Elucidating the role of bone morphogenetic protein-4 in regulating secondary injury mechanisms following traumatic spinal cord injury

By

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Abstract

Traumatic spinal cord injury (SCI) initiates secondary injury cascades that include, but are not limited to, inflammation, ischemia, reactive astrogliosis and apoptosis. These mechanisms contribute to tissue degeneration and limit neural regeneration and repair after injury. To optimize future therapeutic approaches, it is necessary to identify factors that regulate the early stages of these endogenous responses in order to limit their deleterious effects. In a pre-clinical *in vivo* model of clip compressive/contusive SCI in rats, we found that the active form of bone morphogenetic protein-4 (BMP4) is transiently and acutely upregulated after injury. BMP4 is a key signaling protein that regulates embryogenesis and neurodevelopment. BMP4 has also been implicated in modulating glial scarring and remyelination in sub-acute and chronic SCI in rodents. However, the role of BMP4 in early injury cascades after SCI is not well understood.

This work sought to elucidate the impact of BMP4 upregulation in acute SCI by utilizing two BMP inhibitors, noggin and LDN193189. We also utilized primary *in vitro* systems to determine the cell-specific effects of BMP4 on spinal cord neural stem/progenitor cells (NPCs), oligodendrocyte precursor cells (OPCs), astrocytes and neurons. Here, for the first time, we demonstrate that BMP4 promotes cleaved caspase-3 expression in cultured neurons and oligodendrocytes and that acute BMP inhibition attenuates apoptosis and lipid peroxidation after SCI. We also show that BMP4 directly inhibits oligodendrocyte differentiation in NPCs and OPCs *in vitro,* and BMP inhibition is sufficient to promote oligodendrocyte differentiation and preservation in acute SCI. Intriguingly, LDN193189 treatment also increases myelin thickness of remyelinated axons in chronically injured rats. Importantly, BMP4 potentiates the deposition of inhibitory chondroitin sulfate proteoglycans (CSPGs) by reactive astrocytes *in vitro*, and this can be attenuated with noggin or LDN193189 treatments in SCI rats. Despite these early benefits, our

work reveals that acute inhibition of BMP antagonism was not sufficient to improve long-term functional recovery in SCI rats.

Overall, our work provides new insights into the role of BMP4 in acute secondary injury mechanisms of SCI and recognizes BMP4 as a potential target for combinatorial approaches to improve endogenous repair processes in the injured spinal cord.

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This thesis is dedicated to my family

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Contribution of the authors

The contents of the introduction (Chapter 1) were prepared independently for this dissertation, and portions have been submitted for consideration of publication in a peer-reviewed research journal. Components of an article published in a peer-reviewed research journal are presented in Chapter 2, and the contributions of the authors are listed below.

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My contribution to this work includes concept and design, collection and/or assembly of data, data analysis and interpretation, manuscript writing and final approval of manuscript. I contributed to over 80% of the total experimental procedures, data and analysis. Manuscript preparation was done in collaboration with my supervisor, Soheila Karimi-Abdolrezaee. Hardeep Kataria contributed by assisting with primary cell cultures and behavioural analysis in SCI rats. Scott Dyck and Arsalan Alizadeh contributed by performing SCI surgeries. Pandian Nagakannan and Eftekhar Eftekharpour contributed with conceptional and practical guidance for in vivo cell death analysis, and James A. Thliveris performed tissue preparation and sectioning for in vivo semi-thin analysis.

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List of abbreviations

ACVR2b – Activin-A receptor type 2b

AD – Alzheimer's disease

ALS – Amyotrophic lateral sclerosis

AP-1 – Activator protein-1

ARE – Antioxidant response element

ATP – Adenosine tri-phosphate

BAMBI – BMP and activin membrane-bound inhibitor

BDNF – Brain-derived neurotrophic factor

BMP – Bone morphogenetic protein

BMPER – BMP endothelial regulator

BrdU – 5-bromo-2'-deoxyuridine

BSB – Brain spinal barrier

CBP – CREB-binding protein

CCL2 – C-C chemokine ligand 2

ChABC – Chondroitinase ABC

CNP – 2',3'-cyclin-nucleotide 3'-phosphodiesterase

CNS – Central nervous system

CNTF – Ciliary neurotrophic factor

COX-2 – Cyclooxygenase-2

CREB – cAMP response element-binding protein

CSPG – Chondroitin sulfate proteoglycans

CT-1 – Cardiotrophin-1

CtBP – C-terminal binding protein

CXCL1/2 – C-X-C motif chemokine ligand-1 and -2

DAMP – Damage associated molecular pattern

DAPI – 4,6-diamidino-2-phenylindole

dI1-6 – Dorsal and intermediate interneurons (1-6)

DISC – Death-inducing signaling complex

DMSO – Dimethyl sulfoxide

DPI – Days post-injury

DRG – Dorsal root ganglion

E2F – E2 factor

EAE – Experimental autoimmune encephalomyelitis

ECM – Extracellular matrix

EGF – Epidermal growth factor

ER – Endoplasmic reticulum

FASR – Fas-ligand receptor

FBS – Fetal bovine serum

FGF – Fibroblast growth factor

FOP – Fibrodysplasia ossificans progressive

GABA – Gamma aminobutyric acid

GAG – Glycosaminoglycan

GAPDH – Glyceraldehyde-3-phosphate dehydrogenase

GDA^{BMP4} – Glial restricted precursor-derived astrocytes (BMP4-differentiated)

GDNF – Glial derived neurotrophic factor

GFAP – Glial fibrillary acidic protein

GPI – Glycophosphatidylinositol

H&E – Hematoxylin and eosin

HDAC3 – Histone deacetylase-3

HLH – Helix-loop-helix

HMGB1 – High mobility group box factor-1

4-HNE – 4-Hydroxynonenal

HOX – Homeobox

HRP – Horseradish peroxidase

HSPG – Heparin sulfate proteoglycan

HtrA1 – High temperature requirement A-1

Id1/2/4 – Inhibitors of differentiation-1, -2 or -4

IL-1β – Interleukin-1-beta

IL-1R – Interleukin-1 receptor

IL-8 – Interleukin-8

IL-10 – Interleukin-10

I-Smad – Inhibitory Smads (6/7)

JNK – c-Jun N-terminal kinase

LAR – Leukocyte antigen receptor

LFB – Luxol fast blue

LIF – Leukemia inhibitory factor

MAPK – Mitogen-activated protein kinase

MBP – Myelin basic protein

MEF-2A – Myocyte enhancer factor 2A

miRNA - MicroRNA

MMP – Matrix metalloproteinases

MN – Motor neuron

MPSS – Methylprednisolone sodium succinate

Msx2 – Msh homeobox gene-2

NADPH – Nicotinamide adenine dinucleotide phosphate

NF1A – Nuclear factor 1A

NF-κB – Nuclear factor-κB

NGR1/3 – Nogo receptors-1 or -3

NGS – Normal goat serum

NPC – Neural stem/progenitor cell

Nrf2 – Nuclear factor E2-related factor 2

NT3 – Neurotrophin-3

OPC – Oligodendrocyte precursor cell

PARP1 – Poly-ADP-ribose polymerase 1

PD – Parkinson's disease

PFA – Paraformaldehyde

PI3K – Phosphoinositide-3-kinase

PLP – Proteolipid protein

PNN – Perineuronal nets

PTPσ – Protein tyrosine phosphatase-sigma

PVDF – Polyvinyl fluoride

RGC – Retinal ganglion cells

RGM – Repulsive guidance molecules

RNS – Reactive nitrogen species

ROS – Reactive oxygen species

SC – Schwann cells

SCI – Spinal cord injury

SD – Sprague Dawley

Shh – Sonic hedgehog

SIP1 – Smad-interacting protein 1

Smurf1 – Smad ubiquitin regulatory factor-1

SP1 – Specificity protein-1

Srf – Serum response factor

STAT3 – Signal transducer and activator of transcription-3

SVD – Sub-cortical small vessel disease

SVZ – Sub-ventricular zone

TBI – Traumatic brain injury

TGFβ – Transforming growth factor-beta

TGN – Trans-Golgi network

TNFα – Tumour necrosis factor-alpha

TNFR1 – TNFα receptor-1

UTR – Untranslated region

Chapter 1: Introduction

1.1 Pathophysiology of traumatic spinal cord injury

1.1.1 Epidemiology and clinical impact

Spinal cord injury (SCI) can drastically impair sensory and motor functions depending on the level and severity of trauma. SCI patients also experience an imbalance between sympathetic and parasympathetic feedback loops, referred to as autonomic dysreflexia, resulting in hypertension and bradycardia. For most of human history, the cumulative burden of these deficits contributed to a high morbidity and shortened life span for patients. However, recent advances in clinical management of SCI through surgical decompression of the injured spinal cord within the first 24 hr post-injury has improved functional outcome and shortened the duration of hospitalization (Fehlings et al., 2012; Dvorak et al., 2015). Modern advancements in surgical procedures, rehabilitation and long-term care have also improved survival and enabled a handi-accessible lifestyle with relative self-sufficiency in SCI patients. Despite these advances, there is no clinically approved treatment to significantly improve spinal cord repair processes and regain the impaired sensory and motor functions in SCI patients. There are approximately 325,000 Canadians and 27 million individuals globally with SCI (James et al., 2019). SCI is most commonly caused by vehicular or sport-related injuries, falls or violence, with an annual incidence rate of 13-25 new patients per 100,000 individuals (Singh et al., 2014; James et al., 2019). In Canada, the estimated lifetime economic burden for each SCI patient ranges between $1.47-$3.03 million ($2.67 billion/year nationally) depending on the severity of injury (Krueger et al., 2013). Therefore, SCI continues to have major socioeconomic impacts on the patients, their families and the health care system, and new therapeutic strategies are required to further improve the quality of life for SCI patients (Krueger et al., 2013; Williams and Murray, 2015).

1.1.2 Primary spinal cord injury

Traumatic SCI has been divided into two major events: Primary and secondary injury. Primary injury is the tissue damage immediately following physical trauma to the spinal column, predominantly caused by a violent fall or vehicular and sports-related accident (Lee et al., 2014). SCI is classified as either a complete or incomplete injury depending on the degree of tissue sparing at the lesion site, which greatly influences the degree of recovery in patients (Kirshblum et al., 2011). There are several types of experimental SCI models that each have their own distinct advantages for studying pathological responses and repair. For example, the complete or dorsal/lateral transection SCI model is suitable for evaluating axonal regeneration and plasticity (Alizadeh et al., 2019). However, the contusive-compressive SCI model is most clinically relevant as it simulates the most prevalent type of injury observed in human patients (Poon et al., 2007). Physical trauma to the spinal column can cause vertebrae to dislocate or fracture, thereby compressing or lacerating the spinal cord and disrupting homeostatic functions (Sekhon and Fehlings, 2001). The trauma causes neuronal and glial cell death at the impact site, severing of motor and sensory axons, vascular disruption and loss of blood-spinal-barrier (BSB) integrity (Alizadeh et al., 2019). This results in acute neurodegeneration that culminates in the formation of a cavity at the injury site chronically, which is commonly observed in human SCI pathophysiology (Wozniewicz et al., 1983). Rats are advantageous animals for studying SCI pathophysiology as they are a small and economic alternative to larger porcine or primate models (Nardone et al., 2017), and they share similar pathological features (e.g. cavitation) and regenerative capacity observed in primate and human SCI (Kjell and Olson, 2016). In contrast, mice exhibit several histopathological hallmarks that are distinct from rats and humans, including their inflammatory and fibrotic responses (Sroga et al., 2003; Byrnes et al., 2010). Overall, these primary immediate

traumatic events are followed by a plethora of secondary injury mechanisms that contribute to progressive neurodegeneration and limited repair or recovery, which will be discussed in the following section.

1.1.3 Secondary injury mechanisms

Secondary injury mechanisms are cellular processes that contribute to progressive tissue degeneration in sub-acute and chronic stages of SCI; these include ischemia, hemorrhaging, inflammation, glial scarring and cell death (Alizadeh et al., 2019). After injury, there is an efflux of intracellular stressors into the extracellular space from ruptured cells (e.g. glutamate and adenosine tri-phosphate (ATP)), which can aggravate these secondary injury mechanisms and induce cell death in surviving cells (Ankarcrona et al., 1995; Matute et al., 2007). These signals activate resident microglia and astrocytes, which together with recruited peripheral immune cells, potentiate inflammation by releasing pro-inflammatory cytokines, chemokines and free radicals in the lesion (David and Kroner, 2011; Choi et al., 2014). Reactive astrocytes are one of several cell types that construct the glial scar, a dense impenetrable barrier around the lesion that prevents unrestricted diffusion of these cytotoxic factors to neighboring healthy tissue (Wanner et al., 2013). However, surviving neurons and oligodendroglia are still vulnerable to delayed cell death (Springer et al., 1999; Grossman et al., 2001), and contribute to progressive degeneration of neighboring healthy tissue that limits functional recovery after SCI (Alizadeh et al., 2019). The following sections will focus on secondary injury mechanisms and specific cell populations in SCI pathophysiology relevant to my thesis (Fig. 1.1), though a broader overview can be found here (Alizadeh et al., 2019).

1.1.3.1 Neuroinflammation

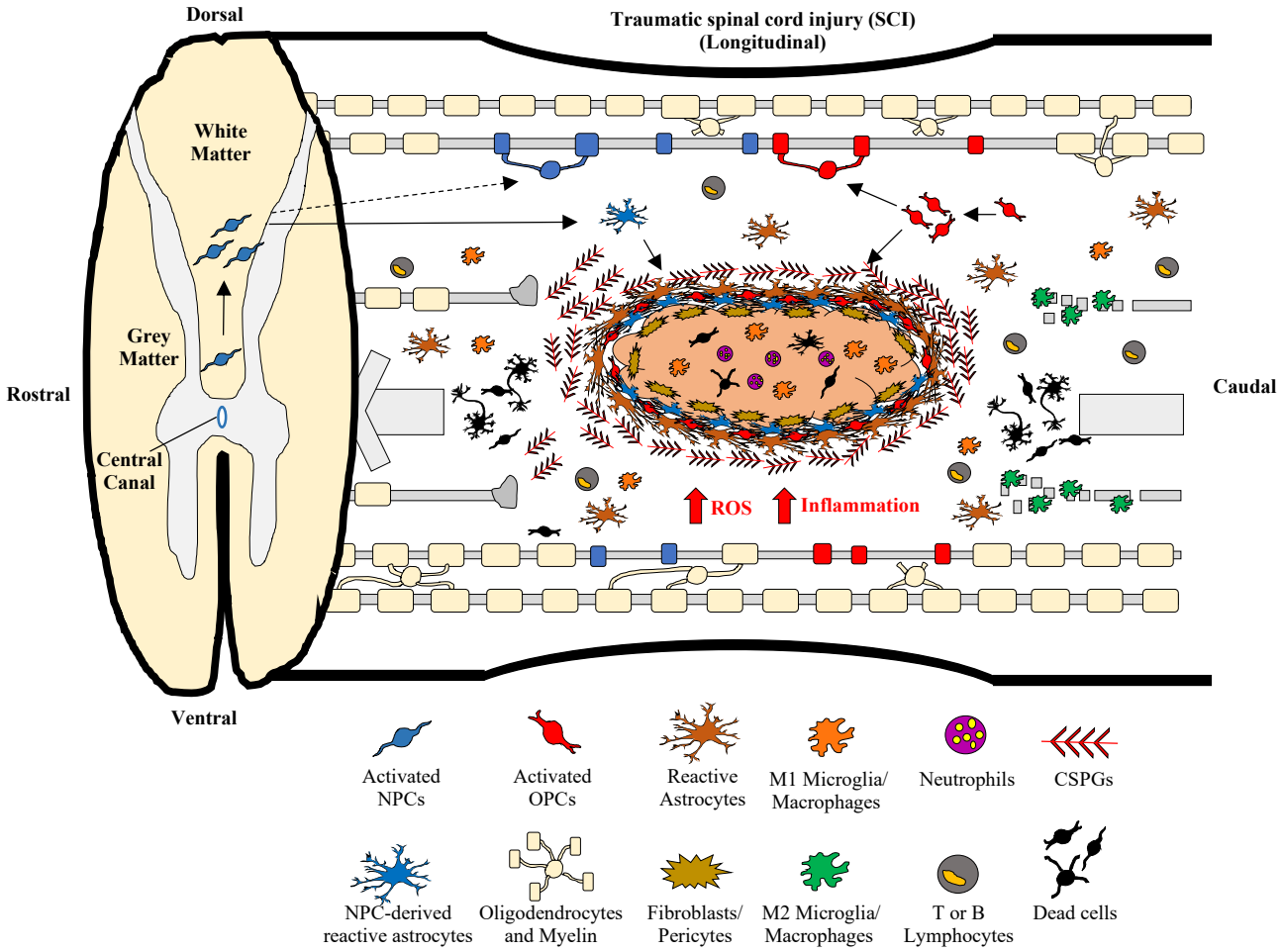
SCI elicits complex innate and adaptive inflammatory responses that are regulated by local and infiltrating cells at the lesion site. In SCI innate immunity is mediated by reactive astrocytes and microglia and infiltrating monocyte-derived macrophages and neutrophils (Alizadeh et al., 2019). These first responders also trigger the adaptive immune response by recruiting and activating T and B lymphocytes (Ankeny and Popovich, 2009). I will briefly discuss the role of innate immunity after traumatic SCI, but the contribution of lymphocytes to SCI neuroinflammation, neuropathy and tissue degeneration is detailed here (Ankeny and Popovich, 2009).

Neutrophils are attracted to the lesion site by pro-inflammatory chemokines released acutely by reactive astrocytes and microglia, including chemokine C-C motif ligand-2 (CCL2) and C-X-C motif chemokine ligand-1 and -2 (CXCL1/2) (Pineau et al., 2010). Reactive astrocytes are a predominant source of these essential chemokines in response to interleukin-1 receptor (IL-1R) activation and downstream MyD88 signaling (Pineau et al., 2010). Neutrophils promote inflammatory cell recruitment and activation by releasing interleukin-8 (IL-8), complement factors and matrix metalloproteinases (MMPs) to degrade the extracellular matrix (ECM) (Baggiolini et al., 1989; Nguyen et al., 2007; Nguyen et al., 2008). They also contribute to a cytotoxic microenvironment by secreting tumor necrosis factor-α (TNFα) and reactive oxidative species (ROS), and indirectly promote neuronal cell death via cell-to-cell contact (Dinkel et al., 2004; Nguyen et al., 2007). Activated neutrophils can contribute to clearing cellular debris via phagocytosis after peripheral nerve injury (Lindborg et al., 2017), but it remains unknown if they have a similar role after SCI.

Microglia are resident immune cells that are uniformly distributed in non-overlapping and dynamic territories in the central nervous system (CNS) (Nimmerjahn et al., 2005). In contrast,

macrophages infiltrate the CNS after injury, and they are either derived from circulating monocytes or peripheral tissues (meninges, choroid plexus, perivascular space) (David and Kroner, 2011). Resting microglia/macrophages can be differentiated by their relative CD45 expression, but they were previously considered indistinguishable after injury based on morphology (David and Kroner, 2011). However, recent studies have characterized a novel microglia-specific surface marker (TMEM119) that can distinguish reactive microglia from macrophages in adult mouse and human CNS (Bennett et al., 2016; Ruan et al., 2020), suggesting that additional defining markers may be identified in the future. Reactive microglia transition from a stellate-to-amoeboid morphology and migrate to the lesion site in response to extracellular nitric oxide (NO), ATP or glutamate (Dibaj et al., 2010; Sieger et al., 2012). Reactive microglia/macrophages are recruited in two distinct waves during sub-acute and chronic stages of injury (Beck et al., 2010), and their phenotypes can shift along a spectrum between pro-inflammatory (M1) and anti-inflammatory (M2a, M2b, M2c) profiles in response to environmental cues (David and Kroner, 2011; Kroner et al., 2014). M1 microglia/macrophages release a host of pro-inflammatory factors, free radicals and MMPs (Cross and Woodroofe, 1999; Pineau and Lacroix, 2007; Bermudez et al., 2016), while M2 microglia/macrophages aid in promoting regeneration by engulfing inhibitory myelin debris or secreting anti-inflammatory cytokines such as IL-10 or transforming growth factor-β (TGFβ) (Zhou et al., 2012; Greenhalgh and David, 2014; Dyck et al., 2018) (Fig. 1.1). The proportion of M1 and M2 microglia/macrophages is relatively equal during the first week after SCI in mice, but they become more pro-inflammatory over time due to the injury microenvironment (Kigerl et al., 2009). Since these cells have an important role in the repair process, it would be therapeutically advantageous to divert microglia/macrophages towards a M2 phenotype rather than limit their infiltration into the lesion.

Figure 1.1. Pathological responses of key cell types after traumatic SCI



The spinal cord is structurally divided into an interior "grey matter", which is highly vascularized and consists of neuronal cell bodies and glia, and an exterior "white matter" where ascending and descending axonal tracts are orientated. Astrocytes and microglia are located throughout the spinal cord, but most of the oligodendrocyte precursor cells (OPCs) and mature oligodendrocytes are found in the white matter. Neural stem/progenitor cells (NPCs) reside in the ependymal cell layer where they infrequently proliferate for self-renewal. Glial cells have several important functions that are necessary to maintain CNS homeostasis, but SCI triggers a drastic change in the microenvironment that initiates several secondary injury mechanisms. Physical trauma severs axons and damages oligodendrocytes at the lesion site, leading to demyelination and functional loss below the level of injury. Reactive astrocytes, microglia and infiltrating macrophages and lymphocytes potentiate inflammation and cell death by secreting pro-inflammatory and stress-inducing factors. Both activated NPCs and OPCs are highly proliferative and migrate towards the lesion after injury; they either succumb to cell death or they are biased to astrocyte differentiation by inhibitory factors in the microenvironment. To reduce further tissue degeneration, the lesion is surrounded by fibrotic/glial scar tissue constructed by reactive endogenous and NPC-derived reactive astrocytes, OPCs, fibroblasts and pericytes.

1.1.3.2 Oxidative stress and lipid peroxidation

Reactive oxygen and nitrogen species (ROS/RNS) are free radicals routinely produced as a mitochondrial by-product of ATP production via the electron transport chain or by nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity (Block et al., 2007; Shadel and Horvath, 2015). Common examples of ROS/RNS include superoxide (O_2^-), hydroxyl radical (OH^-), nitric oxide (NO), peroxynitrite ($ONOO^-$) and hydrogen peroxide (H_2O_2) (Shadel and Horvath, 2015). These free radicals serve a physiological role in the immune system, synapse remodelling and memory formation, and as intracellular second messenger molecules (Massaad and Klann, 2011; Morgan and Liu, 2011; Shadel and Horvath, 2015). Free radicals must be neutralized by antioxidant enzymes to prevent them from disrupting mitochondrial metabolism or damaging/misfolding proteins, lipids and nucleic acids (Cai et al., 2009; Vaishnav et al., 2010; Nunomura et al., 2012). Neurons are particularly susceptible to ROS-mediated cell death as their antioxidant NAD+ levels are quickly depleted under stress, which triggers mitochondria permeabilization and cell death mediated by poly(ADP-ribose) polymerase-1 (PARP1) (Du et al., 2003). Neuronal metabolism is highly dependent on mitochondrial oxidative phosphorylation to generate ATP (Herrero-Mendez et al., 2009), which is why excessive ROS can disrupt neurite outgrowth, glutamate and glucose uptake, and autophagic degradation of damaged proteins and organelles (Keller et al., 1997; Neely et al., 1999; Nagakannan et al., 2016).

SCI triggers a significant increase in oxidative stress that overwhelms anti-oxidant defenses during the first two weeks after injury (Vaziri et al., 2004; Visavadiya et al., 2016). This response is potentiated by inflammatory factors that stimulate ROS production in reactive microglia and astrocytes, neutrophils and macrophages (Bao et al., 2009; Sheng et al., 2013; Parajuli et al., 2015) (Fig. 1.1). The generation of O_2- and NO leads to the spontaneous formation of more potent

oxidizing species (e.g. $ONOO^-$), which initiate a chain reaction of lipid peroxidation that ultimately forms by-products like 4-hydroxynonenal (4-HNE), acrolein or 3-nitrotyrosine (Hall et al., 2016). These peroxidised phospholipids promote apoptotic cell death by accumulating in mitochondrial membranes and significantly impairing cellular respiration and calcium buffering, and by inducing protein misfolding (Singh et al., 2006; Cai et al., 2009; Vaishnav et al., 2010). Administering antioxidants can alleviate oxidative stress and attenuate tissue degeneration, as reviewed earlier (Jia et al., 2012), suggesting it is a viable therapeutic strategy for SCI.

1.1.3.3 Cell death

Acute SCI is characterized by progressive degeneration and loss of neurons and glia (Grossman et al., 2001). Tissue is progressively degenerated as cells are overwhelmed by inflammation, oxidative stress, glutamate and ATP excitotoxicity (Hermann et al., 2001; Du et al., 2003; Wang et al., 2004). Chronic tissue degeneration results in cavitation at the lesion site as observed in rat and human SCI (Poon et al., 2007). There are two general types of cell death that are differentiated by their underlying mechanisms: necrosis and programmed cell death (Galluzzi et al., 2018). Necrosis is the uncontrolled breakdown of cells overwhelmed by traumatic stimuli after injury, while programmed cell death is a regulated process that is divided into subtypes based on their underlying stimuli or mechanism, including apoptosis, autophagy, or ferroptosis (Galluzzi et al., 2018).

Necrosis is the earliest form of cell death observed in neurons and glia impacted by immediate physical trauma or toxic stimuli such as inflammation, ischemic stress and glutamate excitotoxicity (Vanlangenakker et al., 2008). Necrotic cells are characterized by mitochondrial swelling and clustering, and the permeabilization and release of lysosomal and cytoplasmic contents (Golstein

and Kroemer, 2007). A significant consequence of necrosis is the release of pro-inflammatory factors or damage associated molecular patterns (DAMPs), including high mobility group box-1 (HMGB1) and ATP, which can induce apoptotic cell death in neighboring cells (Matute et al., 2007; Kigerl et al., 2018). Apoptosis is programmed cell death mediated by proteolytic caspases and dependent on ATP consumption. It is characterized by condensed chromatin, cytoplasmic shrinkage and nuclear fragmentation, and blebbing/invagination of the plasma membrane to form small vesicles known as apoptotic bodies (Galluzzi et al., 2018). Apoptotic bodies are engulfed by phagocytes and digested via lysosomal degradation, which removes damaged or aged cells with negligible disturbance of neighboring cells (Poon et al., 2014). Apoptosis is stimulated by external or internal pathways triggered by stressors such as inflammation, ROS, DNA damage, or nutrient deprivation (Galluzzi et al., 2018).

The extrinsic apoptotic pathway is initiated by activated death receptors, including Fas-ligand receptor (FASR) or TNF receptor superfamily 1A (TNFR1), which induce the formation of a death-inducing signaling complex (DISC) (Micheau and Tschopp, 2003; Scott et al., 2009). DISC proteolytically cleaves caspase-8 that in turn activates downstream effectors caspase-3 and -7, which directly facilitate protein degradation, DNA fragmentation, organellar breakdown and the formation of apoptotic bodies (Sebbagh et al., 2001; Slee et al., 2001). In contrast, the intrinsic pathway is initiated by cellular stresses that create a disequilibrium between pro-apoptotic BH3-proteins (e.g. BID, BIM, PUMA) and pro- or anti-apoptotic members of the BCL2 protein family (e.g. BAX and BAK or BCL2, respectively) embedded in the mitochondrial outer membrane (Galluzzi et al., 2018). Briefly, activated BAX and BAK oligomerize into pore-like structures that increase permeability of the mitochondrial outer membrane and leak cytochrome C and stored Ca^{2+} into the cytoplasm (Korsmeyer et al., 2000). This process facilitates apoptosis either by activating

a caspase-9-dependent apoptosome or by triggering caspase-independent cell death mechanisms (Galluzzi et al., 2018). As detailed in a later section, neurons and oligodendrocytes are particularly susceptible to cleaved caspase-3-mediated apoptosis within hours after SCI (Springer et al., 1999). Previous studies show that promoting an anti-inflammatory microenvironment or bolstering autophagy can improve neuronal survival and functional recovery in SCI rats (Festoff et al., 2006; Zhou et al., 2009; Tang et al., 2014).

Autophagy is an essential cellular process that facilitates the degradation of ubiquitinated or dysfunctional proteins and organelles for the purpose of recycling substrates or disposing of noxious factors (Galluzzi et al., 2018). Emerging evidence shows that the autophagic process can be disrupted in cells after SCI, either by limiting the formation of autophagosomes or their ability to fuse with protease-rich lysosomes to facilitate degradation (i.e. autophagic flux). For example, there is evidence that autophagic flux is disrupted between 1-7 days post-SCI in mice as indicated by accumulated LC3-I/II (membrane-bound component of autophagosomes) and p62 (recruits ubiquitinated or damaged proteins/organelles to phagophores) (Liu et al., 2015; Muñoz-Galdeano et al., 2018). Interestingly, there are conflicting reports that boosting the autophagic response can positively or negatively impact neuronal survival, tissue preservation and functional recovery in cultured neurons or in SCI mice (Viscomi et al., 2012; Walker et al., 2012; Tang et al., 2014; Wang et al., 2014b; Wang et al., 2018). Muñoz-Galdeano and colleagues recently demonstrated that autophagy was disrupted in neurons, oligodendrocytes, reactive microglia and astrocytes after SCI; however, reactive astrocytes were uniquely resilient as they were able to compensate injury-induced impairment of autophagy by upregulating Beclin-1 (essential initiator of autophagy) (Muñoz-Galdeano et al., 2018). Impaired autophagy can induce apoptosis in neurons in a mechanism that is at least partially mediated via cleaved caspase-12 and endoplasmic reticulum

(ER) stress (Liu et al., 2015), yet autophagic cell death is also prevalent in neurons after SCI in mice (Kanno et al., 2009). Collectively, these findings suggest there is a balance in autophagic flux that must be restored in a cell specific manner under pathological conditions, and that elucidating the mechanisms that regulate the balance and interactions between cell death and autophagy can help develop new neuroprotective and pro-regenerative strategies for SCI.

1.1.3.4 Scar formation

After SCI, the first reparative step is to surround the lesion core in dense scar tissue to limit the spread of damage. Several cell types are recruited to form this physical barrier and drastically remodel the ECM around the lesion. The first stage consists of perivascular fibroblasts and pericytes depositing a matrix of connective tissue (e.g. fibronectin, collagen, laminin) to form a fibrotic scar in the lesion core (Göritz et al., 2011; Soderblom et al., 2013; Zhu et al., 2015a). The fibrotic scar serves as a foundation that guides the construction of the glial scar (Hara et al., 2017), which is formed by pre-existing and precursor-derived reactive astrocytes, microglia and NG2+ oligodendrocyte precursor cells (OPCs) (Adams and Gallo, 2018) (Fig. 1.1). Infiltrating macrophages also regulate the progression of fibrosis and gliosis by expressing MMP13 and transforming growth factor-β (TGFβ) after SCI (Shechter et al., 2011; Zhu et al., 2015b; Song et al., 2019).

Signal transducer and activator of transcription-3 (STAT3) signaling is an essential pathway for regulating reactive astrogliosis (Herrmann et al., 2008). Reactive astrocytes are proliferative, hypertrophic and overlap their processes into a dense barrier surrounding the fibrotic scar and lesion. STAT3-deficient mice have impaired glial scarring and fail to corral inflammatory cells at the lesion site (Wanner et al., 2013), indicating that the glial scar is beneficial as it limits the spread

of inflammation and inhibitory cellular debris to neighboring healthy tissue. When glial scarring is accelerated by increasing astrocytic STAT3-phosphorylation, either by ablating Socs3 (a negative regulator of STAT3 signaling) or down-regulating β-integrin, it results in a more compact lesion and increases the survival of myelinated axons and oligodendrocytes (Okada et al., 2006; Renault-Mihara et al., 2011). In contrast, there is evidence that suppressing reactive astrogliosis by knocking out STAT3 or administering ganciclovir, an anti-viral agent that ablates proliferative reactive astrocytes, increases inflammation and tissue degeneration (Faulkner et al., 2004; Okada et al., 2006).

Scar-forming astrocytes remodel the ECM by upregulating chondroitin sulfate proteoglycans (CSPGs). These are a collection of proteoglycans consisting of a core protein fitted with negatively charged and repetitive glycosaminoglycan (GAG) sidechains (Sherman and Back, 2008). CSPGs can bind to three known receptors: Leukocyte antigen receptor (LAR), protein tyrosine phosphatase-sigma (PTPσ), and Nogo receptors-1 or -3 (NGR1/3) (Shen et al., 2009; Fisher et al., 2011; Dickendesher et al., 2012). CSPGs have an integral role in guiding neuronal growth during development and stabilizing synaptic connections between neurons in perineuronal nets (PNN) (Snow et al., 1990; Corvetti and Rossi, 2005; Dyck and Karimi-Abdolrezaee, 2015). CSPG expression is upregulated after SCI by reactive astrocytes and microglia, activated OPCs, infiltrating Schwann cells (SCs), pericytes and macrophages (Asher et al., 2000; Jones et al., 2002; McTigue et al., 2006; Gao et al., 2010; Hesp et al., 2018). CSPG expression can be induced in reactive astrocytes by several factors including TGFβ, epidermal growth factor (EGF) and interleukin-1β (IL-1β) (Smith and Strunz, 2005), and CSPG subtypes can be differentially expressed and persist chronically up to six months after SCI in rats (Tang et al., 2003). High concentrations of CSPGs are detrimental to SCI recovery as they inhibit axonal regeneration,

oligodendrogenesis and promote a pro-inflammatory microenvironment (Filous et al., 2014; Lang et al., 2015b; Dyck et al., 2018; Dyck et al., 2019). A principal mechanism shared by LAR and PTPσ receptors is RhoA/ROCK-mediated inhibition of Akt and Erk1/2 signaling (Dyck et al., 2015; Ohtake et al., 2016), and this redundancy explains why knocking out either LAR or PTPσ receptor can improve axonal regeneration and locomotor recovery in SCI transgenic mice (Shen et al., 2009; Fry et al., 2010; Fisher et al., 2011; Xu et al., 2015). Evidence suggests that CSPGs inhibit axonal regeneration and induce growth cone collapse via several different mechanisms that include destabilizing neuronal microfilament structures (Arimura et al., 2005; Fisher et al., 2011), inhibiting the secretion of CSPG-degrading proteases (Tran et al., 2018), or inhibiting cortactin-dependent autophagy (Sakamoto et al., 2019; Tran et al., 2020). There are several therapeutic strategies for neutralizing CSPGs including enzymatic digestion by Chondroitinase ABC (ChABC) (Bradbury et al., 2002; Massey et al., 2006; Karimi-Abdolrezaee et al., 2010; Carter et al., 2011; Karimi-Abdolrezaee et al., 2012; Alluin et al., 2014b; DePaul et al., 2017), pharmacological inhibition of TGFβ or LAR and PTPσ receptors (Kohta et al., 2009; Fisher et al., 2011; Lang et al., 2015a; Dyck et al., 2018; Dyck et al., 2019) or inhibiting essential biosynthetic enzymes for CSPGs (Laabs et al., 2007; Keough et al., 2016).

In contrast to previous literature, Anderson and colleagues recently reported a study that concluded the astrocytic scar is essential for promoting axonal regeneration (Anderson et al., 2016). They used two distinct *in vivo* SCI mouse models that eliminated the astrocytic scar and observed worse axonal dieback and spontaneous axonal regeneration (Anderson et al., 2016). When they administered brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT3) via implanted hydrogel, they were only able to stimulate axons to regenerate through and beyond the lesion when the astrocytic scar was intact (Anderson et al., 2016). They argued that reactive

astrocytes are a significant source of several axon-permissive factors (e.g. laminin, fibronectin) and growth supportive CSPGs (e.g. NG2, neuroglycan-C) (Nakanishi et al., 2006; Yang et al., 2006; Anderson et al., 2016). However, the lack of regeneration in the absence of astrocytes could also be attributed to larger lesion volumes and presumably greater infiltration of inflammatory cells (Wanner et al., 2013; Anderson et al., 2016). Importantly, they showed that eliminating scar forming astrocytes had no effect on overall CSPG expression in their SCI models (Anderson et al., 2016), and that specific CSPG sub-types (brevican, aggrecan, neurocan) were upregulated in STAT3-CKO mice relative to wild-type SCI mice (Anderson et al., 2016). NG2 is innately inhibitory to axonal regeneration in the absence of growth supportive matrix such as fibronectin and laminin, which are co-expressed by cells such as reactive astrocytes and NG2+ glia (Dou and Levine, 1994; Ughrin et al., 2003; Busch et al., 2010; Filous et al., 2014). In fact, Anderson and colleagues recently showed that these axon-supporting matrices are necessary to facilitate growth factor-induced axonal regeneration in the presence of astrocytic scar in SCI mice (Anderson et al., 2018). Therefore, further investigation is required to elucidate how inflammation or the distribution of CSPGs and growth supporting matrices may contribute to the lack of axonal regeneration observed in their previous work (Anderson et al., 2016). Overall, emerging evidence suggests that it is therapeutically advantageous to use a combinatorial approach to retain the astrocytic scar while neutralizing inhibitory CSPGs and supplementing neurons with pro-regenerative and chemotactic factors.

1.2 Endogenous cell responses and repair after SCI

1.2.1 Neurons and oligodendrocytes

Physical trauma to the spinal cord induces immediate and progressive degeneration of local neurons and oligodendrocytes as well as long projecting ascending and descending pathways that result in motor, sensory and autonomic impairment. Neurons are particularly susceptible to cleaved caspase-3-mediated apoptosis as early as 1 hr after SCI (Springer et al., 1999). The distal portion of the severed axon undergoes progressive anterograde (Wallerian) degeneration, which is mediated by Ca^{2+}-activated calpain-dependent proteolysis and phagocytosis of myelin debris by macrophages (Ma et al., 2013; Chen et al., 2015). The proximal segment of the axon remains connected to the soma of distal supraspinal neurons and can potentially regenerate by restructuring the cytoskeletal architecture into a growth cone (Hill, 2017). Interestingly, intracellular Ca^{2+} transients and calpain-dependent proteolysis are also required for cytoskeletal restructuring and growth cone formation (Gitler and Spira, 1998; Kamber et al., 2009). The ability of axons to form a competent growth cone can differ based on the age and type of neuron, as reflected by their relative expression of pro-regenerative markers (e.g. GAP-43) (Chierzi et al., 2005). For example, dorsal root ganglion (DRG) neurons are highly regenerative regardless of their stage of development, but retinal ganglion cells (RGCs) are less regenerative and their capacity to form new growth cones declines with age (Chierzi et al., 2005). Unfortunately, there are inhibitory factors that limit axonal outgrowth after SCI such as CSPGs: they ensnare severed axons as dystrophic end bulbs that are targeted for axonal dieback by pro-inflammatory macrophages via cell-to-cell contact and MMP9-dependent mechanisms (Horn et al., 2008; Busch et al., 2009; Evans et al., 2014; Filous et al., 2014). Axonal regeneration is also inhibited by myelin-associated

debris that accumulates from damaged axons and oligodendrocytes (Mukhopadhyay et al., 1994; Chen et al., 2000; Wang et al., 2002).

Oligodendrocytes exhibit a delayed onset (~ 4 hr) in apoptosis after SCI (Springer et al., 1999), and they are observed in both perilesional and distal regions after compressive SCI (T7-10) within 4 days after injury (Li et al., 1999). Oligodendrocyte cell death can be induced by several factors after injury including inflammation or glutamate and ATP excitotoxicity (Selmaj and Raine, 1988; Takahashi et al., 2003; Matute et al., 2007). Progressive loss of oligodendrocytes is detrimental as it causes demyelination of intact axons, which leads to delocalization of nodal Na^+/K^+ channels and increased metabolic stress in neurons (Craner et al., 2003; Karimi-Abdolrezaee et al., 2004; Kiryu-Seo et al., 2010). It should be noted that M2 microglia/macrophages can phagocytose and digest myelin debris, but macrophages are less efficient at degrading internalized myelin and the accumulation of lipids leads to cell death or the formation of pro-inflammatory lipid plaques that persist chronically after injury (Greenhalgh and David, 2014; Wang et al., 2015). In order to preserve axon integrity and function, a viable strategy is to promote oligodendrogenesis and remyelination of damaged axons (detailed in a later section). Therefore, it is imperative that future studies elucidate how to optimize immunomodulation to improve myelin clearance and preservation/regeneration of neurons, axons and oligodendrocytes.

1.2.2 Astrocytes

Astrocytes are highly diverse and the most abundant glial cell in the CNS, and they serve several homeostatic functions including ionic and water balance (Nagelhus and Ottersen, 2013; Bellot-Saez et al., 2017), blood flow (MacVicar and Newman, 2015), synaptic activity (Allen, 2014) and providing metabolic support to neurons and oligodendrocytes (Camargo et al., 2017; Morita et al.,

2019). After injury, astrocytes undergo a gradual but significant change in phenotype referred to as reactive astrogliosis. The heterogeneity and plasticity of reactive astrocytes enables them to shift between positive or negative modulatory roles in relation to the severity or type of injury stimuli and proximity to lesion (Sofroniew, 2009; Zamanian et al., 2012; Hirayama et al., 2015). For example, reactive astrocytes can promote either a pro- or anti-inflammatory phenotype in microglia/macrophages by releasing different inflammatory factors, such as TNFα and IL-1β or interleukin-10 (IL-10) and TGFβ, respectively (Ledeboer et al., 2002; Choi et al., 2014; Norden et al., 2014). Reactive astrocytes can increase ROS production in response to inflammation (Sheng et al., 2013), and they can selectively induce apoptosis in neurons and oligodendrocytes (Kim et al., 2012; Parajuli et al., 2015). However, they also serve several neuroprotective functions in response to injury. For example, they can protect neurons from oxidative stress by increasing nuclear factor E2-related factor 2/antioxidant response element (Nrf2/ARE) signaling (Shih et al., 2003; Wang et al., 2012). Reactive astrocytes can also upregulate neurotrophic or anti-inflammatory factors (Bsibsi et al., 2006), and alleviate contact-mediated cell death by phagocytosing dead cells (Lööv et al., 2012; Iram et al., 2016).

The most characteristic role of pre-existing and neural stem/progenitor cell (NPC)-derived astrocytes is constructing the glial scar. Reactive astrocytes proliferate and migrate towards the lesion site where they adopt a stellate morphology, become hypertrophic and upregulate intermediate filaments such as glial fibrillary acidic protein (GFAP), nestin, vimentin and synemin (Jing et al., 2007). They interdigitate their processes and encapsulate the lesion in dense scar tissue in a STAT3-dependent mechanism (Wanner et al., 2013). Reactive astrocytes remodel the ECM by expressing laminin, collagen IV and several sub-types of inhibitory CSPGs and hyaluronan (Liesi and Kauppila, 2002; Tang et al., 2003; Back et al., 2005; Anderson et al., 2016). As

previously mentioned, the glial scar is beneficial as it limits the spread of inflammation and toxic factors to neighboring healthy tissue, but some of these ECM components inhibit axonal regeneration and remyelination. Overall, reactive astrocytes have a significant role in modulating the injury microenvironment after SCI, and they are an intriguing therapeutic target given their plasticity after SCI. Therefore, it is beneficial to identify factors in the injury microenvironment that regulate reactive astrogliosis.

1.2.3 Neural stem/progenitor cells and oligodendrocyte precursor cells

NPCs and OPCs are precursor populations found in the adult spinal cord. NPCs are relatively quiescent and reside in the ependymal cell layer around the central canal (Barnabé-Heider et al., 2010). Activated NPCs proliferate and migrate towards the lesion when exposed to local elevations in growth-factors, inflammatory cytokines and chemokines after injury (Zai et al., 2005; Ahn et al., 2006; Kang and Kang, 2008). Multipotent NPCs have the innate capacity to differentiate into neurons and oligodendrocytes, as demonstrated *in vitro* (Weiss et al., 1996), which suggests they can potentially facilitate regeneration. However, activated NPCs predominantly differentiate into astrocytes and their overall contribution to gliosis is influenced by the type of SCI (Meletis et al., 2008; Barnabé-Heider et al., 2010). Frisen's group used FoxJ1-CreER transgenic mice to trace the lineage of NPC-derived cells and showed their astrocytes contribute to glial scar formation (Meletis et al., 2008; Barnabé-Heider et al., 2010). NPC-derived astrocytes are localized in the lesion core in regions devoid of CSPGs, in contrast to pre-existing reactive astrocytes in the periphery of the scar (Meletis et al., 2008; Barnabé-Heider et al., 2010). Work from the same group by Sabelström and colleagues also showed that NPC-derived astrocytes form a scar tissue that is critical for tissue preservation and they are a source of several neurotrophic factors after SCI

(Sabelström et al., 2013). Therefore, further investigation is required to elucidate whether these two distinct populations of reactive astrocytes have differential roles in the glial scar and repair process after SCI.

OPCs are predominantly found in the white matter and represent approximately 2-9% of the total cells in the adult rat CNS (Dawson et al., 2003). Adult OPCs self-renew and are a significant source of new oligodendrocytes (Dawson et al., 2003; Barnabé-Heider et al., 2010), but they also have other functions in CNS homeostasis. For example, they project peri-synaptic/nodal processes and express glutamate and gamma-aminobutyric acid (GABA) receptors (Butt et al., 1999; Habermacher et al., 2019), suggesting a communicative neuron-oligodendroglia relationship. OPC proliferation is most robust within days of injury, but it persists chronically and occurs in both perilesional and distal regions after SCI (McTigue et al., 2001; Hesp et al., 2015). It is facilitated by a lack of contact inhibition with neighboring OPCs and increased bioavailability of growth factors (Zai et al., 2005; Hughes et al., 2013; Hesp et al., 2015). Activated OPCs can mature and replace oligodendrocytes to remyelinate damaged axons, but they also help construct the glial scar and secrete MMP9 to facilitate the infiltration of neutrophils following white matter damage (Seo et al., 2013; Hackett et al., 2016; Hackett and Lee, 2016). Their capacity for remyelination can persist chronically in both rat and mice SCI models (Hesp et al., 2015), and OPC differentiation can be regulated by reactive astrocytes (Talbott et al., 2005; Hammond et al., 2014; Hammond et al., 2015; Dyck et al., 2019), M2 microglia/macrophages (Miron et al., 2013), and pericytes (De La Fuente et al., 2017). Damaged axons can also be remyelinated by infiltrating or OPC-derived SCs (Zawadzka et al., 2010; Ma et al., 2018).

There is evidence that promoting remyelination by oligodendrocytes or SCs can improve functional recovery in SCI or demyelinating injury models (Felts and Smith, 1992; Najm et al.,

2015) (Blight and Young, 1989; Govier-Cole et al., 2019). Remyelination is therapeutically beneficial as it improves axonal conductance by reconstructing aberrant localization of Na+/K+ channels within new nodes of Ranvier (Blight and Young, 1989; Felts and Smith, 1992; Black et al., 2006; Eftekharpour et al., 2007). However, a recent study has questioned the importance of spontaneous remyelination by oligodendrocytes (Duncan et al., 2018). Duncan and colleagues used inducible *Myrf*-knock out SCI mice to prevent endogenous remyelination by oligodendrocytes after injury, and found no difference in motor recovery between *Myrf*-knock out and wild-type mice, suggesting that spontaneous remyelination by oligodendrocytes has a limited or negligible role in facilitating motor recovery after SCI (Duncan et al., 2018). They also observed no compensatory increase in SC myelination (Duncan et al., 2018). It should be noted that endogenous remyelination can be bolstered therapeutically by transplanting oligodendroglial precursors (NPCs, OPCs), SCs or olfactory mesenchymal stromal cells, and these approaches have been associated with improved motor function and/or coordination (Takami et al., 2002; Karimi-Abdolrezaee et al., 2006; Cao et al., 2010; Yasuda et al., 2011; Lindsay et al., 2017). These benefits were at least partially attributed to their ability to myelinate damaged axons, as Yasuda and colleagues demonstrated a lack of functional improvement after transplanting NPCs derived from myelin-deficient *Shiverer* mice (Yasuda et al., 2011). It is possible that a threshold of remyelination must be achieved to promote greater functional recovery, and this was not reached by endogenous cells due to inhibitory environmental factors after SCI. For example, there is evidence that oligodendrocyte differentiation and/or remyelination are inhibited by factors such as myelin debris (Kotter et al., 2006; Plemel et al., 2013), CSPGs (Pendleton et al., 2013; Dyck et al., 2015; Dyck et al., 2019), hyaluronan (Back et al., 2005), endothelin-1 (Hammond et al., 2014; Hammond et al., 2015) or bone morphogenetic protein-4 (BMP4) (Samanta and Kessler, 2004;

Sabo et al., 2011; Wang et al., 2011; Govier-Cole et al., 2019). Reactive astrocytes are a significant source of BMP4 in the injured rat spinal cord (Wang et al., 2011), and stimulating BMP-Smad1/5/8 signaling inhibits oligodendrogenesis in both NPCs and OPCs (Samanta and Kessler, 2004; Wang et al., 2011). Recent work has shown that inhibiting BMP-Smad1 signaling is a viable strategy for promoting remyelination after cuprizone-mediated demyelination in mice (Sabo et al., 2011; Govier-Cole et al., 2019). These studies collectively suggest that reactive astrocytes modulate oligodendrogenesis in the injured spinal cord, and activation of BMP signaling may present an underlying mechanism of these effects. This thesis has focused on the role of BMP signaling in SCI. In the following sections, I will describe BMPs in the developing and adult spinal cord.

