *Dev Biol* 2000, 221(1):249-258.

[48] Zhang H, Bradley A: Mice deficient for BMP2 are nonviable and have defects in amnion/chorion and cardiac development. *Development* 1996, 122(10):2977-2986.

[49] Daluiski A, Engstrand T, Bahamonde ME, Gamer LW, Agius E, Stevenson SL, Cox K, Rosen V, Lyons KM: Bone morphogenetic protein-3 is a negative regulator of bone density. *Nat Genet* 2001, 27(1):84-88.

[50] Winnier G, Blessing M, Labosky PA, Hogan BL: Bone morphogenetic protein-4 is required for mesoderm formation and patterning in the mouse. *Genes Dev* 1995, 9(17): 2105-2116.

[51] King JA, Marker PC, Seung KJ, Kingsley DM: BMP5 and the molecular, skeletal, and soft-tissue alterations in short ear mice. *Dev Biol* 1994, 166(1):112-122.

[52] Solloway MJ, Dudley AT, Bikoff EK, Lyons KM, Hogan BL, Robertson EJ: Mice lacking Bmp6 function. *Dev Genet* 1998, 22(4):321-339.

[53] Dupe V, Ghyselinck NB, Thomazy V, Nagy L, Davies PJ, Chambon P, Mark M: Essential roles of retinoic acid signaling in interdigital apoptosis and control of BMP-7 expression in mouse autopods. *Dev Biol* 1999, 208(1):30-43.

[54] Wawersik S, Purcell P, Rauchman M, Dudley AT, Robertson EJ, Maas R: BMP7 acts in murine lens placode development. *Dev Biol* 1999, 207(1):176-188.

[55] Dudley AT, Lyons KM, Robertson EJ: A requirement for bone morphogenetic protein-7 during development of the mammalian kidney and eye. *Genes Dev* 1995, 9(22): 2795-2807.

[56] Luo G, Hofmann C, Bronckers AL, Sohocki M, Bradley A, Karsenty G: BMP-7 is an inducer of nephrogenesis, and is also required for eye development and skeletal patterning. *Genes Dev* 1995, 9(22):2808-2820.

[57] Galloway SD, Maughan RJ: The effects of substrate and fluid provision on thermoregulatory and metabolic responses to prolonged exercise in a hot environment. *J Sports Sci* 2000, 18(5):339-351.

[58] Ying Y, Liu XM, Marble A, Lawson KA, Zhao GQ: Requirement of Bmp8b for the generation of primordial germ cells in the mouse. *Mol Endocrinol* 2000, 14(7): 1053-1063.

[59] Yan C, Wang P, DeMayo J, DeMayo FJ, Elvin JA, Carino C, Prasad SV, Skinner SS, Dunbar BS, Dube JL *et al*: Synergistic roles of bone morphogenetic protein 15 and growth differentiation factor 9 in ovarian function. *Mol Endocrinol* 2001, 15(6):854-866.

[60] Ray BN, Lee NY, How T, Blobe GC: ALK5 phosphorylation of the endoglin cytoplasmic domain regulates Smad1/5/8 signaling and endothelial cell migration. *Carcinogenesis* 2010, 31(3):435-441.

[61] Li DY, Sorensen LK, Brooke BS, Urness LD, Davis EC, Taylor DG, Boak BB, Wendel DP: Defective angiogenesis in mice lacking endoglin. *Science* 1999, 284(5419): 1534-1537.

[62] Mishina Y, Crombie R, Bradley A, Behringer RR: Multiple roles for activin-like kinase-2 signaling during mouse embryogenesis. *Dev Biol* 1999, 213(2):314-326.

[63] Matzuk MM, Kumar TR, Shou W, Coerver KA, Lau AL, Behringer RR, Finegold MJ: Transgenic models to study the roles of inhibins and activins in reproduction, oncogenesis, and development. *Recent Prog Horm Res* 1996, 51:123-154; discussion 155-127.

[64] Ferguson CA, Tucker AS, Christensen L, Lau AL, Matzuk MM, Sharpe PT: Activin is an essential early mesenchymal signal in tooth development that is required for patterning of the murine dentition. *Genes Dev* 1998, 12(16):2636-2649.

[65] Vassalli A, Matzuk MM, Gardner HA, Lee KF, Jaenisch R: Activin/inhibin beta B subunit gene disruption leads to defects in eyelid development and female reproduction. *Genes Dev* 1994, 8(4):414-427.

[66] Brunet LJ, McMahon JA, McMahon AP, Harland RM: Noggin, cartilage morphogenesis, and joint formation in the mammalian skeleton. *Science* 1998, 280(5368):1455-1457.