1.3 Bone morphogenetic proteins

1.3.1 Overview of bone morphogenetic proteins (BMPs)

1.3.1.1 Diversity and function

BMPs were first discovered by Marshall Urist, who showed that demineralized human cortical bone matrix had the innate capacity to induce ectopic bone and cartilage formation weeks following implantation into various tissues of rodents (Urist, 1965; Urist and Strates, 1971). This effect was nullified by pre-treating the implanted matrix with trypsin, suggesting it was mediated by novel proteinaceous factors. In the 1980's, BMPs were purified from bovine and human bones and digested protein fragments were partially sequenced (Sampath and Reddi, 1983; Wang et al., 1988; Wozney et al., 1988; Celeste et al., 1990). These novel BMP1-7 were identified as members of the TGFβ gene superfamily and capable of inducing bone formation (Sampath and Reddi, 1983; Wozney et al., 1988; Celeste et al., 1990; Sampath et al., 1990). BMP proteins are highly conserved and have a similar capacity to induce bone formation in matrices across species including rat,

bovine, monkey and humans (Sampath and Reddi, 1983). Other members of the TGFβ superfamily include TGFβ1-3, glial derived neurotrophic factor (GDNF), inhibins, activins, lefty, nodal and anti-Müllerian hormone (Wang et al., 2014a).

There are 15 BMP isoforms (BMP1-15) sub-divided into distinct subgroups based on their sequence homology (Wang et al., 2014a). BMP2, BMP4, BMP6, BMP7 and BMP9 have the greatest capacity to induce osteogenesis or cartilage formation (Luu et al., 2007). BMP2 and BMP4 have the highest degree of homology as they are derived from a recent gene duplication event (Duret et al., 1994). Homozygous mutants of BMP2 and BMP4 are embryonic lethal due to failures in amnion/chorion and cardiac development or impaired gastrulation and mesoderm formation, respectively (Winnier et al., 1995; Zhang and Bradley, 1996). Mutations in BMP signaling also impair later stages of development in bones, limbs and various organs (Wang et al., 2014a). For example, conditional knockout of cardiac BMP2 leads to heart failure in embryonic mice due to retarded growth and fluid accumulation (Ma et al., 2005), while BMP11 knockout mice exhibit severe defects in kidney formation (Esquela and Lee, 2003). Despite their homology, BMP2 and BMP4 have distinct roles under certain contexts. For example, conditional knock-out studies distinguished BMP2 as a non-redundant factor, relative to BMP4, for regulating proliferation and maturation of chondrocytes and bone fracture healing (Tsuji et al., 2006; Shu et al., 2011). Evidence also suggests that BMP2 and BMP4 differentially regulate hypoxic stress and endothelial response to pulmonary hypertension in mice (Frank et al., 2005; Anderson et al., 2010). Some BMP isoforms serve antagonistic roles or have distinct functions. For example, BMP1 is a novel MMP that is an essential proteolytic enzyme for maturing procollagens I-III, V and XI and laminin (Kessler et al., 1996; Vadon-Le Goff et al., 2015). It also activates TGFβ and other BMP isoforms by directly cleaving their pro-domain complexes or their inhibitors (Marqués et al., 1997; Wardle

et al., 1999; Ge et al., 2005; Ge and Greenspan, 2006; Delolme et al., 2015). In contrast, BMP3 is a divergent and antagonistic isoform that shares only ~40% homology with BMP2 and BMP4-7 (Katoh and Katoh, 2006). BMP3 negatively regulates bone density by limiting osteogenesis and inhibiting differentiation of osteoprogenitors into osteoblasts by binding and inactivating activin A receptor type 2b (ACVR2b) (Daluiski et al., 2001; Kokabu et al., 2012).

1.3.1.2 Transcription and ligand processing

In 1998, Metz and colleagues studied BMP4 cDNA from a *Xenopus* genomic DNA library and showed that it consisted of at least three exons and two introns (Metz et al., 1998). The BMP4 gene is transcribed from one of two alternative promoter sequences, although the proximal element near exon 1 is most critical for BMP4 expression (Metz et al., 1998). Interestingly, the proximal promoter is ubiquitously active in both ventral and dorsal regions (-116 to -96), while the distal promoter (-255 to -116) is more active in ventral regions. This suggests these two distinct promoters have a role in the spatial/temporal regulation of BMP4 expression, in addition to the intron 2 that contains additional auto-regulatory elements (Metz et al., 1998). Later, Ghosh-Choudhury and colleagues demonstrated that the BMP2 gene was similarly structured and identified presumptive binding sites for several transcription factors such as homeodomain (HOX), serum response factor (Srf), specificity protein-1 (SP1), nuclear factor-κB (NF-κB), E2 factor (E2F) and c-Myc (Ghosh-Choudhury et al., 2001). Importantly, there is evidence that BMP2, BMP4 and BMP7 can activate positive and negative feedback mechanisms to amplify their respective expression and that of others during development. For example, it is suggested that maternally-derived BMP2 is important for initiating BMP4 transcription during late blastula/early gastrulation in *Xenopus* embryos (Schuler-Metz et al., 2000), while it has also induced BMP2

mRNA transcription in cultured 2T3 osteoblast cells (Ghosh-Choudhury et al., 1996). Intriguingly, BMPs can differentially regulate the expression of other isoforms via negative and positive feedback loops during chick eye development (Huang et al., 2015). Using pre-lens explants from embryonic chicks (HH8), Huang and colleagues demonstrated that administration of noggin, an endogenous inhibitor of BMPs, significantly increased BMP2 and BMP4 expression, but had no effect on BMP7 (Huang et al., 2015). In contrast, administration of recombinant BMP4 or BMP7 significantly increased BMP7 mRNA transcription in chick embryo explants, while they had the opposite effect on BMP2 and BMP4 mRNA levels (Huang et al., 2015). BMP4 auto-regulatory transcription seems to be dependent on enhancer elements in the 5' untranslated region (UTR) (-206 to -156) and intron 2 (+1815 to +1918), as mutations in these sequences limited BMP4-induced upregulation in BMP4 transcription in *Xenopus* embryos (Schuler-Metz et al., 2000).

Several signaling pathways and transcription factors have been characterized as modulators of BMP transcription (Fig. 1.2). Ghosh-Choudhury and colleagues identified phosphoinositide-3-kinase (PI3K)/Akt signaling as a key pathway for BMP2 autoregulation, as phosphorylated Akt facilitated the nuclear translocation of phosphorylated Smad1/5/8 in a kinase-independent manner in 2T3 osteoblasts (Ghosh-Choudhury et al., 2002). Studies have characterized other positive regulators of BMP2/4 transcription including myocyte enhancer factor 2A (MEF-2A) (Ghosh-Choudhury et al., 2003), Xvent-2 (Schuler-Metz et al., 2000) and C-Jun/Activator Protein-1 (AP-1) (Knöchel et al., 2000). Interestingly, there are conflicting reports as to whether the NF-κB pathway has a promoting or inhibitory influence on BMP transcription. Zhu and colleagues demonstrated that BMP4 transcription is antagonized in TNFα-treated human lung A549 epithelial cells, as activated NF-κB recruited p65/RelA and inhibited transcriptional activator SP1 (Zhu et al., 2007). However, Graham and colleagues later showed that NF-κB can bind directly to BMP2

promoter and act synergistically with BMP2-Smad1 and PI3K/Akt signaling to increase BMP2 expression in TNFα-treated metastatic prostate cancer (C4-2B) cells (Graham et al., 2009). Therefore, it is possible that NF-κB differentially regulates BMP2 and BMP4 transcription, or that its effect may be contextually dependent on other pathways or cell type. In contrast, Wnt8/β-catenin signaling is a pathway that antagonizes BMP4 transcription during corneal and CNS development in *Xenopus* and mouse embryos (Baker et al., 1999; Gomez-Skarmeta et al., 2001; Zhang et al., 2015). This effect is at least partially mediated by induction of Xiro1 expression, a repressor that binds to BMP4 promoter and inhibits transcription (Gomez-Skarmeta et al., 2001). Overall, these findings demonstrate that BMP transcription is tightly regulated during development by several autoregulatory and independent mechanisms, though it remains unclear whether these regulatory pathways are maintained in the adult or injured CNS.

Precursors of BMPs consist of a N-terminal signal peptide, a pro-domain and a C-terminal mature peptide (Harrison et al., 2011). They have highly conserved pro-domains ranging between 165-451 amino acids, although major deviations are observed in BMP3, BMP5, BMP6 and BMP10, BMP11 (Harrison et al., 2011; Shi et al., 2011). Consensus sequence sites between pro- and mature domains are recognized and cleaved by pro-protein convertase subtilisin/kexin-like proteases (e.g. furin, PACE4, PC6/PCB6, PC7) at the cell surface or intracellularly (Susan-Resiga et al., 2011). The amino acid sequence of BMP pro-domains determines their susceptibility to cleavage by a given protease. In *Xenopus* embryos, for example, BMP4 is differentially processed at two distinct sites: S1 (optimal cleavage site near the mature ligand) and S2 (sub-optimal cleavage site in the pro-domain) (Nelsen and Christian, 2009), as illustrated in Fig. 1.2. In the trans-Golgi network (TGN), BMP4 is initially cleaved at S1 site (-RXKR-) by furin, PACE4, PC6 or PC7 to produce a mature peptide (Molloy et al., 1994; Sopory et al., 2006; Nelsen and Christian,

2009). The sequential cleavage at S2 site (-RXXR-) occurs in an acidic paranuclear post-TGN compartment mediated by proteases (Xiang et al., 2000; Nelsen and Christian, 2009). Sopory and colleagues also demonstrated that BMP4 and BMP2 processing in *Xenopus* is dependent on a conserved amino acid sequence between the S1 and S2 sites (i.e. linker domain), which induces conformational changes required for S1 cleavage and secretion of a biologically active ligand (Sopory et al., 2006). These structural elements are not conserved in all isoforms as BMP7, for example, does not have a conserved S1 site or linker domain (Sopory et al., 2006). There is evidence that BMP processing can differ further based on the cell type or tissue region. For example, Susan-Resiga and colleagues showed that furin is the most efficient convertase that cleaves BMP10 at a single motif (-RIRR-) in the heart of adult mice (Susan-Resiga et al., 2011), while BMP4 is cleaved at both S1 and S2 sites equally by furin and PC6, as previously discussed (Nelsen and Christian, 2009). Moreover, previous studies have shown that furin, PACE4 and PC6 mRNA are expressed by neurons and/or glia in the developing and adult rat brain, but they exhibit unique expression profiles in a region or cell-type specific manner (Day et al., 1993; Zheng et al., 1994; Dong et al., 1995; Zheng et al., 1997). For instance, furin mRNA is highly expressed in the hippocampus of adult rats, while PACE4 is absent in this region (Day et al., 1993; Dong et al., 1995). Moreover, furin and PACE4 are expressed in both neurons and glia, but PC6 is expressed only in neurons (Day et al., 1993; Dong et al., 1995).

Interestingly, there is evidence that BMP processing can differentially impact ligand secretion in a species-specific manner. Cui and colleagues showed that selective inactivation of the S2 site, but leaving the linker domain intact, yielded a less biologically active form of BMP4 in *Xenopus* (Cui et al., 2001). A subsequent study showed that deleting both the S2 site and the linker domain in BMP4 yielded a similar result in several mammalian cell lines, but it prevented the secretion of

a biologically active BMP4 ligand in *Xenopus* (Sopory et al., 2006). The S2 site also has a unique regulatory role for BMP4 bioavailability by influencing its half-life and diffusion range (Cui et al., 2001; Ohkawara et al., 2002; Degnin et al., 2004). This is attributable to a high density of hydrophobic residues in BMP pro-domains that, following the cleavage at S1, facilitate non-covalent binding between the cleaved BMP4 pro-domain and mature peptide (Sengle et al., 2008b; Sengle et al., 2008a). Cleavage at the S2 site disrupts this complex and allows the dissociation of the mature BMP4 peptide (Degnin et al., 2004; Nelsen and Christian, 2009). Sengle and colleagues demonstrated that BMP7 is secreted as a stable complex with its pro-domain only for it to be displaced after binding to BMPRII (Sengle et al., 2008a), which suggests BMP pro-domains do not interfere with receptor activation. This contrasts a subsequent study that showed TGFβ is unable to bind its receptor when bound by its pro-domain (Shi et al., 2011). However, if the S2 site is mutated to prevent the dissociation of the BMP pro-domain, the half-life and diffusion range of the bioactive ligand is reduced as this complex is more susceptible to entrapment at the cell surface by heparin sulfate proteoglycans (HSPGs), which triggers internalization and degradation (Degnin et al., 2004; Jiao et al., 2007). Fibrillin microfibrils have a similar role in the developing bone, as they bind and sequester BMP4 and BMP7 to delay osteoblast maturation, and they are critical for proper bone formation (Nistala et al., 2010). Further investigation is required to elucidate whether BMP processing and bioavailability is differentially regulated in cells of the developing or adult CNS.

Figure 1.2. Schematic of BMP4 transcription, processing and signaling

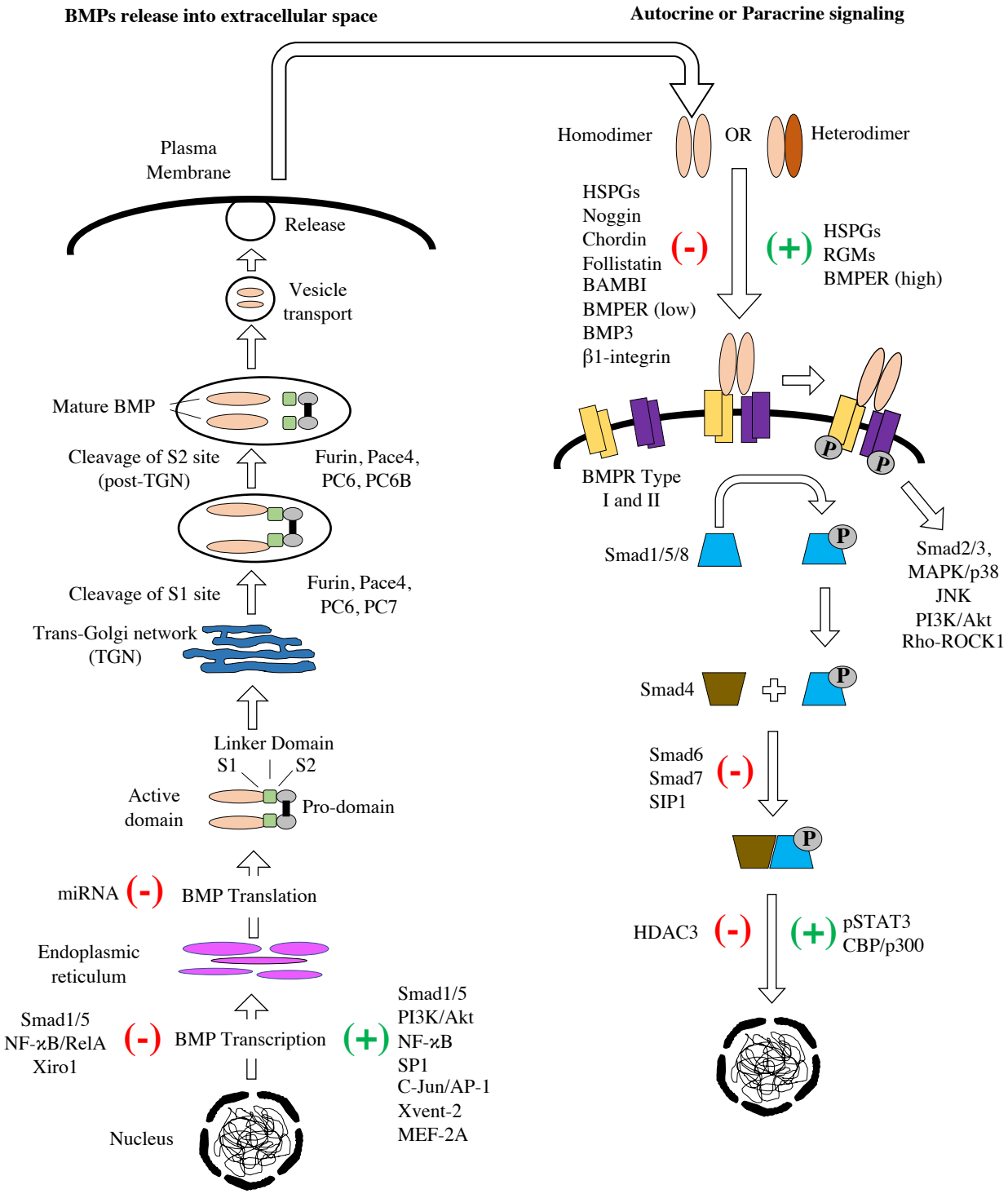


Figure 1.2. Schematic of BMP4 processing and signaling

Expression of BMPs is positively and negatively regulated by several signaling pathways and transcription co-factors, including phosphatidylinositol-3-kinase (PI3K)/Akt, nuclear factor-κB (NF-κB), microRNAs (miRNAs) and autoregulatory BMP-Smad1/5/8. BMPs are synthesized as an inactive dimeric precursor consisting of three domains that are cleaved sequentially at one or two distinct sites (S1 and/or S2), which modulate their bioavailability and range of effect. These cleavage sites are recognized by several pro-protein convertases (furin, PACE4, PC6, PC6B, PC7) that are compartmentalized intracellularly. After S1 site is cleaved in the trans-Golgi network (TGN), the pro-domain remains non-covalently associated with the ligand. The precursor is shuttled to an acidic post-TGN compartment where the S2 site is exposed and cleaved, allowing the pro-domain to dissociate and release the active ligand via vesicular exocytosis. Secreted BMP dimers act as autocrine or paracrine signals whose efficacy is modulated by the extracellular matrix (ECM), ligand antagonists or cell-bound co-receptors. BMPs bind and recruit Type I and constitutively active Type II receptors, which trans-phosphorylate and activate canonical (Smad1/5/8) and non-canonical (Smad2/3, MAPK/p38, JNK, PI3K/Akt, Rho-ROCK1) downstream signaling pathways. Phosphorylated Smad1/5/8 binds with co-factors, such as Smad4, which translocate to the nucleus and elicit changes in gene expression. BMP signaling can modulate cellular behaviour in coordination with both positive and negative intracellular mediators, such as STAT3/CBP/p300 or HDAC3, respectively.

1.3.1.3 Receptors and downstream signaling

There are three BMP Type I receptors (BMPR1a, BMPR1b and ActR-1) and constitutively active Type II receptors (BMPR2, ActR-2A, ActR-2B) (Miyazono et al., 2005). They are transmembrane receptors consisting of three distinct domains, including an intracellular domain with serine/threonine kinase activity (Miyazono et al., 2005). BMP ligands bind to either Type I or II receptors based on their innate receptor affinity. For example, BMP4 binds equally to BMPR1a and BMPR1b, but BMP7 binds less efficiently to BMPR1a (ten Dijke et al., 1994). Ligand binding initiates receptor complex formation between Type I and constitutively active Type II receptors (Fig. 1.2), which trans-phosphorylate each other and activate downstream signaling. BMP ligands can activate canonical (Smad1/5/8) and non-canonical signaling pathways (Smad2/3, mitogen-activated protein kinase (MAPK)/p38, c-Jun N-terminal kinase (JNK), PI3K/Akt or Rho-ROCK1 GTPases) in a cell-dependent manner (Mazerbourg et al., 2004;

Konstantinidis et al., 2011; Tian et al., 2012; Rocher and Singla, 2013). Phosphorylated Smad1/5/8 binds to an essential co-factor Smad4, which together translocate to the nucleus and recruit co-activators p300/CREB-binding protein (CBP) or GCN5 to elicit changes in gene expression (Pearson et al., 1999; Kahata et al., 2004). Targeted downstream signaling pathways can vary depending on the sequence of receptor binding/recruitment or by binding to preformed receptor dimers. For example, Nohe and colleagues demonstrated that BMP2 can activate either Smad1 or MAPK/p38 signaling in COS7 cells *in vitro* depending on whether it binds to pre-formed BMPRI:II heteromeric complex or to BMPRI first (Nohe et al., 2002).

BMP signaling can be regulated at the cell surface by repulsive guidance molecules (RGMa, RGMb/DRAGON, RGMc/hemojuvelin), which are glycophosphatidylinositol (GPI)-anchored proteins that interact with both BMP ligand and receptor to increase the diversity of recruited BMP Type II receptors and potentiate Smad1/5/8 downstream signaling (Babitt et al., 2005; Samad et al., 2005; Babitt et al., 2006). RGMs also facilitate the association of BMP receptor complexes with neogenin, a cell surface protein that increases BMP-Smad1/5/8 signaling during chondrogenesis in mice (Zhou et al., 2010). Despite their previously described role in facilitating BMP4 degradation (Degnin et al., 2004), HSPGs can also increase mature ligand stability and recruit BMPRII to potentiate BMP signaling (Kuo et al., 2010).

1.3.1.4 Inhibitors of BMP signaling

During development, BMP concentration gradients are established by HSPGs and BMP inhibitors such as noggin, chordin and BMP-binding endothelial regulator (BMPER) (Paine-Saunders et al., 2002; Jasuja et al., 2004; Serpe et al., 2008), as illustrated in Fig. 1.2. These inhibitors bind directly to BMP ligands and prevent receptor activation, but it has recently been

shown that high concentrations of noggin can also trigger endocytosis and degradation of BMP4 (Zimmerman et al., 1996; Kelley et al., 2009). BMPER is a secreted BMP-binding protein with a biphasic modulatory role in both *Drosophila* and mouse endothelial cells (Serpe et al., 2008; Kelley et al., 2009). Kelley and colleagues showed that BMP2-mediated signaling was potentiated when the stoichiometric ratio between BMPER and BMP2 was lower than 2:1 (Kelley et al., 2009). But when this ratio was exceeded, it instead promoted clatherin-mediated endocytosis and lysosomal degradation of BMP2 (Kelley et al., 2009). In contrast to RGMs, there are inert BMP pseudo-Type I receptors such as BMP and activin membrane-bound inhibitor (BAMBI), which lacks a cytoplasmic serine/threonine-kinase domain and antagonizes BMP signaling by competitively binding both Type I and II receptors (Onichtchouk et al., 1999). Recent evidence showed BAMBI expression was upregulated in human granulosa-lutein cells when treated with BMP2 (Bai et al., 2017), suggesting this pseudo-receptor can serve a negative auto-feedback role. BMP signaling can also be antagonized at the transcript level by microRNAs (miRNA), which recognize target sequences in the 3'UTR and reduce translation of Smad1 or BMP ligand transcripts (Icli et al., 2013; Kureel et al., 2014). Alternatively, BMP signaling can be antagonized intracellularly by inhibitory Smads (I-Smads), Smad6 and Smad7, or Smad-interacting protein (SIP1). I-Smads antagonize BMP signaling either by binding to Type 1 receptors or to phosphorylated Smad1 to prevent co-localization with Smad4 (Imamura et al., 1997; Hata et al., 1998). Smad6 can also directly repress BMP-targeted gene expression in the nucleus by recruiting transcriptional co-repressor C-terminal binding protein (CtBP) (Lin et al., 2003), Smad ubiquitin regulatory factor-1 (Smurf1) (Murakami et al., 2003) or Tob (Yoshida et al., 2003). SIP1 directly binds and neutralizes Smad1, but it also upregulates Smad7 expression to antagonize both BMP- and TGFβ-mediated Smad signaling (Verschueren et al., 1999; Weng et al., 2012). In contrast,

Smad6 specifically antagonizes BMP-Smad1 signaling by interacting with Smad1 or BMP Type I receptors (Hata et al., 1998; Goto et al., 2007), but only weakly inhibits TGFβ/activin-mediated signaling (Ishisaki et al., 1999; Hanyu et al., 2001). BMP2 and BMP7 induce Smad6 expression *in vitro* in several cell lines via a BMP-responsive element in the Smad6 promoter region that is recognized by phosphorylated Smad1/5 and cAMP response element-binding protein (CREB) (Takase et al., 1998; Ishida et al., 2000; Ionescu et al., 2004). Therefore, I-Smads can provide negative feedback in response to BMP signaling.

1.3.2 Expression and role of BMPs during spinal cord development

1.3.2.1 Significance of BMPs in spinal cord development

Knock-out studies show BMP signaling regulates several stages of embryonic development including gastrulation, left-right asymmetry, skeletal, limb and CNS development (Zhao, 2003). Of note, BMPs have a significant role in regulating the decision to form neural or non-neural tissue. Neural tissue originates from the dorsomedial ectoderm and its formation requires active fibroblast growth factor (FGF) signaling and repressed BMP signaling in vertebrates, which is achieved by a concentration gradient of dorsally-derived noggin and chordin (Piccolo et al., 1996; Zimmerman et al., 1996; Delaune et al., 2005). During gastrulation in *Xenopus*, BMP1 counteracts this gradient by degrading chordin and liberating other BMP isoforms to promote ventralization (Wardle et al., 1999). The induction of primitive streak formation is temporally dependent on chordin-mediated inhibition of BMP4 (Streit et al., 1998), but dorsally-derived BMP4 is essential for subsequent stages of CNS development (neural tube formation and closure) (Liem Jr et al., 1995). Once the neural tube has closed, spinal cord development is spatially governed by distinct signals including

Sonic hedgehog (Shh) ligands (ventral floor plate and notochord), retinoic acid, Wnts and BMP ligands (dorsal roof plate and ectoderm) (del Corral et al., 2003; Liu and Niswander, 2005).

Patterning of the dorsal and intermediate spinal cord requires several BMP isoforms expressed in the dorsal roof plate of the neural tube (Liu and Niswander, 2005). In the developing chick spinal cord, the spatial expression profile for every isoform is not homologous. For example, Andrews and colleagues showed that BMP4 expression was restricted to the roof plate, while BMP7 was expressed in dorsal and intermediate regions (Andrews et al., 2017). The dorsal spinal cord has six parallel layers of dorsal and intermediate interneurons (dI1-6) that interpret and relay different types of information (Lai et al., 2016). Andrews and colleagues recently demonstrated that BMP4, BMP6 and BMP7 differentially modulated their cell cycle progression, termination and phenotype in cultured mouse embryonic stem cells and/or the chick spinal cord (Andrews et al., 2017). Importantly, they demonstrated that increasing the concentration of BMP ligands increased the efficiency of differentiation *in vitro*, but it had no effect on fate selection (Andrews et al., 2017). This indicates that BMPs direct neuronal identity independent of their concentration, in contradiction to previous findings that suggested the duration of BMP exposure can also influence their dorsalized fate (Tozer et al., 2013). This new model is supported by previous findings that showed knocking down of BMP4 expression with short-hairpin RNA (shRNA) reduced the number of dI1 neurons, while BMP7-knock down reduced dI1, dI3 and dI5 neurons in chick spinal cord (Le Dréau et al., 2012). In contrast, the ventral spinal cord consists of five progenitor domains (p3, pMN, p2, p1, p0) that mature into ventral interneurons (V3-0) and motor neurons (MN). BMP antagonism is essential for ventral patterning of the spinal cord, as BMP-Smad1/5/8 signaling antagonizes Shh-induced ventralization of NPCs (Liem et al., 2000;

Sterneckert et al., 2005). This is supported by *in vivo* studies in null mutant mice that showed noggin was essential for proper ventral patterning and somite formation (McMahon et al., 1998).

1.3.2.2 Gliogenic switch: Role of BMP in stem cell development

During later stages of neurodevelopment (E14.5), there is a reduction in neurogenesis as NPCs shift towards gliogenesis. BMP-Smad1/5/8 signaling limits neurogenesis by upregulating inhibitor of differentiation-1 (Id1) expression, which is an inhibitory helix-loop-helix (HLH) factor known to degrade pro-neurogenic factors (Yanagisawa et al., 2001; Viñals et al., 2004). The gliogenic switch is also perpetuated by Notch1 and nuclear factor 1α (NF1A) (Ge et al., 2002; Kang et al., 2012; Mutoh et al., 2012; Bansod et al., 2017), and neuronally-derived cardiotrophin-1 (CT1), ciliary neurotrophic factor (CNTF) and leukemia inhibitor factor (LIF) (Bonni et al., 1997; Ochiai et al., 2001; Barnabé-Heider et al., 2005).

Fibrous and protoplasmic astrocytes are generated consistently from ventral (p1-3) and dorsal (dI5-6) progenitors in mouse spinal cord (Fogarty et al., 2005; Hochstim et al., 2008; Vue et al., 2014). Astrogenesis is governed by several signaling pathways including BMP-Smad1, STAT3, MAPK/Erk and β1-integrin expression (Nakashima et al., 1999; Fukuda et al., 2007; Li et al., 2012; Pan et al., 2014). Smad1 and STAT3 pathways communicate by forming a synergistic complex with coactivator CBP/p300 to promote astrocyte differentiation (Nakashima et al., 1999; Fukuda et al., 2007). However, independent treatment with LIF/noggin or BMP4 can promote two GFAP+ phenotypes reflecting distinct states of maturity. Bonaguidi and colleagues showed that LIF/noggin treatment induced a precursor-like bipolar morphology that increased proliferation and upregulated stem cell marker expression (LeX^+ or $Sox1^+/GFAP^+$) (Bonaguidi et al., 2005). In contrast, BMP4 treatment alone promoted a mature stellate morphology and phenotype while

limiting proliferation (Bonaguidi et al., 2005). Later, it was found BMP4 treatment also increased astrocyte maturity by upregulating aquaporin-4 and S100β expression, and limited cell proliferation by down-regulating EGF receptor expression (Scholze et al., 2014). These data show the importance of LIF/STAT3 and BMP/Smad1 cooperation in facilitating astrocyte development, and that they each have unique regulatory roles in self-renewal and terminal differentiation.

OPCs are generated in two temporally and spatially distinct stages. Knockout studies in *Nkx6.1*-null mice demonstrated that the first wave is derived from pMN at E12, while the second wave originates from dI3-dI5 progenitors between E13.5-15.5 (Liu et al., 2003; Cai et al., 2005). FGF and Shh pathways are ventrally-derived signals that coordinate the timing and progression of oligodendrogenesis in the developing mouse spinal cord (Farreny et al., 2018; Hashimoto et al., 2018). Dorsally-derived BMP-Smad1 signaling antagonizes OPC differentiation in dorsal/intermediate regions of the spinal cord by inducing Id2/Id4 expression, which complex with transcription factors Olig1/2 and co-factor E2A to prevent nuclear translocation and oligogenic gene expression (Samanta and Kessler, 2004). Implanting BMP4-soaked beads restricted ventral expansion and maturation of oligodendrocytes by antagonizing Shh-signaling in embryonic mouse spinal cord explant cultures and *Xenopus* embryos (Miller et al., 2004; See et al., 2004), and they inhibited expression of key myelin proteins including myelin basic protein (MBP), proteolipid protein (PLP) and 2',3'-cyclic nucleotide 3'-phosphodiesterase (CNP) (See et al., 2004). An inhibitory role of BMPs in oligodendrogenesis was further supported when the dorsal expansion of oligogenic precursor cells was facilitated by grafting noggin-overexpressing cells into the developing chick spinal cord (Mekki-Dauriac et al., 2002). Histone deacetylase-3 (HDAC3) also has an important role in limiting the inhibitory effects of BMP and STAT3 signaling on oligogenic precursor cells by competitively binding to p300/CBP and increasing Olig2 and myelin protein

expression (Zhang et al., 2016). Overall, BMP-Smad1 signaling is a key regulator of spinal cord development and precursor maturation, and it potentially exerts similar effects in the healthy or injured adult CNS.

1.3.3 Expression and role of BMP isoforms in adult spinal cord

BMP receptors and ligands are expressed in the adult rat spinal cord, but there is limited insight into their functional significance. In the spinal cord, BMPR1a is expressed ubiquitously at a low intensity, while BMPR1b is expressed relatively higher in the grey matter except for the ependymal cell layer (Miyagi et al., 2012). BMPRII is moderately expressed consistently in both the grey and white matter in the spinal cord (Miyagi et al., 2012). BMP Type I and II receptors co-localize with neurons, astrocytes, oligodendrocytes and microglia, suggesting that BMP responsiveness is ubiquitous in the spinal cord (Miyagi et al., 2012). Seven BMP isoforms have been characterized in the healthy adult rat spinal cord: BMP2-7 and BMP11 (Setoguchi et al., 2001; Miyagi et al., 2012; Kusakawa et al., 2015; Yamashita et al., 2016; Hayashi et al., 2018; Hayashi et al., 2019). Quantitative RT-PCR analysis has also detected BMP9, BMP12 and BMP13 mRNA, but they have not been co-localized to a specific region or cell population (Enzmann et al., 2005).

BMP4 is highly expressed in the ependymal layer and grey matter relative to the white matter, while BMP2 is ubiquitously expressed throughout the healthy spinal cord (Miyagi et al., 2012). BMP2/4 are expressed by neurons, astrocytes, oligodendrocytes and ependymal cells, but not microglia/macrophages (Miyagi et al., 2012). BMP5, BMP6 and BMP11 are similarly expressed and distributed throughout the rat spinal cord (Kusakawa et al., 2015; Hayashi et al., 2018; Hayashi et al., 2019), but of those three, only BMP6 is expressed by oligodendrocytes (Hayashi et al., 2019). *In situ* hybridization also shows BMP7 mRNA expression is limited to oligodendrocytes in

the healthy rat spinal cord (Setoguchi et al., 2001), and antagonistic BMP3 is co-localized to neurons, astrocytes and ependymal cells but not oligodendrocytes (Yamashita et al., 2016). Intriguingly, there is evidence that BMP4 and other isoforms are differentially localized between dorsal laminae, neuropil or different sub-cellular structures (e.g. nuclei, soma, dendrites, axons) (Miyagi et al., 2012; Kusakawa et al., 2015; Yamashita et al., 2016; Hayashi et al., 2018; Hayashi et al., 2019). Further investigation is required to determine the significance of these distributions and if they regulate any neuronal or glial homeostatic functions.

1.3.4 Expression and role of BMPs after SCI

BMP2, BMP4 and BMP7 have distinct temporal and spatial expression patterns after SCI in rodents, which may reflect differences in injury model or severity (Setoguchi et al., 2001; Setoguchi et al., 2004; Chen et al., 2005; Xiao et al., 2010; Park et al., 2013; Hesp et al., 2015; Duran et al., 2017). BMP expression has been identified in mature neurons, oligodendrocytes, astrocytes, ependymal cells and microglia/macrophages after injury (Setoguchi et al., 2001; Chen et al., 2005; Xiao et al., 2010; Park et al., 2013). Previous works suggest BMP4 mRNA or protein expression peak between 2-14 days and may remain elevated up to 35-days after injury in mice (Chen et al., 2005; Xiao et al., 2010; Park et al., 2013). In contrast to mice, a recent transcriptomic study showed that BMP1, BMP2, BMP4-7 and BMP Type I/II receptor mRNA can remain elevated up to 6 months in SCI rats (Duran et al., 2017), suggesting BMP-Smad1 signaling may be more diverse and persist chronically after injury in rats. To my knowledge, there are no studies evaluating BMP6 or BMP8-15 expression and function after SCI, but a few of these isoforms have been described in animal models of Alzheimer's disease (AD), cerebral ischemic reperfusion or

traumatic brain injury (TBI) (Zhang et al., 2006b; Crews et al., 2010; Yan et al., 2012; Feng and Hu, 2018).

BMPs appear to regulate astrocytes through BMPR1 signaling receptors (Fig. 1.3). Evidence shows that conditional knock-out of astrocytic BMPR1a or BMPR1b have significant and opposing effects on glial scar integrity and inflammation after compressive SCI in mice (Sahni et al., 2010). Sahni and colleagues showed that knocking out astrocytic BMPR1b resulted in increased activation of BMPR1a, which accelerated glial scar formation and sequestration of inflammatory cells (Sahni et al., 2010). In contrast, overactivity of BMPR1b signaling impaired glial scar maturation and functional recovery in BMPR1a knockout mice with SCI (Sahni et al., 2010). These outcomes were attributed to their differential regulation of miR-21 processing and astrocyte hypertrophy (Sahni et al., 2010). North and colleagues similarly showed that functional recovery after SCI was impaired in FoxJ1CreER β1-integrin-null mice due to increased activity of BMPR1b-Smad1 and -p38 signaling in activated NPCs and subsequent astrocyte differentiation (North et al., 2015). These findings suggest BMPs are key modulators of astrogenesis and glial scar integrity, but they also influence ECM remodelling. Ectopic delivery of BMP4 induces astrocytic CSPG expression both *in vitro* and following injection into naïve rat spinal cords (Fuller et al., 2007), demonstrating how BMP4 could foster an inhibitory microenvironment that limits repair process (Fig. 1.3).

There are conflicting reports of whether BMPs directly modulate axonal regeneration after SCI. Intrathecal delivery of noggin has improved axonal sprouting in corticospinal tract and locomotor recovery without affecting glial scar integrity in 4 weeks post-SCI rats (Matsuura et al., 2008). However, recent *in vitro* studies showed that BMP-Smad1 signaling promoted neurite outgrowth in explant DRG or hippocampal neuron cultures (E12.5-18.5), and over-expressing

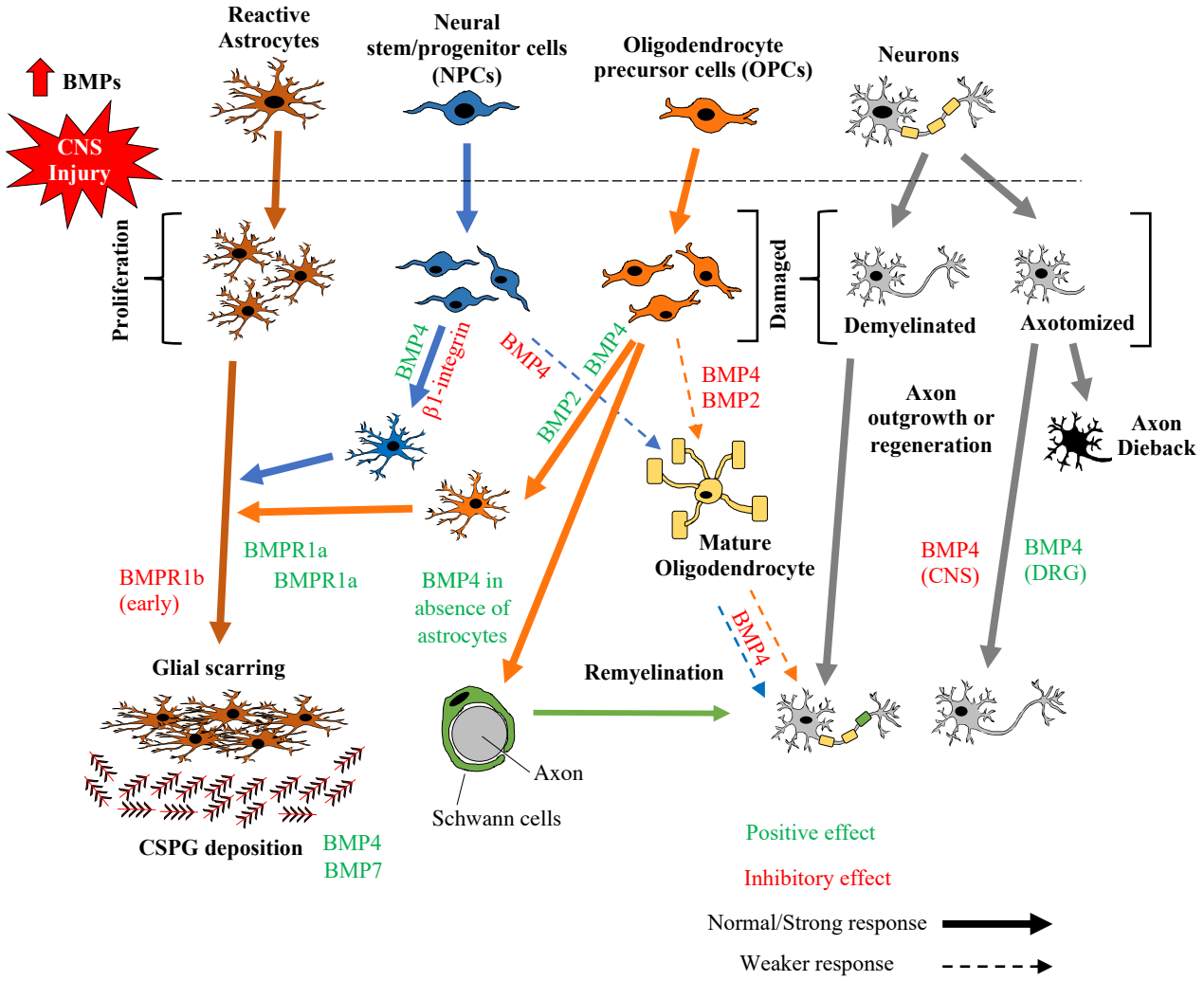
BMP4 in the DRG promoted axon regeneration and improved functional recovery after SCI in mice and rats (Parikh et al., 2011; Farrukh et al., 2019). These observations suggest that distinct neuronal populations may respond differently to BMP signaling.

BMP4 signaling antagonizes the maturation of myelinating oligodendrocytes during development, and evidence suggests it may have a similar role after injury (Fig. 1.3). Wang and colleagues showed that reactive astrocytes were a significant source of BMP2 and BMP4 up to one month after SCI in rats, and their conditioned media induced astrocyte differentiation in cultured OPCs (Wang et al., 2011). There is a correlation between increased BMP4 and Id2/4 mRNA transcription and down-regulated pro-oligogenic markers (Sox11, Hes5) in sub-acute/chronic SCI mice (Hesp et al., 2015). Furthermore, BMP4 has proven to limit OPC maturation and remyelination in models of demyelinating lesions (Sabo et al., 2011; Petersen et al., 2017; Govier-Cole et al., 2019). BMP4 expression is also increased in active and chronically inactive human MS lesions (Harnisch et al., 2019), suggesting BMP4 may be a clinically relevant target to promote remyelination.

The impact of BMP signaling on pathological responses can be influenced by intracellular and extracellular antagonists, as detailed in previous sections (Fig. 1.2). Hampton and colleagues amplified BMP signaling by administering a noggin-neutralizing antibody after spinal stab injury in mice, and they observed a significant increase in the overall number of OPC-derived astrocytes in perilesional tissue (Hampton et al., 2007). However, ectopic delivery of noggin has had limited success in attenuating astrocyte differentiation and gliosis in SCI mice. Xiao and colleagues delivered noggin via mini osmotic pump and found no change in overall GFAP expression despite a reduction in Smad1/5/8 phosphorylation (Xiao et al., 2010). Furthermore, transgenic overexpression of noggin has yielded mixed success in diverting transplanted NPCs or OPCs

towards a mature oligogenic fate and promoting functional recovery in SCI mice (Setoguchi et al., 2004; Enzmann et al., 2005). Interestingly, Park and colleagues administered agmatine, a decarboxylated derivative of arginine, to SCI mice and found it increased BMP2 and BMP7 while down-regulating BMP4 expression in neurons and glia (Park et al., 2013). They found agmatine treatment improved oligodendrogenesis and remyelination, attenuated reactive astrogliosis and neuronal cell death, and improved functional recovery relative to untreated SCI mice (Park et al., 2013). These findings suggest BMP isoforms may have differential roles after injury, and that their selective modulation could be advantageous in future therapeutic strategies.

Figure 1.3. Modulatory effects of BMP signaling on endogenous cell responses after SCI



BMP2, BMP4 and BMP7 expression and signaling are significantly increased after injury. BMPs positively (green) or negatively (red) modulate several endogenous cell types to promote (thick arrow) or inhibit (dotted arrow) responses after injury. BMP4 and/or BMP2 promote astrocyte differentiation in activated neural stem/progenitor cells (NPCs, blue) and oligodendrocyte precursor cells (OPCs, orange). In NPCs, this is facilitated by the dissociation of β1-integrin and BMPR1b, which permits the latter to translocate into lipid rafts and amplify Smad1/5/8 signaling. BMP4 also induces Schwann cell differentiation in activated OPCs in demyelinating lesions devoid of astrocytes. BMP4 and BMP2 also inhibit oligodendrogenesis and remyelination by antagonizing OPC maturation and downregulating their expression of key myelin proteins. BMP signaling regulates glial scar integrity and maturation in a receptor-specific manner, while BMP4 and BMP7 also induce expression of inhibitory chondroitin sulfate proteoglycans (CSPGs) in reactive astrocytes. Further investigation is required to elucidate the role of BMP signaling in regulating axonal regeneration, but evidence suggests BMP4 can inhibit or promote regeneration of corticospinal or sensory axon tracts, respectively.

1.4 Thesis overview

1.4.1 Study rationale

Endogenous cell replacement and repair is limited after SCI due to a dysregulated microenvironment. Emerging evidence shows that endogenous and transplanted NPCs predominantly differentiate into reactive astrocytes unless they are supplemented with therapeutic factors such as growth factors (Karimi-Abdolrezaee et al., 2006; Labombarda et al., 2011; Alluin et al., 2014b), immunomodulatory agents (Festoff et al., 2006; Karimi-Abdolrezaee et al., 2006; Dyck et al., 2018; Kataria et al., 2018), or factors that neutralize or degrade CSPGs and myelin debris (Mi et al., 2007; Karimi-Abdolrezaee et al., 2010; Karimi-Abdolrezaee et al., 2012; Church et al., 2017; Dyck et al., 2019). Reactive astrocytes and the glial scar have a neuroprotective role by forming a physical barrier around the lesion site to sequester inflammatory cells and toxic debris (Sahni et al., 2010; Wanner et al., 2013). However, the glial scar also contains a high concentration of inhibitory CSPGs that limit axon regeneration and oligodendrogenesis (Shen et al., 2009; Pendleton et al., 2013; Lang et al., 2015b; Dyck et al., 2019), while promoting inflammation and cell death in OPCs (Dyck et al., 2018). Better understanding of how endogenous cell responses are regulated in acute SCI will aid in identifying targeted therapies for SCI.

BMP4 is a key morphogen that regulates several stages of CNS development and promotes astrocyte differentiation by antagonizing pro-oligogenic gene expression in precursor cells (Samanta and Kessler, 2004; See et al., 2004). Several studies identify BMP4 as a potential regulator of reactive astrogliosis and inhibitor of remyelination in demyelinating lesions or sub-acute and chronic SCI (Fuller et al., 2007; Sahni et al., 2010; Sabo et al., 2011; Govier-Cole et al., 2019). To date, there has been little insight into the significance of transient BMP4 upregulation on secondary injury mechanisms in acute stages of SCI. Therefore, this thesis sought to elucidate

the role(s) of BMP4 in modulating early cell responses by targeting BMP4 during the acute stage of SCI in rats.

1.4.2 General hypothesis and research objectives

The overall goal of my PhD thesis was to understand the role of an acute and transient upregulation in BMP4 in modulating secondary injury mechanisms and recovery after SCI.

Hypotheses:

1. BMP4 inhibits oligodendrogenesis by limiting survival, differentiation and maturation of activated NPCs and OPCs, thereby limiting the remyelination process.
2. BMP4 potentiates astrocyte differentiation, reactive astrogliosis and CSPG production that directly and indirectly limits remyelination after SCI.

Specific research objectives:

1. Investigate the impact of BMP4 on endogenous precursor cell response in culture and following rat SCI.
2. Investigate the effects of BMP4 on reactive astrogliosis and glial scar formation.
3. Investigate the role of BMP4 on cell death and/or oxidative stress.

These questions were addressed in a published research manuscript that is reported in Chapter 2 of this thesis.

Chapter 2: Acute upregulation of bone morphogenetic protein-4 regulates endogenous cell response and promotes cell death in spinal cord injury

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Author Contributions: My contribution to this work includes concept and design, collection and/or assembly of data, data analysis and interpretation, manuscript writing and final approval of manuscript. I contributed to over 80% of the total experimental procedures, data and analysis. Manuscript preparation was completed in collaboration with my supervisor. Scott Dyck and Arsalan Alizadeh contributed by completing *in vivo* SCI surgeries. Hardeep Kataria assisted with semi-thin g-ratio analysis and OPC cultures, while James Thliveris embedded, sectioned and stained spinal cord semi-sections for analysis. Immunoblotting for cleaved caspase-3 and lipid peroxidation depicted in Figure 6 was completed with guidance from Pandian Nagakannan and Eftekhar Eftekharpour.

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Conflict of Interest: The authors declare no competing financial interests.

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2.1 Abstract

Traumatic spinal cord injury (SCI) elicits a cascade of secondary injury mechanisms that induce profound changes in glia and neurons resulting in their activation, injury or cell death. The resultant imbalanced microenvironment of acute SCI also negatively impacts regenerative processes in the injured spinal cord. Thus, it is imperative to uncover endogenous mechanisms that drive these acute injury events. Here, we demonstrate that the active form of bone morphogenetic protein-4 (BMP4) is robustly and transiently upregulated in acute SCI in rats. BMP4 is a key morphogen in neurodevelopment; however, its role in SCI is not fully defined. Thus, we elucidated the ramification of BMP4 upregulation in a preclinical model of compressive/contusive SCI in the rat by employing noggin, an endogenous antagonist of BMP ligands, and LDN193189, an intracellular inhibitor of BMP signaling. In parallel, we studied cell-specific effects of BMP4 on neural precursor cells (NPCs), oligodendrocyte precursor cells (OPCs), neurons and astrocytes *in vitro*. We demonstrate that activation of BMP4 inhibits differentiation of spinal cord NPCs and OPCs into mature myelin-expressing oligodendrocytes, and acute blockade of BMPs promotes oligodendrogenesis, oligodendrocyte preservation and remyelination after SCI. Importantly, we report for the first time that BMP4 directly induces caspase-3 mediated apoptosis in neurons and oligodendrocytes *in vitro*, and noggin and LDN193189 remarkably attenuate caspase-3 activation and lipid peroxidation in acute SCI. BMP4 also enhances the production of inhibitory chondroitin sulfate proteoglycans (CSPGs) in activated astrocytes *in vitro* and after SCI. Interestingly, our work reveals that despite the beneficial effects of BMP inhibition in acute SCI, neither noggin nor LDN193189 treatment resulted in long-term functional recovery. Collectively, our findings suggest a role for BMP4 in regulating acute secondary injury mechanisms following SCI, and a

potential target for combinatorial approaches to improve endogenous cell response and remyelination.

2.2 Introduction

Traumatic spinal cord injury (SCI) results in an imbalanced microenvironment in the spinal cord within hours and days of injury (Alizadeh et al., 2019). The complex secondary injury mechanisms start with the disruption of vasculature and the blood-spinal cord-barrier (BSB) after primary mechanical SCI that lead to dramatic changes to the cellular and molecular composition of the injured spinal cord (Ahuja et al., 2017; Alizadeh et al., 2019). Response of resident glia and infiltration of leukocytes into the spinal cord induces inflammation, astrogliosis, and oxidative stress causing neuronal and oligodendroglial injury and tissue degeneration (Karimi-Abdolrezaee and Billakanti, 2012; Orr and Gensel, 2018). Reactive astrocytes, in particular, are highly proliferative and surround the lesion to restrict inflammation and noxious stimuli (Wanner et al., 2013). However, activated astrocytes are a major source of inhibitory factors such as chondroitin sulfate proteoglycans (CSPGs), which impede neural regeneration, cell replacement and remyelination and induce oligodendroglial death (Shen et al., 2009; Cregg et al., 2014; Ohtake et al., 2016; Dyck et al., 2019). Oligodendrogenesis is an endogenous repair process after SCI that occurs spontaneously and is important for remyelination (McTigue and Tripathi, 2008; Alizadeh et al., 2015; Hesp et al., 2015). In the adult injured spinal cord, oligodendrogenesis is attributed to the populations of multipotent neural precursor cells (NPCs) and adult oligodendrocyte progenitor cells (OPCs) (Alizadeh et al., 2015). Our group and others have shown that the behaviour of OPCs and NPCs is highly influenced by dysregulated microenvironment of SCI (Wang et al., 2011; Karimi-Abdolrezaee and Eftekharpour, 2012; Karimi-Abdolrezaee et al., 2012; Gauthier et al.,

2013b; Dyck et al., 2019). To this end, efforts have been made to promote the regenerative response of spinal cord precursor cells following SCI by unravelling injury mechanisms that regulate their behaviour within their injury microenvironment.

Emerging evidence suggests that expression of bone morphogenetic protein-4 (BMP4) is highly upregulated in acute SCI lesions and in demyelinating conditions (Fuller et al., 2007; Ara et al., 2008; Xiao et al., 2010; Sandner et al., 2013; Cui et al., 2015; Govier-Cole et al., 2019). BMP4 is a morphogen and a member of the transforming growth factor (TGF)-β superfamily with a well-established role in regulating neural tube patterning and glial differentiation during neurodevelopment. BMPs bind and dimerize with BMP Type I (BMPR1a/R1b) and Type II receptors to activate canonical Smad1/5/8 signaling and lesser studied non-canonical MAPK/Erk1/2 and PI3K/Akt pathways (Gallea et al., 2001; Barneda-Zahonero et al., 2009). In the developing CNS, BMP4 inhibits oligodendrogenesis at a transcriptional level, while promoting astrocyte differentiation in NPC population (D'Alessandro and Wang, 1994; Miller et al., 2004; Samanta and Kessler, 2004). Transplantation of NPCs that express noggin, an endogenous inhibitor of BMPs, promotes differentiation of NPCs into neural lineages after SCI (Setoguchi et al., 2004). Moreover, studies in demyelinating lesions demonstrate that inhibition of BMPs by noggin or LDN193189, an intracellular inhibitor of BMP signaling, promotes oligodendrocyte differentiation and improves chronic remyelination (Sabo et al., 2011; Govier-Cole et al., 2019). Therefore, upregulation of BMP4 after SCI may contribute to a non-permissive niche for oligodendrogenesis and remyelination. Currently, ramifications of BMP4 dysregulation on acute secondary injury mechanisms in SCI remain largely unclear.

In the present study, in a clinically relevant model of contusive/compressive SCI in rats, we have observed a robust and transient upregulation of the active form of BMP4 protein in acute

phase of injury. We have used recombinant BMP4 peptide and two inhibitors of BMP signaling (noggin and LDN193189) to study the role of BMP4 in rat SCI and primary *in vitro* models. We demonstrate that BMP4 negatively regulates the differentiation and maturation of spinal cord NPCs and OPCs into myelin-expressing oligodendrocytes *in vitro*. Inhibition of BMPs in acute SCI can enhance oligodendrogenesis, oligodendrocyte preservation and myelin thickness in the injured spinal cord. Importantly, upregulation of BMP4 is associated with lipid peroxidation and caspase-3 mediated cell death in acute SCI, which can be remarkably ameliorated by noggin and LDN193189 treatments. Moreover, BMP4 promotes astrocyte reactivity and induces their production of inhibitory CSPGs, which can be attenuated by noggin and LDN193189. Despite the beneficial effects of noggin and LDN193189 treatments, our studies indicated no long-term improvement of functional recovery in chronic SCI. Collectively, our study identifies BMP4 as an early regulator of oligodendrogenesis, cell death and astrocyte reactivity after SCI, and a potential target for combinatorial approaches to improve remyelination in the injured spinal cord.

2.3 Materials and methods

2.3.1 Animals and animal care

All animal experimental protocols were approved by the Animal Ethics Committee of the University of Manitoba in accordance with the policies established by the Canadian Council of Animal Care (CCAC). A total of 105 female Sprague Dawley (SD) rats (250g) were used for SCI studies (Table 2.1). For *in vitro* studies, we used 20 postnatal (1-3 days) SD rat pups for primary cortical astrocytes and oligodendrocyte precursor cell cultures, and 4 time-pregnant SD rats for embryonic day 18 (E18) primary cortical neuron cultures. Rats were provided by Central Animal Care at the University of Manitoba. For primary spinal cord neural stem/progenitor cell cultures,

a total of 20 adult mice (6-8 weeks of age, enhanced yellow fluorescent protein (YFP)-expressing transgenic mice [strain129-Tg(ACTB-EYFP)2Nagy/J]) were used from a colony (CAG-EYFP Tg) maintained at a local facility of the University of Manitoba, Winnipeg, Canada. The initial founders of 129-Tg(ACTB-EYFP)2Nagy/J colony were generously provided by Dr. Andras Nagy (Lunenfeld-Tanenbaum Research Institute, Toronto, Ontario, Canada). Animals were housed in standard plastic cages and had easy access to soft paper bedding, food pellets and drinking water under a 12 hr light/dark cycle.

Table 2.1. Number of animals per experimental group in SCI studies

		Assessment			
Time-point	Experimental Group	WB/SB	IHC	Semi-thin	Total
	Uninjured	4	-	-	4
1-day	SCI-Untreated	4			4
3-day	SCI-Untreated	4			4
5-day	SCI-Untreated	4			4
3-day	SCI-Vehicle	4	6	-	10
	SCI-Noggin (1.2ug/day)	5	4	-	9
	SCI-LDN193189 (1.25mg/day)	5	4	-	9
3-day	SCI-Vehicle	4	7	-	11
	SCI-Noggin (2.4ug/day)	4	6	-	10
	SCI-LDN193189 (2.5mg/day)	4	5	-	9
10-weeks	SCI-Vehicle	-	4	5	9
	SCI-Noggin (2.4ug/day)	-	4	5	9
	SCI-LDN193189 (2.5mg/day)	-	4	5	9
	Un-injured	-	-	4	4
				Sum	105

WB/SB, Western or slot blot; **IHC**, immunohistochemistry

2.3.2 Rat model of compressive spinal cord injury

We used a clinically relevant model of clip-compression SCI in adult female rats (Dyck et al., 2018; Gauthier et al., 2013; Karimi-Abdolrezaee et al., 2010; Karimi-Abdolrezaee et al., 2012; Rivlin & Tator, 1977). Briefly, rats were deeply anesthetized under sterile conditions and the

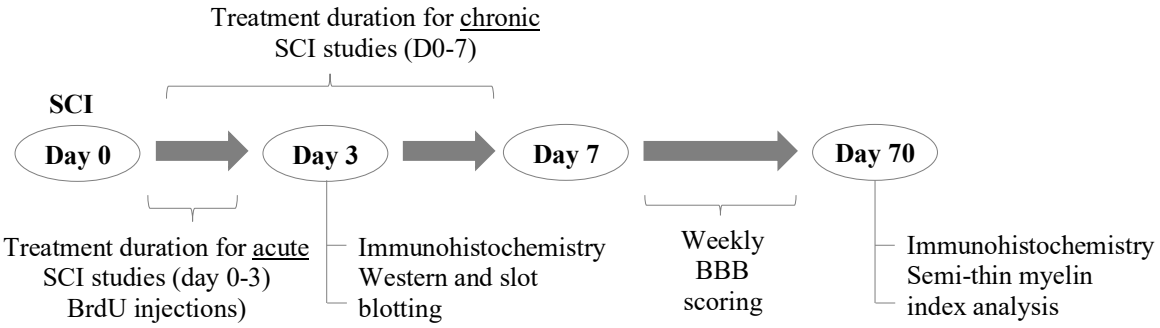
surgical area was shaved and sterilized with 70% ethanol and povidone iodine. The thoracic spinal column (T4-T9) was exposed by a midline incision and retraction of skin and superficial muscle. These animals were laminectomized (T6-8) and injury was induced by spinal cord compression at the level of T7 for 1min using a 35g modified aneurysm clip (University Health Network, Toronto, Ontario, Canada). After the clip was removed and the surgical wounds were sutured, pain was mitigated by a pre-surgical injection of meloxicam (Metacam, Boehringer Ingelheim Burlington, Ontario, Canada, 2mg/kg), a post-surgical injection of buprenorphine (Vetergesic, 0.03 mg/kg Champion Alstoe Animal Health, Whitby, ON, Canada) and three additional doses of buprenorphine with 8 hr intervals. To alleviate post-surgical dehydration, animals received subcutaneous injections of 0.9% saline (5ml). Also, Clavamox® (Amoxicillin plus Clavulanic Acid, Pfizer) was delivered orally via drinking water starting 2 days prior to surgery and up to 3 days post-operation to prevent trauma-induced hematuria and bladder infections. SCI rats received daily inspections for signs of discomfort or illness, and their bladders were expressed three times a day until they regained full urinary control.

2.3.3 SCI Experimental groups and treatments

Animals were randomly assigned to four experimental groups: (1) Uninjured control; (2) SCI/vehicle control; (3) SCI/Noggin (received 1.2-2.4μg/day noggin (R&D Systems, 6057-NG) in vehicle solution containing 0.1% bovine serum albumin, BSA, in 0.1M phosphate buffer saline, PBS intrathecally delivered by mini osmotic pump implanted at time of injury (ALZET 1003D, 3-day); and (4) SCI/LDN193189, (received 1.25-2.5mg/day LDN193189 (Sigma-Aldrich, SML0559) in 100% dimethyl sulfoxide, DMSO, diluted in 0.9% saline through two daily subcutaneous injections). SCI/vehicle control group received noggin and LDN193189 vehicle solution in the same manner. Animals also received one intraperitoneal injection of 5-bromo-2'-

deoxyuridine, BrdU (50mg/kg, prepared in NaOH, filtered sterile) immediately following injury, and additional injections every 12 hr until experimental end-point. A final injection of BrdU was given 2 hr prior to experimental endpoint. Information on experimental groups, endpoints and sample size can be found in Tables 2.1-2.2.

Table 2.2 Experimental paradigm for *in vivo* SCI studies



Experimental groups:

1. Uninjured tissue
2. SCI + Vehicle
3. SCI + Noggin (1.2 or 2.4ug/day for acute studies; 2.4ug/day for chronic studies)
4. SCI + LDN193189 (1.25 or 2.5mg/day for acute studies; 2.5mg/day for chronic studies)

Sample size: N = 3-7 rats/group for acute histological and molecular analyses, N = 9 rats/group for chronic functional analysis (N=4 for histology, N=5 for semi-thin preparation)

2.3.4 Tissue processing

At each experimental endpoint, rats were deeply anesthetized and were transcardially perfused with ice-cold 0.1M PBS. For Western and slot blot analysis, a 5mm length of the spinal cord centered at the injury epicenter was excised and homogenized in 1% NP40 buffer (Calbiochem, 492015) with SIGMAFAST^{TM} protease (Sigma, S8820) and PhosSTOP phosphatase (Roche, 04906845001) inhibitors. For immunohistological analyses, SCI rats were perfused with ice-cold 2.5% paraformaldehyde (PFA) for tissue fixation and submerged in 10% sucrose in 2.5% PFA overnight at 4°C for post-fixation and cryoprotection. Tissues underwent further cryoprotection in

20% sucrose in PBS for two more days at 4°C. A 2cm section of spinal cord centered at the injury epicenter was cut and embedded in optimal cutting temperature compound OCT (Tissue-Tek®, Electron Microscopy Sciences, 102094) and frozen solid over dry ice. Tissues were cross-sectioned by cryostat (Leica Biosystems GmbH) at a thickness of 35µm, and serial sections were mounted onto Superfrost® Plus Micro Slides (Fisher Scientific) and stored at -80°C until immunostaining.

2.3.5 Immunohistochemistry on tissue sections

Frozen tissue sections were processed for immunohistological analysis and Luxol Fast Blue (LFB) and Hematoxylin and Eosin (H&E) staining, as we previously described (Karimi-Abdolrezaee et al., 2012; Alizadeh et al., 2017; Dyck et al., 2018). The sections were incubated with primary antibodies overnight at 4°C (Table 2.3), followed by incubation with appropriate secondary antibodies including goat anti-chicken 488, anti-mouse 568 or anti-rabbit 647 Alexa Fluor (1:400; Invitrogen, Life Technologies Inc, ON, Canada) for 1 hr. In a double immunostaining procedure, the tissue sections were treated with a second primary antibody and then incubated with an appropriate secondary antibody. Following each incubation stage, tissue sections were washed four times with 0.1M PBS for 10min. Finally, all slides were stained with 4,6-diamidino-2-phenylindole (DAPI, Sigma D8417) for 30min at room temperature to identify cell nuclei, washed and cover-slipped with Mowiol mounting media (Sigma, 81381). For BrdU immunodetection, sections underwent acid pretreatment, blocking and antibody incubation as we previously described (Gauthier et al., 2013a; Dyck et al., 2018; Kataria et al., 2018). In a double immunostaining procedure, acid treatment and BrdU immunostaining were conducted last. In our immunohistochemical procedures, the specificity of all antibodies was validated through

implementation of negative controls (omitting primary antibody from staining protocol) and positive controls (cells or tissues known to express the antigen).

Table 2.3. List of antibodies used for protein and immunostaining analysis

Antibody	Targeted Species	Application	Dilution Factor	Source (CAT#)
BMP4	Mouse	WB IHC	1:100	Santa Cruz (sc-12721)
BrdU	Mouse	IHC ICC	1:400 1:600	BD Pharmigen (555627)
βtubulin-III	Mouse	ICC	1:300	Sigma (T8660)
Cleaved Caspase-3	Rabbit	WB ICC	1:1000 1:150	Cell Signaling (9664)
CS56 (CSPGs)	Mouse	SB IHC	1:500 1:150	Sigma (C8035)
E06	Mouse	SB	1:1000	Avanti (330001S)
GAPDH	Rabbit	WB	1:1000	Santa Cruz (sc-47724)
GFAP	Mouse Rabbit Chicken	WB ICC/IHC IHC	1:10,000 1:800/1:500 1:500	Sigma (G3893) DAKO (Z0334) AVES (GFAP)
HMGB1	Rabbit	WB	1:1000	Sigma (H9539)
Iba1	Rabbit Rabbit	WB IHC	1:1000 1:600	WAKO (016-20001) WAKO (019-19741)
IL-1β	Rabbit	WB	1:1000	Serotec (AAR15G)
MBP	Rabbit	ICC	1:500	Millipore (AB980)
Nestin	Chicken	ICC	1:600	AVES (NES)
NeuN	Mouse	IHC	1:150	Chemicon (MAB377)
NF200	Rabbit	IHC	1:500	Sigma (N4142)
O4	Mouse	ICC	1:200	R&D (MAB1326)
Olig2	Rabbit	IHC ICC	1:500 1:1000	Chemicon (AB9610)
PDGFRβ	Rabbit	IHC	1:100	Santa Cruz (sc-432)
pSmad1/5/8	Rabbit	WB ICC	1:1000 1:800	Cell Signaling (D5B10)
TNFα	Rabbit	WB	1:1000	Serotec (AAR33)

WB, Western blot; **SB**, slot blot; **IHC**, immunohistochemistry; **ICC**, immunocytochemistry;

2.3.6 Imaging and immunohistological analysis of SCI tissues

Co-immunostaining of GFAP, CS56 and Iba1 was completed at injury epicenter and perilesional tissue (+/- 1mm) in all SCI groups at 3 days post-injury (dpi) and 10 weeks post-injury (wpi). Immunostaining of BMP4 was assessed up to 6mm rostral and caudal from injury epicenter in both untreated and treated SCI rats at 3dpi. These analyses were conducted based on our previously described protocol (Alizadeh et al., 2017). All tissue sections were processed in parallel under identical conditions and imaged using Zeiss AxioImager M2 fluorescence microscope (Zeiss) with consistent exposure time. Each cross-section was imaged entirely using a 10x objective with Zen tiling software (Zeiss). After background was automatically removed, immunointensity above the threshold was quantified for each tissue cross-section using NIH ImageJ software (imagej.nig.gov), while excluding meninges. Immunointensity was normalized to total area of the sections using the following formula: total immunointensity of tissue section/total area of tissue section. Additionally, qualitative immunostaining of BMP4 was conducted at 2mm caudal to injury epicenter with specific cell populations as labeled by GFAP (astrocytes), NF200 (neurons), Olig2 (oligodendrocyte lineage), Iba1 (microglia/macrophages), and PDGFR-β (pericytes). Representative images were taken using a 63x oil-immersion objective, and co-localization was confirmed by Z-stacking. All tissue quantification was completed by an examiner who was blinded to experimental groups.

2.3.7 Assessment of proliferating and apoptotic cells in SCI tissue

Co-immunostaining of Olig2, GFAP and BrdU was conducted on spinal cord cross sections to evaluate endogenous cell proliferation amongst oligodendroglia and astrocytes in acute (3dpi) SCI. We performed this analysis at approximately 3mm rostral to the injury epicenter where all injured rats had an intact central canal and ependymal layer, the region that endogenous NPCs

reside. In each rat, we quantified an average cell count of approximately 400 DAPI+ cells. All tissue sections were processed in parallel under identical conditions and imaged at 20x magnification using Zeiss AxioImager M2 fluorescence microscope (Zeiss). We quantified proliferating astrocytes (GFAP+/BrdU+) and oligodendrocytes (Olig2+/BrdU+) relative to total BrdU+ cells. We also studied cell preservation in non-BrdU labeled pre-existing astrocytes (GFAP+/BrdU-) or oligodendrocytes (Olig2+/BrdU-) relative to total cells (DAPI+/BrdU-). All analyses were completed by an examiner who was blinded to experimental groups. Co-immunostaining of NeuN (neurons) or APC (oligodendrocytes) with cleaved caspase-3 (ClCasp3) was conducted at 1 and 2mm rostral to injury epicenter in spinal cord cross sections at 3dpi to evaluate apoptosis amongst neurons and oligodendroglia in acute SCI. All tissue sections were processed as described above for assessment of cell proliferation. We quantified apoptotic neurons (NeuN+/ClCasp3+/DAPI+) and oligodendrocytes (APC+/ClCasp3+/DAPI+) relative to total neurons or oligodendrocytes (NeuN+/DAPI+ or APC+/DAPI+). In all cell quantifications, DAPI was used to identify cell nuclei.

2.3.8 Semi-thin sectioning and tissue analysis

Semi-thin tissue analysis was performed in 10-week post-SCI rats as we previously described (Kataria et al., 2018). G-ratio was defined as the ratio between the diameter of the axon and the fiber diameter (including myelin sheath). For each rat, four regions (dorsal column, ventral column and two laterals) were imaged independently at 1mm rostral to injury epicenter using 63x oil-immersion objective with Zen tiling software (Zeiss). Ten digital images were captured of non-overlapping microscope fields (375μm^2) randomly within each region, with a total of forty images per animal. A grid was superimposed over each individual field using grid overlay feature of ImageJ software. The tile width was set to 250mm^2 resulting in 10 equidistant vertical lines. We

counted and assessed the g-ratio of each axon that was crossed by a vertical line, and at least 500 axons were counted for each animal by an examiner blinded to their experimental group.

2.3.9 Neurological assessment by BBB open field locomotor scoring

Basso, Beattie, Bresnahan (BBB) locomotor rating scale was used to evaluate longitudinal hindlimb motor function weekly up to 10wpi. Briefly, SCI rats were placed individually on a flat and enclosed surface and permitted a few minutes to acclimatize to their surroundings. After which, the 22-point (0–21) BBB scale was used to assess hindlimb locomotor recovery including joint movements, stepping ability, coordination, and trunk stability. Rats were observed for 4-5 minutes and given a score denoting the degree of motor function. The tests were performed by two examiners who were blinded to the animals' treatments.

2.3.10 Histological assessment of chronic SCI lesion

For assessing tissue preservation in chronic SCI rats, we used LFB/H&E staining of serial cross sections at 1mm intervals that spanned the injury epicenter by up to 4mm rostrally and caudally, and imaged each section using a 10x objective with Zeiss AxioImager M2 and tiling function of Zeiss Zen software. We quantified tissue preservation using NIH ImageJ software as follows: [(total area – cavity area) / total area] x 100 as we described previously (Alizadeh et al., 2017).

2.3.11 Isolation, culture and treatment of primary spinal cord NPCs

NPCs were isolated from the spinal cords of adult YFP+-transgenic mice as previously described (Meletis et al., 2008; Gauthier et al., 2013a; Dyck et al., 2015). Briefly, mice were deeply anesthetized and euthanized by cervical dislocation. Segments of mouse spinal cord tissue were digested and triturated in papain enzymatic mixture (37°C for 50 min) by fire polished Pasteur

pipette. After a brief centrifugation, cells were resuspended in papain inhibitor solution (Worthington Biochemical Corp., LK003160) and filtered through a 70μm strainer and a 7.5% BSA gradient solution in 0.1M sterile PBS. Cells were resuspended in neurobasal growth media and seeded on uncoated T25 flask. Resulting neurospheres were passaged weekly by mechanical dissociation in the same media.

For BMP4 experimentation, dissociated NPCs from passages 3-7 (P3-7) were plated at 10-20x10^3 cells per well onto 8-well glass multi-chambers (LabTek II, Fisher, 125658) coated with matrigel (VWR, CACB354230). NPCs were maintained in fresh growth media overnight and switched to basal serum (1% fetal bovine serum; FBS) or serum-free and growth factor-free media with or without treatment for 1-, 2-, 5- or 7-days, with half of their media volume refreshed every 2-3 days. Treatments included BMP4 (10-150ng/ml) (rhBMP4; ABM, Z100105; ddH_2O + 0.1% BSA) with or without noggin (50-150ng/ml) or LDN193189 (50nM-1.5μM). Prior to treating cells, noggin and BMP4 were incubated together in media for 10 min at 37°C to optimize associative inhibition. Additionally, cells were pre-treated with LDN193189 alone for 30min at 37°C to promote cellular uptake and binding to BMP Type 1 receptors. After 30 min, the media was completely refreshed with treatment LDN193189 media including BMP4. NPCs proliferation was assessed under control or BMP4 conditions for 48 hrs using BrdU (20μM), which was added to media for the final 24 hrs. To assess cell viability, NPCs were treated with control (basal media, DMSO), and BMP4 (50-150ng/ml) or LDN193189 treatment (50nM-1.5μM) for 24 hrs. We evaluated cell viability either by LIVE/DEAD assay (LIVE/DEAD® Viability/Cytotoxicity Kit; Thermo Fisher, L3224) or WST-8 assay (CCK-8, Dojindo Molecular Technologies Inc., CK04) according to the manufacturer's instructions. Images of 8–10 randomly chosen fields were taken under 20× objective with a Zeiss fluorescent microscope with 494 nm (green, Calcein) and 528

nm (red, EthD-1) excitation filters. For analysis, the percentage of live cells was determined by quantifying the total number of LIVE (green) cells compared with the total number of LIVE (green) and DEAD (red) cells in analyzed fields as we described previously (Dyck et al., 2019). For WST-8 assay, CCK-8 reagent was added directly to NPC media in 96-well plates and incubated at 37°C for 4 hr. Absorbance was measured at 450nm using a Synergy™ H1 hybrid multi-mode 96-well plate reader. Media alone (no cells) served as blank control.

2.3.12 Isolation, culture and activation of primary cortical astrocytes

Primary glial cells were isolated from cortices of postnatal SD rats (P1-3) as we described previously (Alizadeh et al., 2017). Rat pups were deeply anesthetized by isoflurane inhalation, sterilized with 70% ethanol and euthanized by decapitation. Cortices were mechanically dissociated and seeded at low-density in uncoated T75 flasks with 20% horse serum media for one day, after which they were switched to astrocyte DMEM selection media with 5% horse serum (supplemented with 1M sorbitol, L-glutamine, PSN). Astrocytes were maintained for ten days, with half the media refreshed every three days, prior to being switched to glucose media with 5% horse serum until fully confluent (approximately 21 days post-culture). To reduce microglial contamination, astrocyte cultures were shaken for 6 hr at 37°C and media was completely discarded and refreshed to remove floating microglia. Then, purified astrocytes were seeded onto uncoated six-well plates. Once astrocytes reached 70-80% confluency, they were serum-deprived overnight prior to 3-day treatment with either BMP4 (25-100ng/ml), noggin (150ng/ml), LDN193189 (100-250nM), TGFβ (25ng/ml) (rhTGFβ; R&D Systems, 240-B) or interleukin-1β (IL-1β, 50ng/ml) (rrIL-1β; R&D Systems, 501-RL-010) and tumour necrosis factor-α (TNFα,

50ng/ml) (rrTNFα; Shenandoah, 300-18) following protocols as described above for cultured NPCs.

2.3.13 Isolation, culture and treatment of primary cortical oligodendrocyte precursor cells

Primary OPCs were derived from cortices of postnatal SD rats (P1-3), as described previously (Kataria et al., 2018; Dyck et al., 2019). Briefly, the cortices were minced and incubated in papain solution for 1.5 hr at 37°C, and cells were isolated as previously described above for NPCs. The cells were resuspended in DMEM-FBS media (20% FBS, sodium pyruvate (Invitrogen, 11360-070), L-glutamine and PSN) and seeded onto T75 flasks. Media was completely refreshed every 2-3 days up to time of confluency at 10 days post-culture. Confluent mixed cultures were shaken at 200rpm for 1 hr at 37°C, discarding and refreshing media to remove dislodged microglia, and shaking continued overnight (18-20 hr) at 200rpm and 37°C. Suspended cells were collected, passed through a 40μm filter and centrifuged at 1500rpm for 10 min. Cells were seeded at $1x10^4$ cells/cm^2 in OPC DMEM growth media onto PDL-coated 8-well glass multi-chambers and maintained for 7-days in culture, with half the media being refreshed every 2-3 days. To evaluate maturation of OPCs into myelinating oligodendrocytes, cultured OPCs were treated for 7-days with or without BMP4 (10-100ng/ml) in basal media including T3 (3,3′,5-Triiodo-L-thyronine sodium salt; Sigma-Aldrich, T6397). To evaluate cell death, mature oligodendrocytes underwent a 24 hr treatment with identical conditions and protocols as described above.

2.3.14 Isolation, culture and treatment of primary cortical neurons

Primary cortical neurons were derived from cortices of embryonic (E18) SD rat. Cortices were isolated and incubated in papain solution for 15-20 min at 37°C. Then, the supernatant was

discarded and tissues were resuspended in 1ml aCSF and 1x DNase (Sigma, D5025) and mechanically dissociated by fire polished Pasteur pipette. Enzymatic activity was neutralized by adding 1ml FBS and cells were filtered through a 40µm strainer. The cells were diluted in 5ml neuronal media consisting of Neurobasal-A (Gibco, 10888-022), 1% B27 supplement (Gibco, 17504-044), PSN and L-glutamine, followed by a brief centrifugation. Cells were seeded at $1x10^5$ cells/well into PDL-coated 8-well glass multi-chambers and maintained for 7-days in culture, with half the media being refreshed every 2-3 days. Cortical neurons underwent a 3-day treatment, with identical conditions and protocols as described above.

2.3.15 Immunostaining procedures for *in vitro* cultures

Cultured cells were fixed with 3% PFA for 20 min and washed with PBS at room temperature. The cultures were blocked and incubated with antibodies in identical solution described for *in vivo* immunohistological analyses with the exclusion of Triton-X (0.3%) for secondary antibody incubations. The only exceptions were Smad1/5/8 phosphorylation in NPCs and cleaved caspase-3 in cortical neurons and oligodendrocytes. Smad1/5/8 phosphorylation required a 10min incubation with cold methanol (MeOH, 100%) at -20°C following PFA fixation, blocking with 5% normal goat serum (NGS) and 0.3% Triton-X, and pSmad1/5/8 antibody incubation in 1% BSA and 0.3% Triton-X. Cleaved caspase-3 antibody required blocking in 5% NGS and antibody incubation in 1% BSA and 0.3% Triton-X. All relevant details pertaining to primary antibodies are provided in Table 3. For all immunocytological assays, fluorescence images of 4-8 random fields were taken with 10-20x magnification, respectively. Overall, a minimum of 200 cells were counted for each condition.

2.3.16 Western and slot blotting

Protein content was determined in spinal cord tissue, cell lysate and conditioned media by Lowry assay (Sigma, L3540). For Western blotting, a total of 10-40ug per sample was loaded onto 10-15% Tris-glycine gels and transferred onto nitrocellulose or polyvinyl fluoride (PVDF) membranes using Trans-Blot® Turbo Transfer system (BioRad, 2.5V for 10min). Membranes were blocked for 1 hr at room temperature with either 5% non-fat milk or BSA in Tween-20 Tris-buffered saline (0.2% TBST) and incubated for 2 hr at room temperature or overnight at 4°C with primary antibodies diluted in blocking solution (Table 3). Membranes were washed and incubated with horseradish peroxidase-conjugated (HRP) goat anti-mouse or -rabbit secondary antibodies (1:4000) for 1 hr at room temperature. For slot blot analysis, 3-10ug of protein from spinal cord tissue or astrocyte conditioned media was loaded onto nitrocellulose membrane using the Bio-Dot® slot blot system and similar blocking and antibody incubation protocols as described above. Slot blot analysis was used to evaluate CSPGs expression and lipid peroxidation, as it is a more suitable analysis for these markers (Zhang et al., 2014; Alizadeh et al., 2017; Dyck et al., 2018). For Western and slot blot analyses, targeted bands were detected with Clarity™ Western ECL substrate (BioRad, 1705060) using MicroChemi 4.2 (DNR Bio-imaging Systems) following manufacturer's specifications. Densitometry analysis of protein bands was conducted using NIH ImageJ software. To control for variances in protein loading, results were normalized to total protein (Ponceau stain) or glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression.

2.3.17 Statistical analysis and randomization

Statistical analysis was completed for all *in vitro* and *in vivo* assessments using SigmaStat software. Student t-test or One-Way ANOVA followed by Holm-Sidak *post hoc* was used to

determine significance between two or more treatment groups. For SCI immunohistological and behavioural analyses, we employed Two Way ANOVA repeated measures followed by Holm-Sidak *post hoc*. Student test was used to determine significance for semi-thin g-ratio analysis between untreated and treated groups. Significance was noted if $*p<0.05$. All data is expressed as mean $\pm$ standard error of mean (SEM). To ensure integrity of results, proper blinding and randomization was applied for all assessments.

2.4 Results

2.4.1 Active BMP4 expression is transiently upregulated in acute SCI

In a clinically relevant model of mid-thoracic compressive/contusive SCI in the rat (Karimi-Abdolrezaee et al., 2006; Karimi-Abdolrezaee et al., 2010; Alizadeh et al., 2017; Dyck et al., 2018), we demonstrate that SCI results in acute and transient upregulation of BMP4 protein in the injured spinal cord. We conducted a time-point analysis for protein expression of pro- and active forms of BMP4 at 1-, 3- and 5-days post-injury (dpi), which represent acute phase of injury in our rat SCI model. Our Western blot analysis, on spinal cord homogenate of a 5mm length of the spinal cord centered at the injury epicenter, showed temporally distinct expression profiles for pro- and active BMP4 levels. While our analysis of injured spinal cord tissue showed a slight but insignificant increase in expression of pro-BMP4 up to 5dpi relative to uninjured tissue (Fig. 2.1A), there was a pronounced and significant increase in active form of BMP4 protein at 1dpi that peaked at 3dpi (3.2-fold) and returned to its basal levels by 5dpi (Fig. 2.1B). Immunohistological staining of spinal cord cross sections at 3dpi showed that BMP4 expression was widely distributed at the injury epicenter and adjacent segments (Fig. 2.1C). Interestingly, in areas farther from the injury epicenter (3mm rostral and caudal points), upregulated level of BMP4 was predominantly confined

to the gray matter regions. In uninjured segments of the spinal cord (6mm from the injury epicenter), BMP4 expression was negligibly detected. These immunohistochemical data confirmed the specificity of our Western blot data, showing upregulated BMP4 within the injured spinal cord tissue. Our co-immunostaining between BMP4 and various cell markers in the injured spinal cord at 3dpi showed that BMP4 expression was localized predominantly in astrocytes (GFAP+), neurons (NF200+), and oligodendroglia (Olig2+) (Fig. 2.1D-M). There was negligible co-localization of BMP4 with microglia/macrophages (Iba1+) or pericytes (PDGFRβ+) (Fig. 2.1N-S). Co-localization and specificity of BMP4 to each cell-type was verified by Z-stacking analysis, as illustrated with astrocytes (Fig. 2.1G). Our parallel *in vivo* experiments also showed a significant increase of BMP4 protein in conditioned media of activated astrocytes after 72 hr treatment with IL-1β and TNFα (Fig. 2.1T), two prominent pro-inflammatory cytokines that potentiate reactive astrogliosis in acute SCI (Barna et al., 1990; Herx and Yong, 2001; Yang et al., 2005). These *in vivo* and *in vitro* data indicate that activated astrocytes contribute to BMP4 upregulation after SCI. Lastly, we conducted a time-point analysis of phosphorylated Smad1/5/8, the canonical signaling pathway for BMP ligands, in the injured spinal cord tissue by Western blot. We found a significant increase in Smad1/5/8 phosphorylation at 3dpi, which remained elevated up to 5dpi (Fig. 2.1U), confirming increased activation of BMP signaling in acute SCI.

Figure 2.1. Active form of BMP4 protein is transiently upregulated in acute SCI in the rat.

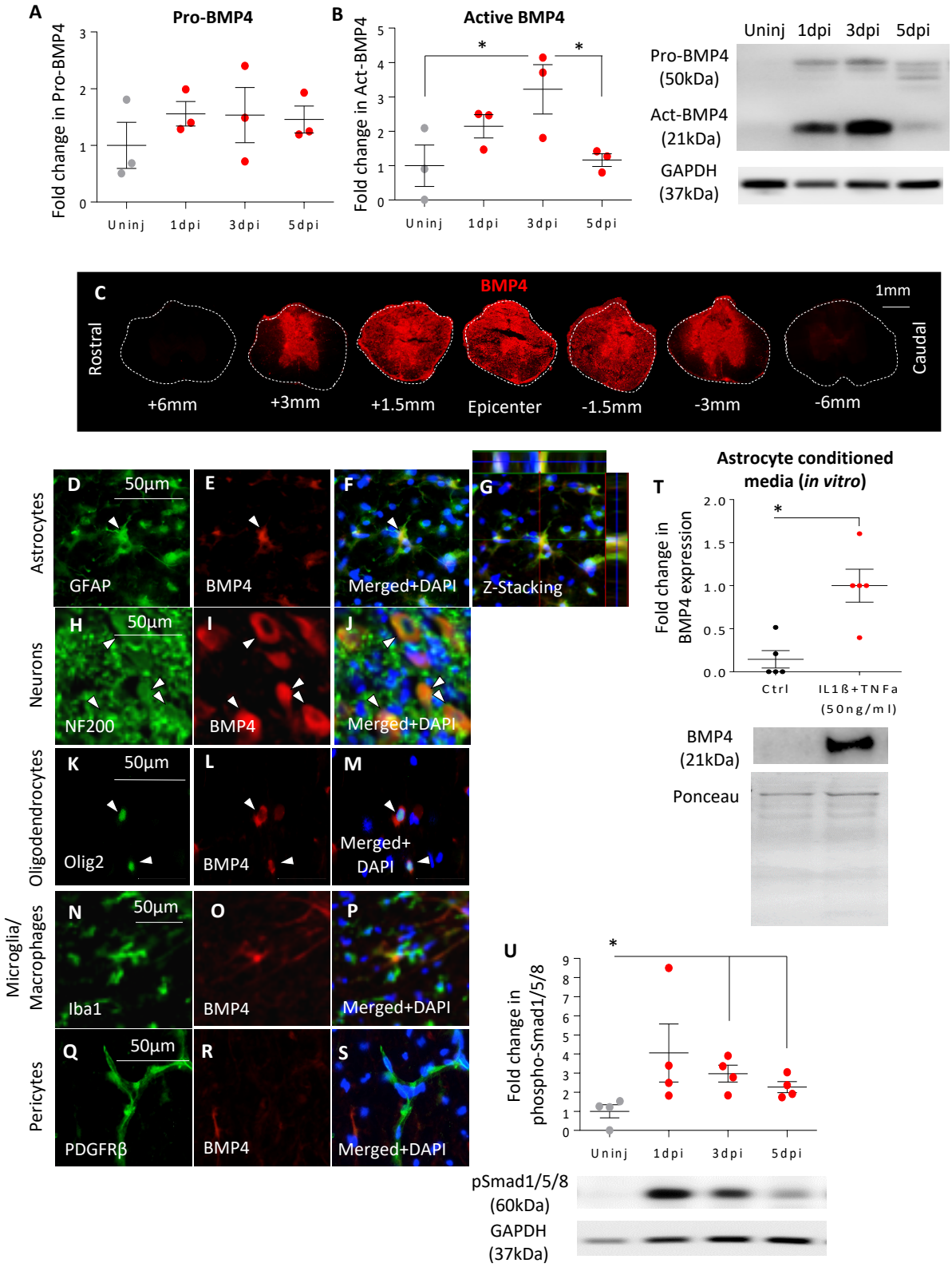


Figure 2.1. Active form of BMP4 protein is transiently upregulated in acute SCI in the rat. (A-B) Temporal expression of pro- and active forms of BMP4 protein were analyzed in rat contusive/compressive SCI at 1-, 3- and 5-days post-SCI using Western blotting on 5mm of spinal cord homogenate centered at the injury epicenter. While pro-BMP4 remained relatively unchanged in all experimental groups compared to uninjured (Uninj) spinal cord (A), active BMP4 was transiently and significantly upregulated between 1-3dpi and returned to its basal levels by 5dpi (B). N=3 rats/time point. Data represented as mean ± SEM expressed as fold change normalized to uninjured group, *$p<0.05$, One Way ANOVA followed by Holm-Sidak *post hoc* test. (C) Immunohistological analysis in serial cross sections (epicenter+/-6mm) of the injured spinal cord confirmed upregulation of BMP4 expression at the epicenter and perilesional areas in both gray and white matter. BMP4 immunointensity was highest at the injury epicenter and declined in more distal sections. (D-S) Representative immunostaining of the injured spinal cord at 3dpi showed co-localization of BMP4 with astrocytes (GFAP+, D-G), neurons (NF200+, H-J), and oligodendroglia (Olig2+, K-M) at 2mm caudal to injury epicenter, while microglia/macrophages (Iba1+, N-P) and pericytes (PDGFRβ+, Q-S) showed negligible expression of BMP4. Representative images of neurons and non-neuronal cells were taken in gray and white matter, respectively. (T) Western blot analysis of BMP4 in conditioned media from primary astrocytes treated with pro-inflammatory cytokines, IL-1β and TNFα (50ng/ml), showed increased BMP4 expression in reactive astrocytes at 3-days post-treatment. N=5 independent experiments. Data represented as mean ± SEM expressed as fold change normalized to treated group. *$p<0.05$, Student t-test. (U) Western blot analysis for phosphorylated Smad1/5/8, the canonical signaling pathway of BMP ligands, in the injured spinal cord tissue showed a significant increase relative to the uninjured spinal cord tissue, which peaked as early as 3dpi and remained elevated up to 5dpi. GAPDH was

used as a loading control for Western blot analysis, and results are shown as fold change relative to uninjured tissues. N=4 rats/time point. Data represented as mean ± SEM expressed as fold change normalized to uninjured group, *p<0.05, Student t-test.

2.4.2 BMP4 reduces proliferation of spinal cord NPCs and promotes their differentiation into astrocytes *in vitro*

In the developing CNS, BMP4 modulates proliferation and astrocyte differentiation of embryonic and adult rodent NPCs from the sub-ventricular zone (SVZ) (D'Alessandro and Wang, 1994; Samanta and Kessler, 2004) and human fetal spinal cord (Weible and Tailoi, 2007). Following SCI, our group and others have shown that activated NPCs are biased towards an astrocytic fate within the injury microenvironment (Meletis et al., 2008; Barnabé-Heider et al., 2010; Karimi-Abdolrezaee et al., 2012). Thus, we asked whether upregulation of BMP4 may direct astrocyte differentiation of endogenous spinal cord precursor cells in the injured spinal cord. To this end, we confirmed that BMP4 significantly reduced proliferation of primary spinal cord derived NPCs *in vitro*, and promoted their differentiation into astrocytes. After 2-day treatment, BMP4 (50-100ng/ml) significantly reduced the percentage of BrdU-labeled proliferating NPCs in a concentration dependent manner (15-26% BrdU+/DAPI+) relative to serum-free control condition (50%) (Fig. 2.2A-D). After 5 days under serum-free control condition, we found that 23% of NPCs differentiated into astrocytes (GFAP+/DAPI+), while 68% remained undifferentiated (Nestin+/DAPI+) (Fig. 2.2E-O). Inversely, BMP4 (50-100ng/ml) significantly

increased the percentage of GFAP+ astrocytes (85-87%) relative to control, with few NPCs remaining undifferentiated Nestin+ (5-9%). For oligodendrogenesis, presence of BMP4 reduced fate specification of NPCs towards an oligodendrocyte lineage (Olig2+). Under baseline control condition (1% FBS), after 7-day differentiation, we found that 10% of NPCs differentiated into Olig2+/DAPI+ cells, which was significantly reduced to 1.9% and 1.3% under 50 and 100ng/ml BMP4, respectively (Fig. 2.2P-X). Interestingly, our analysis of NPC viability using a LIVE/DEAD assay indicated that BMP4 (50-150ng/ml) had no effect on cell survival as the number of live (Calcein-AM+, green) and dead (ethidium homodimer-1+, red) cells remained unchanged after 24 hr relative to control condition under all examined concentrations of BMP4 (50, 100, 150 ng/ml) (Supplemental Fig. 2.1A-F).

Fig. 2.2. BMP4 reduces proliferation and promotes astrocyte differentiation of spinal cord NPCs *in vitro*.

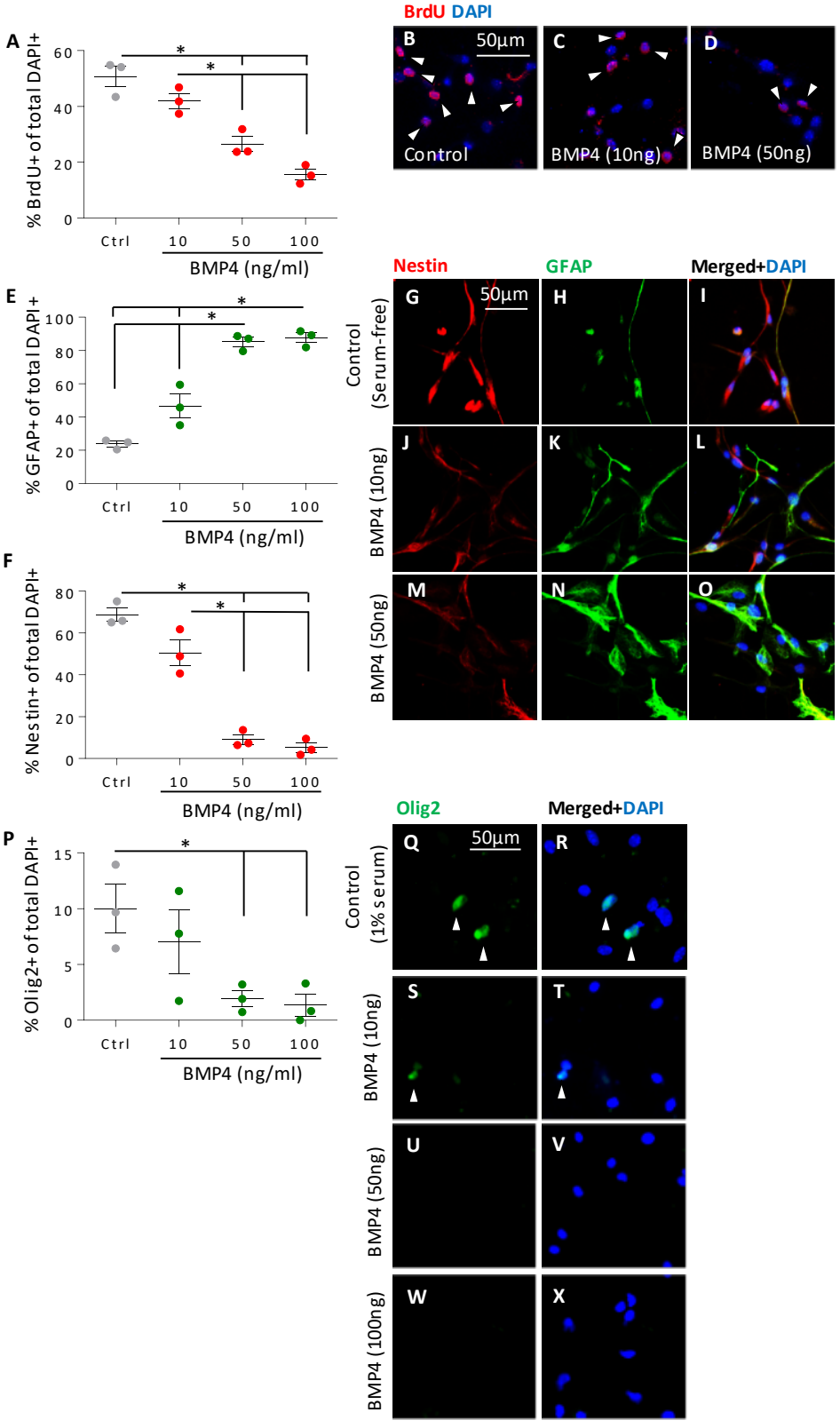
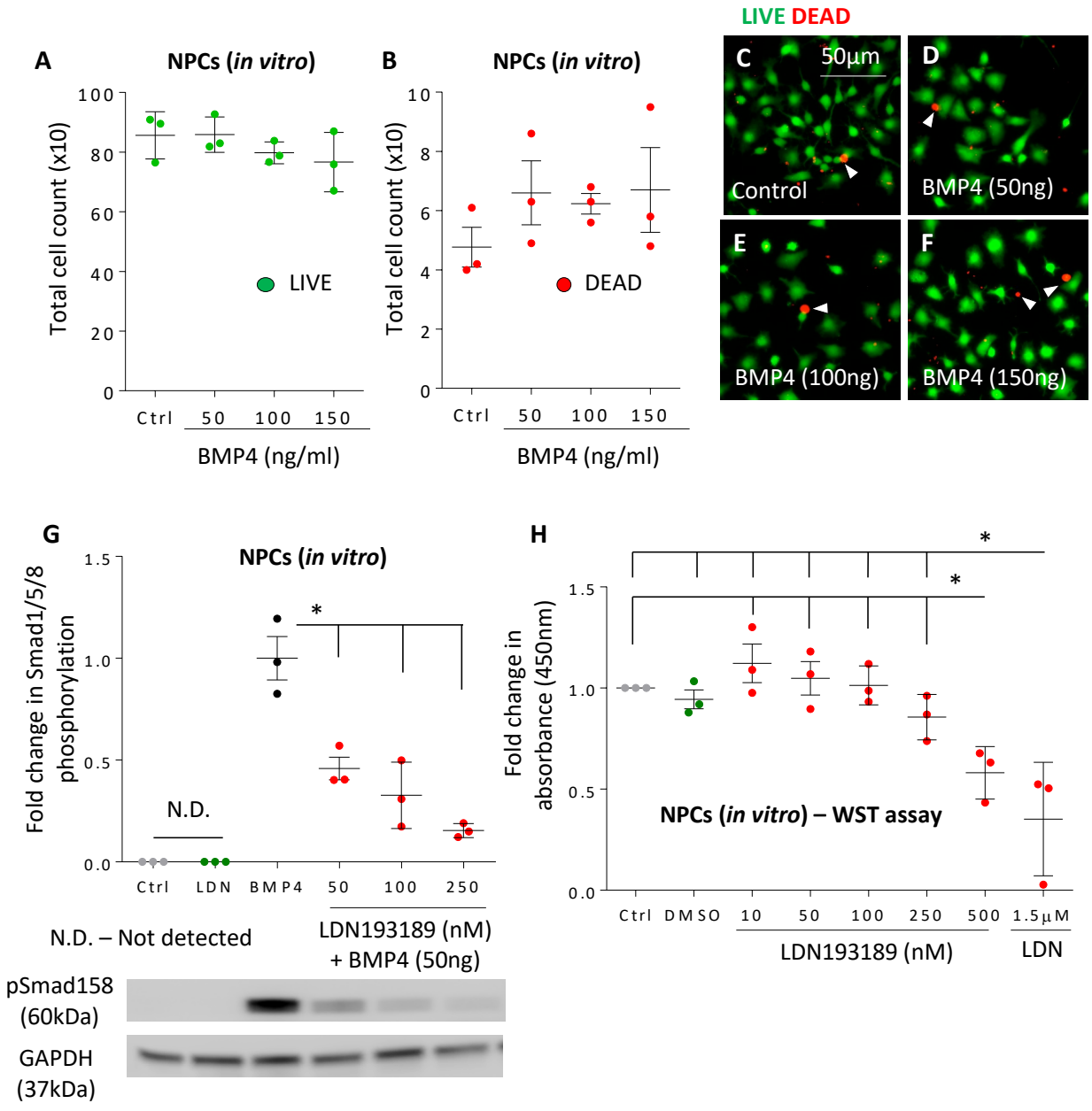


Figure 2.2. BMP4 reduces proliferation and promotes astrocyte differentiation of spinal cord NPCs *in vitro*. Quantitative immunofluorescent analysis of proliferation (A-D) and differentiation (E-X) of primary spinal cord derived NPCs treated with BMP4 (10-100ng/ml) is depicted. (A-D) When NPCs were treated with BMP4 (50-100ng/ml) for 2 days, the percentage of BrdU+ proliferative NPCs to the total DAPI+ (for nuclei) cells was significantly reduced in a concentration dependent manner relative to untreated (control) NPCs. (E-O) BMP4 (50-100ng/ml) increased the percentage of differentiated astrocytes (GFAP+) relative to untreated control NPCs (E), which coincided with a decrease in the percentage of undifferentiated NPCs (GFAP-/Nestin+) (F). (P-X) BMP4 (50-100ng/ml) also decreased the percentage of differentiated oligodendrocytes (GFAP-/Olig2+) relative to control NPCs. All markers were analyzed as a percentage of total cells (DAPI+). N=3 independent experiment. Data represented as mean percentage $\pm$ SEM, *$p<0.05$, One Way ANOVA followed by Holm-Sidak *post hoc* test.