[67] Matzuk MM, Lu N, Vogel H, Sellheyer K, Roop DR, Bradley A: Multiple defects and perinatal death in mice deficient in follistatin. *Nature* 1995, 374(6520):360-363.

[68] Emsley JG, Mitchell BD, Kempermann G, Macklis JD: Adult neurogenesis and repair of the adult CNS with neural progenitors, precursors, and stem cells. *Prog Neurobiol* 2005, 75(5):321-341.

[69] Kazanis I, Lathia J, Moss L, ffrench-Constant C: The neural stem cell microenvironment. In: *StemBook.* Cambridge (MA); 2008.

[70] Ihrie RA, Alvarez-Buylla A: Lake-front property: a unique germinal niche by the lateral ventricles of the adult brain. *Neuron* 2011, 70(4):674-686.

[71] Ming GL, Song H: Adult neurogenesis in the mammalian brain: significant answers and significant questions. *Neuron* 2011, 70(4):687-702.

[72] Vivien D, Ali C: Transforming growth factor-beta signalling in brain disorders. *Cytokine Growth Factor Rev* 2006, 17(1-2):121-128.

[73] ten Dijke P, Arthur HM: Extracellular control of TGFbeta signalling in vascular development and disease. *Nat Rev Mol Cell Biol* 2007, 8(11):857-869.

[74] Flanders KC, Ludecke G, Engels S, Cissel DS, Roberts AB, Kondaiah P, Lafyatis R, Sporn MB, Unsicker K: Localization and actions of transforming growth factor-beta s in the embryonic nervous system. *Development* 1991, 113(1):183-191.

[75] Unsicker K, Meier C, Krieglstein K, Sartor BM, Flanders KC: Expression, localization, and function of transforming growth factor-beta s in embryonic chick spinal cord, hindbrain, and dorsal root ganglia. *J Neurobiol* 1996, 29(2):262-276.

[76] Dobolyi A, Vincze C, Pal G, Lovas G: The neuroprotective functions of transforming growth factor Beta proteins. *Int J Mol Sci* 2012, 13(7):8219-8258.

[77] Flanders KC, Ren RF, Lippa CF: Transforming growth factor-betas in neurodegenerative disease. *Prog Neurobiol* 1998, 54(1):71-85.

[78] Blottner D, Wolf N, Lachmund A, Flanders KC, Unsicker K: TGF-beta rescues target-deprived preganglionic sympathetic neurons in the spinal cord. *Eur J Neurosci* 1996, 8(1):202-210.

[79] De Groot CJ, Montagne L, Barten AD, Sminia P, Van Der Valk P: Expression of transforming growth factor (TGF)-beta1, -beta2, and -beta3 isoforms and TGF-beta type I and type II receptors in multiple sclerosis lesions and human adult astrocyte cultures. *J Neuropathol Exp Neurol* 1999, 58(2):174-187.

[80] Miller MW: Expression of transforming growth factor-beta in developing rat cerebral cortex: effects of prenatal exposure to ethanol. *J Comp Neurol* 2003, 460(3):410-424.

[81] Wachs FP, Winner B, Couillard-Despres S, Schiller T, Aigner R, Winkler J, Bogdahn U, Aigner L: Transforming growth factor-beta1 is a negative modulator of adult neurogenesis. *J Neuropathol Exp Neurol* 2006, 65(4):358-370.

[82] Logan V, Symes: TGF-β superfamily gene expression and induction of the Runx1 transcription factor in adult neurogenic regions after brain injury. *Submitted* 2012.

[83] Werry EL, Enjeti S, Halliday GM, Sachdev PS, Double KL: Effect of age on proliferation-regulating factors in human adult neurogenic regions. *J Neurochem* 2010, 115(4): 956-964.

[84] Artegiani B, Calegari F: Age-related cognitive decline: can neural stem cells help us? *Aging (Albany NY)* 2012, 4(3):176-186.

[85] He Y, Yung A, Wyss-Coray T: TGF-β signaling in newborn neurons is required for their survival and maturation in the adult dentate gyrus. In: *Society for Neuroscience: 2012*. 823.808.

[86] Luo J, Lin AH, Masliah E, Wyss-Coray T: Bioluminescence imaging of Smad signaling in living mice shows correlation with excitotoxic neurodegeneration. *Proc Natl Acad Sci U S A* 2006, 103(48):18326-18331.