Supplemental Figure 2.1. Proof-of-concept data that BMP4 is not cytotoxic to spinal cord NPCs *in vitro* and LDN193189 at 250 nM inhibits BMP4-signaling without significant cytotoxicity to spinal cord NPCs *in vitro*.



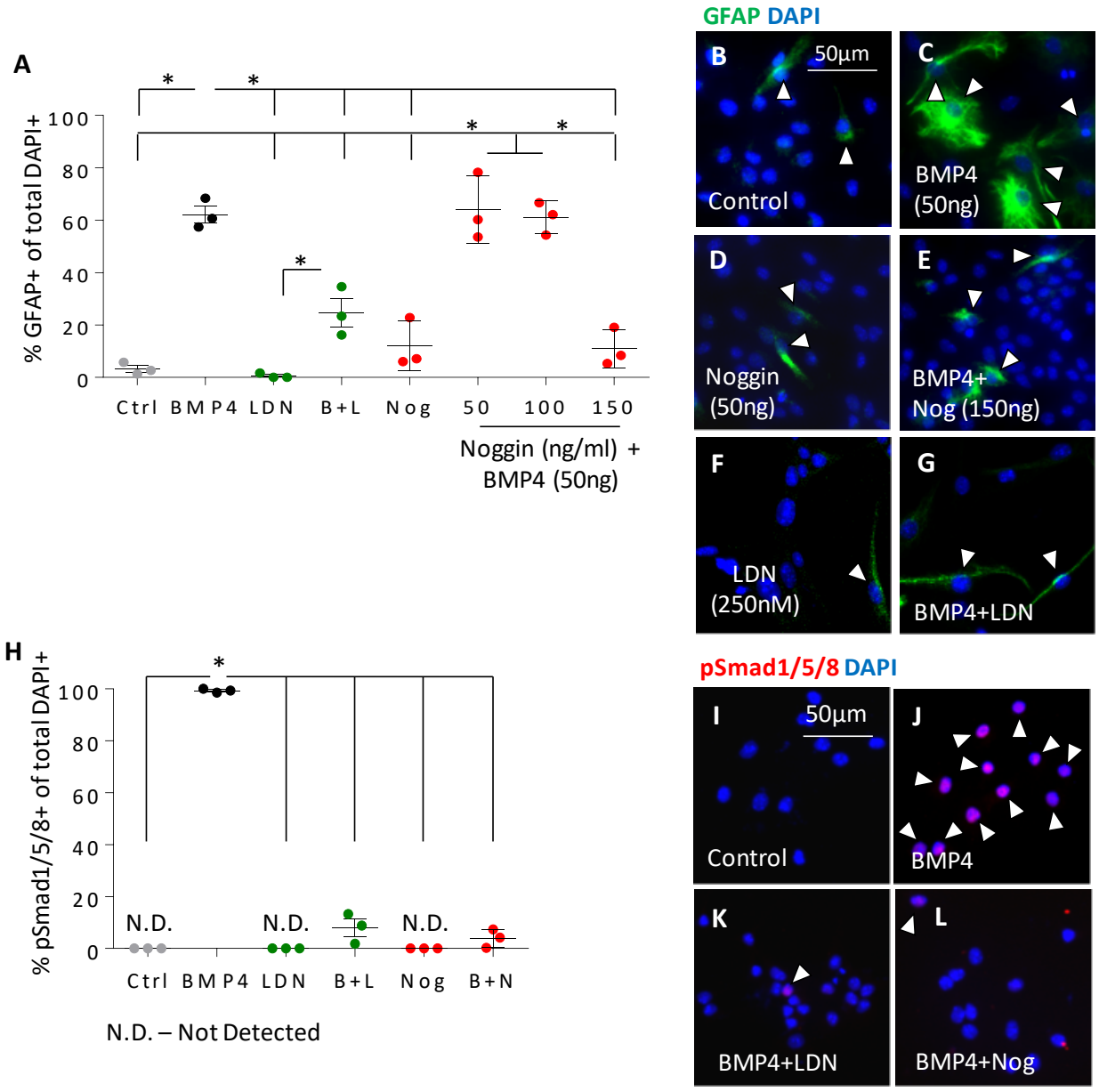
Supplemental Figure 2.1. Proof-of-concept data that BMP4 is not cytotoxic to spinal cord NPCs *in vitro* and LDN193189 at 250 nM inhibits BMP4-signaling without significant cytotoxicity to spinal cord NPCs *in vitro*. (A-F) Cell viability was assessed in spinal cord NPCs after 24 h treatment with BMP4 (50-150 ng/ml) by LIVE/DEAD assay. We found no significant change in the number of live (Calcein AM+, green) or dead cells (ethidium homodimer-1+, red) in BMP4-treated NPCs at any examined concentration relative to untreated control condition. N = 3 independent experiments. (G) Western blot analysis of Smad1/5/8 phosphorylation was performed in cell lysate of spinal cord NPCs treated with BMP4 (50 ng/ml) with or without LDN193189 (50-250 nM) after 1 h treatment. Smad1/5/8 phosphorylation was attenuated in NPCs treated with BMP4 and LDN193189 (50-250 nM) in a dose-dependent manner. N = 3 independent experiments. GAPDH was used as a loading control. (H) Cell viability was evaluated using WST-8 assay in spinal cord NPCs treated with LDN193189 (50 nM-1.5 μM) relative to control (base media) or DMSO vehicle (250 nM) after 24 h treatment. Cell viability was decreased in NPCs treated with LDN193189 at concentrations exceeding 500 nM relative to control. N = 3 independent experiments. Data shown as mean ± SEM expressed as fold change relative to basal control or BMP4-treated groups. *p<.05, One Way ANOVA followed by Holm-Sidak *post hoc* test.

2.4.3 Noggin and LDN193189 attenuate BMP4-mediated astrocyte differentiation and Smad1/5/8 signaling in primary spinal cord derived NPCs *in vitro*

Next, we validated the efficacy of two inhibitors, noggin and LDN193189, in attenuating BMP4-specific effects using our primary spinal cord NPC culture system. Noggin is a well-known tissue inhibitor of BMP ligands (McMahon et al., 1998; Lim et al., 2000), while LDN193189 inhibits BMP signaling by targeting BMP type 1 receptors (Boergermann et al., 2010). Our immunostaining confirmed that noggin and LDN193189 treatments successfully attenuated BMP4 mediated increase of astrocyte differentiation in spinal cord NPCs *in vitro* (Supplemental Fig. 2.2A-G). Co-treatment of noggin (150ng/ml) with BMP4 (50ng/ml) at a ratio of 3:1 was able to reverse BMP4-mediated increase in astrocyte differentiation of NPCs to the control basal level in a concentration-dependent manner (11% GFAP+/DAPI+) relative to BMP4 (50ng/ml) alone (62% GFAP+/DAPI+) (Supplemental Fig. 2.2E). Therefore, this noggin:BMP4 ratio (3:1) was utilized in all *in vitro* analyses. Likewise, LDN193189 (250nM) attenuated BMP4-mediated increase in astrocyte differentiation (24% GFAP+/DAPI+) relative to BMP4 alone (Supplemental Fig. 2.2G). Of note, the concentration of LDN193189 (250nM) was selected based on its efficacy in inhibiting BMP4-mediated astrocyte differentiation and Smad1/5/8 phosphorylation without inducing cytotoxicity (Supplemental Fig. 2.1G-H). Additionally, we confirmed specificity of LDN193189 and noggin treatments in suppressing BMP4 function in NPCs by assessing downstream Smad1/5/8 phosphorylation (Supplemental Fig. 2.2H-L). Our immunocytochemical analysis for Smad1/5/8 phosphorylation revealed that LDN193189 (250nM) or noggin (150ng/ml) co-treatment with BMP4 dramatically reduced the percentage of pSmad1/5/8+/DAPI+ NPCs (8% and 4%, respectively) relative to BMP4 (50ng/ml) alone (99%), confirming their efficacy in inhibiting

BMP4 function (Supplemental Fig. 2.2J-L). Based on these data, noggin and LDN193189 were determined to be suitable pharmacological approaches for targeting BMP4 in at SCI.

Supplemental Figure 2.2. Noggin and LDN193189 attenuate BMP4-mediated astrocyte differentiation and Smad1/5/8 signaling activation in cultured NPCs.



Supplemental Figure 2.2. Noggin and LDN193189 attenuate BMP4-mediated astrocyte differentiation and Smad1/5/8 signaling activation in cultured NPCs. (A-G) Quantitative immunocytochemical analysis was completed in cultures of spinal cord NPCs to evaluate the efficacy of noggin (50, 100, or 150 ng/ml) and LDN193189 (250 nM) in inhibition of BMP4-induced (50 ng/ml) astrocyte differentiation at 5 days post-treatment. (A) BMP4 significantly increased the percentage of differentiated GFAP+ astrocytes relative to basal control. Noggin treatment significantly attenuated BMP4-mediated astrocyte differentiation only when co-treated at a ratio of 3:1 (150 ng/ml noggin: 50 ng/ml BMP4). (E) Representative image indicates noggin-mediated attenuation in astrocyte differentiation of NPCs as confirmed by a decrease in GFAP expressing astrocytes. (G) LDN193189 (250 nM) pre- and co-treatment with BMP4 also significantly attenuated astrocyte differentiation in NPCs relative to BMP4 alone. (H-L) Immunocytochemical analysis confirmed that BMP4 (50 ng/ml) induced Smad1/5/8 phosphorylation in all spinal cord NPCs after 1 h treatment (H), which was significantly attenuated when combined with LDN193189 (250 nM) or noggin (150 ng/ml) (K-L). N = 3 independent experiment. All data represented as mean percentage $\pm$ SEM, *p<.05, One Way ANOVA followed by Holm-Sidak *post hoc* test.

2.4.4 Administration of LDN193189 attenuates active BMP4 expression in acute SCI

We employed noggin and LDN193189 to determine the impact of BMP4 upregulation in acute SCI. We evaluated two doses of noggin (1.2 or 2.4µg/day) and LDN193189 (1.25 or 2.5mg/day) and analyzed the acute outcomes of injury at 3dpi relative to uninjured and vehicle treated SCI groups. Of note, 3dpi represents the timepoint when active BMP4 was found significantly upregulated in our rat SCI (Fig. 2.1B). Our Western blot analyses showed that noggin and LDN193189 treatments differentially modulate expression of active BMP4 in the injured spinal cord (Fig. 2.3A-B). Intriguingly, both doses of LDN193189 treatment significantly attenuated active BMP4 expression by 80% relative to vehicle group, while neither dose of noggin had any effect (Fig. 2.3A-B). Lastly, complementary immunohistochemical analysis reaffirmed that LDN193189 treatment (2.5mg/day) significantly attenuated BMP4 immunointensity in the injured spinal cord at 1.5 mm rostral and all the examined caudal points (1.5, 3 and 4.5 mm) to injury epicenter relative to vehicle SCI group (Fig. 2.3C-S).

Fig. 2.3. Systemic LDN193189 treatment attenuates active BMP4 expression in the injured spinal cord.

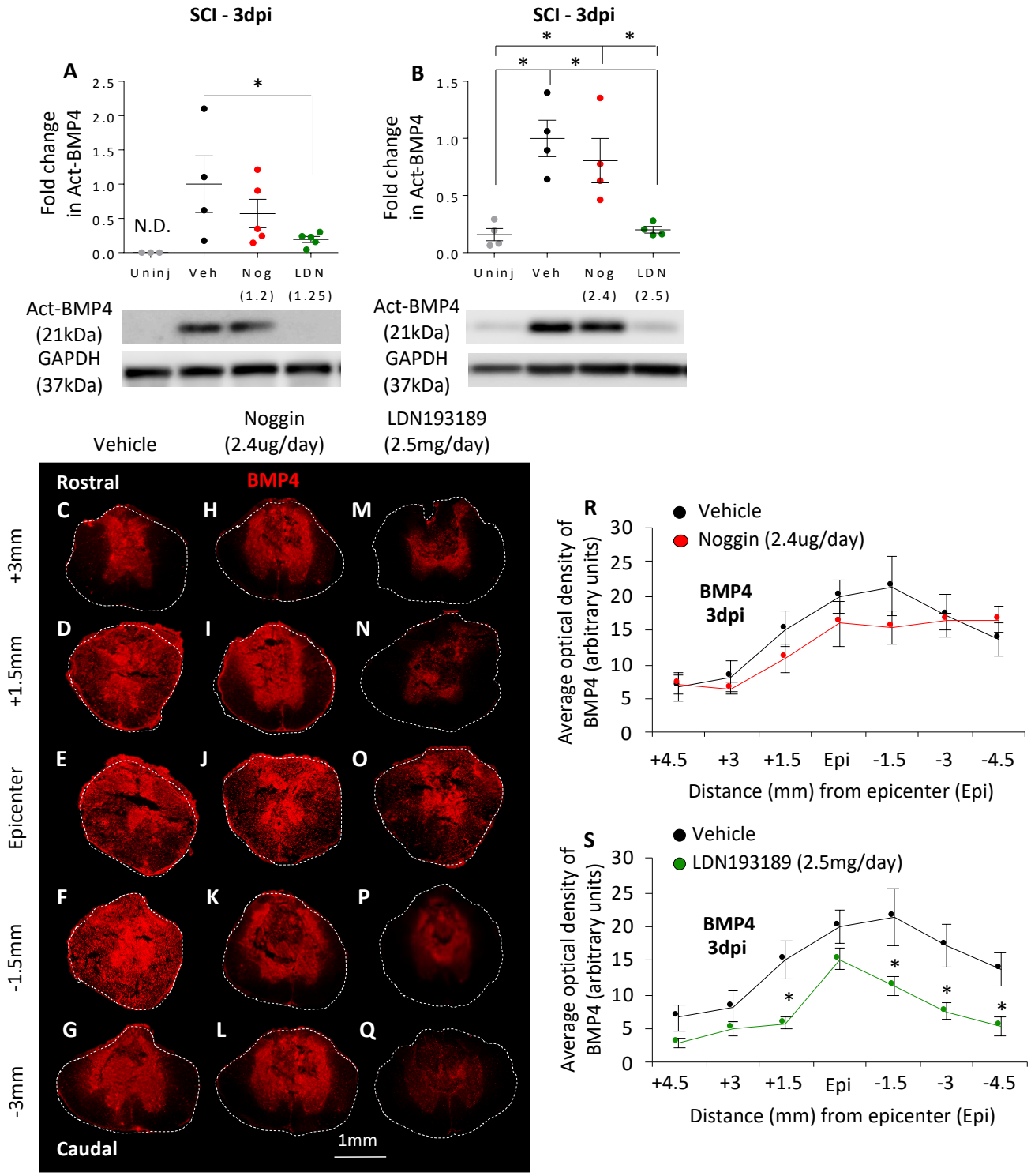


Figure 2.3. Systemic LDN193189 treatment attenuates active BMP4 expression in the injured spinal cord. (A-B) Western blot analysis of active BMP4 protein was performed on tissue homogenate of the injured spinal cord from SCI untreated (Vehicle), SCI/noggin-treated (1.2 or 2.4ug/day) and SCI/LDN193189-treated (1.25 or 2.5mg/day) groups at 3 days post-injury (3dpi) relative to uninjured (Uninj) tissue. Both doses of LDN193189 significantly decreased expression of active BMP4 relative to vehicle group, while noggin had no significant effect (A-B). N=3-5 rats/treatment. Data shown as mean ± SEM expressed as fold change normalized to vehicle group. *p<0.05, One Way ANOVA followed by Holm-Sidak *post hoc* test. (C-S) Quantitative immunostaining of BMP4 was performed on spinal cord tissue serial cross sections (epicenter±4.5mm) of SCI untreated (Vehicle), SCI/noggin-treated (2.4ug/day) and SCI/LDN193189-treated (2.5mg/day) rats at 3dpi. BMP4 immunointensity was significantly reduced in the injured spinal cord tissue with LDN193189 treatment near and caudal to lesion epicenter relative to vehicle group (S). N=5-7 rats/treatment. Data shown as average optical density ± SEM. *p<0.05, Two Way ANOVA followed by Holm-Sidak *post hoc* test.

2.4.5 Noggin and LDN193189 treatments promote oligodendrogenesis in acute rat SCI

BMP4 is known to inhibit oligodendrocyte differentiation in NPCs and maturation of OPCs in the developing CNS (Mekki-Dauriac et al., 2002; Samanta and Kessler, 2004; Bilican et al., 2008). Here, using noggin and LDN193189, we sought to examine whether upregulated level of BMP4 in acute SCI influences oligodendrogenesis in the injured spinal cord. We used thymidine analog BrdU to label cells that had divided during the treatment period after SCI. We quantified the number of BrdU labeled oligodendroglia (BrdU+/Olig2+) and astrocytes (BrdU+/GFAP+) in the injured spinal cord of vehicle, noggin and LDN193189 treated SCI rats at 3dpi (Fig. 2.4A-D). Our analyses identified that noggin (1.2ug/day) treatment significantly increased oligodendrogenesis (Olig2+/BrdU+) by 3.6-fold relative to vehicle group (Fig. 2.4E). LDN193189 (1.25mg/day) and noggin (2.4ug/day) treatments also enhanced preservation of existing non-BrdU labeled oligodendrocytes (Olig2+/BrdU-) by 2.7- and 1.5-fold respectively, compared to vehicle treated group (Fig. 2.4G-H). Our quantification revealed no effect of noggin and LDN193189 treatment on proliferating astrocytes (GFAP+/BrdU+) (Fig. 2.4I-J); however, LDN193189 (2.5mg/day) increased non-proliferating astrocytes (GFAP+/BrdU-) by 1.6-fold relative to untreated (vehicle) SCI rats (Fig. 2.4L). These data suggest upregulation of BMP4 seems to limit oligodendrogenesis in the spinal cord during acute phase of SCI.

Figure 2.4. Administration of noggin and LDN193189 increases oligodendrogenesis in acute SCI.

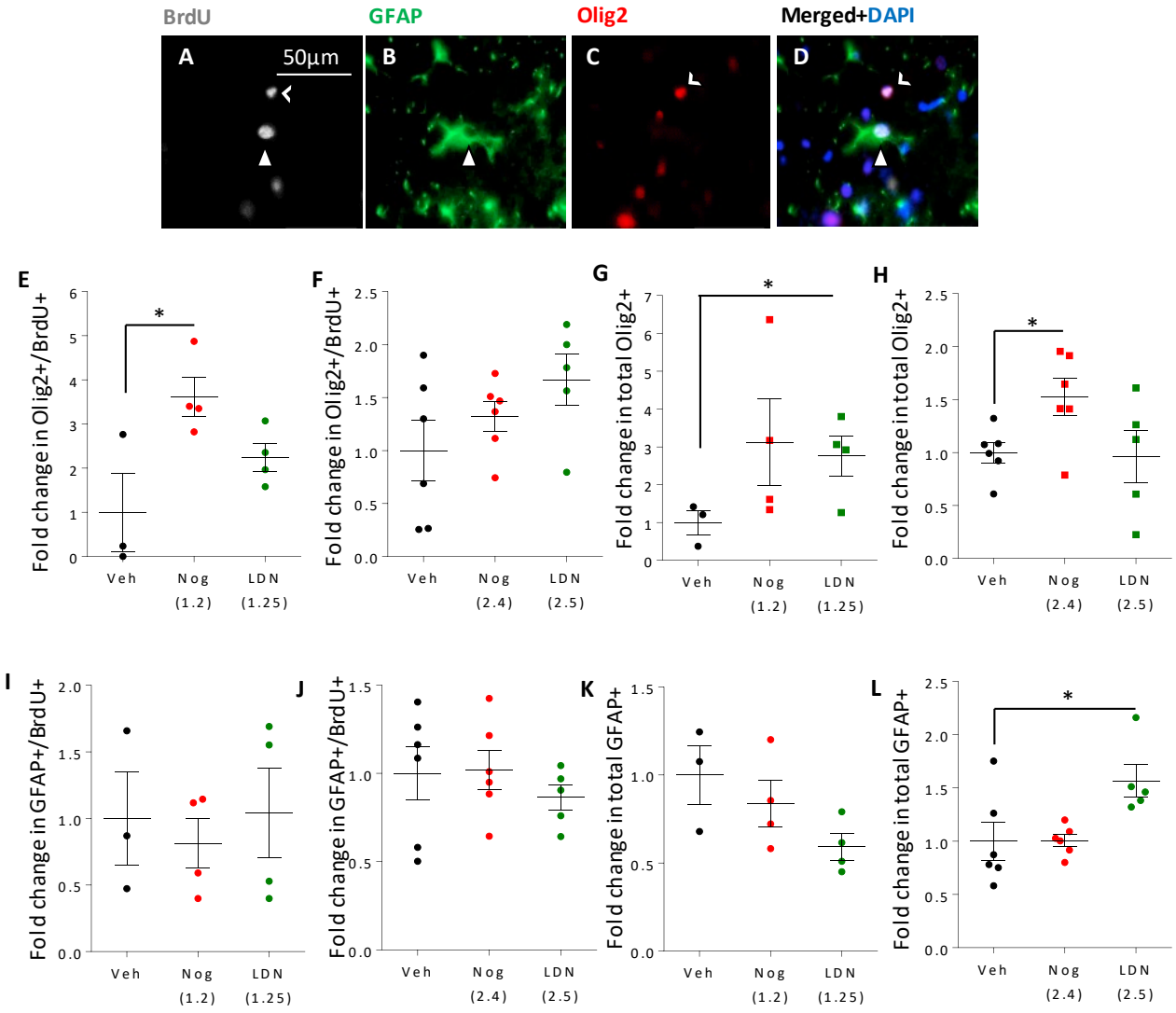


Figure 2.4. Administration of noggin and LDN193189 increases oligodendrogenesis in acute SCI. (A-L) Quantitative immunohistological analysis of endogenous cell differentiation was performed on spinal cord cross sections at 3.5mm rostral to injury epicenter of SCI/Vehicle, SCI/noggin-treated (1.2 or 2.4ug/day) and SCI/LDN193189-treated (1.25 or 2.5mg/day) groups at 3 days post-injury (3dpi). (A-D) Representative immunostaining shows co-localization of BrdU with astrocytes (GFAP+, arrow) or oligodendroglia (Olig2+, flared arrow) in the injured spinal cord. The proportion of BrdU+-labeled oligodendroglia was significantly increased with noggin (1.2ug/day) treatment relative to vehicle group (E). Oligodendrocyte preservation (BrdU-/Olig2+) was also enhanced with LDN193189 (1.25mg/day) and noggin (2.4ug/day) treatments (G-H). We found no significant changes in the proportion of BrdU+ proliferating astrocytes (BrdU+/GFAP+) (I-J), but LDN193189 (2.5mg/day) treatment seemed to increase existing astrocytes (BrdU-/GFAP+) relative to vehicle group (L). N=3-6 rats/treatment. Data represented as mean $\pm$ SEM expressed as fold change normalized to vehicle group. *$p<0.05$. Student t-test between vehicle and each treatment group.

2.4.6 Acute upregulation of BMP4 activity contributes to increased CSPG expression in rat SCI

BMP4 is known for its role in promoting astrocyte differentiation in the developing CNS (Gross et al., 1996). Evidence also suggests BMP4 modulates reactive astrogliosis and integrity of the glial scar in rodent models of SCI and demyelinating spinal cord lesions (Fuller et al., 2007; Sahni et al., 2010). Availability of BMP4 is shown to potentiate expression of CSPGs in mature astrocyte cultures and following direct injection into the rat spinal cord (Fuller et al., 2007). Here, we examined whether upregulated levels of endogenous BMP4 contribute to astrogliosis and production of inhibitory CSPGs in acute SCI. Western blot analysis of astrocytes at 3dpi showed no apparent changes in GFAP expression in the injured spinal cord after LDN193189 and noggin treatment compared to vehicle group (Fig. 2.5A-B). Our slot blot analysis of spinal cord tissue indicated that CSPG production was significantly increased in the SCI lesion in vehicle group at 3dpi relative to uninjured tissue. Interestingly, noggin (1.2ug/day) and LDN193189 (1.25 and 2.5mg/day) treatments attenuated this response by 38%, 57% and 53%, respectively (Fig. 2.5C-D). Furthermore, our complementary immunohistochemical analysis of CSPG protein expression showed that noggin (1.2 and 2.4ug/day) and LDN193189 (1.25mg/day) significantly reduced CSPG immunointensity at the injury epicenter and/or adjacent regions rostrally and caudally relative to vehicle group (Fig. 2.5E-R). In agreement with our SCI experiments, our complementary *in vitro* analysis in primary astrocyte cultures showed that BMP4 (25-100ng/ml) treatment had no significant effects on GFAP expression after 72 hr treatment relative to control (Fig. 2.5S). However, exposure to BMP4 significantly increased CSPG production in astrocytes by 2-2.5-fold (Fig. 2.5T-U), which was entirely returned to its normal levels with noggin (150ng/ml) and LDN193189 (100-250nM) co-treatments (Fig. 2.5T-U). Since BMPs are members

of the same superfamily as TGFβ (Kingsley, 1994), a classical inducer of CSPG production in astrocytes (Schachtrup et al., 2010; Jahan and Hannila, 2015), we next examined whether noggin and LDN193189 had any effects on TGFβ mediated increase of CSPG production in astrocytes (Fig. 2.5V). We found no apparent effects of LDN193189 on TGFβ function, indicating the specificity of BMP4 effects in these experiments. Collectively, our findings suggest that while BMP4 is not a major contributor to astrogliosis, its increase specifically contributes to the upregulation of CSPGs by reactive astrocytes in the acute phase of SCI.

Given that transient upregulation of active BMP4 expression coincides with the start of robust microgliosis and macrophage accumulation in rat SCI (Alizadeh et al., 2018; Dyck et al., 2018), we also examined markers of inflammation at 3dpi in vehicle, noggin- and LDN193189-treated groups relative to uninjured spinal cord. Our Western blot analysis of the injured spinal cord showed a significant increase in Iba1 expression (a marker of microglia/macrophages) in vehicle group relative to uninjured tissue, which was attenuated with LDN193189 (1.25 and 2.5mg/day) treatment by 50% and 46%, respectively, relative to vehicle SCI group (Supplemental Fig. 2.3A-B). Analysis of pro-inflammatory cytokines, tumour necrosis factor-α (TNFα) and interleukin-1β (IL-1β), showed induced expression of these cytokines after SCI in our vehicle group at 3dpi relative to uninjured tissue, as expected (Supplemental Fig. 2.3C-F). However, neither dose of noggin nor LDN193189 had any significant effect on these pro-inflammatory cytokines, suggesting that BMPs do not play a significant role in modulating acute inflammation in rat SCI.

Figure 2.5. Noggin and LDN193189 treatments attenuate injury-induced upregulation of CSPGs in rat SCI.

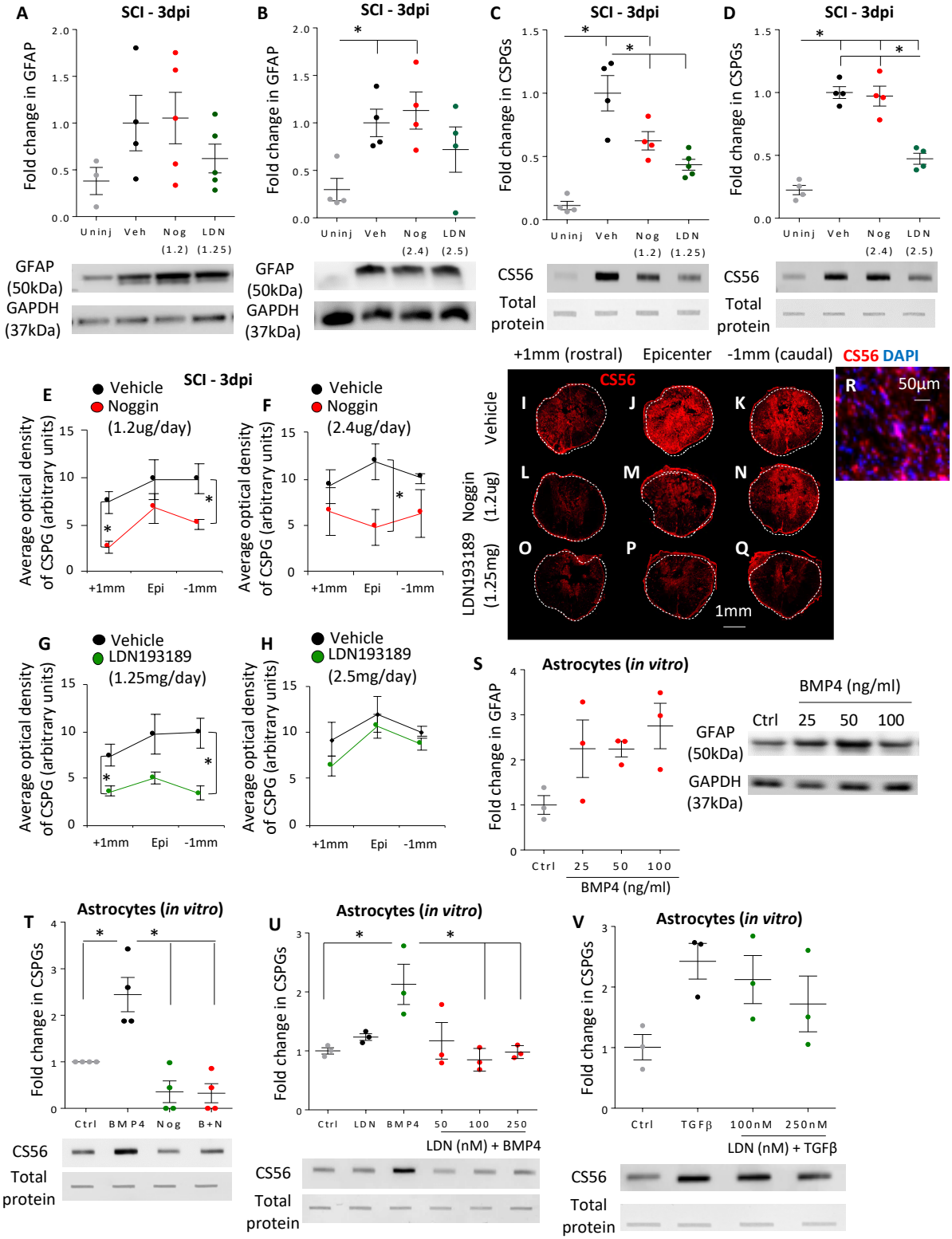
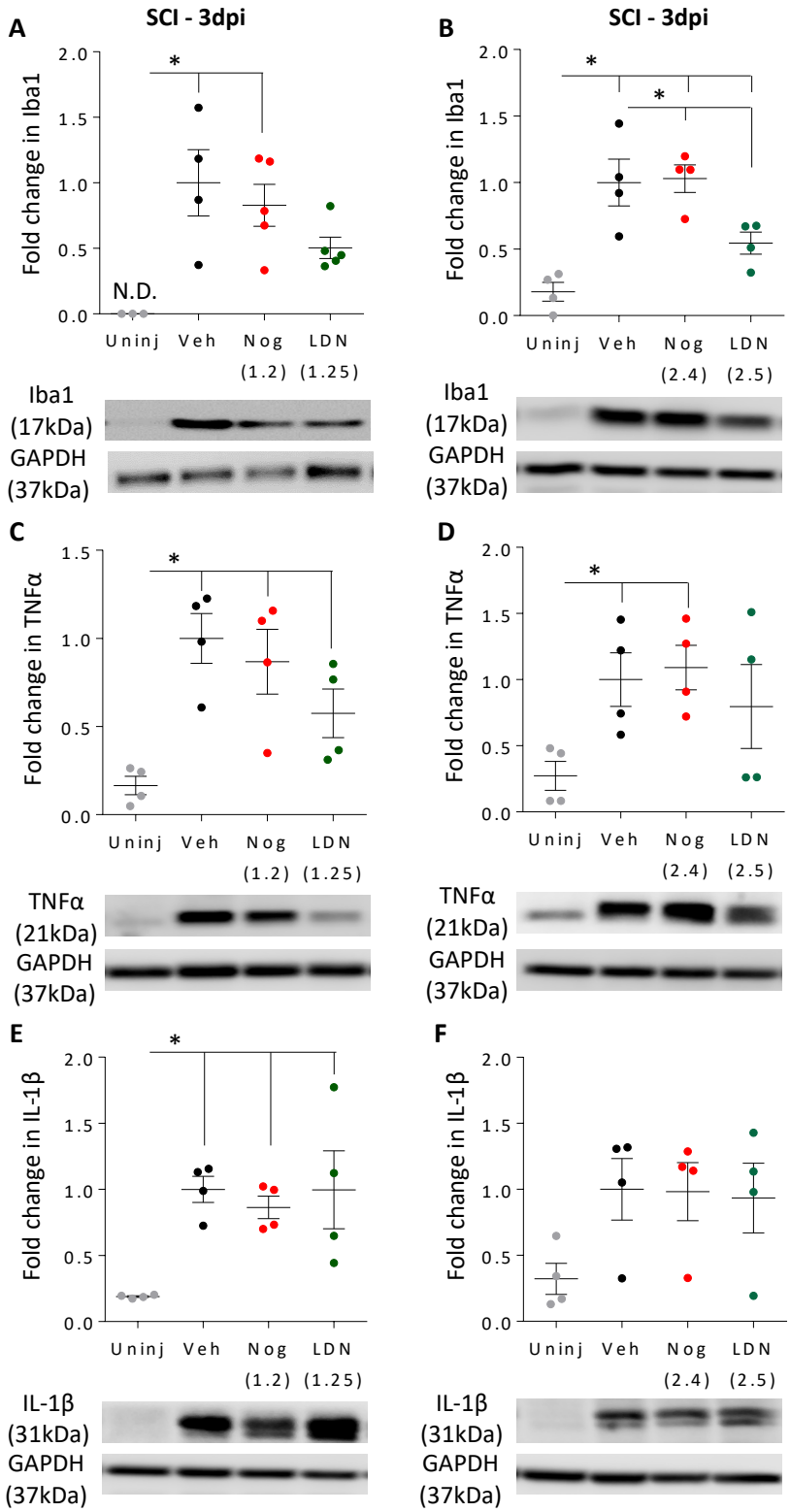


Figure 2.5. Noggin and LDN193189 treatments attenuate injury-induced upregulation of CSPGs in rat SCI. (A-B) Western blot analysis of GFAP expression was performed on tissue homogenate of the injured spinal cord from SCI/Vehicle, SCI/noggin-treated (1.2 or 2.4ug/day) and SCI/LDN193189-treated (1.25 or 2.5mg/day) groups at 3 days post-injury (3dpi) relative to uninjured (Uninj) tissue. GFAP expression was increased in vehicle group at 3dpi relative to uninjured tissue, and this was partially attenuated with LDN193189 (2.5mg/day) treatment to the point that it was not significantly different from the uninjured group (B). N=3-5 rats/treatment. (C-D) Slot blot analysis showed chondroitin sulfate proteoglycan (CSPG) expression was significantly increased in vehicle group at 3dpi relative to uninjured tissue, and it was attenuated with noggin (1.2ug/day) and LDN193189 (1.25 and 2.5mg/day) treatments. N=4-5 rats/treatment. (E-R) Quantitative immunostaining of CSPG was also performed on tissue cross sections (epicenter$\pm$1mm) of all experimental groups at 3dpi. (E-G) CSPG immunointensity was significantly reduced with noggin (1.2 and 2.4ug/day) and LDN193189 (1.25mg/day) treatments at and near injury epicenter relative to vehicle group. N=4-6 rats/treatment. Data shown as mean optical density $\pm$ SEM. *$p<0.05$, Two Way ANOVA followed by Holm-Sidak *post hoc* test. (S) Western blot analysis of GFAP expression in cell lysate of primary astrocytes treated with BMP4 (25-100ng/ml) showed that BMP4 moderately, but insignificantly, elevated GFAP expression at 3-days post-treatment relative to untreated (control) astrocytes. N=3 independent experiments. (T-U) Slot blot analysis of CSPGs in conditioned media of primary astrocytes treated with BMP4 (50ng/ml), noggin (150ng/ml) and LDN193189 (50-250nM) indicated that BMP4 (50ng/ml) potentiated CSPG expression in astrocytes after 3-days treatment, and noggin (150ng/ml) and LDN193189 (100-250nM) co-treatments significantly attenuated this BMP-mediated response. (V) Proof-of-concept data that LDN193189 (100-250nM) had no significant effects on TGFβ

(25ng/ml) mediated increase in CSPG expression in astrocytes. N=3-4 independent experiments. GAPDH expression or total protein (ponceau) were used as a loading control for protein analysis, in cell lysate and conditioned media, respectively. Data is shown as mean ± SEM expressed as fold change normalized to basal control. *$p<0.05$, One Way ANOVA followed by Holm-Sidak *post hoc* test.

Supplemental Figure 2.3. Inhibition of BMPs does not have a significant effect on inflammation in acute SCI in the rat.



Supplemental Figure 2.3. Inhibition of BMPs does not have a significant effect on inflammation in acute SCI in the rat. (A-F) Western blot analysis of Iba1 and pro-inflammatory cytokine expression was performed on tissue homogenate of the injured spinal cord from SCI untreated (Vehicle), SCI/noggin-treated (1.2 or 2.4 μg/day) and SCI/LDN193189-treated (1.25 or 2.5 mg/day) groups at 3 days post-injury (3dpi) relative to uninjured (Uninj) tissue. (A-B) Iba1 expression increased in vehicle group at 3 dpi relative to uninjured tissue, but this response was attenuated with LDN193189 (2.5 mg/day) treatment. (C-F) Western blot analysis showed increased expression of pro-inflammatory cytokines, TNFα and IL-1β, at 3 dpi in vehicle group relative to uninjured tissue. Neither noggin nor LDN193189 had any significant effects on TNFα and IL-1β expression. N = 4-5 rats/treatment. GAPDH was used as a loading control. Data is shown as mean $\pm$ SEM expressed as fold change normalized to vehicle group. *p<.05, One Way ANOVA followed by Holm-Sidak *post hoc* test.

2.4.7 Administration of noggin and LDN193189 attenuates cell death and lipid peroxidation in rat SCI

SCI results in neuronal and glial apoptosis in the acute injury microenvironment (Liu et al., 1997; Dyck et al., 2019). Neurodevelopmental studies suggest that BMP4 has a pro-apoptotic role in embryonic neuroblasts, ventral progenitor cells and neural crest cells (Graham et al., 1994; Gomes and Kessler, 2001; Israsena and Kessler, 2002). Therefore, we examined whether upregulated levels of endogenous BMP4 contribute to cell death in acute SCI. Western blot analysis showed induced expression of pro-apoptotic cleaved caspase-3 expression in vehicle SCI group at 3dpi compared to uninjured tissue (Fig. 2.6A-B). This response was significantly attenuated with noggin (1.2ug/day) and LDN193189 (1.25 and 2.5mg/day) treatments relative to vehicle group by 75%, 100% and 64%, respectively (Fig. 2.6A-B). We further investigated the effects of noggin and LDN193189 on lipid peroxidation in acute SCI (Springer et al., 1997). Our slot blot analysis of spinal cord tissue showed increased expression of E06, a marker of lipid peroxidation, in vehicle group at 3dpi relative to uninjured tissue (Fig. 2.6C-D). Both noggin (1.2ug/day) and LDN193189 (1.25mg/day) treatments significantly attenuated this response by 38% and 57% respectively, relative to vehicle group (Fig. 2.6C). Analysis of high mobility group box-1 (HMGB1) expression, a global marker of neuronal injury and necrosis (Kawabata et al., 2010), showed increased expression in vehicle group at 3dpi relative to uninjured tissue. However, neither noggin nor LDN193189 treatments had any significant effect (Fig. 2.6E-F).

Neurons and oligodendrocytes are both susceptible to apoptotic cell death after SCI (Springer et al., 1999; Dyck et al., 2019). To study cell specific effects of BMP4, we quantified the number of apoptotic neurons (NeuN+/ClCasp3+) and mature oligodendrocytes (APC+/ClCasp3+) between 1 and 2mm rostral of injury epicenter in vehicle, noggin- and LDN193189-treated SCI

rats at 3dpi (Fig. 2.6G-P). Our analysis showed a decreasing trend in apoptotic neurons with LDN193189 (1.25 and 2.5mg/day) treatment relative to vehicle group; although it was not statistically significant (Fig. 2.6G-H). Neither noggin nor LDN193189 treatments had any apparent effect on oligodendrocyte apoptosis in our assessments (Fig. 2.6L-M). However, our complementary *in vitro* analysis showed that BMP4 (50-150ng/ml) increased cleaved caspase-3 expression in both primary oligodendrocytes and neurons *in vitro* after 24- and 72-hr treatments, respectively, relative to control (Fig. 2.7A-T), and this response was attenuated with noggin (3:1 ratio) co-treatment (Fig. 2.7A,K). BMP4 did not have a significant effect on cleaved caspase-3 expression in cortical neurons after 24-hr treatment (data not shown). Altogether, these data suggest that upregulation of BMP4 is a contributing factor to cell death and lipid peroxidation in acute SCI.

Figure 2.6. Noggin and LDN193189 treatments reduce cell death and lipid peroxidation in rat SCI.

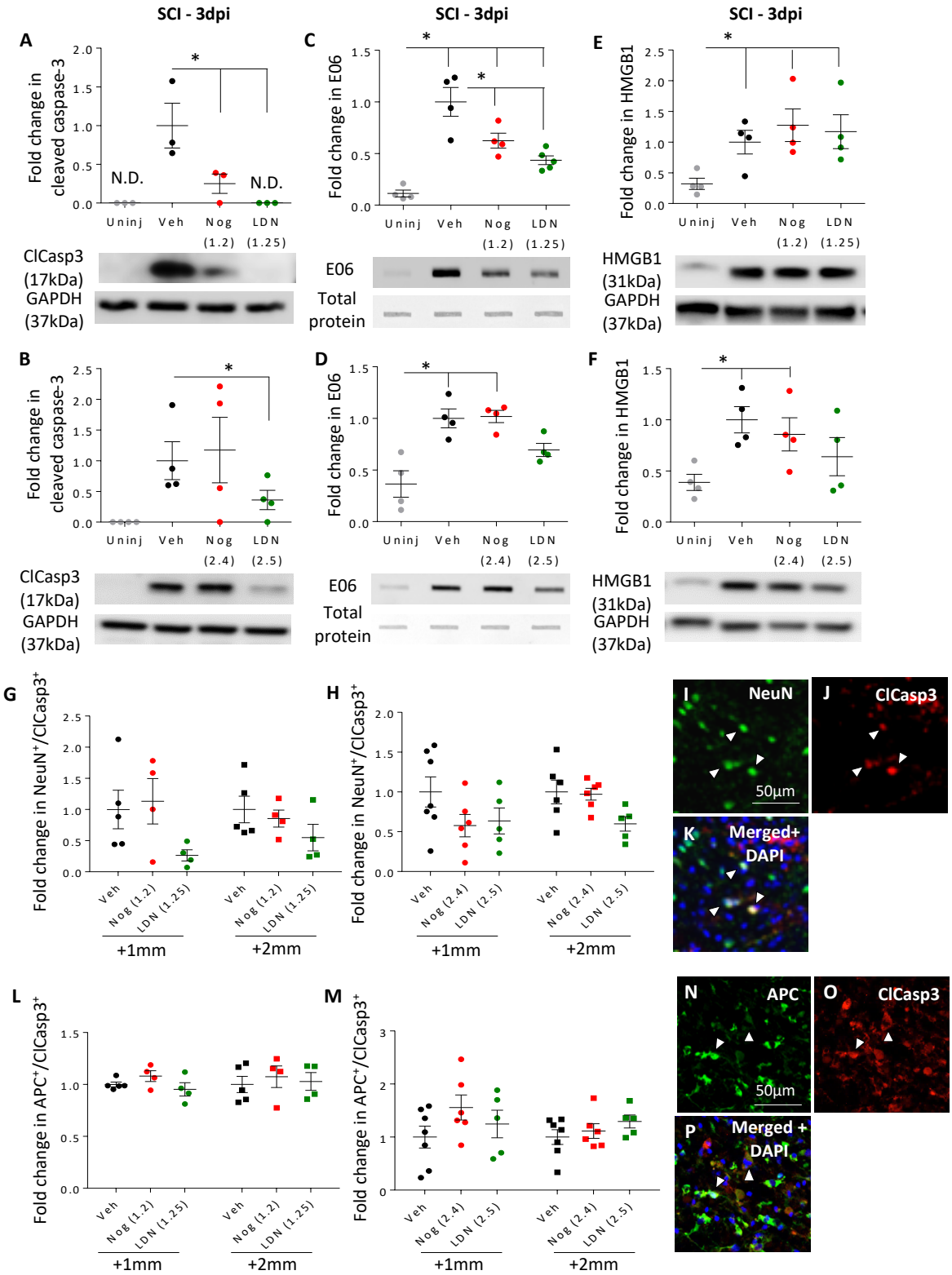


Figure 2.6. Noggin and LDN193189 treatments reduce cell death and lipid peroxidation in rat SCI. (A-B) Western and slot blot analysis of cell death and oxidative stress were performed on tissue homogenate of the injured spinal cord from SCI/vehicle, SCI/noggin- (1.2 or 2.4ug/day) and SCI/LDN193189-treated (1.25 or 2.5mg/day) groups at 3 days post-injury (3dpi) relative to uninjured (Uninj) tissue. (A-B) Expression of cleaved caspase-3 expression, a cellular marker of apoptosis, was increased in vehicle group at 3dpi relative to uninjured tissue, and this response was attenuated with noggin (1.2ug/day) and LDN193189 (1.25 and 2.5mg/day) treatments. N=3-4 rats/treatment. (C-D) Slot blot analysis showed noggin (1.2ug/day) and LDN193189 (1.25mg) treatments attenuated E06 expression, a marker of lipid peroxidation and oxidative stress, relative to vehicle group (C). N=4-5 rats/treatment. (E-F) Western blot analysis showed noggin (1.2 or 2.4ug/day) and LDN193189 (1.25 or 2.5mg/day) had no significant effect on high mobility group box-1 (HMGB1) expression, a marker of necrosis and neuronal apoptosis, relative to vehicle group. N=3-4 rats/treatment. *$p<0.05$, One Way ANOVA followed by Holm-Sidak *post hoc* test. GAPDH was used for normalization as a loading control for all Western blot analysis. Total protein (ponceau) was used for normalization as a loading control for slot blot analysis. (G-P) Quantitative immunohistological analysis of neuronal (NeuN+/ClCasp3+) and oligodendrocyte (APC+/ClCasp3+) cell death was performed on spinal cord cross sections at 1 and 2mm rostral to injury epicenter in all experimental groups at 3dpi. LDN193189 treatments reduced the proportion of apoptotic neurons (NeuN+/ClCasp3+), though significance was not reached relative to vehicle group (G-H). Neither noggin nor LDN193189 treatments had any effect on oligodendrocytes cell death (APC+/ClCasp3+) relative to vehicle group at 3dpi (L-M). N=4-7 rats/treatment. Data is shown as mean $\pm$ SEM expressed as fold change normalized to vehicle group.

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Figure 2.7. BMP4 promotes caspase-3-mediated apoptosis in oligodendrocytes and neurons *in vitro*.

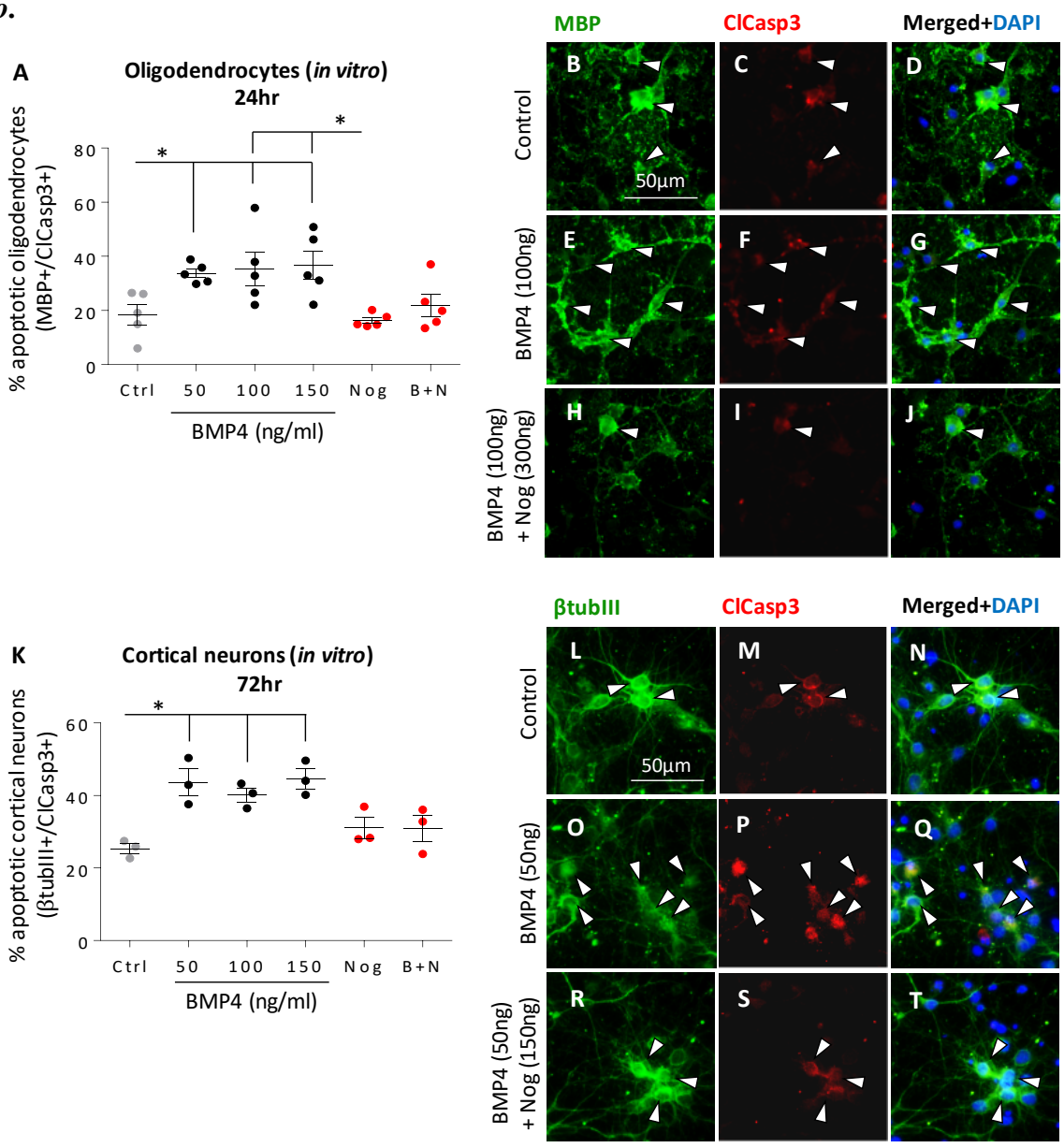


Figure 2.7. BMP4 promotes caspase-3-mediated apoptosis in oligodendrocytes and neurons *in vitro*. (A-T) Immunocytological analysis of cleaved caspase-3-mediated apoptosis in cultured primary rat mature oligodendrocytes and cortical neurons following treatment with BMP4 (50, 100 or 150ng/ml) with or without noggin (150 or 300ng/ml). BMP4 significantly increased cleaved caspase-3 expression in mature oligodendrocytes (MBP+/ClCasp3+) and neurons (βtubIII+/ClCasp3+) after 1- and 3-day treatments, respectively, relative to basal control (A, K). This response was effectively attenuated by noggin co-treatment (3:1 ratio). N=3-5/condition. All data is shown as mean percentage ± SEM. *p<0.05, One Way ANOVA followed by Holm-Sidak *post hoc* test.

2.4.8 Administration of LDN193189 enhances myelin thickness in chronic SCI

Given our data showing that inhibition of BMPs promote oligodendrogenesis and oligodendrocyte preservation at acute stage of SCI, we next sought to determine the long-term effects of noggin and LDN193189 on axonal preservation and remyelination in chronic SCI. We delivered noggin (2.4 μg/day) and LDN193189 (2.5 mg/day) during the first week of SCI, when BMP4 is upregulated, and studied axonal density and myelin g-ratio in semi-thin sections of the injured spinal cord at 10 weeks post-injury (wpi) (Fig. 2.8A-D). As expected, axonal density was significantly decreased in vehicle treated SCI group (46 axons/mm^2) relative to uninjured rats (214 axons/mm^2). However, noggin (55 axons/mm^2) or LDN193189 (51 axons/mm^2) treatment had no

beneficial effects on axonal density (Fig. 2.8E-F). For remyelination, we studied the mean g-ratio, defined as the ratio of axon to fiber (axon + myelin) diameter, in white matter of the injured and healthy spinal cord. As expected, injured spinal cord axons showed myelin thinning after injury as indicated by a significant increase in their mean g-ratio in vehicle group (0.752±0.003) relative to axons in the uninjured spinal cord (0.69±0.007, dotted line) (Fig. 2.8G-H). Importantly, LDN193189 treatment resulted in a significant reduction in mean g-ratio (*i.e.* thicker myelin) (0.729±0.007) relative to vehicle group (Fig. 2.8H), indicating an overall increase in remyelination. However, we found no significant difference in mean g-ratio between noggin and vehicle groups. We also analyzed a scatter plot of g-ratio as a function of axon diameter for individual myelinated axons but found there was no significant correlation between axon diameter and g-ratio across treatment groups (Fig. 2.8I-J). Of note, our analysis of frequency distribution of g-ratio (between 0.6-0.86) showed there was a significant reduction in percentage of thinly myelinated axons (ratio >0.81), and an increasing trend in thicker myelinated axons (0.61-0.7), in LDN193189-treated rats relative to vehicle group (Fig. 2.8K-L). Our complementary *in vitro* studies in primary cultures of oligodendrocytes also showed that BMP4 (100ng/ml) reduced maturation of oligodendrocytes (16% reduction) to myelin-expressing cells (O4+/MBP+) relative to control after 7-days in culture (Fig. 2.8M-O). These data suggest early inhibition of BMPs with LDN193189 treatment appears to improve remyelination of preserved axons in chronic SCI.

Figure 2.8. LDN193189 improves myelin thickness in chronic SCI in the rat.

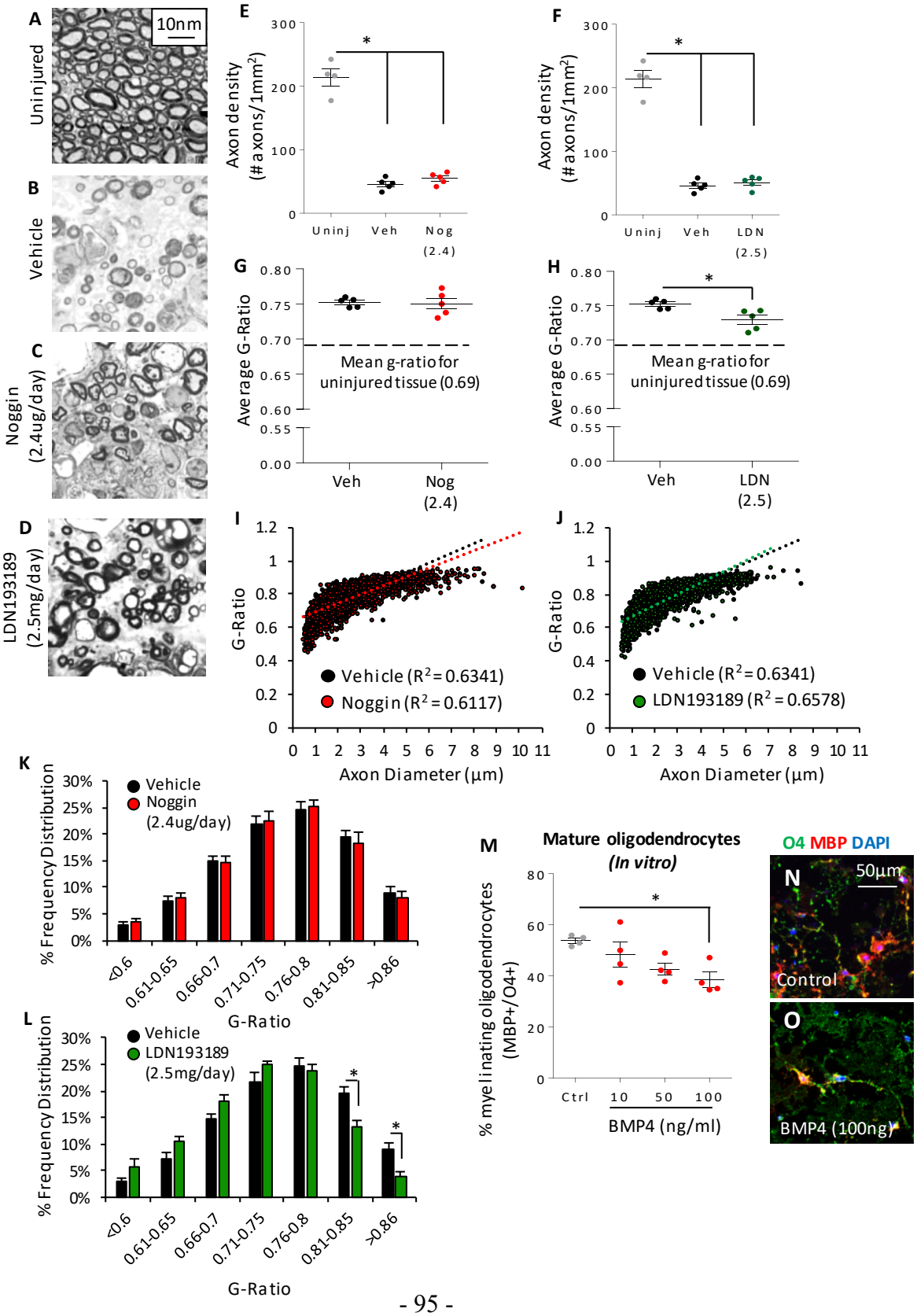


Figure 2.8. LDN193189 improves myelin thickness in chronic SCI in the rat. (A-D) Representative semi-thin sections stained with toluidine blue show the extent of myelination at 1mm rostral point from lesion epicenter in SCI/vehicle, SCI/noggin- (2.4ug/day) and SCI/LDN193189-treated (2.5mg/day) groups at 10 weeks post-injury (wpi) relative to uninjured tissue sections. (E-F) Quantitative analysis of axonal density showed a significant reduction in axon count in all experimental SCI groups relative to uninjured tissues, which remained unchanged with noggin or LDN193189 treatment. (G-H) Quantitative g-ratio analysis showed that mean g-ratio of myelinated axons increased after SCI in all experimental groups relative to uninjured tissue (dotted line), suggestive of axon demyelination and remyelination. LDN193189 treatment significantly decreased mean g-ratio (*i.e.* increase in myelin thickness) relative to vehicle group (H). *$p<0.05$, Student t-test. G-ratio was defined as the ratio of axon diameter to fibre diameter (axon + myelin). (I-J) Scatter plots illustrate the relationship between g-ratios of individual myelinated axons and their respective axon size. The linear regression of g-ratios is represented by the sloped dotted line for vehicle (black), noggin (red) and LDN193189 (green) groups. There was no significant correlation between axon diameter and g-ratio across treatment groups. (K-L) Analysis of frequency distribution of g-ratios between 0.6-0.86 (interval of 0.05) showed a significant decrease in thinly myelinated axons (>0.81), and an increasing trend in thicker myelinated axons (0.61-0.65, $P=0.065$; 0.66-0.7, $P=0.05$), in LDN193189-treated rats relative to vehicle group. N=4-5 rats/treatment. All data shown as mean $\pm$ SEM. *$p<0.05$. Statistical analysis by Student t-test. (M-O) Quantitative immunofluorescent analysis of maturation in primary cortical OPCs treated with BMP4 (10-100ng/ml) is depicted. When OPCs were matured in presence of BMP4 (100ng/ml) for 7 days, the percentage of myelin-expressing oligodendrocytes (MBP+/O4+) was significantly reduced relative to untreated (control) OPCs (M). N=4 independent

experiments. Data represented as mean percentage ± SEM. *p<0.05. One Way ANOVA followed by Holm-Sidak *post hoc* test.

2.4.9 Noggin and LDN193189 treatments do not provide long-term benefits on functional recovery or tissue preservation in chronic SCI

Considering the early benefits of noggin or LDN193189 treatment after SCI, we also evaluated long-term functional recovery, tissue preservation and glial scarring in vehicle, noggin- and LDN193189-treated SCI rats at 10wpi. We performed weekly assessment of locomotion using open field BBB analysis over a 10-week period after SCI. Our data showed that SCI rats reached a plateau with an average BBB score of 8-9 by 5 or 6wpi (Fig. 2.9A-B) and there was no difference in hindlimb motor recovery across vehicle, noggin- and LDN193189-treated SCI rats during our 10-week BBB assessment. We also assessed the degree of tissue preservation in SCI groups using morphometric analysis of spinal cord tissue cross sections stained with LFB+HE (Fig. 2.9C-Q). Generally, all SCI groups exhibited less than 14% preserved tissue at the injury epicenter at 10wpi. We found no difference in tissue preservation among vehicle, noggin- and LDN193189-treated groups (Fig. 2.9R-S). Lastly, our immunohistochemical analysis of GFAP and CSPG in chronically injured spinal cord showed no difference among vehicle, noggin- and LDN193189-treated SCI groups (Supplemental Fig. 2.4A-V), suggesting that the formation of the glial scar can progress chronically despite the early attenuation of CSPG deposition by inhibition of BMPs.

Altogether, these findings indicate that targeting the acute upregulation of BMP4 as a solitary treatment does not result in significant long-term improvement in the recovery of function and scar formation in chronic SCI.

Figure 2.9. Acute BMP inhibition has no long-term beneficial effect on functional recovery or tissue preservation in chronic SCI.

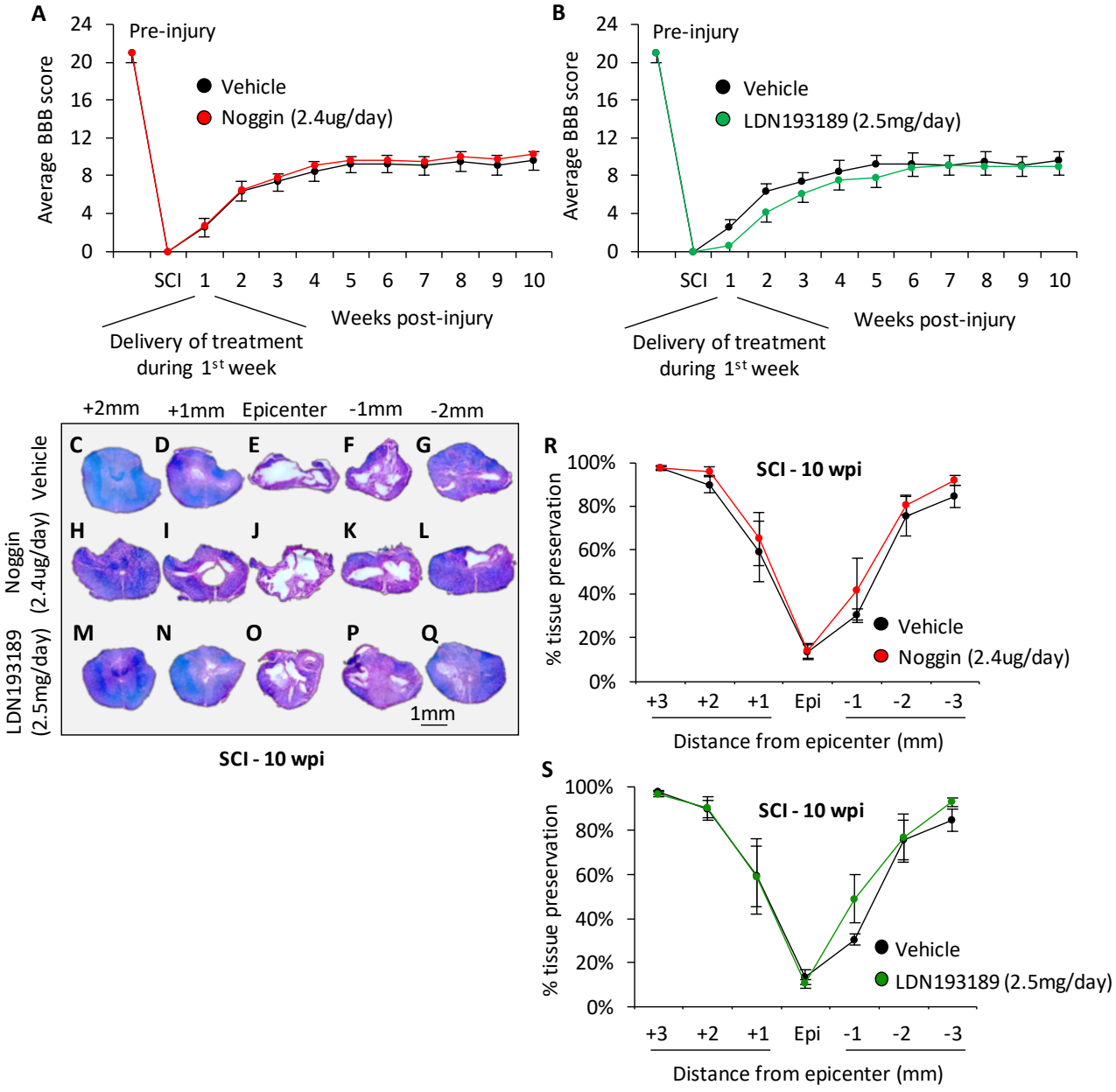
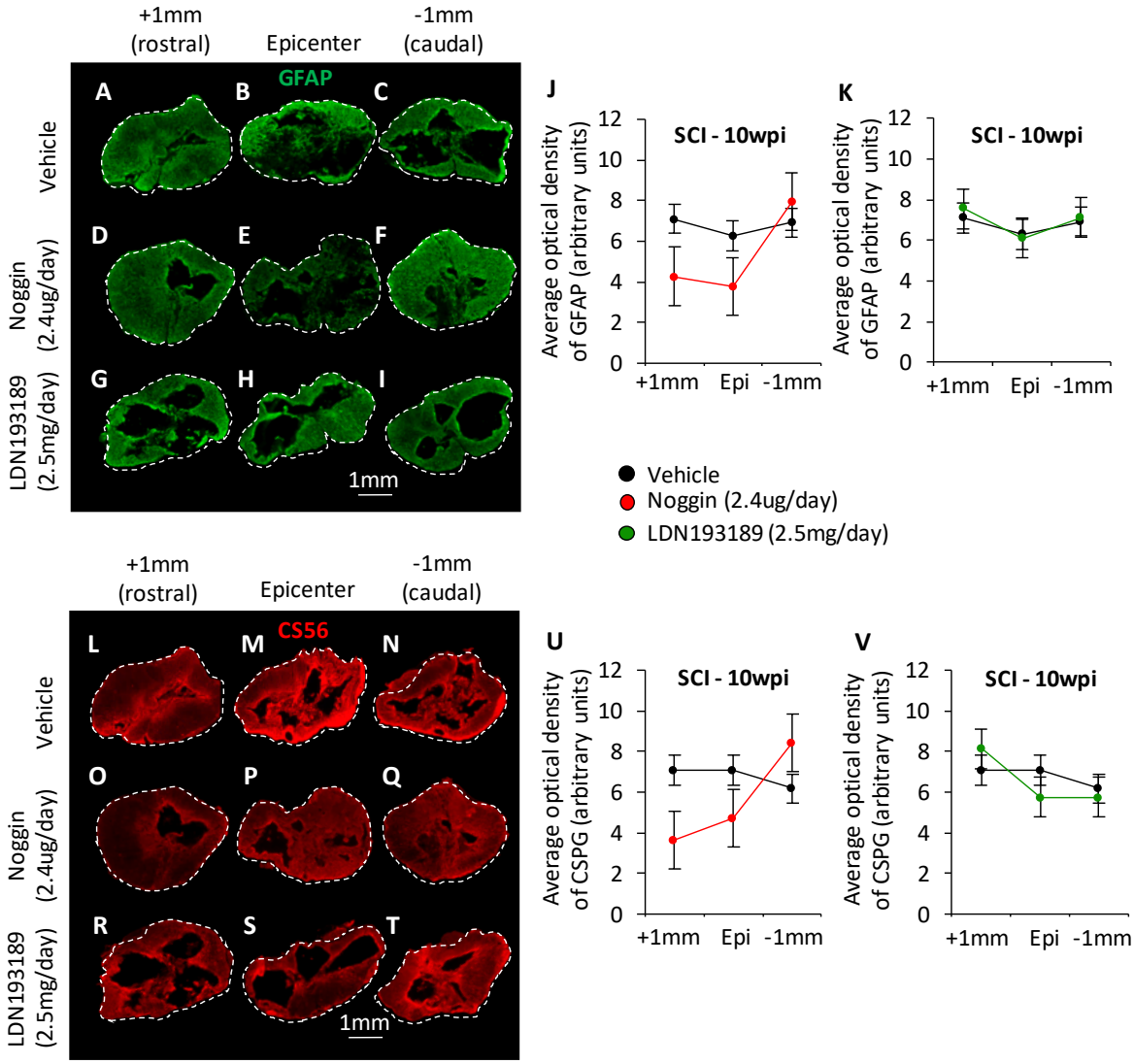


Figure 2.9. Acute BMP inhibition has no long-term beneficial effect on functional recovery or tissue preservation in chronic SCI. (A-B) Hindlimb locomotor function was assessed every week by BBB open field scale in SCI untreated (vehicle), SCI/noggin-treated (2.4ug/day) and SCI/LDN193189-treated (2.5mg/day) groups up to 10 weeks post-injury (wpi). Treatments were delivered during the first week post-injury, which coincided with acute and transient upregulation of active BMP4. Weekly assessments showed all experimental groups reached a plateau in score (8.8-9.6) between 5-6wpi and noggin or LDN193189 treatments did not improve the injury-baseline BBB score. (C-S) Analysis of tissue preservation using LFB-H&E co-stained serial cross sections (epicenter+/-3mm) showed no improvement in tissue integrity with noggin or LDN193189 treatments relative to vehicle group (R-S). N=9 rats/treatment for functional analysis and N=4 rats/treatment for tissue preservation analysis. Data represented as mean $\pm$ SEM, *$p<0.05$, Two Way ANOVA followed by Holm-Sidak *post hoc* test.

Supplemental Figure 2.4. Acute inhibition of BMPs has no effect on reactive astrogliosis in the chronic SCI rat.



Supplemental Figure 2.4: Acute inhibition of BMPs has no effect on reactive astrogliosis in the chronic SCI rat. (A-V) Quantitative immunostaining of GFAP and CSPG was performed in spinal cord tissue cross sections (epicenter±1mm) of SCI untreated (vehicle), SCI/noggin-treated (2.4 μg/day) and SCI/LDN193189-treated (2.5 mg/day) groups at 10 weeks post-injury. There was no significant difference in GFAP or CS56 immunointensity with noggin or LDN193189 treatments relative to vehicle group. N = 4 rats/treatment. Data shown as average optical density ± SEM.

2.5 Discussion

Acute SCI represents a complex pathophysiology that results in a dysregulated microenvironment. Currently, there are gaps in our understanding of the underlying endogenous mechanisms that contribute to the secondary injury cascade after SCI. In the present study, we demonstrate that SCI triggers an acute and transient upregulation of active form of BMP4. In a clinically relevant model of compressive/contusive SCI in the rat, pharmacological inhibition of BMPs using noggin and LDN193189 promoted preservation of oligodendrocytes and oligodendrogenesis. Our direct *in vitro* assessments suggest that BMP4 promotes astrocyte differentiation, while limiting oligodendrogenesis of spinal cord NPCs. BMP4 also impedes maturation of OPCs into myelinating oligodendrocytes. Intriguingly, we provide new evidence that BMP4 promotes caspase-3 mediated cell death in oligodendrocytes and neurons *in vitro* and

targeting BMPs in SCI remarkably attenuates the injury-induced expression of cleaved caspase-3 and lipid peroxidation. Importantly, increase in active BMP4 contributes to upregulation of inhibitory CSPGs in SCI, and this effect appears to be through direct modulation of reactive astrocytes. While acute inhibition of BMPs does not translate to long-term neurological recovery and tissue preservation, it seems to promote myelin thickness in chronically injured spinal cord white matter. Altogether, our findings have uncovered a role for BMP4 as an endogenous modulator of acute events after SCI.

BMP4 is a key regulator of neural stem cell differentiation and spinal cord patterning in neurodevelopment. BMP4 blocks neuronal and oligodendroglial differentiation by inducing the expression of helix-loop-helix transcriptional inhibitors of differentiation-1 (Id1) and Id2/4 in NPCs in the developing CNS (Yanagisawa et al., 2001; Samanta and Kessler, 2004), while it potentiates astrocyte differentiation through Smad1/5/8 and STAT3-p300 cross-talk signaling (Nakashima et al., 1999; Hong and Song, 2014). BMP4 can also drive astrogenesis amongst OPCs by inhibiting their differentiation and maturation into myelinating oligodendrocytes (See et al., 2004; Cheng et al., 2007; Wang et al., 2011). In the healthy adult rat spinal cord, BMP4 and its receptors are widely expressed (Miyagi et al., 2012). In agreement with our studies in rat SCI, increased expression of BMP4 has been also reported in SCI mice and in lysolecithin (LPC)-induced demyelination in rats (Fuller et al., 2007; Sahni et al., 2010; Xiao et al., 2010; Wang et al., 2011; Hesp et al., 2015). However, our study is the first to make a distinction between the pro- and biologically active form of BMP4. Like other members of TGFβ superfamily, an inactive BMP4 precursor undergoes post-translational processing by various pro-protein convertases, which recognize two distinct and sequentially cleaved sites within the pro-domain that modulate BMP4 ligand activity and range (Cui et al., 2001; Nelsen and Christian, 2009). In *Xenopus* oocytes,

it was demonstrated that the pro-domain of BMP4 is first cleaved at an optimal cleavage motif (-R-X-R/K-R-, S1 site) to yield a biologically active dimer of 35-kD pro-domain and 15-kD mature BMP4 peptides linked by non-covalent association (Degnin et al., 2004). The pro-domain is additionally cleaved and produces a small linker domain and large (32-kD) pro-domain (Sopory et al., 2006), which liberates the mature BMP4 ligand and increases its bioactivity (Degnin et al., 2004). We found a robust upregulation of active BMP4 between 1-3 days post-SCI, which is associated with increased phosphorylation of BMP canonical Smad1/5/8 pathway. Pro-BMP4 protein expression remains relatively stable, which may reflect a transient increase in BMP4 processing after injury. We identified astrocytes, oligodendrocytes and neurons as main sources of BMP4 in the injured spinal cord. While the underlying cause of transient BMP4 upregulation after SCI requires further elucidations, current evidence suggests that activated astrocytes contribute to BMP4 upregulation in SCI. Astrocytes expressed BMP4 in the injured spinal cord and *in vitro* when treated with IL-1β and TNFα, two pro-inflammatory cytokines relevant to acute SCI in rats. Our findings are in agreement with previous studies that showed astrocytes were significant sources of BMP2/4 when isolated from rats with contusive SCI (Wang et al., 2011). We found that microglia/macrophages did not express BMP4 in SCI, and upon activation *in vitro* (data not shown). Miyagi and colleagues also previously showed that microglia/macrophages do not express BMP2/4 in the adult rat spinal cord (Miyagi et al., 2012). However, expression of BMP4 has been reported in activated microglia/macrophages in demyelinating lesions of experimental autoimmune encephalitis (EAE) and contusive SCI in mice (Ara et al., 2008; Xiao et al., 2010). These data suggest that contribution of microglia/macrophages to BMP4 upregulation in SCI needs further investigation.

We demonstrate that inhibition of BMPs with either noggin or LDN193189 protects oligodendrocytes and enhances generation of new oligodendrocytes after SCI. Oligodendrogenesis is attributed to activation of both NPCs and OPCs in SCI (Meletis et al., 2008; Barnabé-Heider et al., 2010; Hesp et al., 2015). Our direct *in vitro* investigations confirmed that BMP4 modulates both NPCs and OPCs. In spinal cord NPCs, BMP4 limits oligodendrogenesis while promoting astrocyte differentiation. In addition to oligodendrogenesis, our *in vitro* studies clearly showed the negative impact of BMP4 on maturation of oligodendrocytes into a myelinating phenotype, which is in agreement with previous work (See et al., 2004; Wang et al., 2011). Thus, our studies collectively suggest a role for BMP4 as a negative regulator of oligodendrogenesis in acute SCI. However, it is noteworthy to highlight that our BrdU phenotyping assessment was not able to differentiate the effects of BMP4 on oligogenic capacity of NPCs verses OPCs in our pre-clinical rat SCI, as both populations are proliferative and would incorporate BrdU after injury. To allow this differentiation, transgenic mouse models are required in future investigations for lineage tracing of NPCs and OPCs. Nonetheless, our long-term SCI studies showed inhibition of BMP signaling promotes remyelination in the chronically injured spinal cord. Improved remyelination may have resulted from enhanced preservation and/or oligodendrogenesis that we observed at the acute stage. Emerging evidence from demyelinating CNS lesions have also shown promising effects of noggin and LDN193189 treatments on remyelination (Sabo et al., 2011; Govier-Cole et al., 2019). Furthermore, recent work shows elevated expression of noggin in remyelinating human MS lesions relative to chronic inactive demyelinated lesions (Harnisch et al., 2019). It is plausible that BMP inhibition may improve remyelination through Schwann cells. It has been shown that Schwann cells can be transdifferentiated from OPCs through a BMP4-Wnt-Sosctdc1-dependent mechanism in demyelinating lesions of rodents (Ulanska-Poutanen et al., 2018); however, it is

only detected in regions relatively devoid of astrocytes (Talbott et al., 2006; Ulanska-Poutanen et al., 2018). Whether BMPs exert the same effects in SCI requires further investigation to differentially characterize the role of BMP4 in regulating oligodendrocyte verses Schwann cell myelination.

BMP4 promoted fate specification of adult derived spinal cord NPCs towards an astrocytic lineage *in vitro*; however, noggin and LDN193189 treatments had no significant effect on the number of proliferating BrdU positive astrocytes in the injured spinal cord. This finding may reflect the low number of NPC derived astrocytes in the injured spinal cord among the highly proliferative population of pre-existing astrocytes that undergo reactive astrogliosis and incorporate BrdU. As we discussed earlier, genetic tracing of NPCs is required to determine the explicit role of BMP4 in regulating astrogenesis of NPCs after SCI. Based on our rat SCI data, neither noggin nor LDN193189 treatments affected overall GFAP expression after SCI, suggesting that inhibition of BMPs does not prevent astrogliosis *per se.* Previous work also suggested that noggin would be insufficient to attenuate reactive astrogliosis in sub-acute SCI (Matsuura et al., 2008; Xiao et al., 2010). Similarly, neither noggin nor LDN193189 treatments influenced the presence of microglia/macrophages in acute SCI and had no effects on the expression of pro-inflammatory TNFα and IL-1β. Thus, our current evidence does not suggest any apparent role for BMP4 in acute neuroinflammation.

Increased BMP4 expression appears to be an underlying mechanism of CSPG upregulation in acute SCI through modulation of astrocyte reactivity. Although GFAP expression of astrocytes remained unchanged under noggin and LDN193189 treatments, BMP inhibition resulted in a significant reduction in CSPGs after SCI. In pure cultures of astrocytes in absence of any other injury stimuli, exposure to BMP4 was sufficient to significantly induce CSPG release by

astrocytes. Of note, CSPGs are major inhibitory components of the extracellular matrix in SCI with several deleterious effects (Karimi-Abdolrezaee et al., 2010; Karimi-Abdolrezaee and Billakanti, 2012; Karimi-Abdolrezaee et al., 2012; Dyck et al., 2015; Lang et al., 2015b; Dyck et al., 2018; Dyck et al., 2019). More relevantly, our recent SCI studies unraveled that activation of CSPGs and their signaling receptors promotes caspase-3 mediated cell death and limits oligodendrogenesis and maturation of oligodendrocytes in the injured spinal cord (Dyck et al., 2019). Therefore, it is plausible that improved oligodendrogenesis following BMP inhibition could be, in part, due to reduction in CSPGs expression in SCI. Moreover, decrease in cleaved caspase-3 under noggin and LDN193189 treatments may also reflect their effects in reducing CSPGs in SCI. Taken together, BMP4 seems to modulate oligodendrocytes replacement and survival through both direct and indirect mechanisms that need further investigations.

Despite the early promising effects of BMP inhibition in SCI, we observed no long-term functional benefits in rats that received noggin and LDN193189 treatments. Our results are in contrast with another study that observed significant improvements in BBB scores of rats that received noggin treatment at a slightly higher dose for 2 weeks after contusive SCI (Matsuura et al., 2008). The authors attributed this improvement to increased axonal sprouting in the corticospinal tract. However, this study contrasts another report that showed AAV-mediated overexpression of BMP4-Smad1 signaling can promote axonal sprouting in primary adult dorsal root ganglion (DRG) neurons and neurite outgrowth in explant cultures of embryonic (E18.5) hippocampal neurons (Parikh et al., 2011), and potentiate sensory axon regeneration in SCI mice and rats (Parikh et al., 2011; Farrukh et al., 2019). AAV-BMP4-treated rats also exhibited improved electrophysiology and performance at horizontal ladder walking (Farrukh et al., 2019). The lack of improved functional recovery in our studies could potentially reflect limitations of our

in vivo strategy with utilizing pan-BMP inhibitors. The commonly used noggin and LDN193189 do not specifically target BMP4. Noggin is a highly potent inhibitor of BMP-2 and -4 (Zimmerman et al., 1996; Gazzerro et al., 1998), but it also has lower affinity for BMP-5, -6, -7, -13 and -14 in both *in vitro* or *in vivo* models (Chang and Hemmati-Brivanlou, 1999; Baade Ro et al., 2004; Seemann et al., 2009; Song et al., 2010). LDN193189 is a competitive inhibitor that binds to intracellular kinase domains of BMP Type 1 receptors, with some inhibition of ALK1 and ALK2 receptors (Yu et al., 2008b), and attenuates BMP-2, -6 and -14 mediated downstream Smad and non-Smad signaling (Boergermann et al., 2010). Therefore, general inhibition of BMPs may impede beneficial actions of other BMP isoforms. Previous work by de Rivero Vaccari and colleagues indeed showed that BMP7 improves neuronal preservation by increasing MAPK-p38 signaling and NMDAR expression in the ventral horn of rats with SCI (de Rivero Vaccari et al., 2009), while it also improved functional recovery and increased neurogenesis in a rat stroke model induced by middle cerebral artery occlusion (MCAO) (Chou et al., 2006). Moreover, both BMP-6 and -7 can attenuate apoptosis in primary rat cerebellar granular neurons under nutrient-deficient or excitotoxic conditions (Yabe et al., 2002). Transplantation of astrocytes derived from BMP4-differentiated embryonic mouse and human glial restricted precursors (GRPs) promotes axonal regeneration and functional recovery (Davies et al., 2008; Davies et al., 2011). Additionally, conditional knockout of astrocytic BMPR1a or BMPR1b exert opposing effects on glial scar, resulting in impaired or accelerated scar formation and inflammatory sequestration, which influences axon preservation and functional recovery in SCI in mice (Sahni et al., 2010). These controversial findings suggest that BMP/BMPR1a signaling can be also neuroprotective and pro-regenerative and that future studies may benefit from selective strategies minimizing the impact on beneficial BMP mediated responses in SCI. Alternative BMP4-specific strategies may use anti-

BMP4 blocking antibodies or targeted siRNA knockdown that have been employed *in vitro* (Wislet-Gendebien et al., 2004) or in developing *Xenopus* and mouse *in vivo* (Gratsch et al., 2003; Miller et al., 2004). However, the feasibility and efficacy of BMP4 inhibition using these strategies in adult SCI rats has yet to be examined.

In conclusion, our present work identifies BMP4 as an early modulator of secondary injury mechanisms after SCI. We demonstrate that acute inhibition of BMPs can enhance oligodendrogenesis, oligodendrocyte preservation and remyelination. Importantly, we have uncovered that BMP4 induces lipid peroxidation and apoptosis in neurons and oligodendrocytes directly, and potentially by indirect mechanisms through induction of CSPGs by reactive astrocytes. Despite the beneficial effects of targeting BMP4 in acute SCI, this strategy did not translate into long-term functional recovery in rats with chronic SCI. This is likely due to the presence of other inhibitory factors that drive injury mechanisms in subacute and chronic SCI. Given our new findings, we propose that acute inhibition of BMPs may serve as an early neuroprotective strategy in combinatorial strategies for SCI.

2.6 Acknowledgements

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Chapter 3: Discussion

3.1 General overview of findings

This thesis sought to evaluate the impact of BMP4 upregulation on secondary injury mechanisms and specifically on endogenous cell response after traumatic SCI. We employed a pre-clinical rat model of compressive/contusive SCI and targeted BMP4 by administering noggin or LDN193189 (two general inhibitors of BMP signaling). We used parallel *in vitro* assays with primary cultures to evaluate the effects of BMP4 on spinal cord NPCs and cortical OPCs, astrocytes and neurons. First, we observed a dramatic upregulation in active BMP4 expression between 1-3 days after SCI, which returned to basal levels after 5 days (Hart et al., 2019); intriguingly, this response was neutralized with LDN193189 treatment (Hart et al., 2019). We showed that BMP4 limited proliferation and oligodendrogenesis in NPCs, and instead diverted them towards an astrocytic fate *in vitro* (Hart et al., 2019). BMP4 also limited the maturation of OPCs into myelin-expressing oligodendrocytes (Hart et al., 2019), but had no effect on their proliferation (data not shown). Administration of BMP inhibitors promoted oligodendrogenesis and oligodendrocyte preservation within days of injury and fostered chronic remyelination after SCI (Hart et al., 2019). We also found that BMP4 induced astrocytic CSPG expression *in vitro* and in the injured spinal cord, as its expression was attenuated acutely by noggin or LDN193189 treatment (Hart et al., 2019). We showed BMP inhibition can attenuate cleaved caspase-3 expression and lipid peroxidation in SCI rats (Hart et al., 2019), identifying a novel role of BMP4 in modulating apoptosis and oxidative stress. Our *in vitro* assays confirmed BMP4 induces caspase-3 mediated apoptosis in cortical neurons and mature oligodendrocytes, but neither of these populations were significantly rescued *in vivo* with noggin or LDN193189 treatment (Hart et al., 2019). Despite the beneficial effects detailed above, acute delivery of noggin and LDN193189 had

no effect on inflammation or tissue preservation, glial scarring and behavioural recovery in chronic SCI rats. Overall, our findings show that BMP4 is a contributing factor that modulates several key injury mechanisms in acute stages of SCI and may be considered in future combinatorial approaches to improve neuroprotection and remyelination.

3.2 General discussion

3.2.1 BMP4 and endogenous cell response in SCI

Previous works have characterized BMP4 expression in several models of CNS injuries, including demyelinating lesions (Ara et al., 2008), SCI (Chen et al., 2005; Hesp et al., 2015), sub-cortical small vessel disease (SVD) (Uemura et al., 2018), and AD (Li et al., 2008). Our *in vivo* analysis showed that the active form of BMP4 is transiently and robustly increased within days of injury, and that it is most prevalent in proximity to the injury epicentre (Hart et al., 2019). Interestingly, we found that LDN193189 treatment attenuated this response (Hart et al., 2019), which may suggest the existence of a potential auto-regulatory feedback loop that governs BMP4 expression or processing after SCI in rats. This is supported by previous works demonstrating that BMP ligands have the capacity to induce transcription of BMP2, BMP4 and furin under *in vitro* and *in vivo* conditions (Ghosh-Choudhury et al., 1996; Schuler-Metz et al., 2000; Ghosh-Choudhury et al., 2001; Ghosh-Choudhury et al., 2002; Chang et al., 2015). A recent publication by Chen and colleagues demonstrated that knocking out high temperature requirement A-1 (HtrA1), a BMP4-induced serine protease expressed by reactive astrocytes, can impact both BMP4 ligand processing and stability after TBI in mice (Chen et al., 2018). Therefore, it is possible that LDN193189 attenuates the expression of BMP4 processing enzymes such as HtrA1 or furin (Hajebrahimi et al., 2008).

BMP-Smad1 signaling is a key developmental pathway that promotes gliogenesis among spinal cord progenitor cells (See et al., 2007). BMP4 antagonizes oligodendrogenesis in NPCs and OPCs by upregulating Id2/4 expression, which attenuates the expression of oligogenic factors such as Olig1/2 or myelin-associated proteins (Yanagisawa et al., 2001; Samanta and Kessler, 2004; See et al., 2004; Cheng et al., 2007). In agreement, our *in vitro* assays showed recombinant BMP4 antagonized oligodendrocyte differentiation and maturation in both NPCs and OPCs (Hart et al., 2019). It should be noted that these effects may be BMP4-specific, as knocking out both BMP Type I receptors has been shown to decrease the number of astrocytes and oligodendrocytes in the developing spinal cord of mice (See et al., 2007). This suggests that some BMP isoforms may be neutral or have a pro-oligogenic effect. Nevertheless, we found that BMP inhibition increased oligodendrogenesis and oligodendrocyte preservation acutely after SCI (Hart et al., 2019). Intriguingly, we also found acute and transient delivery of LDN193189 improved remyelination (i.e. myelin thickness) in chronic stages of SCI (Hart et al., 2019). Previous works have detailed similar benefits by inhibiting BMP-Smad1 signaling in models of contusive SCI and demyelinating lesions (Sabo et al., 2011; Petersen et al., 2017; Govier-Cole et al., 2019; Li et al., 2019).

Transplantation studies have demonstrated that NPCs and OPCs can promote remyelination of damaged axons, which correlates with improved functional recovery after SCI (Karimi-Abdolrezaee et al., 2006; Cao et al., 2010; Yasuda et al., 2011; Nagoshi et al., 2018; Nori et al., 2018) . In our study, however, neither of our treatment groups showed any improvement in long-term functional recovery relative to vehicle-SCI rats, despite LDN193189 treatment enhancing remyelination (Hart et al., 2019). These outcomes could be explained by the difference in therapeutic approaches, as those previous studies transplanted large volumes of exogenous cells

while our study used approaches to promote activation of endogenously present precursor cells after injury. Moreover, transplanted NPCs and OPCs are significant sources of growth factors, immunomodulatory factors and supplement endogenous remyelination (Zhang et al., 2006a; Yasuda et al., 2011). These studies also provided combinatorial treatments with NPC or OPC transplantation that further improved the injury microenvironment (e.g. growth factors, immunosuppression, CSPG degradation). Future studies are warranted to investigate whether combining acute BMP inhibition with one or more of these alternative strategies may elicit long-term functional recovery in SCI rats.

Alternatively, acute BMP inhibition could have a negative impact on the responsiveness of SCs after SCI. SCs can enter the spinal cord and remyelinate damaged axons and persist chronically in parallel with oligodendrocytes after injury (Guest et al., 2005). Existing *in vitro* evidence suggests that BMP2 and BMP4 can also directly transdifferentiate adult OPCs into SCs (Crang et al., 2004), which is observed *in vivo* in demyelinating lesions devoid of astrocytes (Ulanska-Poutanen et al., 2018). A recent study has emphasized a role of OPC-derived SCs in spontaneous remyelination and functional recovery after SCI (Bartus et al., 2019). Therefore, it is possible that acute BMP inhibition inadvertently impaired SC-derived recruitment or remyelination and limited the potential for functional recovery, which was not addressed in this thesis.

3.2.2 BMP4 and reactive astrogliosis in SCI

In our study, we confirmed that recombinant BMP4 increased astrocyte differentiation in cultured NPCs, and this effect was successfully attenuated with noggin or LDN193189 co-treatments (Hart et al., 2019). However, our *in vivo* analysis showed that neither treatment changed

the number of new astrocytes (BrdU+/GFAP+) generated after SCI (Hart et al., 2019). It is possible that our *in vivo* analysis was confounded by pre-existing reactive astrocytes, which are highly proliferative after SCI (Wanner et al., 2013). Setoguchi and colleagues showed that overexpressing noggin in transplanted NPCs only rescued a fraction from differentiating into astrocytes (~15% GFAP-) in SCI mice (Setoguchi et al., 2004). These findings also suggest that astrogenesis can be sustained by activating BMP-independent pathways.

BMP-Smad1 signaling is one of several signaling pathways that regulate reactive astrogliosis and glial scar formation. For example, STAT3 is an essential transcription factor that promotes proliferation, hypertrophy and GFAP expression in scar-forming astrocytes (Herrmann et al., 2008; LeComte et al., 2015). While we did not evaluate STAT3 phosphorylation in our study, Xiao and colleagues previously showed that noggin treatment had no effect on STAT3 phosphorylation in SCI mice (Xiao et al., 2010). Inflammation is a key inducer of reactive astrogliosis and STAT3 signaling in acute SCI (Herx and Yong, 2001; Okada et al., 2004; Liddelow et al., 2017). We showed that LDN193189 treatment reduced total Iba1 expression (a marker of inflammatory microglia/macrophages) relative to the vehicle SCI group, but the expression of pro-inflammatory cytokines TNFα and IL-1β were unaffected by noggin or LDN193189 treatments (Hart et al., 2019). Another group showed anti-inflammatory TGFβ can also induce astrocyte differentiation in NPCs in a Smad2/3-dependent manner (Giannakopoulou et al., 2011). Therefore, it is plausible that BMP signaling does not contribute to inflammatory cell recruitment or immunomodulation after SCI in rats, and that the acute inflammatory microenvironment reinforced glial scarring even in the presence of BMP antagonists. However, studies in other systems outside the CNS have provided contradicting evidence that BMP signaling can promote inflammatory cell recruitment and serve in an immunomodulatory role (Rocher and Singla, 2013; Helbing et al., 2017; Martínez

et al., 2017; Pardali et al., 2018). Helbing and colleagues previously demonstrated that endothelial cells upregulate BMP4 expression in response to inflammation, and that BMP4 facilitated the infiltration of blood-derived leukocytes under *in vitro* and *in vivo* conditions by destabilizing endothelial tight junctions (Helbing et al., 2017). Of note, this *in vitro* model showed that BMP4 alone was not a chemotactic factor, as it did not increase leukocyte transmigration in transwell chambers without endothelial cells (Helbing et al., 2017). In contrast, Pardali and colleagues later showed *in vitro* that BMP2 induced chemotaxis in human monocytes, and that it upregulated the expression of cell-adhesion proteins in endothelial cells that facilitated the attachment and infiltration of inflammatory cells (Pardali et al., 2018). Moreover, there is evidence that BMP4 and BMP7 or BMP2 can promote or antagonize an anti-inflammatory M2 phenotype in monocyte/macrophages, respectively (Rocher and Singla, 2013; Martínez et al., 2017; Pardali et al., 2018). In consideration of these findings, further investigation is required to clarify the immunomodulatory role of BMP signaling in the acute inflammatory response after SCI in rats.

Of note, there may be a risk that inhibiting BMPs could develop a less supportive phenotype in NPC-derived astrocytes. A few studies argue that glial restricted precursor-derived and BMP4-differentiated astrocytes (GDA^{BMP4}) have a pro-regenerative or neuroprotective phenotype after transplantation into models of SCI and Parkinson's disease (PD), respectively, as detailed here (Haas et al., 2012; Proschel et al., 2014; Shih et al., 2014). GDA^{BMP4} are a unique source of periostin (ECM protein), for example, which supports axon regeneration *in vivo* and neurite outgrowth in the presence of myelin debris or CSPGs *in vitro* (Shih et al., 2014). However, further investigation is required to determine if these pro-regenerative GDA^{BMP4} are generated endogenously and if their absence would have a significant impact on recovery after SCI.

Glial scar formation is modulated by several distinct signaling pathways (Kang and Hébert, 2011). Our study showed that acute BMP inhibition had no effect on total GFAP expression in acute or chronic SCI rats, and BMP4 alone was insufficient to significantly induce GFAP expression in cultured astrocytes (Hart et al., 2019). These findings suggest that BMP4 may not be important for reactive astrogliosis and glial scar formation after SCI. This notion is also supported by other studies that showed intrathecal delivery of noggin had no effect on GFAP immunointensity in SCI mice or rats (Matsuura et al., 2008; Xiao et al., 2010). However, BMP signaling is shown to modulate the integrity of the glial scar in a Type I receptor-specific manner (Sahni et al., 2010). Briefly, astrocytic BMPR1a activation accelerates glial scar formation and sequestration of inflammatory cells, while BMPR1b has detrimental effects on both measures and functional recovery in SCI mice (Sahni et al., 2010). Interestingly, Chen and colleagues showed that BMP4 induces the expression of HtrA1 via BMPR1a in forebrain astrocytes, which is necessary for effective wound closure and resolution of inflammation after TBI (Chen et al., 2018). It is possible that a BMP4-BMPR1a-HtrA1 cascade has a similar beneficial role in modulating glial scarring after SCI in rats.