[87] Buckwalter MS, Yamane M, Coleman BS, Ormerod BK, Chin JT, Palmer T, Wyss-Coray T: Chronically increased transforming growth factor-beta1 strongly inhibits hippocampal neurogenesis in aged mice. *Am J Pathol* 2006, 169(1):154-164.

[88] Abdipranoto-Cowley A, Park JS, Croucher D, Daniel J, Henshall S, Galbraith S, Mervin K, Vissel B: Activin A is essential for neurogenesis following neurodegeneration. *Stem Cells* 2009, 27(6):1330-1346.

[89] Bottner M, Dubal DB, Rau SW, Suzuki S, Wise PM: Stroke injury in rats causes an increase in activin A gene expression which is unaffected by oestradiol treatment. *J Neuroendocrinol* 2006, 18(2):97-103.

[90] Lewen A, Soderstrom S, Hillered L, Ebendal T: Expression of serine/threonine kinase receptors in traumatic brain injury. *Neuroreport* 1997, 8(2):475-479.

[91] Ageta H, Murayama A, Migishima R, Kida S, Tsuchida K, Yokoyama M, Inokuchi K: Activin in the brain modulates anxiety-related behavior and adult neurogenesis. *PLoS One* 2008, 3(4):e1869.

[92] Gokoffski KK, Wu HH, Beites CL, Kim J, Kim EJ, Matzuk MM, Johnson JE, Lander AD, Calof AL: Activin and GDF11 collaborate in feedback control of neuroepithelial stem cell proliferation and fate. *Development* 2011, 138(19):4131-4142.

[93] Wang Y, Symes AJ: Smad3 deficiency reduces neurogenesis in adult mice. *J Mol Neurosci* 2010, 41(3):383-396.

[94] Charytoniuk DA, Traiffort E, Pinard E, Issertial O, Seylaz J, Ruat M: Distribution of bone morphogenetic protein and bone morphogenetic protein receptor transcripts in the rodent nervous system and up-regulation of bone morphogenetic protein receptor type II in hippocampal dentate gyrus in a rat model of global cerebral ischemia. *Neuroscience* 2000, 100(1):33-43.

[95] Miyagi M, Mikawa S, Hasegawa T, Kobayashi S, Matsuyama Y, Sato K: Bone morphogenetic protein receptor expressions in the adult rat brain. *Neuroscience* 2011, 176:93-109.

[96] Lim DA, Tramontin AD, Trevejo JM, Herrera DG, Garcia-Verdugo JM, Alvarez-Buylla A: Noggin antagonizes BMP signaling to create a niche for adult neurogenesis. *Neuron* 2000, 28(3):713-726.

[97] Mira H, Andreu Z, Suh H, Lie DC, Jessberger S, Consiglio A, San Emeterio J, Hortiguela R, Marques-Torrejon MA, Nakashima K *et al*: Signaling through BMPR-IA regulates quiescence and long-term activity of neural stem cells in the adult hippocampus. *Cell Stem Cell* 2010, 7(1):78-89.

[98] Sato T, Mikawa S, Sato K: BMP2 expression in the adult rat brain. *J Comp Neurol* 2010, 518(22):4513-4530.

[99] Mikawa S, Wang C, Sato K: Bone morphogenetic protein-4 expression in the adult rat brain. *J Comp Neurol* 2006, 499(4):613-625.

[100] Bonaguidi MA, Peng CY, McGuire T, Falciglia G, Gobeske KT, Czeisler C, Kessler JA: Noggin expands neural stem cells in the adult hippocampus. *J Neurosci* 2008, 28(37): 9194-9204.

[101] Fan X, Xu H, Huang Y, Cai W: A combined in situ hybridization and RT-PCR method to detect spatial and temporal patterns of Noggin gene expression in embryonic and postnatal rat hippocampus. *Brain Res Brain Res Protoc* 2004, 13(2):99-105.

[102] Gobeske KT, Das S, Bonaguidi MA, Weiss C, Radulovic J, Disterhoft JF, Kessler JA: BMP signaling mediates effects of exercise on hippocampal neurogenesis and cognition in mice. *PLoS One* 2009, 4(10):e7506.

[103] Lenzlinger PM, Morganti-Kossmann MC, Laurer HL, McIntosh TK: The duality of the inflammatory response to traumatic brain injury. *Mol Neurobiol* 2001, 24(1-3): 169-181.

[104] Annes JP, Munger JS, Rifkin DB: Making sense of latent TGFbeta activation. *J Cell Sci* 2003, 116(Pt 2):217-224.

[105] Jankowsky JL, Patterson PH: The role of cytokines and growth factors in seizures and their sequelae. *Prog Neurobiol* 2001, 63(2):125-149.

[106] McCoy MK, Tansey MG: TNF signaling inhibition in the CNS: implications for normal brain function and neurodegenerative disease. *J Neuroinflammation* 2008, 5:45.

[107] Logan A, Frautschy SA, Gonzalez AM, Sporn MB, Baird A: Enhanced expression of transforming growth factor beta 1 in the rat brain after a localized cerebral injury. *Brain Res* 1992, 587(2):216-225.

[108] Doyle KP, Cekanaviciute E, Mamer LE, Buckwalter MS: TGFbeta signaling in the brain increases with aging and signals to astrocytes and innate immune cells in the weeks after stroke. *J Neuroinflammation* 2010, 7:62.

[109] Stoll G, Schroeter M, Jander S, Siebert H, Wollrath A, Kleinschnitz C, Bruck W: Lesion-associated expression of transforming growth factor-beta-2 in the rat nervous system: evidence for down-regulating the phagocytic activity of microglia and macrophages. *Brain Pathol* 2004, 14(1):51-58.

[110] Dobbertin A, Schmid P, Gelman M, Glowinski J, Mallat M: Neurons promote macrophage proliferation by producing transforming growth factor-beta2. *J Neurosci* 1997, 17(14):5305-5315.

[111] Vincze C, Pal G, Wappler EA, Szabo ER, Nagy ZG, Lovas G, Dobolyi A: Distribution of mRNAs encoding transforming growth factors-beta1, -2, and -3 in the intact rat

brain and after experimentally induced focal ischemia. *J Comp Neurol* 2010, 518(18): 3752-3770.

[112] Villapol S, Yaszemski AK, Logan TT, Sanchez-Lemus E, Saavedra JM, Symes AJ: Candesartan, an Angiotensin II AT(1)-Receptor Blocker and PPAR-gamma Agonist, Reduces Lesion Volume and Improves Motor and Memory Function After Traumatic Brain Injury in Mice. *Neuropsychopharmacology* 2012.

[113] Villapol S, Flanders K, Symes AJ: TGF-β family proteins expression after traumatic brain injury in mice. unpublished.

[114] Lai M, Sirimanne E, Williams CE, Gluckman PD: Sequential patterns of inhibin subunit gene expression following hypoxic-ischemic injury in the rat brain. *Neuroscience* 1996, 70(4):1013-1024.

[115] Cate HS, Sabo JK, Merlo D, Kemper D, Aumann TD, Robinson J, Merson TD, Emery B, Perreau VM, Kilpatrick TJ: Modulation of bone morphogenic protein signalling alters numbers of astrocytes and oligodendroglia in the subventricular zone during cuprizone-induced demyelination. *J Neurochem* 2010, 115(1):11-22.

[116] Wang Y, Moges H, Bharucha Y, Symes A: Smad3 null mice display more rapid wound closure and reduced scar formation after a stab wound to the cerebral cortex. *Exp Neurol* 2007, 203(1):168-184.

[117] Wiessner C, Gehrmann J, Lindholm D, Topper R, Kreutzberg GW, Hossmann KA: Expression of transforming growth factor-beta 1 and interleukin-1 beta mRNA in rat brain following transient forebrain ischemia. *Acta Neuropathol* 1993, 86(5):439-446.

[118] Lehrmann E, Kiefer R, Finsen B, Diemer NH, Zimmer J, Hartung HP: Cytokines in cerebral ischemia: expression of transforming growth factor beta-1 (TGF-beta 1) mRNA in the postischemic adult rat hippocampus. *Exp Neurol* 1995, 131(1):114-123.

[119] Ma M, Ma Y, Yi X, Guo R, Zhu W, Fan X, Xu G, Frey WH, 2nd, Liu X: Intranasal delivery of transforming growth factor-beta1 in mice after stroke reduces infarct volume and increases neurogenesis in the subventricular zone. *BMC Neurosci* 2008, 9:117.

[120] Becker KJ, McCarron RM, Ruetzler C, Laban O, Sternberg E, Flanders KC, Hallenbeck JM: Immunologic tolerance to myelin basic protein decreases stroke size after transient focal cerebral ischemia. *Proc Natl Acad Sci U S A* 1997, 94(20):10873-10878.

[121] Wang X, Yue TL, White RF, Barone FC, Feuerstein GZ: Transforming growth factor-beta 1 exhibits delayed gene expression following focal cerebral ischemia. *Brain Res Bull* 1995, 36(6):607-609.