Importantly, we showed that BMP inhibition attenuated the expression of inhibitory CSPGs during acute stage of SCI (Hart et al., 2019). Our complementary *in vitro* analysis also confirmed that BMP4 induced CSPG expression in cultured astrocytes (Hart et al., 2019). We further demonstrated the specificity of LDN193189 treatment in blocking BMPs *in vitro* as it had no effect on CSPG expression in TGFβ-treated astrocytes (Hart et al., 2019). Together, these findings suggest upregulation of BMP4 contributes to a less permissive SCI microenvironment by potentiating early CSPG deposition after SCI. This hypothesis is supported by previous work that showed BMP4 and BMP7 promote CSPG expression when injected into the naïve rat spinal cord

(Fuller et al., 2007). However, it was recently shown that BMP4-inducible HtrA1 antagonizes TGFβ activation and suppresses neurocan expression (a subtype of CSPGs) in cultured astrocytes (Chen et al., 2018). Consequently, BMP4 may differentially regulate distinct sub-types of CSPGs in naïve and reactive astrocytes, but this requires further clarification in the context of SCI. In our study, while acute BMP inhibition reduced deposits of CSPGs during the acute phase of SCI, it had no impact on long-term CSPG deposition in the chronic glial scar (Hart et al., 2019). This was not unexpected, as CSPG production is a sustained, longstanding process in SCI and upregulation of BMP4 is one of many factors that contributes to CSPG production and glial scar formation after SCI. Thus, acute and transient inhibition of BMP4 is not evidently sufficient to suppress long-term production of CSPGs. This also suggests that additional strategies are necessary to maintain improvements chronically in future combinatorial approaches.

3.2.3 BMP4 and cell death in SCI

A major finding of our study is that acute BMP inhibition attenuated apoptosis following SCI, and that availability of BMP4 directly induced cleaved caspase-3 expression in cultured cortical neurons and oligodendrocytes (Hart et al., 2019). Our study also demonstrated that noggin and LDN193189 treatments attenuated lipid peroxidation, which is a by-product of oxidative stress and promotes apoptosis after SCI (Xu et al., 2005).

BMP4 is a pro-apoptotic factor during certain stages of embryogenesis, limb and eye development (Graham et al., 1994; Yokouchi et al., 1996; Trousse et al., 2001; Musto et al., 2015). BMP4 induces the expression of Msh homeobox gene-2 (Msx2), a key transcriptional repressor that promotes apoptosis in embryonic stem cells and NPCs during neurodevelopment (Marazzi et al., 1997; Israsena and Kessler, 2002). Msx2 is also upregulated in the grey and white matter at 4 days post-SCI in mice, and it co-localizes with neurons and astrocytes (Chen et al., 2005).

However, it remains unclear if Msx2 is similarly pro-apoptotic in these adult cells after injury. There have been few and conflicting reports of BMP4 modulating cell death in the healthy or injured adult CNS. Previously, Sabo and colleagues showed that administering BMP4 increased cleaved caspase-3 mediated apoptosis near demyelinating lesions in the corpus callosum of mice, but this response did not reach statistical significance (Sabo et al., 2011). In contrast, Chou and colleagues reported that BMP4 can improve survival of embryonic motor neurons in response to glutamate excitotoxicity (Chou et al., 2013).

Neurons and oligodendrocytes are two key populations prone to cell death after SCI, and their loss contributes directly to functional impairment (Shuman et al., 1997; Yong et al., 1998). Our *in vitro* assay showed BMP4 can directly induce cleaved caspase-3 expression in cultured cortical neurons and mature oligodendrocytes (Hart et al., 2019). Moreover, our assessments in SCI showed noggin and LDN193189 reduce the overall expression of SCI-induced cleaved caspase-3 when assessed with immunoblotting. However, cell specific immunohistological analysis of cleaved caspase-3 expression in neurons or oligodendroglia showed no significant effects of noggin and LDN193189. Of note, we observed a decreasing trend in neuronal apoptosis with LDN193189 treatment relative to vehicle-treated SCI rats, but it was not statistically significant (Hart et al., 2019). These findings suggest that BMP4 can induce cell death in neurons and oligodendrocytes as shown in our direct *in vitro* assay. However, BMP4 is one of several possible modulators of apoptotic cell death in the acute injury microenvironment after SCI, and apparently its inhibition is not sufficient to significantly block apoptosis in these cells, at least with the dose of noggin and LDN193189 that we used in our studies. For example, STAT3 signaling is a regulatory pathway that increases lysosomal protease expression and facilitates lysosomal-mediated cell death in the mammary gland (Kreuzaler et al., 2011; Martínez-Fábregas et al., 2018),

and lysosomal dysregulation is a contributor to cell death after SCI (Ellis et al., 2005; Liu et al., 2018). Therefore, STAT3 could be a potential target in future combinatorial studies, along with BMP4, that aim to optimize neuroprotection acutely after SCI.

Based on the literature, BMP signaling may increase oxidative stress and cell death by restricting iron efflux in neurons. Iron is a highly reactive metal ion that serves several catalytic functions, but its bioavailability is tightly regulated to limit ROS production and cellular damage. Systemic iron homeostasis is regulated primarily in the liver by hepatocytes which, in response to inflammation, release hepcidin (a peptide hormone) in a BMP-Smad1-dependent manner (Steinbicker et al., 2011). Steinbicker and colleagues demonstrated that administering LDN193189 attenuated hepcidin expression by hepatocytes *in vitro* and in IL-6-treated mice (Steinbicker et al., 2011). Hepcidin regulates iron bioavailability by binding to iron transporters (e.g. FPN1) at the cell surface, triggering their internalization and degradation (Nemeth et al., 2004). A recent study showed that hepcidin expression is upregulated in the spinal cord of experimental autoimmune encephalomyelitis (EAE) mice (Zarruk et al., 2015). BMP4 expression is also upregulated by glial cells and macrophages in the spinal cord of EAE mice (Ara et al., 2008), as well as in active and chronically inactive MS lesions in humans (Harnisch et al., 2019). As previously described, BMP-Smad1 signaling has been characterized as antagonistic to oligodendrogenesis and remyelination after injury. However, there has been no defined role for BMPs in regulating hepcidin expression or iron homeostasis after traumatic SCI or EAE. *In vitro* and *in vivo* studies show inflammation induces hepcidin expression in brain-derived reactive astrocytes (Urrutia et al., 2013; You et al., 2017), which in turn limits neuronal capacity to release iron and triggers apoptosis in LPS-treated mice (You et al., 2017). In contrast, Zhou and colleagues showed that hepcidin is also

neuroprotective to hemin-treated cortical neurons (Zhou et al., 2017), suggesting a BMP-hepcidin cascade can be beneficial under a hyperferremic microenvironment.

While our study focused on neuronal and oligodendrocyte cell death due to their therapeutic relevance in SCI, endothelial cells are also lost at the lesion site early after injury (Casella et al., 2006). Kiyono and Shibuya demonstrated that BMP4 induces Msx1/2 expression and promotes apoptosis in human umbilical vein endothelial cells (Kiyono and Shibuya, 2003). Previous studies also showed that BMP4 increases ROS production in mammalian endothelial cells by activating NAPDH oxidase or upregulating cyclooxygenase-2 (COX-2) via BMPR1a and MAPK/p38 and JNK signaling, which promote cellular dysfunction, DNA fragmentation and cleaved caspase-3 mediated apoptosis (Wong et al., 2010; Tian et al., 2012). BMP4-induced ROS also oxidizes proteins by reacting with metal ions in human or rodent endothelial cell cultures, and these carbonylated proteins either serve as signaling molecules or are targeted for proteasome-dependent degradation (Wong et al., 2008; Wong et al., 2014). Wong and colleagues noted that several degraded proteins were integral enzymes for glycolysis, ATP production and protein folding (Wong et al., 2014), which could also contribute to BMP4-mediated cellular dysfunction or cell death. Previous studies demonstrated that BMP7 and BMP2 also induce ROS production in sympathetic neurons and osteoblasts by activating Nox2 and Nox4, respectively (Mandal et al., 2010; Chandrasekaran et al., 2015). Therefore, future studies are necessary to further elucidate the role of BMPs and their upstream and downstream partners in cell death and oxidative stress after SCI.

3.3 Study limitations

As with all scientific research, there were limitations in our work that could be addressed to appropriately interpret the results and provide directions for future investigations and optimizations.

One limitation in our study is that we sought to evaluate the role of BMP4 *in vivo* by administering noggin or LDN193189 (general BMP signaling inhibitors) in SCI rats. Thereby, it is likely that these inhibitors are antagonizing all BMP isoforms in the SCI microenvironment, some of which have demonstrated neuroprotective effects after SCI (i.e. BMP7) (de Rivero Vaccari et al., 2009; Wang et al., 2016). Despite their lack of specificity, noggin and LDN193189 have been utilized widely in the literature to target BMP4 *in vitro* and *in vivo* (Sabo et al., 2011; Uemura et al., 2018; Govier-Cole et al., 2019). Furthermore, we complemented our SCI studies with *in vitro* analyses that confirmed the direct effects of exogenous BMP4 peptide on neural cells. In these *in vitro* studies, both noggin and LDN193189 treatments were successful in significantly or completely attenuating BMP4-dependent responses. Therefore, we believe that our culture experiments provide sufficient evidence that supports a role for BMP4 upregulation in regulating acute SCI mechanisms. To specifically address the role of BMP4 in SCI, BMP4 knockout mice are necessary, which will be discussed in the following section as future directions. It is worthwhile to mention that unlike mouse SCI, rat compressive/contusive SCI is a highly relevant model for therapeutic development. Therefore, the rat preclinical model was selected for these studies due to its therapeutic values.

Another limitation in our study is that it did not identify the mechanism(s) by which BMP4 modulated apoptosis after SCI. We showed that BMP signaling has a role in promoting cleaved caspase-3 expression and lipid peroxidation in SCI rats, and BMP4 alone increased cleaved

caspase-3-mediated apoptosis in primary cortical neuron and oligodendrocyte cultures (Hart et al., 2019). These findings identify BMP4 as a potential modulator of cell death after SCI and alludes to oxidative stress as a likely mediator. As mentioned above, genetic models are required to better understand molecule mechanisms underpinning BMP4 effects.

Lastly, our study implemented an *in vivo* cell differentiation model using BrdU incorporation after SCI. Our group has previously used this technique to approximate precursor cell fate as activated NPCs and OPCs are highly proliferative after SCI (Karimi-Abdolrezaee et al., 2012; Dyck et al., 2019), however it is unable to explicitly discern the origins of new oligodendrocytes and astrocytes. Another strategy would be to use transgenic nestin-Cre^{ERT2} and PDGFRα-Cre^{ERT2} mice to trace NPCs and OPCs, respectively, in in SCI studies (Faiz et al., 2015; Baxi et al., 2017). However, as mentioned above, this strategy is less therapeutically relevant because rats, but not mice, share an overall pathological response that is comparable to human SCI patients (Jakeman et al., 2000; Metz et al., 2000; Sroga et al., 2003). Therefore, while we were unable to distinguish between NPC and OPC-derived cells after SCI, we characterized the effect of acute BMP inhibition on total oligodendrogenesis in a clinically relevant model of SCI and used *in vitro* assays to evaluate the direct effects of BMP4 on NPC and OPC differentiation individually. Our laboratory has recently established nestin-Cre^{ERT2} and PDGFRα-Cre^{ERT2} mice lines that can be used to address these questions in future studies.

3.4 Future directions

My PhD thesis has characterized BMP4 as an early modulator of oligodendrogenesis and glial scarring in SCI rats, and we identified a novel role for BMP4 in modulating cell death and lipid peroxidation in the acute SCI microenvironment. Based on these results, we propose that BMP4

antagonism could serve as a treatment in a combinatorial approach to synergistically promote neurological recovery.

Several steps can be taken next to further clarify the regulatory role of BMP4 after SCI. For our *in vivo* analysis, the first logical step would be to determine if/how tissue and behavioural outcomes are affected by concurrent administration of noggin and LDN193189 treatments. Given that these treatments differ in mode of delivery (intrathecal vs. systemic) and action (ligand vs. receptor binding) (Zimmerman et al., 1996; Sanvitale et al., 2013), it is possible that they could act synergistically to target BMP-Smad1 signaling. Future studies could also use alternative strategies to acquire more BMP4-specific information about the acute SCI microenvironment by using BMP4 specific siRNA, BMP4-neutralizing antibodies or conditional BMP4-KO transgenic models. A group recently developed a pair of llama-derived BMP4-specific antibodies that exhibit higher specificity and efficacy in *in vitro* systems comparable to other commercially available antibodies, noggin and LDN193189 treatments (Calpe et al., 2016). This may present a future opportunity for collaboration. Also, as previously described, our study could benefit from complementary *in vivo* fate mapping of NPC and OPC lineages in noggin or LDN193189-treated transgenic nestin-Cre^{ERT2} and PDGFRα-Cre^{ERT2} SCI mice.

Next, it would be reasonable to evaluate how acute BMP inhibition impacts SC differentiation and recruitment after SCI (Blight and Young, 1989; Felts and Smith, 1992), and whether BMP4 upregulation influences the relative contribution of oligodendrocytes (MBP+ or PLP+) and SCs (P0+ or MPZ+) to remyelination after SCI. SCs are shown to contribute to remyelination of damaged axons in human SCI patients (Guest et al., 2005).

Lastly, further investigations can be built on our findings to elucidate the mechanism by which BMP4 modulates oxidative stress and apoptosis after SCI. One candidate target would be iron

overload. As discussed earlier, the literature suggests that BMP4 may promote neuronal apoptosis by increasing iron overload (Steinbicker et al., 2011; Urrutia et al., 2013; Zarruk et al., 2015; You et al., 2017). This could be confirmed in our *in vivo* model by evaluating whether acute BMP inhibition has any effect on hepcidin and/or iron transporter expression via Western blot analysis. Furthermore, recombinant BMP4 can be delivered intrathecally (with or without iron chelators) to detect changes in endogenous ROS production and apoptosis in the naïve rat spinal cord.

3.5 Clinical implications

Traumatic SCI is a debilitating form of injury that affects nearly 86,000 Canadians, and there are approximately 180,000 new patients globally every year (Noonan et al., 2012; Lee et al., 2014). There are currently no therapeutic strategies that protect or repair damaged tissue and significantly improve recovery in SCI patients. The most effective strategy to minimize tissue damage is to surgically decompress the spinal cord as early as possible after injury (Fehlings et al., 2012; Dvorak et al., 2015). Acute administration of methylprednisolone sodium succinate (MPSS) (an anti-inflammatory steroid) is the only clinically-approved treatment for SCI patients, and it was originally hailed as a viable therapeutic strategy to improve neurological recovery (Bracken et al., 1990; Bracken et al., 1997; Bracken, 2001). However, the efficacy and long-term benefits of this treatment has been questioned by experimental and clinical studies in the last two decades (Rabchevsky et al., 2002; Evaniew et al., 2016; Fehlings et al., 2017). For example, meta-analyses of MPSS administration in acute traumatic SCI patients suggest it may increase their risk of developing gastrointestinal bleeds or infection (Aguayo, 2015; Evaniew et al., 2016). Given the complexity of the SCI microenvironment, it is likely that a multi-faceted approach is required to target several barriers to repair at once (Karimi-Abdolrezaee et al., 2006; Karimi-Abdolrezaee et

al., 2010; Alluin et al., 2014a; DePaul et al., 2017; Liu et al., 2017; Nori et al., 2018). Therefore, identifying new targets that modulate one or more secondary injury mechanisms is required to optimize future therapeutic strategies.

Our study used a clinically relevant model of compressive/contusive SCI in rats, which exhibits a pathophysiology resembling that of human SCI patients (Metz et al., 2000; Poon et al., 2007). Our model showed that BMP4 is transiently upregulated within days of SCI (Hart et al., 2019), and it has previously been shown to limit oligodendrogenesis and remyelination in demyelinating lesions (Cate et al., 2010; Sabo et al., 2011; Wang et al., 2011; Govier-Cole et al., 2019), and modulate glial scar integrity and axonal regeneration after SCI (Matsuura et al., 2008; Sahni et al., 2010; Farrukh et al., 2019). Considering the relevance of early therapeutic intervention, our study has illustrated the potential of acute and transient BMP inhibition as part of future combinatorial approaches to improve functional recovery in SCI patients. For example, acute BMP inhibition may foster a more permissive SCI microenvironment and bolster regenerative processes if combined with growth factors, immunomodulators and/or transplanted NPCs or OPCs.

In the last two decades, several pre-clinical studies have identified BMP signaling as a viable therapeutic target in a plethora of systemic pathologies, including fibrodysplasia ossificans progressive (FOP) (Shore et al., 2006; Yu et al., 2008a), cancer (Balboni et al., 2013; Owens et al., 2015), anemia (Steinbicker et al., 2011; Mayeur et al., 2015) and atherosclerosis (Derwall et al., 2012; Saeed et al., 2012). Relative to our study, other groups have described similar detrimental roles of BMP4 in other CNS disorders such as MS (Harnisch et al., 2019), amyotrophic lateral sclerosis (ALS) (Shijo et al., 2018), AD (Li et al., 2008), stroke (Samanta et al., 2010) and age-related neurological decline (Meyers et al., 2016). Of note, these pre-clinical studies demonstrate the potential impact of developing pharmacological tools to safely antagonize BMP signaling.

Unfortunately, there is currently no BMP4 antagonist that is clinically approved for use in treating human patients. However, several patents have been filed for LDN193189 and other pyrazolo-(1,5-α)-pyrimidine analogs due to their potent and specific inhibition of BMP receptors (Hopkins, 2016), which increases the clinical relevance of our current study. We demonstrated that systemic delivery of LDN193189 can attenuate BMP4 expression in the injured rat spinal cord, and it can directly or indirectly limit BMP-mediated responses during the acute stage of injury. Another study also demonstrated that intravenous or oral administration of LDN193189 can yield detectable levels of the molecule in circulating blood serum and inhibit BMP-Smad1/5/8 signaling in the liver of mice (Mayeur et al., 2015). The efficacy of systemic delivery for LDN193189 is promising for future therapeutic strategies as non-invasive strategies are often more attractive than local or viral delivery. Overall, our study identifies BMP4 as a critical modulator of endogenous cell responses and survival in early stages of SCI, and presents LDN193189-mediated inhibition of BMP4 as a potential therapeutic strategy for other neurodegenerative diseases that share similar pathological hallmarks of SCI.

Chapter 4: References

4.1 References

Adams KL, Gallo V (2018) The diversity and disparity of the glial scar. Nature Neuroscience 21:9-15.

Aguayo JMDT (2015) Side effects of steroid use in patients with traumatic spinal cord injury. Coluna/Columna 14:45-49.

Ahn YH, Bae Yeon Y, Lee G, Kang Mee K, Kang SK (2006) Molecular insights of the injured lesions of rat spinal cords: Inflammation, apoptosis, and cell survival. Biochemical and Biophysical Research Communications 348:560-570.

Ahuja CS, Wilson JR, Nori S, Kotter MRN, Druschel C, Curt A, Fehlings MG (2017) Traumatic spinal cord injury. Nature Reviews Disease Primers 3:17018.

Alizadeh A, Dyck SM, Karimi-Abdolrezaee S (2015) Myelin damage and repair in pathologic CNS: challenges and prospects. Frontiers in molecular neuroscience 8:35.

Alizadeh A, Dyck SM, Karimi-Abdolrezaee S (2019) Traumatic Spinal Cord Injury: An Overview of Pathophysiology, Models and Acute Injury Mechanisms. Frontiers in Neurology 10.

Alizadeh A, Santhosh KT, Kataria H, Gounni AS, Karimi-Abdolrezaee S (2018) Neuregulin-1 elicits a regulatory immune response following traumatic spinal cord injury. J Neuroinflammation 15:53.

Alizadeh A, Dyck SM, Kataria H, Shahriary GM, Nguyen DH, Santhosh KT, Karimi-Abdolrezaee S (2017) Neuregulin-1 positively modulates glial response and improves neurological recovery following traumatic spinal cord injury. Glia 65:1152-1175.

Allen NJ (2014) Astrocyte Regulation of Synaptic Behavior. Annual Review of Cell and Developmental Biology 30:439-463.

Alluin O, Delivet-Mongrain H, Gauthier M-K, Fehlings MG, Rossignol S, Karimi-Abdolrezaee S (2014a) Examination of the combined effects of chondroitinase ABC, growth factors and locomotor training following compressive spinal cord injury on neuroanatomical plasticity and kinematics. PLoS One 9.

Alluin O, Delivet-Mongrain H, Gauthier MK, Fehlings MG, Rossignol S, Karimi-Abdolrezaee S (2014b) Examination of the combined effects of chondroitinase ABC, growth factors and locomotor training following compressive spinal cord injury on neuroanatomical plasticity and kinematics. PloS one 9:e111072.

Anderson L, Lowery JW, Frank DB, Novitskaya T, Jones M, Mortlock DP, Chandler RL, Caestecker MPd (2010) Bmp2 and Bmp4 exert opposing effects in hypoxic pulmonary hypertension. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology 298:R833-R842.

Anderson MA, Burda JE, Ren Y, Ao Y, O'Shea TM, Kawaguchi R, Coppola G, Khakh BS, Deming TJ, Sofroniew MV (2016) Astrocyte scar formation aids central nervous system axon regeneration. Nature 532:195-200.

Anderson MA, O'Shea TM, Burda JE, Ao Y, Barlatey SL, Bernstein AM, Kim JH, James ND, Rogers A, Kato B, Wollenberg AL, Kawaguchi R, Coppola G, Wang C, Deming TJ, He Z, Courtine G, Sofroniew MV (2018) Required growth facilitators propel axon regeneration across complete spinal cord injury. Nature 561:396-400.

Andrews MG, del Castillo LM, Ochoa-Bolton E, Yamauchi K, Smogorzewski J, Butler SJ (2017) BMPs direct sensory interneuron identity in the developing spinal cord using signal-specific not morphogenic activities. Elife 6:e30647.

Ankarcrona M, Dypbukt JM, Bonfoco E, Zhivotovsky B, Orrenius S, Lipton SA, Nicotera P (1995) Glutamate-induced neuronal death: A succession of necrosis or apoptosis depending on mitochondrial function. Neuron 15:961-973.

Ankeny DP, Popovich PG (2009) Mechanisms and implications of adaptive immune responses after traumatic spinal cord injury. Neuroscience 158:1112-1121.

Ara J, See J, Mamontov P, Hahn A, Bannerman P, Pleasure D, Grinspan JB (2008) Bone morphogenetic proteins 4, 6, and 7 are up-regulated in mouse spinal cord during experimental autoimmune encephalomyelitis. Journal of Neuroscience Research 86:125-135.

Arimura N, Ménager C, Kawano Y, Yoshimura T, Kawabata S, Hattori A, Fukata Y, Amano M, Goshima Y, Inagaki M, Morone N, Usukura J, Kaibuchi K (2005) Phosphorylation by Rho Kinase Regulates CRMP-2 Activity in Growth Cones. Molecular and Cellular Biology 25:9973-9984.

Asher RA, Morgenstern DA, Fidler PS, Adcock KH, Oohira A, Braistead JE, Levine JM, Margolis RU, Rogers JH, Fawcett JW (2000) Neurocan Is Upregulated in Injured Brain and in Cytokine-Treated Astrocytes. The Journal of Neuroscience 20:2427-2438.

Baade Ro T, Utne Holt R, Brenne A-T, Hjorth-Hansen H, Waage A, Hjertner O, Sundan A, Borset M (2004) Bone morphogenetic protein-5, -6 and -7 inhibit growth and induce apoptosis in human myeloma cells. Oncogene 23:3024-3032.

Babitt JL, Zhang Y, Samad TA, Xia Y, Tang J, Campagna JA, Schneyer AL, Woolf CJ, Lin HY (2005) Repulsive Guidance Molecule (RGMa), a DRAGON Homologue, Is a Bone Morphogenetic Protein Co-receptor. Journal of Biological Chemistry 280:29820-29827.

Babitt JL, Huang FW, Wrighting DM, Xia Y, Sidis Y, Samad TA, Campagna JA, Chung RT, Schneyer AL, Woolf CJ, Andrews NC, Lin HY (2006) Bone morphogenetic protein signaling by hemojuvelin regulates hepcidin expression. Nature genetics 38:531-539.

Back SA, Tuohy TMF, Chen H, Wallingford N, Craig A, Struve J, Luo NL, Banine F, Liu Y, Chang A, Trapp BD, Bebo BF, Rao MS, Sherman LS (2005) Hyaluronan accumulates in demyelinated lesions and inhibits oligodendrocyte progenitor maturation. Nature Medicine 11:966-972.

Baggiolini M, Walz A, Kunkel S (1989) Neutrophil-activating peptide-1/interleukin 8, a novel cytokine that activates neutrophils. The Journal of clinical investigation 84:1045-1049.

Bai L, Chang H-M, Cheng J-C, Klausen C, Chu G, Leung PCK, Yang G (2017) SMAD1/5 mediates bone morphogenetic protein 2-induced up-regulation of BAMBI expression in human granulosa-lutein cells. Cellular Signalling 37:52-61.

Baker JC, Beddington RSP, Harland RM (1999) Wnt signaling in Xenopus embryos inhibits Bmp4 expression and activates neural development. Genes & Development 13:3149-3159.

Balboni AL, Hutchinson JA, DeCastro AJ, Cherukuri P, Liby K, Sporn MB, Schwartz GN, Wells WA, Sempere LF, Yu PB, DiRenzo J (2013) ΔNp63α-Mediated Activation of Bone Morphogenetic Protein Signaling Governs Stem Cell Activity and Plasticity in Normal and Malignant Mammary Epithelial Cells. Cancer Research 73:1020-1030.

Bansod S, Kageyama R, Ohtsuka T (2017) Hes5 regulates the transition timing of neurogenesis and gliogenesis in mammalian neocortical development. Development 144:3156-3167.

Bao F, Bailey CS, Gurr KR, Bailey SI, Rosas-Arellano MP, Dekaban GA, Weaver LC (2009) Increased oxidative activity in human blood neutrophils and monocytes after spinal cord injury. Experimental Neurology 215:308-316.

Barna BP, Estes ML, Jacobs BS, Hudson S, Ransohoff RM (1990) Human astrocytes proliferate in response to tumor necrosis factor alpha. Journal of Neuroimmunology 30:239-243.

Barnabé-Heider F, Wasylnka JA, Fernandes KJL, Porsche C, Sendtner M, Kaplan DR, Miller FD (2005) Evidence that Embryonic Neurons Regulate the Onset of Cortical Gliogenesis via Cardiotrophin-1. Neuron 48:253-265.

Barnabé-Heider F, Göritz C, Sabelström H, Takebayashi H, Pfrieger FW, Meletis K, Frisén J (2010) Origin of New Glial Cells in Intact and Injured Adult Spinal Cord. Cell Stem Cell 7:470-482.

Barneda-Zahonero B, Miñano-Molina A, Badiola N, Fadó R, Xifró X, Saura CA, Rodríguez-Alvarez J (2009) Bone Morphogenetic Protein-6 Promotes Cerebellar Granule Neurons Survival by Activation of the MEK/ERK/CREB Pathway. Molecular Biology of the Cell 20:5051-5063.

Bartus K, Burnside ER, Galino J, James ND, Bennett DLH, Bradbury EJ (2019) ErbB receptor signaling directly controls oligodendrocyte progenitor cell transformation and spontaneous remyelination after spinal cord injury. Glia 67:1036-1046.

Baxi EG, DeBruin J, Jin J, Strasburger HJ, Smith MD, Orthmann-Murphy JL, Schott JT, Fairchild AN, Bergles DE, Calabresi PA (2017) Lineage tracing reveals dynamic changes in oligodendrocyte precursor cells following cuprizone-induced demyelination. Glia 65:2087-2098.

Beck KD, Nguyen HX, Galvan MD, Salazar DL, Woodruff TM, Anderson AJ (2010) Quantitative analysis of cellular inflammation after traumatic spinal cord injury: evidence for a multiphasic inflammatory response in the acute to chronic environment. Brain.

Bellot-Saez A, Kékesi O, Morley JW, Buskila Y (2017) Astrocytic modulation of neuronal excitability through K+ spatial buffering. Neuroscience & Biobehavioral Reviews 77:87-97.

Bennett ML, Bennett FC, Liddelow SA, Ajami B, Zamanian JL, Fernhoff NB, Mulinyawe SB, Bohlen CJ, Adil A, Tucker A, Weissman IL, Chang EF, Li G, Grant GA, Hayden Gephart MG, Barres BA (2016) New tools for studying microglia in the mouse and human CNS. Proceedings of the National Academy of Sciences 113:E1738-E1746.

Bermudez S, Khayrullina G, Zhao Y, Byrnes KR (2016) NADPH oxidase isoform expression is temporally regulated and may contribute to microglial/macrophage polarization after spinal cord injury. Molecular and Cellular Neuroscience 77:53-64.

Bilican B, Fiore-Heriche C, Compston A, Allen ND, Chandran S (2008) Induction of Olig2+ Precursors by FGF Involves BMP Signalling Blockade at the Smad Level. PLoS ONE 3:e2863.

Black JA, Waxman SG, Smith KJ (2006) Remyelination of dorsal column axons by endogenous Schwann cells restores the normal pattern of Nav1.6 and Kv1.2 at nodes of Ranvier. Brain 129:1319-1329.

Blight AR, Young W (1989) Central axons in injured cat spinal cord recover electrophysiological function following remyelination by Schwann cells. Journal of the Neurological Sciences 91:15-34.

Block ML, Zecca L, Hong J-S (2007) Microglia-mediated neurotoxicity: uncovering the molecular mechanisms. Nature Reviews Neuroscience 8:57-69.

Boergermann JH, Kopf J, Yu PB, Knaus P (2010) Dorsomorphin and LDN-193189 inhibit BMP-mediated Smad, p38 and Akt signalling in C2C12 cells. The International Journal of Biochemistry & Cell Biology 42:1802-1807.

Bonaguidi MA, McGuire T, Hu M, Kan L, Samanta J, Kessler JA (2005) LIF and BMP signaling generate separate and discrete types of GFAP-expressing cells. Development 132:5503-5514.

Bonni A, Sun Y, Nadal-Vicens M, Bhatt A, Frank DA, Rozovsky I, Stahl N, Yancopoulos GD, Greenberg ME (1997) Regulation of Gliogenesis in the Central Nervous System by the JAK-STAT Signaling Pathway. Science 278:477-483.

Bracken MB (2001) Methylprednisolone and Acute Spinal Cord Injury: An Update of the Randomized Evidence. Spine 26:S47-S54.

Bracken MB, Shepard MJ, Collins WF, Holford TR, Young W, Baskin DS, Eisenberg HM, Flamm E, Leo-Summers L, Maroon J (1990) A randomized, controlled trial of methylprednisolone or naloxone in the treatment of acute spinal-cord injury. Results of the Second National Acute Spinal Cord Injury Study. The New England journal of medicine 322:1405-1411.

Bracken MB, Shepard MJ, Holford TR, Leo-Summers L, Aldrich EF, Fazl M, Fehlings M, Herr DL, Hitchon PW, Marshall LF (1997) Administration of methylprednisolone for 24 or 48 hours or tirilazad mesylate for 48 hours in the treatment of acute spinal cord injury: results of the third national acute spinal cord injury randomized controlled trial. Jama 277:1597-1604.

Bradbury EJ, Moon LDF, Popat RJ, King VR, Bennett GS, Patel PN, Fawcett JW, McMahon SB (2002) Chondroitinase ABC promotes functional recovery after spinal cord injury. Nature 416:636-640.

Bsibsi M, Persoon-Deen C, Verwer RWH, Meeuwsen S, Ravid R, Van Noort JM (2006) Toll-like receptor 3 on adult human astrocytes triggers production of neuroprotective mediators. Glia 53:688-695.

Busch SA, Horn KP, Silver DJ, Silver J (2009) Overcoming Macrophage-Mediated Axonal Dieback Following CNS Injury. The Journal of Neuroscience 29:9967-9976.

Busch SA, Horn KP, Cuascut FX, Hawthorne AL, Bai L, Miller RH, Silver J (2010) Adult NG2+ cells are permissive to neurite outgrowth and stabilize sensory axons during macrophage-induced axonal dieback after spinal cord injury. The Journal of neuroscience : the official journal of the Society for Neuroscience 30:255-265.

Butt AM, Duncan A, Hornby MF, Kirvell SL, Hunter A, Levine JM, Berry M (1999) Cells expressing the NG2 antigen contact nodes of Ranvier in adult CNS white matter. Glia 26:84-91.
Byrnes KR, Fricke ST, Faden AI (2010) Neuropathological differences between rats and mice after spinal cord injury. Journal of Magnetic Resonance Imaging 32:836-846.
Cai J, Bhatnagar A, Pierce WM (2009) Protein Modification by Acrolein: Formation and Stability of Cysteine Adducts. Chemical Research in Toxicology 22:708-716.
Cai J, Qi Y, Hu X, Tan M, Liu Z, Zhang J, Li Q, Sander M, Qiu M (2005) Generation of Oligodendrocyte Precursor Cells from Mouse Dorsal Spinal Cord Independent of Nkx6 Regulation and Shh Signaling. Neuron 45:41-53.
Calpe S, Correia ACP, Sancho-Serra MDC, Krishnadath KK (2016) Comparison of newly developed anti-bone morphogenetic protein 4 llama-derived antibodies with commercially available BMP4 inhibitors. mAbs 8:678-688.
Camargo N, Goudriaan A, van Deijk A-LF, Otte WM, Brouwers JF, Lodder H, Gutmann DH, Nave K-A, Dijkhuizen RM, Mansvelder HD, Chrast R, Smit AB, Verheijen MHG (2017) Oligodendroglial myelination requires astrocyte-derived lipids. PLOS Biology 15:e1002605.
Cao Q, He Q, Wang Y, Cheng X, Howard RM, Zhang Y, DeVries WH, Shields CB, Magnuson DSK, Xu X-M, Kim DH, Whittemore SR (2010) Transplantation of Ciliary Neurotrophic Factor-Expressing Adult Oligodendrocyte Precursor Cells Promotes Remyelination and Functional Recovery after SpinalCord Injury. The Journal of Neuroscience 30:2989-3001.
Carter LM, McMahon SB, Bradbury EJ (2011) Delayed treatment with Chondroitinase ABC reverses chronic atrophy of rubrospinal neurons following spinal cord injury. Experimental Neurology 228:149-156.
Casella GTB, Bunge MB, Wood PM (2006) Endothelial cell loss is not a major cause of neuronal and glial cell death following contusion injury of the spinal cord. Experimental Neurology 202:8-20.
Cate HS, Sabo JK, Merlo D, Kemper D, Aumann TD, Robinson J, Merson TD, Emery B, Perreau VM, Kilpatrick TJ (2010) Modulation of bone morphogenic protein signalling alters numbers of astrocytes and oligodendroglia in the subventricular zone during cuprizone-induced demyelination. Journal of Neurochemistry 115:11-22.
Celeste AJ, Iannazzi JA, Taylor RC, Hewick RM, Rosen V, Wang EA, Wozney JM (1990) Identification of transforming growth factor beta family members present in bone-inductive protein purified from bovine bone. Proceedings of the National Academy of Sciences 87:9843-9847.
Chandrasekaran V, Lea C, Sosa JC, Higgins D, Lein PJ (2015) Reactive oxygen species are involved in BMP-induced dendritic growth in cultured rat sympathetic neurons. Molecular and Cellular Neuroscience 67:116-125.
Chang C, Hemmati-Brivanlou A (1999) Xenopus GDF6, a new antagonist of noggin and a partner of BMPs. Development 126:3347-3357.
Chang H-M, Cheng J-C, Klausen C, Leung PCK (2015) Recombinant BMP4 and BMP7 Increase Activin A Production by Up-Regulating Inhibin βA Subunit and Furin Expression in Human Granulosa-Lutein Cells. The Journal of Clinical Endocrinology & Metabolism 100:E375-E386.
Chen J, Leong S-Y, Schachner M (2005) Differential expression of cell fate determinants in neurons and glial cells of adult mouse spinal cord after compression injury. European Journal of Neuroscience 22:1895-1906.
Chen J, Van Gulden S, McGuire TL, Fleming AC, Oka C, Kessler JA, Peng C-Y (2018) BMP-responsive Protease HtrA1 is Differentially Expressed in Astrocytes and Regulates Astrocytic Development and Injury Response. The Journal of Neuroscience.
Chen MS, Huber AB, van der Haar ME, Frank M, Schnell L, Spillmann AA, Christ F, Schwab ME (2000) Nogo-A is a myelin-associated neurite outgrowth inhibitor and an antigen for monoclonal antibody IN-1. Nature 403:434-439.
Chen P, Piao X, Bonaldo P (2015) Role of macrophages in Wallerian degeneration and axonal regeneration after peripheral nerve injury. Acta Neuropathologica 130:605-618.

Cheng X, Wang Y, He Q, Qiu M, Whittemore SR, Cao Q (2007) Bone Morphogenetic Protein Signaling and Olig1/2 Interact to Regulate the Differentiation and Maturation of Adult Oligodendrocyte Precursor Cells. STEM CELLS 25:3204-3214.

Chierzi S, Ratto GM, Verma P, Fawcett JW (2005) The ability of axons to regenerate their growth cones depends on axonal type and age, and is regulated by calcium, cAMP and ERK. European Journal of Neuroscience 21:2051-2062.

Choi SS, Lee HJ, Lim I, Satoh J-i, Kim SU (2014) Human Astrocytes: Secretome Profiles of Cytokines and Chemokines. PLOS ONE 9:e92325.

Chou H-J, Lai D-M, Huang C-W, McLennan IS, Wang H-D, Wang P-Y (2013) BMP4 Is a Peripherally-Derived Factor for Motor Neurons and Attenuates Glutamate-Induced Excitotoxicity In Vitro. PLOS ONE 8:e58441.

Chou J, Harvey BK, Chang C-F, Shen H, Morales M, Wang Y (2006) Neuroregenerative effects of BMP7 after stroke in rats. Journal of the Neurological Sciences 240:21-29.

Church JS, Milich LM, Lerch JK, Popovich PG, McTigue DM (2017) E6020, a synthetic TLR4 agonist, accelerates myelin debris clearance, Schwann cell infiltration, and remyelination in the rat spinal cord. Glia 65:883-899.

Corvetti L, Rossi F (2005) Degradation of Chondroitin Sulfate Proteoglycans Induces Sprouting of Intact Purkinje Axons in the Cerebellum of the Adult Rat. The Journal of Neuroscience 25:7150-7158.

Craner MJ, Lo AC, Black JA, Waxman SG (2003) Abnormal sodium channel distribution in optic nerve axons in a model of inflammatory demyelination. Brain 126:1552-1561.

Crang AJ, Gilson JM, Li W-W, Blakemore WF (2004) The remyelinating potential and in vitro differentiation of MOG-expressing oligodendrocyte precursors isolated from the adult rat CNS. European Journal of Neuroscience 20:1445-1460.

Cregg JM, DePaul MA, Filous AR, Lang BT, Tran A, Silver J (2014) Functional regeneration beyond the glial scar. Exp Neurol 253:197-207.

Crews L, Adame A, Patrick C, DeLaney A, Pham E, Rockenstein E, Hansen L, Masliah E (2010) Increased BMP6 Levels in the Brains of Alzheimer's Disease Patients and APP Transgenic Mice Are Accompanied by Impaired Neurogenesis. The Journal of Neuroscience 30:12252-12262.

Cross AK, Woodroofe MN (1999) Chemokine modulation of matrix metalloproteinase and TIMP production in adult rat brain microglia and a human microglial cell line in vitro. Glia 28:183-189.

Cui Y, Hackenmiller R, Berg L, Jean F, Nakayama T, Thomas G, Christian JL (2001) The activity and signaling range of mature BMP-4 is regulated by sequential cleavage at two sites within the prodomain of the precursor. Genes & Development 15:2797-2802.

Cui ZS, Zhao P, Jia CX, Liu HJ, Qi R, Cui JW, Cui JH, Peng Q, Lin B, Rao YJ (2015) Local expression and role of BMP-2/4 in injured spinal cord. Genetics and molecular research : GMR 14:9109-9117.

D'Alessandro JS, Wang EA (1994) Bone Morphogenetic Proteins Inhibit Proliferation, Induce Reversible Differentiation and Prevent Cell Death in Astrocyte Lineage Cells. Growth Factors 11:45-52.

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

David S, Kroner A (2011) Repertoire of microglial and macrophage responses after spinal cord injury. Nat Rev Neurosci 12:388-399.

Davies JE, Pröschel C, Zhang N, Noble M, Mayer-Pröschel M, Davies SJ (2008) Transplanted astrocytes derived from BMP- or CNTF-treated glial-restricted precursors have opposite effects on recovery and allodynia after spinal cord injury. Journal of Biology 7:24.

Davies SJA, Shih C-H, Noble M, Mayer-Proschel M, Davies JE, Proschel C (2011) Transplantation of Specific Human Astrocytes Promotes Functional Recovery after Spinal Cord Injury. PloS one 6:e17328.

Dawson MRL, Polito A, Levine JM, Reynolds R (2003) NG2-expressing glial progenitor cells: an abundant and widespread population of cycling cells in the adult rat CNS. Molecular and Cellular Neuroscience 24:476-488.

Day R, Schafer MKH, Cullinan WE, Watson SJ, Chrétien M, Seidah NG (1993) Region specific expression of furin mRNA in the rat brain. Neuroscience Letters 149:27-30.
De La Fuente AG et al. (2017) Pericytes Stimulate Oligodendrocyte Progenitor Cell Differentiation during CNS Remyelination. Cell Reports 20:1755-1764.
de Rivero Vaccari JP, Marcillo A, Nonner D, Dietrich WD, Keane RW (2009) Neuroprotective effects of bone morphogenetic protein 7 (BMP7) treatment after spinal cord injury. Neuroscience Letters 465:226-229.
Degnin C, Jean F, Thomas G, Christian JL (2004) Cleavages within the Prodomain Direct Intracellular Trafficking and Degradation of Mature Bone Morphogenetic Protein-4. Molecular Biology of the Cell 15:5012-5020.
del Corral RD, Olivera-Martinez I, Goriely A, Gale E, Maden M, Storey K (2003) Opposing FGF and Retinoid Pathways Control Ventral Neural Pattern, Neuronal Differentiation, and Segmentation during Body Axis Extension. Neuron 40:65-79.
Delaune E, Lemaire P, Kodjabachian L (2005) Neural induction in Xenopus requires early FGF signalling in addition to BMP inhibition. Development 132:299-310.
Delolme F, Anastasi C, Alcaraz LB, Mendoza V, Vadon-Le Goff S, Talantikite M, Capomaccio R, Mevaere J, Fortin L, Mazzocut D, Damour O, Zanella-Cléon I, Hulmes DJS, Overall CM, Valcourt U, Lopez-Casillas F, Moali C (2015) Proteolytic control of TGF-β co-receptor activity by BMP-1/tolloid-like proteases revealed by quantitative iTRAQ proteomics. Cellular and Molecular Life Sciences 72:1009-1027.
DePaul MA, Lin C-Y, Silver J, Lee Y-S (2017) Combinatory repair strategy to promote axon regeneration and functional recovery after chronic spinal cord injury. Scientific Reports 7:9018.
Derwall M, Malhotra R, Lai CS, Beppu Y, Aikawa E, Seehra JS, Zapol WM, Bloch KD, Yu PB (2012) Inhibition of Bone Morphogenetic Protein Signaling Reduces Vascular Calcification and Atherosclerosis. Arteriosclerosis, Thrombosis, and Vascular Biology 32:613-622.
Dibaj P, Nadrigny F, Steffens H, Scheller A, Hirrlinger J, Schomburg ED, Neusch C, Kirchhoff F (2010) NO mediates microglial response to acute spinal cord injury under ATP control in vivo. Glia 58:1133-1144.
Dickendesher TL, Baldwin KT, Mironova YA, Koriyama Y, Raiker SJ, Askew KL, Wood A, Geoffroy CG, Zheng B, Liepmann CD, Katagiri Y, Benowitz LI, Geller HM, Giger RJ (2012) NgR1 and NgR3 are receptors for chondroitin sulfate proteoglycans. Nat Neurosci 15:703-712.
Dinkel K, Dhabhar FS, Sapolsky RM (2004) Neurotoxic effects of polymorphonuclear granulocytes on hippocampal primary cultures. Proceedings of the National Academy of Sciences 101:331-336.
Dong W, Marcinkiewicz M, Vieau D, Chretien M, Seidah N, Day R (1995) Distinct mRNA expression of the highly homologous convertases PC5 and PACE4 in the rat brain and pituitary. The Journal of Neuroscience 15:1778-1796.
Dou C, Levine J (1994) Inhibition of neurite growth by the NG2 chondroitin sulfate proteoglycan. The Journal of Neuroscience 14:7616-7628.
Du L, Zhang X, Han YY, Burke NA, Kochanek PM, Watkins SC, Graham SH, Carcillo JA, Szabó C, Clark RSB (2003) Intra-mitochondrial Poly(ADP-ribosylation) Contributes to NAD+ Depletion and Cell Death Induced by Oxidative Stress. Journal of Biological Chemistry 278:18426-18433.
Duncan GJ, Manesh SB, Hilton BJ, Assinck P, Liu J, Moulson A, Plemel JR, Tetzlaff W (2018) Locomotor recovery following contusive spinal cord injury does not require oligodendrocyte remyelination. Nature Communications 9:3066.
Duran RC-D, Yan H, Zheng Y, Huang X, Grill R, Kim DH, Cao Q, Wu JQ (2017) The systematic analysis of coding and long non-coding RNAs in the sub-chronic and chronic stages of spinal cord injury. Scientific Reports 7:41008.
Duret L, Mouchiroud D, Gouy M (1994) HOVERGEN: a database of homologous vertebrate genes. Nucleic Acids Research 22:2360-2365.

Dvorak MF et al. (2015) The influence of time from injury to surgery on motor recovery and length of hospital stay in acute traumatic spinal cord injury: an observational Canadian cohort study. Journal of neurotrauma 32:645-654.

Dyck SM, Karimi-Abdolrezaee S (2015) Chondroitin sulfate proteoglycans: Key modulators in the developing and pathologic central nervous system. Experimental Neurology 269:169-187.

Dyck SM, Kataria H, Akbari-Kelachayeh K, Silver J, Karimi-Abdolrezaee S (2019) LAR and PTPσ receptors are negative regulators of oligodendrogenesis and oligodendrocyte integrity in spinal cord injury. Glia 67:125-145.

Dyck SM, Alizadeh A, Santhosh KT, Proulx EH, Wu C-L, Karimi-Abdolrezaee S (2015) Chondroitin Sulfate Proteoglycans Negatively Modulate Spinal Cord Neural Precursor Cells by Signaling Through LAR and RPTPσ and Modulation of the Rho/ROCK Pathway. STEM CELLS 33:2550-2563.

Dyck SM, Kataria H, Alizadeh A, Santhosh KT, Lang B, Silver J, Karimi-Abdolrezaee S (2018) Perturbing chondroitin sulfate proteoglycan signaling through LAR and PTPσ receptors promotes a beneficial inflammatory response following spinal cord injury. Journal of Neuroinflammation 15:90.

Eftekharpour E, Karimi-Abdolrezaee S, Wang J, El Beheiry H, Morshead C, Fehlings MG (2007) Myelination of congenitally dysmyelinated spinal cord axons by adult neural precursor cells results in formation of nodes of Ranvier and improved axonal conduction. J Neurosci 27:3416-3428.

Ellis RC, O'Steen WA, Hayes RL, Nick HS, Wang KKW, Anderson DK (2005) Cellular localization and enzymatic activity of cathepsin B after spinal cord injury in the rat. Experimental Neurology 193:19-28.

Enzmann GU, Benton RL, Woock JP, Howard RM, Tsoulfas P, Whittemore SR (2005) Consequences of noggin expression by neural stem, glial, and neuronal precursor cells engrafted into the injured spinal cord. Experimental Neurology 195:293-304.

Esquela AF, Lee Se-J (2003) Regulation of metanephric kidney development by growth/differentiation factor 11. Developmental Biology 257:356-370.

Evaniew N, Belley-Côté EP, Fallah N, Noonan VK, Rivers CS, Dvorak MF (2016) Methylprednisolone for the treatment of patients with acute spinal cord injuries: a systematic review and meta-analysis. Journal of neurotrauma 33:468-481.

Evans TA, Barkauskas DS, Myers JT, Hare EG, You JQ, Ransohoff RM, Huang AY, Silver J (2014) High-resolution intravital imaging reveals that blood-derived macrophages but not resident microglia facilitate secondary axonal dieback in traumatic spinal cord injury. Experimental Neurology 254:109-120.

Faiz M, Sachewsky N, Gascón S, Bang KWA, Morshead Cindi M, Nagy A (2015) Adult Neural Stem Cells from the Subventricular Zone Give Rise to Reactive Astrocytes in the Cortex after Stroke. Cell Stem Cell 17:624-634.

Farreny M-A, Agius E, Bel-Vialar S, Escalas N, Khouri-Farah N, Soukkarieh C, Danesin C, Pituello F, Cochard P, Soula C (2018) FGF signaling controls Shh-dependent oligodendroglial fate specification in the ventral spinal cord. Neural Development 13:3.

Farrukh F, Davies E, Berry M, Logan A, Ahmed Z (2019) BMP4/Smad1 Signalling Promotes Spinal Dorsal Column Axon Regeneration and Functional Recovery After Injury. Molecular Neurobiology.

Faulkner JR, Herrmann JE, Woo MJ, Tansey KE, Doan NB, Sofroniew MV (2004) Reactive Astrocytes Protect Tissue and Preserve Function after Spinal Cord Injury. The Journal of Neuroscience 24:2143-2155.