[122] Vivien D, Bernaudin M, Buisson A, Divoux D, MacKenzie ET, Nouvelot A: Evidence of type I and type II transforming growth factor-beta receptors in central nervous tissues: changes induced by focal cerebral ischemia. *J Neurochem* 1998, 70(6):2296-2304.

[123] Ishibashi S: [Immunomodulation by inducing tolerance to E-selectin and adult neurogenesis after stroke]. *Rinsho Shinkeigaku* 2010, 50(11):882-885.

[124] Martinez G, Carnazza ML, Di Giacomo C, Sorrenti V, Vanella A: Expression of bone morphogenetic protein-6 and transforming growth factor-beta1 in the rat brain after a mild and reversible ischemic damage. *Brain Res* 2001, 894(1):1-11.

[125] Haqqani AS, Nesic M, Preston E, Baumann E, Kelly J, Stanimirovic D: Characterization of vascular protein expression patterns in cerebral ischemia/reperfusion using laser capture microdissection and ICAT-nanoLC-MS/MS. *FASEB J* 2005, 19(13): 1809-1821.

[126] Li JJ, Lu J, Kaur C, Sivakumar V, Wu CY, Ling EA: Effects of hypoxia on expression of transforming growth factor-beta1 and its receptors I and II in the amoeboid microglial cells and murine BV-2 cells. *Neuroscience* 2008, 156(3):662-672.

[127] Huang RQ, Cheng HL, Zhao XD, Dai W, Zhuang Z, Wu Y, Liu Y, Shi JX: Preliminary study on the effect of trauma-induced secondary cellular hypoxia in brain injury. *Neurosci Lett* 2010, 473(1):22-27.

[128] Yin J, Sakamoto K, Zhang H, Ito Z, Imagama S, Kishida S, Natori T, Sawada M, Matsuyama Y, Kadomatsu K: Transforming growth factor-beta1 upregulates keratan sulfate and chondroitin sulfate biosynthesis in microglias after brain injury. *Brain Res* 2009, 1263:10-22.

[129] Acarin L, Gonzalez B, Castellano B: Neuronal, astroglial and microglial cytokine expression after an excitotoxic lesion in the immature rat brain. *Eur J Neurosci* 2000, 12(10):3505-3520.

[130] Mehta PS, Bruccoleri A, Brown HW, Harry GJ: Increase in brain stem cytokine mRNA levels as an early response to chemical-induced myelin edema. *J Neuroimmunol* 1998, 88(1-2):154-164.

[131] Bruccoleri A, Brown H, Harry GJ: Cellular localization and temporal elevation of tumor necrosis factor-alpha, interleukin-1 alpha, and transforming growth factor-beta 1 mRNA in hippocampal injury response induced by trimethyltin. *J Neurochem* 1998, 71(4):1577-1587.

[132] Krieglstein K, Strelau J, Schober A, Sullivan A, Unsicker K: TGF-beta and the regulation of neuron survival and death. *J Physiol Paris* 2002, 96(1-2):25-30.

[133] Boche D, Cunningham C, Gauldie J, Perry VH: Transforming growth factor-beta 1-mediated neuroprotection against excitotoxic injury in vivo. *J Cereb Blood Flow Metab* 2003, 23(10):1174-1182.

[134] Kim SH, Lim DJ, Chung YG, Cho TH, Lim SJ, Kim WJ, Suh JK: Expression of TNF-alpha and TGF-beta 1 in the rat brain after a single high-dose irradiation. *J Korean Med Sci* 2002, 17(2):242-248.

[135] Morgan TE, Nichols NR, Pasinetti GM, Finch CE: TGF-beta 1 mRNA increases in macrophage/microglial cells of the hippocampus in response to deafferentation and kainic acid-induced neurodegeneration. *Exp Neurol* 1993, 120(2):291-301.

[136] Aronica E, van Vliet EA, Mayboroda OA, Troost D, da Silva FH, Gorter JA: Upregulation of metabotropic glutamate receptor subtype mGluR3 and mGluR5 in reactive astrocytes in a rat model of mesial temporal lobe epilepsy. *Eur J Neurosci* 2000, 12(7): 2333-2344.

[137] Jarvela JT, Lopez-Picon FR, Plysjuk A, Ruohonen S, Holopainen IE: Temporal profiles of age-dependent changes in cytokine mRNA expression and glial cell activation after status epilepticus in postnatal rat hippocampus. *J Neuroinflammation* 2011, 8:29.