Fehlings MG, Wilson JR, Harrop JS, Kwon BK, Tetreault LA, Arnold PM, Singh JM, Hawryluk G, Dettori JR (2017) Efficacy and Safety of Methylprednisolone Sodium Succinate in Acute Spinal Cord Injury: A Systematic Review. Global Spine Journal 7:116S-137S.

Fehlings MG, Vaccaro A, Wilson JR, Singh A, W Cadotte D, Harrop JS, Aarabi B, Shaffrey C, Dvorak M, Fisher C, Arnold P, Massicotte EM, Lewis S, Rampersaud R (2012) Early versus delayed decompression for traumatic cervical spinal cord injury: results of the Surgical Timing in Acute Spinal Cord Injury Study (STASCIS). PloS one 7:e32037-e32037.

Felts PA, Smith KJ (1992) Conduction properties of central nerve fibers remyelinated by Schwann cells. Brain research 574:178-192.
Feng Y, Hu Y (2018) Bone morphogenetic protein 9 serves a protective role in response to ischemic-reperfusion in the brain by promoting ERK activation. Molecular medicine reports 17:2845-2852.
Festoff BW, Ameenuddin S, Arnold PM, Wong A, Santacruz KS, Citron BA (2006) Minocycline neuroprotects, reduces microgliosis, and inhibits caspase protease expression early after spinal cord injury. Journal of Neurochemistry 97:1314-1326.
Filous AR, Tran A, Howell CJ, Busch SA, Evans TA, Stallcup WB, Kang SH, Bergles DE, Lee S-i, Levine JM, Silver J (2014) Entrapment via Synaptic-Like Connections between NG2 Proteoglycan+ Cells and Dystrophic Axons in the Lesion Plays a Role in Regeneration Failure after Spinal Cord Injury. The Journal of Neuroscience 34:16369-16384.
Fisher D, Xing B, Dill J, Li H, Hoang HH, Zhao Z, Yang X-L, Bachoo R, Cannon S, Longo FM, Sheng M, Silver J, Li S (2011) Leukocyte Common Antigen-Related Phosphatase Is a Functional Receptor for Chondroitin Sulfate Proteoglycan Axon Growth Inhibitors. The Journal of Neuroscience 31:14051-14066.
Fogarty M, Richardson WD, Kessaris N (2005) A subset of oligodendrocytes generated from radial glia in the dorsal spinal cord. Development 132:1951-1959.
Frank DB, Abtahi A, Yamaguchi DJ, Manning S, Shyr Y, Pozzi A, Baldwin HS, Johnson JE, Caestecker MPd (2005) Bone Morphogenetic Protein 4 Promotes Pulmonary Vascular Remodeling in Hypoxic Pulmonary Hypertension. Circulation Research 97:496-504.
Fry EJ, Chagnon MJ, López-Vales R, Tremblay ML, David S (2010) Corticospinal tract regeneration after spinal cord injury in receptor protein tyrosine phosphatase sigma deficient mice. Glia 58:423-433.
Fukuda S, Abematsu M, Mori H, Yanagisawa M, Kagawa T, Nakashima K, Yoshimura A, Taga T (2007) Potentiation of Astrogliogenesis by STAT3-Mediated Activation of Bone Morphogenetic Protein-Smad Signaling in Neural Stem Cells. Molecular and Cellular Biology 27:4931-4937.
Fuller ML, DeChant AK, Rothstein B, Caprariello A, Wang R, Hall AK, Miller RH (2007) Bone morphogenetic proteins promote gliosis in demyelinating spinal cord lesions. Annals of Neurology 62:288-300.
Gallea S, Lallemand F, Atfi A, Rawadi G, Ramez V, Spinella-Jaegle S, Kawai S, Faucheu C, Huet L, Baron R, Roman-Roman S (2001) Activation of mitogen-activated protein kinase cascades is involved in regulation of bone morphogenetic protein-2-induced osteoblast differentiation in pluripotent C2C12 cells. Bone 28:491-498.
Galluzzi L et al. (2018) Molecular mechanisms of cell death: recommendations of the Nomenclature Committee on Cell Death 2018. Cell Death & Differentiation 25:486-541.
Gao Q, Lu J, Huo Y, Baby N, Ling EA, Dheen ST (2010) NG2, a member of chondroitin sulfate proteoglycans family mediates the inflammatory response of activated microglia. Neuroscience 165:386-394.
Gauthier M-K, Kosciuczyk K, Tapley L, Karimi-Abdolrezaee S (2013a) Dysregulation of the neuregulin-1–ErbB network modulates endogenous oligodendrocyte differentiation and preservation after spinal cord injury. European Journal of Neuroscience 38:2693-2715.
Gauthier MK, Kosciuczyk K, Tapley L, Karimi-Abdolrezaee S (2013b) Dysregulation of the neuregulin-1-ErbB network modulates endogenous oligodendrocyte differentiation and preservation after spinal cord injury. Eur J Neurosci 38:2693-2715.
Gazzerro E, Gangji V, Canalis E (1998) Bone morphogenetic proteins induce the expression of noggin, which limits their activity in cultured rat osteoblasts. The Journal of Clinical Investigation 102:2106-2114.
Ge G, Greenspan DS (2006) BMP1 controls TGFβ1 activation via cleavage of latent TGFβ-binding protein. The Journal of Cell Biology 175:111-120.

Ge G, Hopkins DR, Ho W-B, Greenspan DS (2005) GDF11 Forms a Bone Morphogenetic Protein 1-Activated Latent Complex That Can Modulate Nerve Growth Factor-Induced Differentiation of PC12 Cells. Molecular and Cellular Biology 25:5846-5858.

Ge W, Martinowich K, Wu X, He F, Miyamoto A, Fan G, Weinmaster G, Sun YE (2002) Notch signaling promotes astrogliogenesis via direct CSL-mediated glial gene activation. Journal of Neuroscience Research 69:848-860.

Ghosh-Choudhury N, Abboud SL, Mahimainathan L, Chandrasekar B, Choudhury GG (2003) Phosphatidylinositol 3-Kinase Regulates Bone Morphogenetic Protein-2 (BMP-2)-induced Myocyte Enhancer Factor 2A-dependent Transcription of BMP-2 Gene in Cardiomyocyte Precursor Cells. Journal of Biological Chemistry 278:21998-22005.

Ghosh-Choudhury N, Abboud SL, Nishimura R, Celeste A, Mahimainathan L, Choudhury GG (2002) Requirement of BMP-2-induced Phosphatidylinositol 3-Kinase and Akt Serine/Threonine Kinase in Osteoblast Differentiation and Smad-dependent BMP-2 Gene Transcription. Journal of Biological Chemistry 277:33361-33368.

Ghosh-Choudhury N, Ghosh Choudhury G, Harris MA, Wozney J, Mundy GR, Abboud SL, Harris SE (2001) Autoregulation of Mouse BMP-2 Gene Transcription Is Directed by the Proximal Promoter Element. Biochemical and Biophysical Research Communications 286:101-108.

Ghosh-Choudhury N, Windle JJ, Koop BA, Harris MA, Guerrero DL, Wozney JM, Mundy GR, Harris SE (1996) Immortalized murine osteoblasts derived from BMP 2-T-antigen expressing transgenic mice. Endocrinology 137:331-339.

Giannakopoulou A, Grigoriadis N, Polyzoidou E, Lourbopoulos A, Michaloudi E, Papadopoulos GC (2011) Time-dependent fate of transplanted neural precursor cells in experimental autoimmune encephalomyelitis mice. Experimental Neurology 230:16-26.

Gitler D, Spira ME (1998) Real time imaging of calcium-induced localized proteolytic activity after axotomy and its relation to growth cone formation. Neuron 20:1123-1135.

Golstein P, Kroemer G (2007) Cell death by necrosis: towards a molecular definition. Trends in Biochemical Sciences 32:37-43.

Gomes WA, Kessler JA (2001) Msx-2 and p21 Mediate the Pro-Apoptotic but Not the Anti-Proliferative Effects of BMP4 on Cultured Sympathetic Neuroblasts. Developmental Biology 237:212-221.

Gomez-Skarmeta J, de La Calle-Mustienes E, Modolell J (2001) The Wnt-activated Xiro1 gene encodes a repressor that is essential for neural development and downregulates Bmp4. Development 128:551-560.

Göritz C, Dias DO, Tomilin N, Barbacid M, Shupliakov O, Frisén J (2011) A Pericyte Origin of Spinal Cord Scar Tissue. Science 333:238-242.

Goto K, Kamiya Y, Imamura T, Miyazono K, Miyazawa K (2007) Selective Inhibitory Effects of Smad6 on Bone Morphogenetic Protein Type I Receptors. Journal of Biological Chemistry 282:20603-20611.

Govier-Cole AE, Wood RJ, Fletcher JL, Gonsalvez DG, Merlo D, Cate HS, Murray SS, Xiao J (2019) Inhibiting bone morphogenetic protein 4 type I receptor signaling promotes remyelination by potentiating oligodendrocyte differentiation. eneuro:ENEURO.0399-0318.2019.

Graham A, Francis-West P, Brickell P, Lumsden A (1994) The signalling molecule BMP4 mediates apoptosis in the rhombencephalic neural crest. Nature 372:684-686.

Graham TR, Odero-Marah VA, Chung LW, Agrawal KC, Davis R, Abdel-Mageed AB (2009) PI3K/Akt-dependent transcriptional regulation and activation of BMP-2-Smad signaling by NF-κB in metastatic prostate cancer cells. The Prostate 69:168-180.

Gratsch TE, De Boer LS, O'Shea KS (2003) RNA inhibition of BMP-4 gene expression in postimplantation mouse embryos. genesis 37:12-17.

Greenhalgh AD, David S (2014) Differences in the Phagocytic Response of Microglia and Peripheral Macrophages after Spinal Cord Injury and Its Effects on Cell Death. The Journal of Neuroscience 34:6316-6322.

Gross RE, Mehler MF, Mabie PC, Zang Z, Santschi L, Kessler JA (1996) Bone Morphogenetic Proteins Promote Astroglial Lineage Commitment by Mammalian Subventricular Zone Progenitor Cells. Neuron 17:595-606.

Grossman SD, Rosenberg LJ, Wrathall JR (2001) Temporal–Spatial Pattern of Acute Neuronal and Glial Loss after Spinal Cord Contusion. Experimental Neurology 168:273-282.

Guest JD, Hiester ED, Bunge RP (2005) Demyelination and Schwann cell responses adjacent to injury epicenter cavities following chronic human spinal cord injury. Experimental Neurology 192:384-393.

Haas C, Neuhuber B, Yamagami T, Rao M, Fischer I (2012) Phenotypic analysis of astrocytes derived from glial restricted precursors and their impact on axon regeneration. Experimental Neurology 233:717-732.

Habermacher C, Angulo MC, Benamer N (2019) Glutamate versus GABA in neuron–oligodendroglia communication. Glia 67:2092-2106.

Hackett AR, Lee JK (2016) Understanding the NG2 Glial Scar after Spinal Cord Injury. Frontiers in Neurology 7.

Hackett AR, Lee D-H, Dawood A, Rodriguez M, Funk L, Tsoulfas P, Lee JK (2016) STAT3 and SOCS3 regulate NG2 cell proliferation and differentiation after contusive spinal cord injury. Neurobiology of Disease 89:10-22.

Hajebrahimi Z, Mowla SJ, Movahedin M, Tavallaei M (2008) Gene expression alterations of neurotrophins, their receptors and prohormone convertases in a rat model of spinal cord contusion. Neuroscience Letters 441:261-266.

Hall ED, Wang JA, Bosken JM, Singh IN (2016) Lipid peroxidation in brain or spinal cord mitochondria after injury. Journal of Bioenergetics and Biomembranes 48:169-174.

Hammond Timothy R, McEllin B, Morton Paul D, Raymond M, Dupree J, Gallo V (2015) Endothelin-B Receptor Activation in Astrocytes Regulates the Rate of Oligodendrocyte Regeneration during Remyelination. Cell Reports 13:2090-2097.

Hammond Timothy R, Gadea A, Dupree J, Kerninon C, Nait-Oumesmar B, Aguirre A, Gallo V (2014) Astrocyte-Derived Endothelin-1 Inhibits Remyelination through Notch Activation. Neuron 81:588-602.

Hampton DW, Asher RA, Kondo T, Steeves JD, Ramer MS, Fawcett JW (2007) A potential role for bone morphogenetic protein signalling in glial cell fate determination following adult central nervous system injury in vivo. European Journal of Neuroscience 26:3024-3035.

Hanyu A, Ishidou Y, Ebisawa T, Shimanuki T, Imamura T, Miyazono K (2001) The N domain of Smad7 is essential for specific inhibition of transforming growth factor-β signaling. Journal of Cell Biology 155:1017-1028.

Hara M, Kobayakawa K, Ohkawa Y, Kumamaru H, Yokota K, Saito T, Kijima K, Yoshizaki S, Harimaya K, Nakashima Y, Okada S (2017) Interaction of reactive astrocytes with type I collagen induces astrocytic scar formation through the integrin–N-cadherin pathway after spinal cord injury. Nature Medicine 23:818.

Harnisch K, Teuber-Hanselmann S, Macha N, Mairinger F, Fritsche L, Soub D, Meinl E, Junker A (2019) Myelination in Multiple Sclerosis Lesions Is Associated with Regulation of Bone Morphogenetic Protein 4 and Its Antagonist Noggin. International journal of molecular sciences 20:154.

Harrison CA, Al-Musawi SL, Walton KL (2011) Prodomains regulate the synthesis, extracellular localisation and activity of TGF-β superfamily ligands. Growth Factors 29:174-186.

Hart CG, Dyck SM, Kataria H, Alizadeh A, Nagakannan P, Thliveris JA, Eftekharpour E, Karimi-Abdolrezaee S (2019) Acute upregulation of bone morphogenetic protein-4 regulates endogenous cell response and promotes cell death in spinal cord injury. Experimental Neurology 325:113163.

Hashimoto H, Jiang W, Yoshimura T, Moon K-H, Bok J, Ikenaka K (2018) Strong sonic hedgehog signaling in the mouse ventral spinal cord is not required for oligodendrocyte precursor cell (OPC) generation but is necessary for correct timing of its generation. Neurochemistry International 119:178-183.

Hata A, Lagna G, Massagué J, Hemmati-Brivanlou A (1998) Smad6 inhibits BMP/Smad1 signaling by specifically competing with the Smad4 tumor suppressor. Genes & Development 12:186-197.
Hayashi Y, Mikawa S, Masumoto K, Katou F, Sato K (2018) GDF11 expression in the adult rat central nervous system. Journal of Chemical Neuroanatomy 89:21-36.
Hayashi Y, Mikawa S, Ogawa C, Masumoto K, Katou F, Sato K (2019) BMP6 expression in the adult rat central nervous system. Journal of Chemical Neuroanatomy 98:41-54.
Helbing T, Arnold L, Wiltgen G, Hirschbihl E, Gabelmann V, Hornstein A, Esser JS, Diehl P, Grundmann S, Busch H-J, Fink K, Bode C, Moser M (2017) Endothelial BMP4 Regulates Leukocyte Diapedesis and Promotes Inflammation. Inflammation 40:1862-1874.
Hermann GE, Rogers RC, Bresnahan JC, Beattie MS (2001) Tumor Necrosis Factor-α Induces cFOS and Strongly Potentiates Glutamate-Mediated Cell Death in the Rat Spinal Cord. Neurobiology of Disease 8:590-599.
Herrero-Mendez A, Almeida A, Fernández E, Maestre C, Moncada S, Bolaños JP (2009) The bioenergetic and antioxidant status of neurons is controlled by continuous degradation of a key glycolytic enzyme by APC/C–Cdh1. Nature Cell Biology 11:747-752.
Herrmann JE, Imura T, Song B, Qi J, Ao Y, Nguyen TK, Korsak RA, Takeda K, Akira S, Sofroniew MV (2008) STAT3 is a Critical Regulator of Astrogliosis and Scar Formation after Spinal Cord Injury. The Journal of Neuroscience 28:7231-7243.
Herx LM, Yong VW (2001) Interleukin-1β is Required for the Early Evolution of Reactive Astrogliosis Following CNS Lesion. Journal of Neuropathology & Experimental Neurology 60:961-971.
Hesp ZC, Goldstein EA, Miranda CJ, Kaspar BK, McTigue DM (2015) Chronic Oligodendrogenesis and Remyelination after Spinal Cord Injury in Mice and Rats. The Journal of Neuroscience 35:1274-1290.
Hesp ZC, Yoseph RY, Suzuki R, Jukkola P, Wilson C, Nishiyama A, McTigue DM (2018) Proliferating NG2-Cell-Dependent Angiogenesis and Scar Formation Alter Axon Growth and Functional Recovery After Spinal Cord Injury in Mice. The Journal of Neuroscience 38:1366-1382.
Hill CE (2017) A view from the ending: Axonal dieback and regeneration following SCI. Neuroscience Letters 652:11-24.
Hirayama Y, Ikeda-Matsuo Y, Notomi S, Enaida H, Kinouchi H, Koizumi S (2015) Astrocyte-Mediated Ischemic Tolerance. The Journal of Neuroscience 35:3794-3805.
Hochstim C, Deneen B, Lukaszewicz A, Zhou Q, Anderson DJ (2008) Identification of Positionally Distinct Astrocyte Subtypes whose Identities Are Specified by a Homeodomain Code. Cell 133:510-522.
Hong S, Song M-R (2014) STAT3 but Not STAT1 Is Required for Astrocyte Differentiation. PloS one 9:e86851.
Hopkins CR (2016) Inhibitors of the bone morphogenetic protein (BMP) signaling pathway: a patent review (2008-2015). Expert Opinion on Therapeutic Patents 26:1115-1128.
Horn KP, Busch SA, Hawthorne AL, van Rooijen N, Silver J (2008) Another Barrier to Regeneration in the CNS: Activated Macrophages Induce Extensive Retraction of Dystrophic Axons through Direct Physical Interactions. The Journal of Neuroscience 28:9330-9341.
Huang J, Liu Y, Filas B, Gunhaga L, Beebe DC (2015) Negative and positive auto-regulation of BMP expression in early eye development. Developmental Biology 407:256-264.
Hughes EG, Kang SH, Fukaya M, Bergles DE (2013) Oligodendrocyte progenitors balance growth with self-repulsion to achieve homeostasis in the adult brain. Nature Neuroscience 16:668.
Icli B, Wara AKM, Moslehi J, Sun X, Plovie E, Cahill M, Marchini Julio F, Schissler A, Padera Robert F, Shi J, Cheng H-W, Raghuram S, Arany Z, Liao R, Croce K, MacRae C, Feinberg Mark W (2013) MicroRNA-26a Regulates Pathological and Physiological Angiogenesis by Targeting BMP/SMAD1 Signaling. Circulation Research 113:1231-1241.
Imamura T, Takase M, Nishihara A, Oeda E, Hanai J-i, Kawabata M, Miyazono K (1997) Smad6 inhibits signalling by the TGF-[beta] superfamily. Nature 389:622-626.

Ionescu AM, Drissi H, Schwarz EM, Kato M, Puzas JE, McCance DJ, Rosier RN, Zuscik MJ, O'Keefe RJ (2004) CREB Cooperates with BMP-stimulated Smad signaling to enhance transcription of the Smad6 promoter. Journal of Cellular Physiology 198:428-440.

Iram T, Ramirez-Ortiz Z, Byrne MH, Coleman UA, Kingery ND, Means TK, Frenkel D, El Khoury J (2016) Megf10 Is a Receptor for C1Q That Mediates Clearance of Apoptotic Cells by Astrocytes. The Journal of Neuroscience 36:5185-5192.

Ishida W, Hamamoto T, Kusanagi K, Yagi K, Kawabata M, Takehara K, Sampath TK, Kato M, Miyazono K (2000) Smad6 Is a Smad1/5-induced Smad Inhibitor: CHARACTERIZATION OF BONE MORPHOGENETIC PROTEIN-RESPONSIVE ELEMENT IN THE MOUSE Smad6 PROMOTER. Journal of Biological Chemistry 275:6075-6079.

Ishisaki A, Yamato K, Hashimoto S, Nakao A, Tamaki K, Nonaka K, ten Dijke P, Sugino H, Nishihara T (1999) Differential Inhibition of Smad6 and Smad7 on Bone Morphogenetic Protein- and Activin-mediated Growth Arrest and Apoptosis in B Cells. Journal of Biological Chemistry 274:13637-13642.

Israsena N, Kessler JA (2002) Msx2 and p21CIP1/WAF1 mediate the proapoptotic effects of bone morphogenetic protein-4 on ventricular zone progenitor cells. Journal of Neuroscience Research 69:803-809.

Jahan N, Hannila SS (2015) Transforming growth factor β-induced expression of chondroitin sulfate proteoglycans is mediated through non-Smad signaling pathways. Experimental Neurology 263:372-384.

Jakeman LB, Guan Z, Wei P, Ponnappan R, Dzwonczyk R, Popovich PG, Stokes BT (2000) Traumatic spinal cord injury produced by controlled contusion in mouse. Journal of neurotrauma 17:299-319.

James SL et al. (2019) Global, regional, and national burden of traumatic brain injury and spinal cord injury, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. The Lancet Neurology 18:56-87.

Jasuja R, Allen BL, Pappano WN, Rapraeger AC, Greenspan DS (2004) Cell-surface Heparan Sulfate Proteoglycans Potentiate Chordin Antagonism of Bone Morphogenetic Protein Signaling and Are Necessary for Cellular Uptake of Chordin. Journal of Biological Chemistry 279:51289-51297.

Jia Z, Zhu H, Li J, Wang X, Misra H, Li Y (2012) Oxidative stress in spinal cord injury and antioxidant-based intervention. Spinal Cord 50:264-274.

Jiao X, Billings PC, O'Connell MP, Kaplan FS, Shore EM, Glaser DL (2007) Heparan Sulfate Proteoglycans (HSPGs) Modulate BMP2 Osteogenic Bioactivity in C2C12 Cells. Journal of Biological Chemistry 282:1080-1086.

Jing R, Wilhelmsson U, Goodwill W, Li L, Pan Y, Pekny M, Skalli O (2007) Synemin is expressed in reactive astrocytes in neurotrauma and interacts differentially with vimentin and GFAP intermediate filament networks. Journal of Cell Science 120:1267-1277.

Jones LL, Yamaguchi Y, Stallcup WB, Tuszynski MH (2002) NG2 Is a Major Chondroitin Sulfate Proteoglycan Produced after Spinal Cord Injury and Is Expressed by Macrophages and Oligodendrocyte Progenitors. The Journal of Neuroscience 22:2792-2803.

Kahata K, Hayashi M, Asaka M, Hellman U, Kitagawa H, Yanagisawa J, Kato S, Imamura T, Miyazono K (2004) Regulation of transforming growth factor-β and bone morphogenetic protein signalling by transcriptional coactivator GCN5. Genes to Cells 9:143-151.

Kamber D, Erez H, Spira ME (2009) Local calcium-dependent mechanisms determine whether a cut axonal end assembles a retarded endbulb or competent growth cone. Experimental Neurology 219:112-125.

Kang MK, Kang SK (2008) Interleukin-6 induces proliferation in adult spinal cord-derived neural progenitors via the JAK2/STAT3 pathway with EGF-induced MAPK phosphorylation. Cell Proliferation 41:377-392.

Kang P, Lee Hyun K, Glasgow Stacey M, Finley M, Donti T, Gaber Zachary B, Graham Brett H, Foster Aaron E, Novitch Bennett G, Gronostajski Richard M, Deneen B (2012) Sox9 and NFIA

Coordinate a Transcriptional Regulatory Cascade during the Initiation of Gliogenesis. Neuron 74:79-94.
Kang W, Hébert JM (2011) Signaling Pathways in Reactive Astrocytes, a Genetic Perspective. Molecular Neurobiology 43:147-154.
Kanno H, Ozawa H, Sekiguchi A, Itoi E (2009) Spinal cord injury induces upregulation of Beclin 1 and promotes autophagic cell death. Neurobiology of Disease 33:143-148.
Karimi-Abdolrezaee S, Eftekharpour E (2012) Stem cells and spinal cord injury repair. Adv Exp Med Biol 760:53-73.
Karimi-Abdolrezaee S, Billakanti R (2012) Reactive Astrogliosis after Spinal Cord Injury—Beneficial and Detrimental Effects. Molecular Neurobiology 46:251-264.
Karimi-Abdolrezaee S, Eftekharpour E, Fehlings MG (2004) Temporal and spatial patterns of Kv1.1 and Kv1.2 protein and gene expression in spinal cord white matter after acute and chronic spinal cord injury in rats: implications for axonal pathophysiology after neurotrauma. Eur J Neurosci 19:577-589.
Karimi-Abdolrezaee S, Schut D, Wang J, Fehlings MG (2012) Chondroitinase and growth factors enhance activation and oligodendrocyte differentiation of endogenous neural precursor cells after spinal cord injury. PloS one 7:e37589.
Karimi-Abdolrezaee S, Eftekharpour E, Wang J, Morshead CM, Fehlings MG (2006) Delayed Transplantation of Adult Neural Precursor Cells Promotes Remyelination and Functional Neurological Recovery after Spinal Cord Injury. The Journal of Neuroscience 26:3377-3389.
Karimi-Abdolrezaee S, Eftekharpour E, Wang J, Schut D, Fehlings MG (2010) Synergistic effects of transplanted adult neural stem/progenitor cells, chondroitinase, and growth factors promote functional repair and plasticity of the chronically injured spinal cord. J Neurosci 30:1657-1676.
Kataria H, Alizadeh A, Shahriary GM, Saboktakin Rizi S, Henrie R, Santhosh KT, Thliveris JA, Karimi-Abdolrezaee S (2018) Neuregulin-1 promotes remyelination and fosters a pro-regenerative inflammatory response in focal demyelinating lesions of the spinal cord. Glia 66:538-561.
Katoh Y, Katoh M (2006) Comparative integromics on BMP/GDF family. International journal of molecular medicine 17:951-955.
Kawabata H, Setoguchi T, Yone K, Souda M, Yoshida H, Kawahara K-i, Maruyama I, Komiya S (2010) High Mobility Group Box 1 Is Upregulated After Spinal Cord Injury and Is Associated With Neuronal Cell Apoptosis. Spine 35:1109-1115.
Keller JN, Pang Z, Geddes JW, Begley JG, Germeyer A, Waeg G, Mattson MP (1997) Impairment of Glucose and Glutamate Transport and Induction of Mitochondrial Oxidative Stress and Dysfunction in Synaptosomes by Amyloid β-Peptide: Role of the Lipid Peroxidation Product 4-Hydroxynonenal. Journal of Neurochemistry 69:273-284.
Kelley R, Ren R, Pi X, Wu Y, Moreno I, Willis M, Moser M, Ross M, Podkowa M, Attisano L, Patterson C (2009) A concentration-dependent endocytic trap and sink mechanism converts Bmper from an activator to an inhibitor of Bmp signaling. The Journal of Cell Biology 184:597-609.
Keough MB, Rogers JA, Zhang P, Jensen SK, Stephenson EL, Chen T, Hurlbert MG, Lau LW, Rawji KS, Plemel JR, Koch M, Ling C-C, Yong VW (2016) An inhibitor of chondroitin sulfate proteoglycan synthesis promotes central nervous system remyelination. Nature Communications 7:11312.
Kessler E, Takahara K, Biniaminov L, Brusel M, Greenspan DS (1996) Bone Morphogenetic Protein-1: The Type I Procollagen C-Proteinase. Science 271:360-362.
Kigerl KA, Lai W, Wallace LM, Yang H, Popovich PG (2018) High mobility group box-1 (HMGB1) is increased in injured mouse spinal cord and can elicit neurotoxic inflammation. Brain, Behavior, and Immunity 72:22-33.
Kigerl KA, Gensel JC, Ankeny DP, Alexander JK, Donnelly DJ, Popovich PG (2009) Identification of two distinct macrophage subsets with divergent effects causing either neurotoxicity or regeneration in the injured mouse spinal cord. J Neurosci 29.
Kim S, Steelman AJ, Zhang Y, Kinney HC, Li J (2012) Aberrant Upregulation of Astroglial Ceramide Potentiates Oligodendrocyte Injury. Brain Pathology 22:41-57.

Kingsley DM (1994) The TGF-beta superfamily: new members, new receptors, and new genetic tests of function in different organisms. Genes & development 8:133-146.
Kirshblum SC, Burns SP, Biering-Sorensen F, Donovan W, Graves DE, Jha A, Johansen M, Jones L, Krassioukov A, Mulcahey MJ, Schmidt-Read M, Waring W (2011) International standards for neurological classification of spinal cord injury (Revised 2011). The Journal of Spinal Cord Medicine 34:535-546.
Kiryu-Seo S, Ohno N, Kidd GJ, Komuro H, Trapp BD (2010) Demyelination Increases Axonal Stationary Mitochondrial Size and the Speed of Axonal Mitochondrial Transport. The Journal of Neuroscience 30:6658-6666.
Kiyono M, Shibuya M (2003) Bone Morphogenetic Protein 4 Mediates Apoptosis of Capillary Endothelial Cells during Rat Pupillary Membrane Regression. Molecular and Cellular Biology 23:4627-4636.
Kjell J, Olson L (2016) Rat models of spinal cord injury: from pathology to potential therapies. Disease Models & Mechanisms 9:1125-1137.
Knöchel S, Schuler-Metz A, Knöchel W (2000) c-Jun (AP-1) activates BMP-4 transcription in Xenopus embryos. Mechanisms of Development 98:29-36.
Kohta M, Kohmura E, Yamashita T (2009) Inhibition of TGF-β1 promotes functional recovery after spinal cord injury. Neuroscience Research 65:393-401.
Kokabu S, Gamer L, Cox K, Lowery J, Tsuji K, Raz R, Economides A, Katagiri T, Rosen V (2012) BMP3 suppresses osteoblast differentiation of bone marrow stromal cells via interaction with Acvr2b. Molecular endocrinology 26:87-94.
Konstantinidis G, Moustakas A, Stournaras C (2011) Regulation of Myosin Light Chain Function by BMP Signaling Controls Actin Cytoskeleton Remodeling. Cellular Physiology and Biochemistry 28:1031-1044.
Korsmeyer SJ, Wei MC, Saito M, Weiler S, Oh KJ, Schlesinger PH (2000) Pro-apoptotic cascade activates BID, which oligomerizes BAK or BAX into pores that result in the release of cytochrome c. Cell Death & Differentiation 7:1166-1173.
Kotter MR, Li W-W, Zhao C, Franklin RJM (2006) Myelin Impairs CNS Remyelination by Inhibiting Oligodendrocyte Precursor Cell Differentiation. The Journal of Neuroscience 26:328-332.
Kreuzaler PA, Staniszewska AD, Li W, Omidvar N, Kedjouar B, Turkson J, Poli V, Flavell RA, Clarkson RWE, Watson CJ (2011) Stat3 controls lysosomal-mediated cell death in vivo. Nature Cell Biology 13:303-309.
Kroner A, Greenhalgh AD, Zarruk JG, Passos Dos Santos R, Gaestel M, David S (2014) TNF and increased intracellular iron alter macrophage polarization to a detrimental M1 phenotype in the injured spinal cord. Neuron 83.
Krueger H, Noonan V, Trenaman L, Joshi P, Rivers CS (2013) The economic burden of traumatic spinal cord injury in Canada. Chronic diseases and injuries in Canada 33.
Kuo W-J, Digman MA, Lander AD (2010) Heparan Sulfate Acts as a Bone Morphogenetic Protein Coreceptor by Facilitating Ligand-induced Receptor Hetero-oligomerization. Molecular Biology of the Cell 21:4028-4041.
Kureel J, Dixit M, Tyagi AM, Mansoori MN, Srivastava K, Raghuvanshi A, Maurya R, Trivedi R, Goel A, Singh D (2014) miR-542-3p suppresses osteoblast cell proliferation and differentiation, targets BMP-7 signaling and inhibits bone formation. Cell Death &Amp; Disease 5:e1050.
Kusakawa Y, Mikawa S, Sato K (2015) BMP5 expression in the adult rat brain. Neuroscience 284:972-987.
Laabs TL, Wang H, Katagiri Y, McCann T, Fawcett JW, Geller HM (2007) Inhibiting Glycosaminoglycan Chain Polymerization Decreases the Inhibitory Activity of Astrocyte-Derived Chondroitin Sulfate Proteoglycans. The Journal of Neuroscience 27:14494-14501.
Labombarda F, González S, Lima A, Roig P, Guennoun R, Schumacher M, De Nicola AF (2011) Progesterone attenuates astro- and microgliosis and enhances oligodendrocyte differentiation following spinal cord injury. Experimental Neurology 231:135-146.

Lai HC, Seal RP, Johnson JE (2016) Making sense out of spinal cord somatosensory development. Development 143:3434-3448.
Lang BT, Cregg JM, DePaul MA, Tran AP, Xu K, Dyck SM, Madalena KM, Brown BP, Weng Y-L, Li S, Karimi-Abdolrezaee S, Busch SA, Shen Y, Silver J (2015a) Modulation of the proteoglycan receptor PTP[sgr] promotes recovery after spinal cord injury. Nature 518:404-408.
Lang BT, Cregg JM, DePaul MA, Tran AP, Xu K, Dyck SM, Madalena KM, Brown BP, Weng YL, Li S, Karimi-Abdolrezaee S, Busch SA, Shen Y, Silver J (2015b) Modulation of the proteoglycan receptor PTPsigma promotes recovery after spinal cord injury. Nature.
Le Dréau G, Garcia-Campmany L, Rabadán MA, Ferronha T, Tozer S, Briscoe J, Martí E (2012) Canonical BMP7 activity is required for the generation of discrete neuronal populations in the dorsal spinal cord. Development 139:259-268.
LeComte MD, Shimada IS, Sherwin C, Spees JL (2015) Notch1–STAT3–ETBR signaling axis controls reactive astrocyte proliferation after brain injury. Proceedings of the National Academy of Sciences 112:8726-8731.
Ledeboer A, Brevé JJP, Wierinckx A, Van Der Jagt S, Bristow AF, Leysen JE, Tilders FJH, Van Dam A-M (2002) Expression and regulation of interleukin-10 and interleukin-10 receptor in rat astroglial and microglial cells. European Journal of Neuroscience 16:1175-1185.
Lee BB, Cripps RA, Fitzharris M, Wing PC (2014) The global map for traumatic spinal cord injury epidemiology: update 2011, global incidence rate. Spinal Cord 52:110-116.
Li D, Tang J, Xu H, Fan X, Bai Y, Yang L (2008) Decreased hippocampal cell proliferation correlates with increased expression of BMP4 in the APPswe/PS1ΔE9 mouse model of Alzheimer's disease. Hippocampus 18:692-698.
Li G, Fan Z-K, Gu G-F, Jia Z-Q, Zhang Q-Q, Dai J-Y, He S-S (2019) Epidural Spinal Cord Stimulation Promotes Motor Functional Recovery by Enhancing Oligodendrocyte Survival and Differentiation and by Protecting Myelin after Spinal Cord Injury in Rats. Neuroscience Bulletin.
Li GL, Farooque M, Holtz A, Olsson Y (1999) Apoptosis of oligodendrocytes occurs for long distances away from the primary injury after compression trauma to rat spinal cord. Acta Neuropathologica 98:473-480.
Li X, Newbern JM, Wu Y, Morgan-Smith M, Zhong J, Charron J, Snider WD (2012) MEK Is a Key Regulator of Gliogenesis in the Developing Brain. Neuron 75:1035-1050.
Liddelow SA et al. (2017) Neurotoxic reactive astrocytes are induced by activated microglia. Nature 541:481.
Liem Jr KF, Tremml G, Roelink H, Jessell TM (1995) Dorsal differentiation of neural plate cells induced by BMP-mediated signals from epidermal ectoderm. Cell 82:969-979.
Liem KF, Jessell TM, Briscoe J (2000) Regulation of the neural patterning activity of sonic hedgehog by secreted BMP inhibitors expressed by notochord and somites. Development 127:4855-4866.
Liesi P, Kauppila T (2002) Induction of Type IV Collagen and Other Basement-Membrane-Associated Proteins after Spinal Cord Injury of the Adult Rat May Participate in Formation of the Glial Scar. Experimental Neurology 173:31-45.
Lim DA, Tramontin AD, Trevejo JM, Herrera DG, García-Verdugo JM, Alvarez-Buylla A (2000) Noggin Antagonizes BMP Signaling to Create a Niche for Adult Neurogenesis. Neuron 28:713-726.
Lin X, Liang Y-Y, Sun B, Liang M, Shi Y, Brunicardi FC, Shi Y, Feng X-H (2003) Smad6 Recruits Transcription Corepressor CtBP To Repress Bone Morphogenetic Protein-Induced Transcription. Molecular and Cellular Biology 23:9081-9093.
Lindborg JA, Mack M, Zigmond RE (2017) Neutrophils Are Critical for Myelin Removal in a Peripheral Nerve Injury Model of Wallerian Degeneration. The Journal of Neuroscience 37:10258-10277.
Lindsay SL, Toft A, Griffin J, M. M. Emraja A, Barnett SC, Riddell JS (2017) Human olfactory mesenchymal stromal cell transplants promote remyelination and earlier improvement in gait co-ordination after spinal cord injury. Glia 65:639-656.
Liu A, Niswander LA (2005) Bone morphogenetic protein signalling and vertebrate nervous system development. Nature Reviews Neuroscience 6:945-954.

Liu R, Cai J, Hu X, Tan M, Qi Y, German M, Rubenstein J, Sander M, Qiu M (2003) Region-specific and stage-dependent regulation of Olig gene expression and oligodendrogenesis by Nkx6.1 homeodomain transcription factor. Development 130:6221-6231.
Liu S, Sarkar C, Dinizo M, Faden AI, Koh EY, Lipinski MM, Wu J (2015) Disrupted autophagy after spinal cord injury is associated with ER stress and neuronal cell death. Cell Death & Disease 6:e1582.
Liu S, Li Y, Choi HMC, Sarkar C, Koh EY, Wu J, Lipinski MM (2018) Lysosomal damage after spinal cord injury causes accumulation of RIPK1 and RIPK3 proteins and potentiation of necroptosis. Cell Death & Disease 9:476.
Liu S, Sandner B, Schackel T, Nicholson L, Chtarto A, Tenenbaum L, Puttagunta R, Müller R, Weidner N, Blesch A (2017) Regulated viral BDNF delivery in combination with Schwann cells promotes axonal regeneration through capillary alginate hydrogels after spinal cord injury. Acta Biomaterialia 60:167-180.
Liu XZ, Xu XM, Hu R, Du C, Zhang SX, McDonald JW, Dong HX, Wu YJ, Fan GS, Jacquin MF, Hsu CY, Choi DW (1997) Neuronal and Glial Apoptosis after Traumatic Spinal Cord Injury. The Journal of Neuroscience 17:5395-5406.
Lööv C, Hillered L, Ebendal T, Erlandsson A (2012) Engulfing Astrocytes Protect Neurons from Contact-Induced Apoptosis following Injury. PLoS ONE 7:e33090.
Luu HH, Song W-X, Luo X, Manning D, Luo J, Deng Z-L, Sharff KA, Montag AG, Haydon RC, He T-C (2007) Distinct roles of bone morphogenetic proteins in osteogenic differentiation of mesenchymal stem cells. Journal of Orthopaedic Research 25:665-677.
Ma D, Wang B, Zawadzka M, Gonzalez G, Wu Z, Yu B, Rawlins EL, Franklin RJM, Zhao C (2018) A Subpopulation of Foxj1-Expressing, Nonmyelinating Schwann Cells of the Peripheral Nervous System Contribute to Schwann Cell Remyelination in the Central Nervous System. The Journal of Neuroscience 38:9228-9239.
Ma L, Lu M-F, Schwartz RJ, Martin JF (2005) Bmp2 is essential for cardiac cushion epithelial-mesenchymal transition and myocardial patterning. Development 132:5601-5611.
Ma M, Ferguson TA, Schoch KM, Li J, Qian Y, Shofer FS, Saatman KE, Neumar RW (2013) Calpains mediate axonal cytoskeleton disintegration during Wallerian degeneration. Neurobiology of Disease 56:34-46.
MacVicar BA, Newman EA (2015) Astrocyte Regulation of Blood Flow in the Brain. Cold Spring Harbor Perspectives in Biology 7.
Mandal Chandi C, Ganapathy S, Gorin Y, Mahadev K, Block K, Abboud Hanna E, Harris Stephen E, Ghosh-Choudhury G, Ghosh-Choudhury N (2010) Reactive oxygen species derived from Nox4 mediate BMP2 gene transcription and osteoblast differentiation. Biochemical Journal 433:393-402.
Marazzi G, Wang Y, Sassoon D (1997) Msx2 Is a Transcriptional Regulator in the BMP4-Mediated Programmed Cell Death Pathway. Developmental Biology 186:127-138.
Marqués G, Musacchio M, Shimell MJ, Wünnenberg-Stapleton K, Cho KWY, O'Connor MB (1997) Production of a DPP Activity Gradient in the Early Drosophila Embryo through the Opposing Actions of the SOG and TLD Proteins. Cell 91:417-426.
Martínez VG, Rubio C, Martínez-Fernández M, Segovia C, López-Calderón F, Garín MI, Teijeira A, Munera-Maravilla E, Varas A, Sacedón R, Guerrero F, Villacampa F, de la Rosa F, Castellano D, López-Collazo E, Paramio JM, Vicente Á, Dueñas M (2017) BMP4 Induces M2 Macrophage Polarization and Favors Tumor Progression in Bladder Cancer. Clinical Cancer Research 23:7388-7399.
Martínez-Fábregas J, Prescott A, van Kasteren S, Pedrioli DL, McLean I, Moles A, Reinheckel T, Poli V, Watts C (2018) Lysosomal protease deficiency or substrate overload induces an oxidative-stress mediated STAT3-dependent pathway of lysosomal homeostasis. Nature Communications 9:5343.
Massaad CA, Klann E (2011) Reactive oxygen species in the regulation of synaptic plasticity and memory. Antioxidants & redox signaling 14:2013-2054.

Massey JM, Hubscher CH, Wagoner MR, Decker JA, Amps J, Silver J, Onifer SM (2006) Chondroitinase ABC Digestion of the Perineuronal Net Promotes Functional Collateral Sprouting in the Cuneate Nucleus after Cervical Spinal Cord Injury. The Journal of Neuroscience 26:4406-4414.

Matsuura I, Taniguchi J, Hata K, Saeki N, Yamashita T (2008) BMP inhibition enhances axonal growth and functional recovery after spinal cord injury. Journal of Neurochemistry 105:1471-1479.

Matute C, Torre I, Pérez-Cerdá F, Pérez-Samartín A, Alberdi E, Etxebarria E, Arranz AM, Ravid R, Rodríguez-Antigüedad A, Sánchez-Gómez M, Domercq M (2007) P2X7 Receptor Blockade Prevents ATP Excitotoxicity in Oligodendrocytes and Ameliorates Experimental Autoimmune Encephalomyelitis. The Journal of Neuroscience 27:9525-9533.

Mayeur C, Kolodziej SA, Wang A, Xu X, Lee A, Yu PB, Shen J, Bloch KD, Bloch DB (2015) Oral administration of a bone morphogenetic protein type I receptor inhibitor prevents the development of anemia of inflammation. Haematologica 100:e68-e71.

Mazerbourg S, Klein C, Roh J, Kaivo-Oja N, Mottershead DG, Korchynskyi O, Ritvos O, Hsueh AJW (2004) Growth Differentiation Factor-9 Signaling Is Mediated by the Type I Receptor, Activin Receptor-Like Kinase 5. Molecular Endocrinology 18:653-665.

McMahon JA, Takada S, Zimmerman LB, Fan C-M, Harland RM, McMahon AP (1998) Noggin-mediated antagonism of BMP signaling is required for growth and patterning of the neural tube and somite. Genes & Development 12:1438-1452.

McTigue DM, Tripathi RB (2008) The life, death, and replacement of oligodendrocytes in the adult CNS. J Neurochem 107:1-19.

McTigue DM, Wei P, Stokes BT (2001) Proliferation of NG2-Positive Cells and Altered Oligodendrocyte Numbers in the Contused Rat Spinal Cord. The Journal of Neuroscience 21:3392-3400.

McTigue DM, Tripathi R, Wei P (2006) NG2 Colocalizes With Axons and Is Expressed by a Mixed Cell Population in Spinal Cord Lesions. Journal of Neuropathology & Experimental Neurology 65:406-420.

Mekki-Dauriac S, Agius E, Kan P, Cochard P (2002) Bone morphogenetic proteins negatively control oligodendrocyte precursor specification in the chick spinal cord. Development 129:5117-5130.

Meletis K, Barnabé-Heider F, Carlén M, Evergren E, Tomilin N, Shupliakov O, Frisén J (2008) Spinal Cord Injury Reveals Multilineage Differentiation of Ependymal Cells. PLoS Biol 6:e182.

Metz A, Knöchel S, Büchler P, Köster M, Knöchel W (1998) Structural and functional analysis of the BMP-4 promoter in early embryos of Xenopus laevis. Mechanisms of Development 74:29-39.

Metz GA, Curt A, Van De Meent H, Klusman I, Schwab ME, Dietz V (2000) Validation of the weight-drop contusion model in rats: a comparative study of human spinal cord injury. Journal of neurotrauma 17:1-17.

Meyers EA, Gobeske KT, Bond AM, Jarrett JC, Peng C-Y, Kessler JA (2016) Increased bone morphogenetic protein signaling contributes to age-related declines in neurogenesis and cognition. Neurobiology of Aging 38:164-175.

Mi S, Hu B, Hahm K, Luo Y, Kam Hui ES, Yuan Q, Wong WM, Wang L, Su H, Chu T-H, Guo J, Zhang W, So K-F, Pepinsky B, Shao Z, Graff C, Garber E, Jung V, Wu EX, Wu W (2007) LINGO-1 antagonist promotes spinal cord remyelination and axonal integrity in MOG-induced experimental autoimmune encephalomyelitis. Nature Medicine 13:1228-1233.

Micheau O, Tschopp J (2003) Induction of TNF Receptor I-Mediated Apoptosis via Two Sequential Signaling Complexes. Cell 114:181-190.

Miller RH, Dinsio K, Wang R, Geertman R, Maier CE, Hall AK (2004) Patterning of spinal cord oligodendrocyte development by dorsally derived BMP4. Journal of Neuroscience Research 76:9-19.

Miron VE, Boyd A, Zhao JW, Yuen TJ, Ruckh JM, Shadrach JL (2013) M2 microglia and macrophages drive oligodendrocyte differentiation during CNS remyelination. Nat Neurosci 16.

Miyagi M, Mikawa S, Sato T, Hasegawa T, Kobayashi S, Matsuyama Y, Sato K (2012) BMP2, BMP4, noggin, BMPRIA, BMPRIB, and BMPRII are differentially expressed in the adult rat spinal cord. Neuroscience 203:12-26.

Miyazono K, Maeda S, Imamura T (2005) BMP receptor signaling: Transcriptional targets, regulation of signals, and signaling cross-talk. Cytokine & Growth Factor Reviews 16:251-263.
Molloy SS, Thomas L, VanSlyke JK, Stenberg PE, Thomas G (1994) Intracellular trafficking and activation of the furin proprotein convertase: localization to the TGN and recycling from the cell surface. The EMBO Journal 13:18-33.
Morgan MJ, Liu Z-g (2011) Crosstalk of reactive oxygen species and NF-κB signaling. Cell research 21:103-115.
Morita M, Ikeshima-Kataoka H, Kreft M, Vardjan N, Zorec R, Noda M (2019) Metabolic plasticity of astrocytes and aging of the brain. International journal of molecular sciences 20:941.
Mukhopadhyay G, Doherty P, Walsh FS, Crocker PR, Filbin MT (1994) A novel role for myelin-associated glycoprotein as an inhibitor of axonal regeneration. Neuron 13:757-767.
Muñoz-Galdeano T, Reigada D, del Águila Á, Velez I, Caballero-López MJ, Maza RM, Nieto-Díaz M (2018) Cell Specific Changes of Autophagy in a Mouse Model of Contusive Spinal Cord Injury. Frontiers in Cellular Neuroscience 12.
Murakami G, Watabe T, Takaoka K, Miyazono K, Imamura T (2003) Cooperative Inhibition of Bone Morphogenetic Protein Signaling by Smurf1 and Inhibitory Smads. Molecular Biology of the Cell 14:2809-2817.
Musto A, Navarra A, Vocca A, Gargiulo A, Minopoli G, Romano S, Romano MF, Russo T, Parisi S (2015) miR-23a, miR-24 and miR-27a protect differentiating ESCs from BMP4-induced apoptosis. Cell Death Differ 22:1047-1057.
Mutoh T, Sanosaka T, Ito K, Nakashima K (2012) Oxygen Levels Epigenetically Regulate Fate Switching of Neural Precursor Cells via Hypoxia-Inducible Factor 1α-Notch Signal Interaction in the Developing Brain. STEM CELLS 30:561-569.
Nagakannan P, Iqbal MA, Yeung A, Thliveris JA, Rastegar M, Ghavami S, Eftekharpour E (2016) Perturbation of redox balance after thioredoxin reductase deficiency interrupts autophagy-lysosomal degradation pathway and enhances cell death in nutritionally stressed SH-SY5Y cells. Free Radical Biology and Medicine 101:53-70.
Nagelhus EA, Ottersen OP (2013) Physiological Roles of Aquaporin-4 in Brain. Physiological Reviews 93:1543-1562.
Nagoshi N, Khazaei M, Ahlfors J-E, Ahuja CS, Nori S, Wang J, Shibata S, Fehlings MG (2018) Human Spinal Oligodendrogenic Neural Progenitor Cells Promote Functional Recovery After Spinal Cord Injury by Axonal Remyelination and Tissue Sparing. STEM CELLS Translational Medicine 7:806-818.
Najm FJ et al. (2015) Drug-based modulation of endogenous stem cells promotes functional remyelination in vivo. Nature 522:216-220.
Nakanishi K, Aono S, Hirano K, Kuroda Y, Ida M, Tokita Y, Matsui F, Oohira A (2006) Identification of neurite outgrowth-promoting domains of neuroglycan C, a brain-specific chondroitin sulfate proteoglycan, and involvement of phosphatidylinositol 3-kinase and protein kinase C signaling pathways in neuritogenesis. The Journal of biological chemistry 281:24970-24978.
Nakashima K, Yanagisawa M, Arakawa H, Kimura N, Hisatsune T, Kawabata M, Miyazono K, Taga T (1999) Synergistic Signaling in Fetal Brain by STAT3-Smad1 Complex Bridged by p300. Science 284:479-482.
Nardone R, Florea C, Höller Y, Brigo F, Versace V, Lochner P, Golaszewski S, Trinka E (2017) Rodent, large animal and non-human primate models of spinal cord injury. Zoology 123:101-114.
Neely MD, Sidell KR, Graham DG, Montine TJ (1999) The Lipid Peroxidation Product 4-Hydroxynonenal Inhibits Neurite Outgrowth, Disrupts Neuronal Microtubules, and Modifies Cellular Tubulin. Journal of Neurochemistry 72:2323-2333.
Nelsen SM, Christian JL (2009) Site-specific Cleavage of BMP4 by Furin, PC6, and PC7. Journal of Biological Chemistry 284:27157-27166.