[138] Schachtrup C, Ryu JK, Helmrick MJ, Vagena E, Galanakis DK, Degen JL, Margolis RU, Akassoglou K: Fibrinogen triggers astrocyte scar formation by promoting the availability of active TGF-beta after vascular damage. *J Neurosci* 2010, 30(17): 5843-5854.

[139] Wyss-Coray T, Mucke L: Inflammation in neurodegenerative disease--a double-edged sword. *Neuron* 2002, 35(3):419-432.

[140] Wyss-Coray T, Feng L, Masliah E, Ruppe MD, Lee HS, Toggas SM, Rockenstein EM, Mucke L: Increased central nervous system production of extracellular matrix components and development of hydrocephalus in transgenic mice overexpressing transforming growth factor-beta 1. *Am J Pathol* 1995, 147(1):53-67.

[141] Fee DB, Sewell DL, Andresen K, Jacques TJ, Piaskowski S, Barger BA, Hart MN, Fabry Z: Traumatic brain injury increases TGF beta RII expression on endothelial cells. *Brain Res* 2004, 1012(1-2):52-59.

[142] Chang CF, Lin SZ, Chiang YH, Morales M, Chou J, Lein P, Chen HL, Hoffer BJ, Wang Y: Intravenous administration of bone morphogenetic protein-7 after ischemia improves motor function in stroke rats. *Stroke* 2003, 34(2):558-564.

[143] Harvey BK, Hoffer BJ, Wang Y: Stroke and TGF-beta proteins: glial cell line-derived neurotrophic factor and bone morphogenetic protein. *Pharmacol Ther* 2005, 105(2): 113-125.

[144] Hampton DW, Asher RA, Kondo T, Steeves JD, Ramer MS, Fawcett JW: A potential role for bone morphogenetic protein signalling in glial cell fate determination following adult central nervous system injury in vivo. *Eur J Neurosci* 2007, 26(11):3024-3035.

[145] Chou J, Harvey BK, Chang CF, Shen H, Morales M, Wang Y: Neuroregenerative effects of BMP7 after stroke in rats. *J Neurol Sci* 2006, 240(1-2):21-29.

[146] Mukerji SS, Katsman EA, Wilber C, Haner NA, Selman WR, Hall AK: Activin is a neuronal survival factor that is rapidly increased after transient cerebral ischemia and hypoxia in mice. *J Cereb Blood Flow Metab* 2007, 27(6):1161-1172.

[147] Zheng F, Adelsberger H, Muller MR, Fritschy JM, Werner S, Alzheimer C: Activin tunes GABAergic neurotransmission and modulates anxiety-like behavior. *Mol Psychiatry* 2009, 14(3):332-346.

[148] Tretter YP, Hertel M, Munz B, ten Bruggencate G, Werner S, Alzheimer C: Induction of activin A is essential for the neuroprotective action of basic fibroblast growth factor in vivo. *Nat Med* 2000, 6(7):812-815.

[149] Christensen KV, Leffers H, Watson WP, Sanchez C, Kallunki P, Egebjerg J: Levetiracetam attenuates hippocampal expression of synaptic plasticity-related immediate early and late response genes in amygdala-kindled rats. *BMC Neurosci* 2010, 11:9.

[150] Parent JM, Lowenstein DH: Seizure-induced neurogenesis: are more new neurons good for an adult brain? *Prog Brain Res* 2002, 135:121-131.

[151] Thored P, Heldmann U, Gomes-Leal W, Gisler R, Darsalia V, Taneera J, Nygren JM, Jacobsen SE, Ekdahl CT, Kokaia Z *et al*: Long-term accumulation of microglia with proneurogenic phenotype concomitant with persistent neurogenesis in adult subventricular zone after stroke. *Glia* 2009, 57(8):835-849.

[152] Arvidsson A, Collin T, Kirik D, Kokaia Z, Lindvall O: Neuronal replacement from endogenous precursors in the adult brain after stroke. *Nat Med* 2002, 8(9):963-970.

[153] Urrea C, Castellanos DA, Sagen J, Tsoulfas P, Bramlett HM, Dietrich WD: Widespread cellular proliferation and focal neurogenesis after traumatic brain injury in the rat. *Restor Neurol Neurosci* 2007, 25(1):65-76.

[154] Zhang RL, Zhang ZG, Chopp M: Neurogenesis in the adult ischemic brain: generation, migration, survival, and restorative therapy. *Neuroscientist* 2005, 11(5):408-416.

[155] Massouh M, Saghatelyan A: De-routing neuronal precursors in the adult brain to sites of injury: role of the vasculature. *Neuropharmacology* 2010, 58(6):877-883.

[156] Ekdahl CT: Microglial activation - tuning and pruning adult neurogenesis. *Front Pharmacol* 2012, 3:41.

[157] Monje ML, Toda H, Palmer TD: Inflammatory blockade restores adult hippocampal neurogenesis. *Science* 2003, 302(5651):1760-1765.

[158] Aarum J, Sandberg K, Haeberlein SL, Persson MA: Migration and differentiation of neural precursor cells can be directed by microglia. *Proc Natl Acad Sci U S A* 2003, 100(26):15983-15988.

[159] Ziv Y, Ron N, Butovsky O, Landa G, Sudai E, Greenberg N, Cohen H, Kipnis J, Schwartz M: Immune cells contribute to the maintenance of neurogenesis and spatial learning abilities in adulthood. *Nat Neurosci* 2006, 9(2):268-275.

[160] Cacci E, Ajmone-Cat MA, Anelli T, Biagioni S, Minghetti L: In vitro neuronal and glial differentiation from embryonic or adult neural precursor cells are differently affected by chronic or acute activation of microglia. *Glia* 2008, 56(4):412-425.

[161] Nakanishi M, Niidome T, Matsuda S, Akaike A, Kihara T, Sugimoto H: Microglia-derived interleukin-6 and leukaemia inhibitory factor promote astrocytic differentiation of neural stem/progenitor cells. *Eur J Neurosci* 2007, 25(3):649-658.

[162] Makwana M, Jones LL, Cuthill D, Heuer H, Bohatschek M, Hristova M, Friedrichsen S, Ormsby I, Bueringer D, Koppius A *et al*: Endogenous transforming growth factor beta 1 suppresses inflammation and promotes survival in adult CNS. *J Neurosci* 2007, 27(42):11201-11213.

[163] Battista D, Ferrari CC, Gage FH, Pitossi FJ: Neurogenic niche modulation by activated microglia: transforming growth factor beta increases neurogenesis in the adult dentate gyrus. *Eur J Neurosci* 2006, 23(1):83-93.

[164] Ma YP, Ma MM, Ge S, Guo RB, Zhang HJ, Frey WH, 2nd, Xu GL, Liu XF: Intranasally delivered TGF-beta1 enters brain and regulates gene expressions of its receptors in rats. *Brain Res Bull* 2007, 74(4):271-277.

[165] Mathieu P, Piantanida AP, Pitossi F: Chronic expression of transforming growth factor-beta enhances adult neurogenesis. *Neuroimmunomodulation* 2010, 17(3):200-201.

[166] Graciarena M, Depino AM, Pitossi FJ: Prenatal inflammation impairs adult neurogenesis and memory related behavior through persistent hippocampal TGFbeta1 downregulation. *Brain Behav Immun* 2010, 24(8):1301-1309.

[167] Samanta J, Alden T, Gobeske K, Kan L, Kessler JA: Noggin protects against ischemic brain injury in rodents. *Stroke* 2010, 41(2):357-362.

[168] Setoguchi T, Nakashima K, Takizawa T, Yanagisawa M, Ochiai W, Okabe M, Yone K, Komiya S, Taga T: Treatment of spinal cord injury by transplantation of fetal neural precursor cells engineered to express BMP inhibitor. *Exp Neurol* 2004, 189(1):33-44.

[169] Jablonska B, Aguirre A, Raymond M, Szabo G, Kitabatake Y, Sailor KA, Ming GL, Song H, Gallo V: Chordin-induced lineage plasticity of adult SVZ neuroblasts after demyelination. *Nat Neurosci* 2010, 13(5):541-550.

[170] Dow AL, Russell DS, Duman RS: Regulation of activin mRNA and Smad2 phosphorylation by antidepressant treatment in the rat brain: effects in behavioral models. *J Neurosci* 2005, 25(20):4908-4916.

[171] Caraci F, Battaglia G, Bruno V, Bosco P, Carbonaro V, Giuffrida ML, Drago F, Sortino MA, Nicoletti F, Copani A: TGF-beta1 pathway as a new target for neuroprotection in Alzheimer's disease. *CNS Neurosci Ther* 2011, 17(4):237-249.

[172] Li LY, Li JL, Zhang HM, Yang WM, Wang K, Fang Y, Wang Y: TGFbeta1 Treatment Reduces Hippocampal Damage, Spontaneous Recurrent Seizures, and Learning Memory Deficits in Pilocarpine-Treated Rats. *J Mol Neurosci* 2012.

[173] El Khoury J, Luster AD: Mechanisms of microglia accumulation in Alzheimer's disease: therapeutic implications. *Trends Pharmacol Sci* 2008, 29(12):626-632.

[174] Florio P, Gazzolo D, Luisi S, Petraglia F: Activin A in brain injury. *Adv Clin Chem* 2007, 43:117-130.

[175] Luisi S, Florio P, Reis FM, Petraglia F: Expression and secretion of activin A: possible physiological and clinical implications. *Eur J Endocrinol* 2001, 145(3):225-236.

[176] Goings GE, Sahni V, Szele FG: Migration patterns of subventricular zone cells in adult mice change after cerebral cortex injury. *Brain Res* 2004, 996(2):213-226.

[177] Sun J, Zhou W, Sha B, Yang Y: Ischemia induced neural stem cell proliferation and differentiation in neonatal rat involved vascular endothelial growth factor and transforming growth factor-beta pathways. *Brain Dev* 2010, 32(3):191-200.

[178] Cao QL, Zhang YP, Howard RM, Walters WM, Tsoulfas P, Whittemore SR: Pluripotent stem cells engrafted into the normal or lesioned adult rat spinal cord are restricted to a glial lineage. *Exp Neurol* 2001, 167(1):48-58.

[179] Wang Y, Cheng X, He Q, Zheng Y, Kim DH, Whittemore SR, Cao QL: Astrocytes from the contused spinal cord inhibit oligodendrocyte differentiation of adult oligodendrocyte precursor cells by increasing the expression of bone morphogenetic proteins. *J Neurosci* 2011, 31(16):6053-6058.

[180] Chang CF, Morales M, Chou J, Chen HL, Hoffer B, Wang Y: Bone morphogenetic proteins are involved in fetal kidney tissue transplantation-induced neuroprotection in stroke rats. *Neuropharmacology* 2002, 43(3):418-426.

[181] Voumvourakis KI, Antonelou R, Kitsos DK, Stamboulis E, Tsiodras S: TGF-beta/BMPs: crucial crossroad in neural autoimmune disorders. *Neurochem Int* 2011, 59(5):542-550.

[182] Loane DJ, Faden AI: Neuroprotection for traumatic brain injury: translational challenges and emerging therapeutic strategies. *Trends Pharmacol Sci* 2010, 31(12):596-604.

[183] Marklund N, Hillered L: Animal modelling of traumatic brain injury in preclinical drug development: where do we go from here? *Br J Pharmacol* 2011, 164(4):1207-1229.

[184] Wang Y, Lin SZ, Chiou AL, Williams LR, Hoffer BJ: Glial cell line-derived neurotrophic factor protects against ischemia-induced injury in the cerebral cortex. *J Neurosci* 1997, 17(11):4341-4348.

[185] Yagi T, Jikihara I, Fukumura M, Watabe K, Ohashi T, Eto Y, Hara M, Maeda M: Rescue of ischemic brain injury by adenoviral gene transfer of glial cell line-derived neurotrophic factor after transient global ischemia in gerbils. *Brain Res* 2000, 885(2):273-282.

[186] Seidenfaden R, Desoeuvre A, Bosio A, Virard I, Cremer H: Glial conversion of SVZ-derived committed neuronal precursors after ectopic grafting into the adult brain. *Mol Cell Neurosci* 2006, 32(1-2):187-198.

[187] Joannides AJ, Webber DJ, Raineteau O, Kelly C, Irvine KA, Watts C, Rosser AE, Kemp PJ, Blakemore WF, Compston A *et al*: Environmental signals regulate lineage

choice and temporal maturation of neural stem cells from human embryonic stem cells. *Brain* 2007, 130(Pt 5):1263-1275.

[188] Teng FY, Hor CH, Tang BL: Emerging cues mediating astroglia lineage restriction of progenitor cells in the injured/diseased adult CNS. *Differentiation* 2009, 77(2):121-127.

[189] Gomes WA, Mehler MF, Kessler JA: Transgenic overexpression of BMP4 increases astroglial and decreases oligodendroglial lineage commitment. *Dev Biol* 2003, 255(1): 164-177.

[190] Bain JM, Ziegler A, Yang Z, Levison SW, Sen E: TGFbeta1 stimulates the over-production of white matter astrocytes from precursors of the "brain marrow" in a rodent model of neonatal encephalopathy. *PLoS One* 2010, 5(3):e9567.

[191] Loane DJ, Byrnes KR: Role of microglia in neurotrauma. *Neurotherapeutics* 2010, 7(4): 366-377.

[192] Nishiyama A: Glial progenitor cells in normal and pathological states. *Keio J Med* 1998, 47(4):205-208.