Nemeth E, Tuttle MS, Powelson J, Vaughn MB, Donovan A, Ward DM, Ganz T, Kaplan J (2004) Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. Science (New York, NY) 306:2090-2093.

Nguyen HX, O'Barr TJ, Anderson AJ (2007) Polymorphonuclear leukocytes promote neurotoxicity through release of matrix metalloproteinases, reactive oxygen species, and TNF-α. Journal of Neurochemistry 102:900-912.

Nguyen HX, Galvan MD, Anderson AJ (2008) Characterization of early and terminal complement proteins associated with polymorphonuclear leukocytes in vitro and in vivo after spinal cord injury. Journal of Neuroinflammation 5:26.

Nimmerjahn A, Kirchhoff F, Helmchen F (2005) Resting Microglial Cells Are Highly Dynamic Surveillants of Brain Parenchyma in Vivo. Science 308:1314-1318.

Nistala H, Lee-Arteaga S, Smaldone S, Siciliano G, Carta L, Ono RN, Sengle G, Arteaga-Solis E, Levasseur R, Ducy P, Sakai LY, Karsenty G, Ramirez F (2010) Fibrillin-1 and -2 differentially modulate endogenous TGF-β and BMP bioavailability during bone formation. The Journal of Cell Biology 190:1107-1121.

Nohe A, Hassel S, Ehrlich M, Neubauer F, Sebald W, Henis YI, Knaus P (2002) The Mode of Bone Morphogenetic Protein (BMP) Receptor Oligomerization Determines Different BMP-2 Signaling Pathways. Journal of Biological Chemistry 277:5330-5338.

Noonan VK, Fingas M, Farry A, Baxter D, Singh A, Fehlings MG, Dvorak MF (2012) Incidence and Prevalence of Spinal Cord Injury in Canada: A National Perspective. Neuroepidemiology 38:219-226.

Norden DM, Fenn AM, Dugan A, Godbout JP (2014) TGFβ produced by IL-10 redirected astrocytes attenuates microglial activation. Glia 62:881-895.

Nori S, Khazaei M, Ahuja CS, Yokota K, Ahlfors J-E, Liu Y, Wang J, Shibata S, Chio J, Hettiaratchi MH, Führmann T, Shoichet MS, Fehlings MG (2018) Human Oligodendrogenic Neural Progenitor Cells Delivered with Chondroitinase ABC Facilitate Functional Repair of Chronic Spinal Cord Injury. Stem Cell Reports 11:1433-1448.

North HA, Pan L, McGuire TL, Brooker S, Kessler JA (2015) β1-Integrin Alters Ependymal Stem Cell BMP Receptor Localization and Attenuates Astrogliosis after Spinal Cord Injury. The Journal of Neuroscience 35:3725-3733.

Nunomura A, Moreira P, Castellani R, Lee H-g, Zhu X, Smith M, Perry G (2012) Oxidative Damage to RNA in Aging and Neurodegenerative Disorders. Neurotoxicity Research 22:231-248.

Ochiai W, Yanagisawa M, Takizawa T, Nakashima K, Taga T (2001) Astrocyte differentiation of fetal neuroepithelial cells involving cardiotrophin-1-induced activation of STAT3. Cytokine 14:264-271.

Ohkawara B, Iemura S-i, ten Dijke P, Ueno N (2002) Action Range of BMP Is Defined by Its N-Terminal Basic Amino Acid Core. Current Biology 12:205-209.

Ohtake Y, Wong D, Abdul-Muneer PM, Selzer ME, Li S (2016) Two PTP receptors mediate CSPG inhibition by convergent and divergent signaling pathways in neurons. Scientific Reports 6:37152.

Okada S, Nakamura M, Mikami Y, Shimazaki T, Mihara M, Ohsugi Y, Iwamoto Y, Yoshizaki K, Kishimoto T, Toyama Y, Okano H (2004) Blockade of interleukin-6 receptor suppresses reactive astrogliosis and ameliorates functional recovery in experimental spinal cord injury. Journal of Neuroscience Research 76:265-276.

Okada S, Nakamura M, Katoh H, Miyao T, Shimazaki T, Ishii K, Yamane J, Yoshimura A, Iwamoto Y, Toyama Y, Okano H (2006) Conditional ablation of Stat3 or Socs3 discloses a dual role for reactive astrocytes after spinal cord injury. Nat Med 12:829-834.

Onichtchouk D, Chen Y-G, Dosch R, Gawantka V, Delius H, Massague´ J, Niehrs C (1999) Silencing of TGF-β signalling by the pseudoreceptor BAMBI. Nature 401:480-485.

Orr MB, Gensel JC (2018) Spinal Cord Injury Scarring and Inflammation: Therapies Targeting Glial and Inflammatory Responses. Neurotherapeutics : the journal of the American Society for Experimental NeuroTherapeutics 15:541-553.

Owens P, Pickup MW, Novitskiy SV, Giltnane JM, Gorska AE, Hopkins CR, Hong CC, Moses HL (2015) Inhibition of BMP signaling suppresses metastasis in mammary cancer. Oncogene 34:2437-2449.
Paine-Saunders S, Viviano BL, Economides AN, Saunders S (2002) Heparan Sulfate Proteoglycans Retain Noggin at the Cell Surface: A POTENTIAL MECHANISM FOR SHAPING BONE MORPHOGENETIC PROTEIN GRADIENTS. Journal of Biological Chemistry 277:2089-2096.
Pan L, North HA, Sahni V, Jeong SJ, McGuire TL, Berns EJ, Stupp SI, Kessler JA (2014) β1-Integrin and integrin linked kinase regulate astrocytic differentiation of neural stem cells. PloS one 9:e104335-e104335.
Parajuli B, Horiuchi H, Mizuno T, Takeuchi H, Suzumura A (2015) CCL11 enhances excitotoxic neuronal death by producing reactive oxygen species in microglia. Glia 63:2274-2284.
Pardali E, Makowski L-M, Leffers M, Borgscheiper A, Waltenberger J (2018) BMP-2 induces human mononuclear cell chemotaxis and adhesion and modulates monocyte-to-macrophage differentiation. Journal of Cellular and Molecular Medicine 22:5429-5438.
Parikh P, Hao Y, Hosseinkhani M, Patil SB, Huntley GW, Tessier-Lavigne M, Zou H (2011) Regeneration of axons in injured spinal cord by activation of bone morphogenetic protein/Smad1 signaling pathway in adult neurons. Proceedings of the National Academy of Sciences 108:E99–E107.
Park YM, Lee WT, Bokara KK, Seo SK, Park SH, Kim JH, Yenari MA, Park KA, Lee JE (2013) The multifaceted effects of agmatine on functional recovery after spinal cord injury through Modulations of BMP-2/4/7 expressions in neurons and glial cells. PloS one 8:e53911.
Pearson KL, Hunter T, Janknecht R (1999) Activation of Smad1-mediated transcription by p300/CBP. Biochimica et Biophysica Acta (BBA) - Gene Structure and Expression 1489:354-364.
Pendleton JC, Shamblott MJ, Gary DS, Belegu V, Hurtado A, Malone ML, McDonald JW (2013) Chondroitin sulfate proteoglycans inhibit oligodendrocyte myelination through PTPσ. Experimental Neurology 247:113-121.
Petersen MA et al. (2017) Fibrinogen Activates BMP Signaling in Oligodendrocyte Progenitor Cells and Inhibits Remyelination after Vascular Damage. Neuron 96:1003-1012.e1007.
Piccolo S, Sasai Y, Lu B, De Robertis EM (1996) Dorsoventral Patterning in Xenopus: Inhibition of Ventral Signals by Direct Binding of Chordin to BMP-4. Cell 86:589-598.
Pineau I, Lacroix S (2007) Proinflammatory cytokine synthesis in the injured mouse spinal cord: Multiphasic expression pattern and identification of the cell types involved. Journal of Comparative Neurology 500:267-285.
Pineau I, Sun L, Bastien D, Lacroix S (2010) Astrocytes initiate inflammation in the injured mouse spinal cord by promoting the entry of neutrophils and inflammatory monocytes in an IL-1 receptor/MyD88-dependent fashion. Brain, Behavior, and Immunity 24:540-553.
Plemel JR, Manesh SB, Sparling JS, Tetzlaff W (2013) Myelin inhibits oligodendroglial maturation and regulates oligodendrocytic transcription factor expression. Glia 61:1471-1487.
Poon IKH, Lucas CD, Rossi AG, Ravichandran KS (2014) Apoptotic cell clearance: basic biology and therapeutic potential. Nature Reviews Immunology 14:166-180.
Poon PC, Gupta D, Shoichet MS, Tator CH (2007) Clip Compression Model Is Useful for Thoracic Spinal Cord Injuries: Histologic and Functional Correlates. Spine 32:2853-2859.
Proschel C, Stripay JL, Shih CH, Munger JC, Noble MD (2014) Delayed transplantation of precursor cell-derived astrocytes provides multiple benefits in a rat model of Parkinsons.
Rabchevsky AG, Fugaccia I, Sullivan PG, Blades DA, Scheff SW (2002) Efficacy of methylprednisolone therapy for the injured rat spinal cord. Journal of Neuroscience Research 68:7-18.
Renault-Mihara F, Katoh H, Ikegami T, Iwanami A, Mukaino M, Yasuda A, Nori S, Mabuchi Y, Tada H, Shibata S, Saito K, Matsushita M, Kaibuchi K, Okada S, Toyama Y, Nakamura M, Okano H (2011) Beneficial compaction of spinal cord lesion by migrating astrocytes through glycogen synthase kinase-3 inhibition. EMBO Molecular Medicine 3:682-696.
Rocher C, Singla DK (2013) SMAD-PI3K-Akt-mTOR Pathway Mediates BMP-7 Polarization of Monocytes into M2 Macrophages. PLOS ONE 8:e84009.

Ruan C, Sun L, Kroshilina A, Beckers L, De Jager P, Bradshaw EM, Hasson SA, Yang G, Elyaman W (2020) A novel Tmem119-tdTomato reporter mouse model for studying microglia in the central nervous system. Brain, Behavior, and Immunity 83:180-191.

Sabelström H, Stenudd M, Réu P, Dias DO, Elfineh M, Zdunek S, Damberg P, Göritz C, Frisén J (2013) Resident neural stem cells restrict tissue damage and neuronal loss after spinal cord injury in mice. Science (New York, NY) 342:637-640.

Sabo JK, Aumann TD, Merlo D, Kilpatrick TJ, Cate HS (2011) Remyelination Is Altered by Bone Morphogenic Protein Signaling in Demyelinated Lesions. The Journal of Neuroscience 31:4504-4510.

Saeed O, Otsuka F, Polavarapu R, Karmali V, Weiss D, Davis T, Rostad B, Pachura K, Adams L, Elliott J, Taylor WR, Narula J, Kolodgie F, Virmani R, Hong CC, Finn AV (2012) Pharmacological Suppression of Hepcidin Increases Macrophage Cholesterol Efflux and Reduces Foam Cell Formation and Atherosclerosis. Arteriosclerosis, Thrombosis, and Vascular Biology 32:299-307.

Sahni V, Mukhopadhyay A, Tysseling V, Hebert A, Birch D, Mcguire TL, Stupp SI, Kessler JA (2010) BMPR1a and BMPR1b Signaling Exert Opposing Effects on Gliosis after Spinal Cord Injury. The Journal of Neuroscience 30:1839-1855.

Sakamoto K, Ozaki T, Ko Y-C, Tsai C-F, Gong Y, Morozumi M, Ishikawa Y, Uchimura K, Nadanaka S, Kitagawa H, Zulueta MML, Bandaru A, Tamura J-i, Hung S-C, Kadomatsu K (2019) Glycan sulfation patterns define autophagy flux at axon tip via PTPRσ-cortactin axis. Nature Chemical Biology 15:699-709.

Samad TA, Rebbapragada A, Bell E, Zhang Y, Sidis Y, Jeong S-J, Campagna JA, Perusini S, Fabrizio DA, Schneyer AL, Lin HY, Brivanlou AH, Attisano L, Woolf CJ (2005) DRAGON, a Bone Morphogenetic Protein Co-receptor. Journal of Biological Chemistry 280:14122-14129.

Samanta J, Kessler JA (2004) Interactions between ID and OLIG proteins mediate the inhibitory effects of BMP4 on oligodendroglial differentiation. Development 131:4131-4142.

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

Sampath TK, Reddi AH (1983) Homology of bone-inductive proteins from human, monkey, bovine, and rat extracellular matrix. Proceedings of the National Academy of Sciences 80:6591-6595.

Sampath TK, Coughlin JE, Whetstone RM, Banach D, Corbett C, Ridge RJ, Ozkaynak E, Oppermann H, Rueger DC (1990) Bovine osteogenic protein is composed of dimers of OP-1 and BMP-2A, two members of the transforming growth factor-beta superfamily. Journal of Biological Chemistry 265:13198-13205.

Sandner B, Rivera FJ, Caioni M, Nicholson L, Eckstein V, Bogdahn U, Aigner L, Blesch A, Weidner N (2013) Bone morphogenetic proteins prevent bone marrow stromal cell-mediated oligodendroglial differentiation of transplanted adult neural progenitor cells in the injured spinal cord. Stem cell research 11:758-771.

Sanvitale CE, Kerr G, Chaikuad A, Ramel M-C, Mohedas AH, Reichert S, Wang Y, Triffitt JT, Cuny GD, Yu PB, Hill CS, Bullock AN (2013) A New Class of Small Molecule Inhibitor of BMP Signaling. PLoS ONE 8:e62721.

Schachtrup C, Ryu JK, Helmrick MJ, Vagena E, Galanakis DK, Degen JL, Margolis RU, Akassoglou K (2010) Fibrinogen Triggers Astrocyte Scar Formation by Promoting the Availability of Active TGF-β after Vascular Damage. The Journal of Neuroscience 30:5843-5854.

Scholze AR, Foo LC, Mulinyawe S, Barres BA (2014) BMP Signaling in Astrocytes Downregulates EGFR to Modulate Survival and Maturation. PloS one 9:e110668.

Schuler-Metz A, Knöchel S, Kaufmann E, Knöchel W (2000) The Homeodomain Transcription Factor Xvent-2 Mediates Autocatalytic Regulation of BMP-4 Expression in XenopusEmbryos. Journal of Biological Chemistry 275:34365-34374.

Scott FL, Stec B, Pop C, Dobaczewska MK, Lee JJ, Monosov E, Robinson H, Salvesen GS, Schwarzenbacher R, Riedl SJ (2009) The Fas–FADD death domain complex structure unravels signalling by receptor clustering. Nature 457:1019-1022.

Sebbagh M, Renvoizé C, Hamelin J, Riché N, Bertoglio J, Bréard J (2001) Caspase-3-mediated cleavage of ROCK I induces MLC phosphorylation and apoptotic membrane blebbing. Nature Cell Biology 3:346-352.

See J, Mamontov P, Ahn K, Wine-Lee L, Crenshaw EB, Grinspan JB (2007) BMP signaling mutant mice exhibit glial cell maturation defects. Molecular and Cellular Neuroscience 35:171-182.

See J, Zhang X, Eraydin N, Mun S-B, Mamontov P, Golden JA, Grinspan JB (2004) Oligodendrocyte maturation is inhibited by bone morphogenetic protein. Molecular and Cellular Neuroscience 26:481-492.

Seemann P, Brehm A, König J, Reissner C, Stricker S, Kuss P, Haupt J, Renninger S, Nickel J, Sebald W, Groppe JC, Plöger F, Pohl J, Schmidt-von Kegler M, Walther M, Gassner I, Rusu C, Janecke AR, Dathe K, Mundlos S (2009) Mutations in GDF5 Reveal a Key Residue Mediating BMP Inhibition by NOGGIN. PLOS Genetics 5:e1000747.

Sekhon LH, Fehlings MG (2001) Epidemiology, demographics, and pathophysiology of acute spinal cord injury. Spine 26:S2-S12.

Selmaj KW, Raine CS (1988) Tumor necrosis factor mediates myelin and oligodendrocyte damage in vitro. Annals of neurology 23:339-346.

Sengle G, Ono RN, Lyons KM, Bächinger HP, Sakai LY (2008a) A New Model for Growth Factor Activation: Type II Receptors Compete with the Prodomain for BMP-7. Journal of Molecular Biology 381:1025-1039.

Sengle G, Charbonneau NL, Ono RN, Sasaki T, Alvarez J, Keene DR, Bächinger HP, Sakai LY (2008b) Targeting of Bone Morphogenetic Protein Growth Factor Complexes to Fibrillin. Journal of Biological Chemistry 283:13874-13888.

Seo JH, Miyamoto N, Hayakawa K, Pham L-DD, Maki T, Ayata C, Kim K-W, Lo EH, Arai K (2013) Oligodendrocyte precursors induce early blood-brain barrier opening after white matter injury. The Journal of Clinical Investigation 123:782-786.

Serpe M, Umulis D, Ralston A, Chen J, Olson DJ, Avanesov A, Othmer H, O'Connor MB, Blair SS (2008) The BMP-Binding Protein Crossveinless 2 Is a Short-Range, Concentration-Dependent, Biphasic Modulator of BMP Signaling in Drosophila. Developmental Cell 14:940-953.

Setoguchi T, Yone K, Matsuoka E, Takenouchi H, Nakashima K, Sakou T, Komiya S, Izumo S (2001) Traumatic injury-induced BMP7 expression in the adult rat spinal cord. Brain Research 921:219-225.

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

Shadel Gerald S, Horvath Tamas L (2015) Mitochondrial ROS Signaling in Organismal Homeostasis. Cell 163:560-569.

Shechter R, Raposo C, London A, Sagi I, Schwartz M (2011) The Glial Scar-Monocyte Interplay: A Pivotal Resolution Phase in Spinal Cord Repair. PLOS ONE 6:e27969.

Shen Y, Tenney AP, Busch SA, Horn KP, Cuascut FX, Liu K, He Z, Silver J, Flanagan JG (2009) PTPσ Is a Receptor for Chondroitin Sulfate Proteoglycan, an Inhibitor of Neural Regeneration. Science 326:592-596.

Sheng W, Hu S, Feng A, Rock RB (2013) Reactive Oxygen Species from Human Astrocytes Induced Functional Impairment and Oxidative Damage. Neurochemical Research 38:2148-2159.

Sherman LS, Back SA (2008) A 'GAG' reflex prevents repair of the damaged CNS. Trends in Neurosciences 31:44-52.

Shi M, Zhu J, Wang R, Chen X, Mi L, Walz T, Springer TA (2011) Latent TGF-β structure and activation. Nature 474:343.

Shih AY, Johnson DA, Wong G, Kraft AD, Jiang L, Erb H, Johnson JA, Murphy TH (2003) Coordinate Regulation of Glutathione Biosynthesis and Release by Nrf2-Expressing Glia Potently Protects Neurons from Oxidative Stress. The Journal of Neuroscience 23:3394-3406.

Shih C-H, Lacagnina M, Leuer-Bisciotti K, Pröschel C (2014) Astroglial-Derived Periostin Promotes Axonal Regeneration after Spinal Cord Injury. The Journal of Neuroscience 34:2438-2443.
Shijo T, Warita H, Suzuki N, Ikeda K, Mitsuzawa S, Akiyama T, Ono H, Nishiyama A, Izumi R, Kitajima Y, Aoki M (2018) Antagonizing bone morphogenetic protein 4 attenuates disease progression in a rat model of amyotrophic lateral sclerosis. Experimental Neurology 307:164-179.
Shore EM, Xu M, Feldman GJ, Fenstermacher DA, Cho T-J, Choi IH, Connor JM, Delai P, Glaser DL, LeMerrer M, Morhart R, Rogers JG, Smith R, Triffitt JT, Urtizberea JA, Zasloff M, Brown MA, Kaplan FS (2006) A recurrent mutation in the BMP type I receptor ACVR1 causes inherited and sporadic fibrodysplasia ossificans progressiva. Nature Genetics 38:525-527.
Shu B, Zhang M, Xie R, Wang M, Jin H, Hou W, Tang D, Harris SE, Mishina Y, O'Keefe RJ, Hilton MJ, Wang Y, Chen D (2011) BMP2, but not BMP4, is crucial for chondrocyte proliferation and maturation during endochondral bone development. Journal of Cell Science 124:3428-3440.
Shuman SL, Bresnahan JC, Beattie MS (1997) Apoptosis of microglia and oligodendrocytes after spinal cord contusion in rats. Journal of Neuroscience Research 50:798-808.
Sieger D, Moritz C, Ziegenhals T, Prykhozhij S, Peri F (2012) Long-Range Ca2+ Waves Transmit Brain-Damage Signals to Microglia. Developmental Cell 22:1138-1148.
Singh A, Tetreault L, Kalsi-Ryan S, Nouri A, Fehlings MG (2014) Global prevalence and incidence of traumatic spinal cord injury. Clinical epidemiology 6:309-331.
Singh IN, Sullivan PG, Deng Y, Mbye LH, Hall ED (2006) Time Course of Post-Traumatic Mitochondrial Oxidative Damage and Dysfunction in a Mouse Model of Focal Traumatic Brain Injury: Implications for Neuroprotective Therapy. Journal of Cerebral Blood Flow & Metabolism 26:1407-1418.
Slee EA, Adrain C, Martin SJ (2001) Executioner Caspase-3, -6, and -7 Perform Distinct, Non-redundant Roles during the Demolition Phase of Apoptosis. Journal of Biological Chemistry 276:7320-7326.
Smith GM, Strunz C (2005) Growth factor and cytokine regulation of chondroitin sulfate proteoglycans by astrocytes. Glia 52:209-218.
Snow DM, Steindler DA, Silver J (1990) Molecular and cellular characterization of the glial roof plate of the spinal cord and optic tectum: A possible role for a proteoglycan in the development of an axon barrier. Developmental Biology 138:359-376.
Soderblom C, Luo X, Blumenthal E, Bray E, Lyapichev K, Ramos J, Krishnan V, Lai-Hsu C, Park KK, Tsoulfas P, Lee JK (2013) Perivascular Fibroblasts Form the Fibrotic Scar after Contusive Spinal Cord Injury. The Journal of Neuroscience 33:13882-13887.
Sofroniew MV (2009) Molecular dissection of reactive astrogliosis and glial scar formation. Trends in Neurosciences 32:638-647.
Song G, Yang R, Zhang Q, Chen L, Huang D, Zeng J, Yang C, Zhang T (2019) TGF-β Secretion by M2 Macrophages Induces Glial Scar Formation by Activating Astrocytes In Vitro. Journal of Molecular Neuroscience 69:324-332.
Song K, Krause C, Shi S, Patterson M, Suto R, Grgurevic L, Vukicevic S, van Dinther M, Falb D, ten Dijke P, Alaoui-Ismaili MH (2010) Identification of a Key Residue Mediating Bone Morphogenetic Protein (BMP)-6 Resistance to Noggin Inhibition Allows for Engineered BMPs with Superior Agonist Activity. Journal of Biological Chemistry 285:12169-12180.
Sopory S, Nelsen SM, Degnin C, Wong C, Christian JL (2006) Regulation of Bone Morphogenetic Protein-4 Activity by Sequence Elements within the Prodomain. Journal of Biological Chemistry 281:34021-34031.
Springer JE, Azbill RD, Knapp PE (1999) Activation of the caspase-3 apoptotic cascade in traumatic spinal cord injury. Nature medicine 5:943-946.
Springer JE, Azbill RD, Mark RJ, Begley JG, Waeg G, Mattson MP (1997) 4-Hydroxynonenal, a Lipid Peroxidation Product, Rapidly Accumulates Following Traumatic Spinal Cord Injury and Inhibits Glutamate Uptake. Journal of Neurochemistry 68:2469-2476.
Sroga JM, Jones TB, Kigerl KA, McGaughy VM, Popovich PG (2003) Rats and mice exhibit distinct inflammatory reactions after spinal cord injury. Journal of Comparative Neurology 462:223-240.

Steinbicker AU, Sachidanandan C, Vonner AJ, Yusuf RZ, Deng DY, Lai CS, Rauwerdink KM, Winn JC, Saez B, Cook CM, Szekely BA, Roy CN, Seehra JS, Cuny GD, Scadden DT, Peterson RT, Bloch KD, Yu PB (2011) Inhibition of bone morphogenetic protein signaling attenuates anemia associated with inflammation. Blood 117:4915-4923.

Sterneckert JL, Hill CM, Palmer R, Gearhart JD (2005) Bone Morphogenetic Proteins Produced by Cells within Embryoid Bodies Inhibit Ventral Directed Differentiation by Sonic Hedgehog. Cloning and Stem Cells 7:27-34.

Streit A, Lee KJ, Woo I, Roberts C, Jessell TM, Stern CD (1998) Chordin regulates primitive streak development and the stability of induced neural cells, but is not sufficient for neural induction in the chick embryo. Development 125:507-519.

Susan-Resiga D, Essalmani R, Hamelin J, Asselin M-C, Benjannet S, Chamberland A, Day R, Szumska D, Constam D, Bhattacharya S, Prat A, Seidah NG (2011) Furin Is the Major Processing Enzyme of the Cardiac-specific Growth Factor Bone Morphogenetic Protein 10. Journal of Biological Chemistry 286:22785-22794.

Takahashi JL, Giuliani F, Power C, Imai Y, Yong VW (2003) Interleukin-1β promotes oligodendrocyte death through glutamate excitotoxicity. Annals of Neurology 53:588-595.

Takami T, Oudega M, Bates ML, Wood PM, Kleitman N, Bunge MB (2002) Schwann Cell But Not Olfactory Ensheathing Glia Transplants Improve Hindlimb Locomotor Performance in the Moderately Contused Adult Rat Thoracic Spinal Cord. The Journal of Neuroscience 22:6670-6681.

Takase M, Imamura T, Sampath TK, Takeda K, Ichijo H, Miyazono K, Kawabata M (1998) Induction of Smad6 mRNA by Bone Morphogenetic Proteins. Biochemical and Biophysical Research Communications 244:26-29.

Talbott JF, Loy DN, Liu Y, Qiu MS, Bunge MB, Rao MS, Whittemore SR (2005) Endogenous Nkx2.2+/Olig2+ oligodendrocyte precursor cells fail to remyelinate the demyelinated adult rat spinal cord in the absence of astrocytes. Experimental Neurology 192:11-24.

Talbott JF, Cao Q, Enzmann GU, Benton RL, Achim V, Cheng XX, Mills MD, Rao MS, Whittemore SR (2006) Schwann Cell-Like Differentiation by Adult Oligodendrocyte Precursor Cells Following Engraftment into the Demyelinated Spinal Cord is BMP-Dependent. Glia 54:147.

Tang P, Hou H, Zhang L, Lan X, Mao Z, Liu D, He C, Du H, Zhang L (2014) Autophagy Reduces Neuronal Damage and Promotes Locomotor Recovery via Inhibition of Apoptosis After Spinal Cord Injury in Rats. Molecular Neurobiology 49:276-287.

Tang X, Davies JE, Davies SJA (2003) Changes in distribution, cell associations, and protein expression levels of NG2, neurocan, phosphacan, brevican, versican V2, and tenascin-C during acute to chronic maturation of spinal cord scar tissue. Journal of Neuroscience Research 71:427-444.

ten Dijke P, Yamashita H, Sampath TK, Reddi AH, Estevez M, Riddle DL, Ichijo H, Heldin CH, Miyazono K (1994) Identification of type I receptors for osteogenic protein-1 and bone morphogenetic protein-4. Journal of Biological Chemistry 269:16985-16988.

Tian XY, Yung LH, Wong WT, Liu J, Leung FP, Liu L, Chen Y, Kong SK, Kwan KM, Ng SM, Lai PBS, Yung LM, Yao X, Huang Y (2012) Bone morphogenic protein-4 induces endothelial cell apoptosis through oxidative stress-dependent p38MAPK and JNK pathway. Journal of Molecular and Cellular Cardiology 52:237-244.

Tozer S, Le Dréau G, Marti E, Briscoe J (2013) Temporal control of BMP signalling determines neuronal subtype identity in the dorsal neural tube. Development 140:1467-1474.

Tran AP, Warren PM, Silver J (2020) Regulation of autophagy by inhibitory CSPG interactions with receptor PTPσ and its impact on plasticity and regeneration after spinal cord injury. Experimental Neurology 328:113276.

Tran AP, Sundar S, Yu M, Lang BT, Silver J (2018) Modulation of receptor protein tyrosine phosphatase sigma increases chondroitin sulfate proteoglycan degradation through Cathepsin B secretion to enhance axon outgrowth. The Journal of Neuroscience.

Trousse F, Esteve P, Bovolenta P (2001) BMP4 Mediates Apoptotic Cell Death in the Developing Chick Eye. The Journal of Neuroscience 21:1292-1301.

Tsuji K, Bandyopadhyay A, Harfe BD, Cox K, Kakar S, Gerstenfeld L, Einhorn T, Tabin CJ, Rosen V (2006) BMP2 activity, although dispensable for bone formation, is required for the initiation of fracture healing. Nature Genetics 38:1424-1429.

Uemura MT, Ihara M, Maki T, Nakagomi T, Kaji S, Uemura K, Matsuyama T, Kalaria RN, Kinoshita A, Takahashi R (2018) Pericyte-derived bone morphogenetic protein 4 underlies white matter damage after chronic hypoperfusion. Brain Pathology 28:521-535.

Ughrin YM, Chen ZJ, Levine JM (2003) Multiple Regions of the NG2 Proteoglycan Inhibit Neurite Growth and Induce Growth Cone Collapse. The Journal of Neuroscience 23:175-186.

Ulanska-Poutanen J, Mieczkowski J, Zhao C, Konarzewska K, Kaza B, Pohl HBF, Bugajski L, Kaminska B, Franklin RJM, Zawadzka M (2018) Injury-induced perivascular niche supports alternative differentiation of adult rodent CNS progenitor cells. eLife 7:e30325.

Urist MR (1965) Bone: formation by autoinduction. Science (New York, NY) 150:893-899.

Urist MR, Strates BS (1971) Bone Morphogenetic Protein. Journal of Dental Research 50:1392-1406.

Urrutia P, Aguirre P, Esparza A, Tapia V, Mena NP, Arredondo M, González-Billault C, Núñez MT (2013) Inflammation alters the expression of DMT1, FPN1 and hepcidin, and it causes iron accumulation in central nervous system cells. Journal of Neurochemistry 126:541-549.

Vadon-Le Goff S, Hulmes DJS, Moali C (2015) BMP-1/tolloid-like proteinases synchronize matrix assembly with growth factor activation to promote morphogenesis and tissue remodeling. Matrix Biology 44-46:14-23.

Vaishnav RA, Singh IN, Miller DM, Hall ED (2010) Lipid peroxidation-derived reactive aldehydes directly and differentially impair spinal cord and brain mitochondrial function. Journal of neurotrauma 27:1311-1320.

Vanlangenakker N, Vanden Berghe T, Krysko DV, Festjens N, Vandenabeele P (2008) Molecular mechanisms and pathophysiology of necrotic cell death. Current molecular medicine 8:207-220.

Vaziri ND, Lee Y-S, Lin C-Y, Lin VW, Sindhu RK (2004) NAD(P)H oxidase, superoxide dismutase, catalase, glutathione peroxidase and nitric oxide synthase expression in subacute spinal cord injury. Brain Research 995:76-83.

Verschueren K, Remacle JE, Collart C, Kraft H, Baker BS, Tylzanowski P, Nelles L, Wuytens G, Su M-T, Bodmer R, Smith JC, Huylebroeck D (1999) SIP1, a Novel Zinc Finger/Homeodomain Repressor, Interacts with Smad Proteins and Binds to 5′-CACCT Sequences in Candidate Target Genes. Journal of Biological Chemistry 274:20489-20498.

Viñals F, Reiriz J, Ambrosio S, Bartrons R, Rosa JL, Ventura F (2004) BMP-2 decreases Mash1 stability by increasing Id1 expression. The EMBO Journal 23:3527-3537.

Visavadiya NP, Patel SP, VanRooyen JL, Sullivan PG, Rabchevsky AG (2016) Cellular and subcellular oxidative stress parameters following severe spinal cord injury. Redox Biology 8:59-67.

Viscomi MT, D'Amelio M, Cavallucci V, Latini L, Bisicchia E, Nazio F, Fanelli F, Maccarrone M, Moreno S, Cecconi F, Molinari M (2012) Stimulation of autophagy by rapamycin protects neurons from remote degeneration after acute focal brain damage. Autophagy 8:222-235.

Vue TY, Kim EJ, Parras CM, Guillemot F, Johnson JE (2014) Ascl1 controls the number and distribution of astrocytes and oligodendrocytes in the gray matter and white matter of the spinal cord. Development 141:3721-3731.

Walker CL, Walker MJ, Liu N-K, Risberg EC, Gao X, Chen J, Xu X-M (2012) Systemic Bisperoxovanadium Activates Akt/mTOR, Reduces Autophagy, and Enhances Recovery following Cervical Spinal Cord Injury. PLOS ONE 7:e30012.

Wang EA, Rosen V, Cordes P, Hewick RM, Kriz MJ, Luxenberg DP, Sibley BS, Wozney JM (1988) Purification and characterization of other distinct bone-inducing factors. Proceedings of the National Academy of Sciences 85:9484-9488.

Wang KC, Koprivica V, Kim JA, Sivasankaran R, Guo Y, Neve RL, He Z (2002) Oligodendrocyte-myelin glycoprotein is a Nogo receptor ligand that inhibits neurite outgrowth. Nature 417:941-944.

Wang P, Lin C, Wu S, Huang K, Wang Y, Bao X, Zhang F, Huang Z, Teng H (2018) Inhibition of Autophagy is Involved in the Protective Effects of Ginsenoside Rb1 on Spinal Cord Injury. Cellular and Molecular Neurobiology 38:679-690.

Wang RN, Green J, Wang Z, Deng Y, Qiao M, Peabody M, Zhang Q, Ye J, Yan Z, Denduluri S, Idowu O, Li M, Shen C, Hu A, Haydon RC, Kang R, Mok J, Lee MJ, Luu HL, Shi LL (2014a) Bone Morphogenetic Protein (BMP) signaling in development and human diseases. Genes & Diseases 1:87-105.

Wang X, Xu J-M, Wang Y-P, Yang L, Li Z-J (2016) Protective effects of BMP-7 against tumor necrosis factor α-induced oligodendrocyte apoptosis. International Journal of Developmental Neuroscience 53:10-17.

Wang X, Cao K, Sun X, Chen Y, Duan Z, Sun L (2015) Macrophages in spinal cord injury: phenotypic and functional change from exposure to myelin debris. Glia 63.

Wang X, de Rivero Vaccari JP, Wang H, Diaz P, German R, Marcillo AE, Keane RW (2012) Activation of the nuclear factor E2-related factor 2/antioxidant response element pathway is neuroprotective after spinal cord injury. Journal of neurotrauma 29:936-945.

Wang X, Arcuino G, Takano T, Lin J, Peng WG, Wan P, Li P, Xu Q, Liu QS, Goldman SA, Nedergaard M (2004) P2X7 receptor inhibition improves recovery after spinal cord injury. Nature Medicine 10:821-827.

Wang Y, Cheng X, He Q, Zheng Y, Kim DH, Whittemore SR, Cao QL (2011) Astrocytes from the Contused Spinal Cord Inhibit Oligodendrocyte Differentiation of Adult Oligodendrocyte Precursor Cells by Increasing the Expression of Bone Morphogenetic Proteins. The Journal of Neuroscience 31:6053-6058.

Wang Z-Y, Lin J-H, Muharram A, Liu W-G (2014b) Beclin-1-mediated autophagy protects spinal cord neurons against mechanical injury-induced apoptosis. Apoptosis 19:933-945.

Wanner IB, Anderson MA, Song B, Levine J, Fernandez A, Gray-Thompson Z, Ao Y, Sofroniew MV (2013) Glial Scar Borders Are Formed by Newly Proliferated, Elongated Astrocytes That Interact to Corral Inflammatory and Fibrotic Cells via STAT3-Dependent Mechanisms after Spinal Cord Injury. The Journal of Neuroscience 33:12870-12886.

Wardle FC, Welch JV, Dale L (1999) Bone morphogenetic protein 1 regulates dorsal-ventral patterning in early Xenopus embryos by degrading chordin, a BMP4 antagonist. Mechanisms of Development 86:75-85.

Weible MW, Tailoi C-L (2007) Phenotypic characterization of neural stem cells from human fetal spinal cord: Synergistic effect of LIF and BMP4 to generate astrocytes. Glia 55:1156-1168.

Weiss S, Dunne C, Hewson J, Wohl C, Wheatley M, Peterson AC, Reynolds BA (1996) Multipotent CNS Stem Cells Are Present in the Adult Mammalian Spinal Cord and Ventricular Neuroaxis. The Journal of Neuroscience 16:7599-7609.

Weng Q, Chen Y, Wang H, Xu X, Yang B, He Q, Shou W, Chen Y, Higashi Y, van den Berghe V, Seuntjens E, Kernie Steven G, Bukshpun P, Sherr Elliott H, Huylebroeck D, Lu QR (2012) Dual-Mode Modulation of Smad Signaling by Smad-Interacting Protein Sip1 Is Required for Myelination in the Central Nervous System. Neuron 73:713-728.

Williams R, Murray A (2015) Prevalence of Depression After Spinal Cord Injury: A Meta-Analysis. Archives of Physical Medicine and Rehabilitation 96:133-140.

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

Wislet-Gendebien S, Bruyère F, Hans G, Leprince P, Moonen G, Rogister B (2004) Nestin-positive mesenchymal stem cells favour the astroglial lineage in neural progenitors and stem cells by releasing active BMP4. BMC Neuroscience 5:33.

Wong CM, Zhang Y, Huang Y (2014) Bone morphogenic protein-4-induced oxidant signaling via protein carbonylation for endothelial dysfunction. Free Radical Biology and Medicine 75:178-190.

Wong CM, Cheema AK, Zhang L, Suzuki YJ (2008) Protein carbonylation as a novel mechanism in redox signaling. Circulation research 102:310-318.

Wong WT, Tian XY, Chen Y, Leung FP, Liu L, Lee HK, Ng CF, Xu A, Yao X, Vanhoutte PM, Tipoe GL, Huang Y (2010) Bone Morphogenic Protein-4 Impairs Endothelial Function Through Oxidative Stress-Dependent Cyclooxygenase-2 Upregulation. Circulation Research 107:984-991.

Wozney J, Rosen V, Celeste A, Mitsock L, Whitters M, Kriz R, Hewick R, Wang E (1988) Novel regulators of bone formation: molecular clones and activities. Science 242:1528-1534.

Wozniewicz B, Filipowicz K, Swiderska SK, Deraka K (1983) Pathophysiological mechanism of traumatic cavitation of the spinal cord. Spinal Cord 21:312-317.

Xiang Y, Molloy SS, Thomas L, Thomas G (2000) The PC6B Cytoplasmic Domain Contains Two Acidic Clusters That Direct Sorting to Distinct trans-Golgi Network/Endosomal Compartments. Molecular Biology of the Cell 11:1257-1273.

Xiao Q, Du Y, Wu W, Yip HK (2010) Bone morphogenetic proteins mediate cellular response and, together with Noggin, regulate astrocyte differentiation after spinal cord injury. Experimental Neurology 221:353-366.

Xu B, Park D, Ohtake Y, Li H, Hayat U, Liu J, Selzer ME, Longo FM, Li S (2015) Role of CSPG receptor LAR phosphatase in restricting axon regeneration after CNS injury. Neurobiology of Disease 73:36-48.

Xu W, Chi L, Xu R, Ke Y, Luo C, Cai J, Qiu M, Gozal D, Liu R (2005) Increased production of reactive oxygen species contributes to motor neuron death in a compression mouse model of spinal cord injury. Spinal Cord 43:204-213.

Yabe T, Samuels I, Schwartz JP (2002) Bone morphogenetic proteins BMP-6 and BMP-7 have differential effects on survival and neurite outgrowth of cerebellar granule cell neurons. Journal of Neuroscience Research 68:161-168.

Yamashita K, Mikawa S, Sato K (2016) BMP3 expression in the adult rat CNS. Brain Research 1643:35-50.

Yan Y, Gong P, Jin W, Xu J, Wu X, Xu T, Hang Q, Fu H, Kei K, Gao Y (2012) The cell-specific upregulation of bone morphogenetic protein-10 (BMP-10) in a model of rat cortical brain injury. Journal of Molecular Histology 43:543-552.

Yanagisawa M, Takizawa T, Ochiai W, Uemura A, Nakashima K, Taga T (2001) Fate alteration of neuroepithelial cells from neurogenesis to astrocytogenesis by bone morphogenetic proteins. Neuroscience Research 41:391-396.

Yang L, Jones NR, Blumbergs PC, Van Den Heuvel C, Moore EJ, Manavis J, Sarvestani GT, Ghabriel MN (2005) Severity-dependent expression of pro-inflammatory cytokines in traumatic spinal cord injury in the rat. Journal of Clinical Neuroscience 12:276-284.

Yang Z, Suzuki R, Daniels SB, Brunquell CB, Sala CJ, Nishiyama A (2006) NG2 Glial Cells Provide a Favorable Substrate for Growing Axons. The Journal of Neuroscience 26:3829-3839.

Yasuda A, Tsuji O, Shibata S, Nori S, Takano M, Kobayashi Y, Takahashi Y, Fujiyoshi K, Hara CM, Miyawaki A, Okano HJ, Toyama Y, Nakamura M, Okano H (2011) Significance of Remyelination by Neural Stem/Progenitor Cells Transplanted into the Injured Spinal Cord. STEM CELLS 29:1983-1994.

Yokouchi Y, Sakiyama J, Kameda T, Iba H, Suzuki A, Ueno N, Kuroiwa A (1996) BMP-2/-4 mediate programmed cell death in chicken limb buds. Development 122:3725-3734.

Yong C, Arnold PM, Zoubine MN, Citron BA, Watanabe I, Berman NE, Festoff BW (1998) Apoptosis in cellular compartments of rat spinal cord after severe contusion injury. Journal of neurotrauma 15:459-472.

Yoshida Y, von Bubnoff A, Ikematsu N, Blitz IL, Tsuzuku JK, Yoshida EH, Umemori H, Miyazono K, Yamamoto T, Cho KWY (2003) Tob proteins enhance inhibitory Smad-receptor interactions to repress BMP signaling. Mechanisms of Development 120:629-637.

You L-H, Yan C-Z, Zheng B-J, Ci Y-Z, Chang S-Y, Yu P, Gao G-F, Li H-Y, Dong T-Y, Chang Y-Z (2017) Astrocyte hepcidin is a key factor in LPS-induced neuronal apoptosis. Cell Death &Amp; Disease 8:e2676.

Yu PB, Deng DY, Lai CS, Hong CC, Cuny GD, Bouxsein ML, Hong DW, McManus PM, Katagiri T, Sachidanandan C, Kamiya N, Fukuda T, Mishina Y, Peterson RT, Bloch KD (2008a) BMP type I receptor inhibition reduces heterotopic ossification. Nature Medicine 14:1363-1369.

Yu PB, Deng DY, Lai CS, Hong CC, Cuny GD, Bouxsein ML, Hong DW, McManus PM, Katagiri T, Sachidanandan C, Kamiya N, Fukuda T, Mishina Y, Peterson RT, Bloch KD (2008b) BMP type I receptor inhibition reduces heterotopic ossification. Nature Medicine 14:1363.

Zai LJ, Yoo S, Wrathall JR (2005) Increased growth factor expression and cell proliferation after contusive spinal cord injury. Brain Research 1052:147-155.

Zamanian JL, Xu L, Foo LC, Nouri N, Zhou L, Giffard RG, Barres BA (2012) Genomic Analysis of Reactive Astrogliosis. The Journal of Neuroscience 32:6391-6410.

Zarruk JG, Berard JL, Passos dos Santos R, Kroner A, Lee J, Arosio P, David S (2015) Expression of iron homeostasis proteins in the spinal cord in experimental autoimmune encephalomyelitis and their implications for iron accumulation. Neurobiology of Disease 81:93-107.

Zawadzka M, Rivers LE, Fancy SPJ, Zhao C, Tripathi R, Jamen F, Young K, Goncharevich A, Pohl H, Rizzi M, Rowitch DH, Kessaris N, Suter U, Richardson WD, Franklin RJM (2010) CNS-Resident Glial Progenitor/Stem Cells Produce Schwann Cells as well as Oligodendrocytes during Repair of CNS Demyelination. Cell Stem Cell 6:578-590.

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

Zhang L, He X, Liu L, Jiang M, Zhao C, Wang H, He D, Zheng T, Zhou X, Hassan A, Ma Z, Xin M, Sun Z, Lazar Mitchell A, Goldman Steven A, Olson Eric N, Lu QR (2016) Hdac3 Interaction with p300 Histone Acetyltransferase Regulates the Oligodendrocyte and Astrocyte Lineage Fate Switch. Developmental Cell 36:316-330.

Zhang M, Wang S, Mao L, Leak RK, Shi Y, Zhang W, Hu X, Sun B, Cao G, Gao Y, Xu Y, Chen J, Zhang F (2014) Omega-3 Fatty Acids Protect the Brain against Ischemic Injury by Activating Nrf2 and Upregulating Heme Oxygenase 1. The Journal of Neuroscience 34:1903-1915.

Zhang Y, Yeh L-K, Zhang S, Call M, Yuan Y, Yasunaga M, Kao WW-Y, Liu C-Y (2015) Wnt/β-catenin signaling modulates corneal epithelium stratification via inhibition of Bmp4 during mouse development. Development 142:3383-3393.

Zhang YW, Denham J, Thies RS (2006a) Oligodendrocyte progenitor cells derived from human embryonic stem cells express neurotrophic factors. Stem cells and development 15:943-952.

Zhang Z, Trautmann K, Artelt M, Burnet M, Schluesener HJ (2006b) Bone morphogenetic protein-6 is expressed early by activated astrocytes in lesions of rat traumatic brain injury. Neuroscience 138:47-53.

Zhao G-Q (2003) Consequences of knocking out BMP signaling in the mouse. genesis 35:43-56.

Zheng M, Seidah NG, Pintar JE (1997) The developmental expression in the rat CNS and peripheral tissues of proteases PC5 and PACE4 mRNAs: comparison with other proprotein processing enzymes. Developmental biology 181:268-283.

Zheng M, Streck R, Scott R, Seidah N, Pintar J (1994) The developmental expression in rat of proteases furin, PC1, PC2, and carboxypeptidase E: implications for early maturation of proteolytic processing capacity. The Journal of Neuroscience 14:4656-4673.

Zhou X, Spittau B, Krieglstein K (2012) TGFβ signalling plays an important role in IL4-induced alternative activation of microglia. Journal of Neuroinflammation 9:210.

Zhou Y-F, Zhang C, Yang G, Qian Z-M, Zhang M-W, Ma J, Zhang F-L, Ke Y (2017) Hepcidin Protects Neuron from Hemin-Mediated Injury by Reducing Iron. Frontiers in Physiology 8.

Zhou Z, Peng X, Insolera R, Fink DJ, Mata M (2009) IL-10 promotes neuronal survival following spinal cord injury. Experimental Neurology 220:183-190.

Zhou Z, Xie J, Lee D, Liu Y, Jung J, Zhou L, Xiong S, Mei L, Xiong W-C (2010) Neogenin Regulation of BMP-Induced Canonical Smad Signaling and Endochondral Bone Formation. Developmental Cell 19:90-102.

Zhu N-L, Li C, Huang HH, Sebald M, Londhe VA, Heisterkamp N, Warburton D, Bellusci S, Minoo P (2007) TNF-alpha represses transcription of human Bone Morphogenetic Protein-4 in lung epithelial cells. Gene 393:70-80.

Zhu Y, Soderblom C, Trojanowsky M, Lee D-H, Lee JK (2015a) Fibronectin matrix assembly after spinal cord injury. Journal of neurotrauma 32:1158-1167.

Zhu Y, Soderblom C, Krishnan V, Ashbaugh J, Bethea JR, Lee JK (2015b) Hematogenous macrophage depletion reduces the fibrotic scar and increases axonal growth after spinal cord injury. Neurobiology of Disease 74:114-125.

Zimmerman LB, De Jesús-Escobar JM, Harland RM (1996) The Spemann Organizer Signal noggin Binds and Inactivates Bone Morphogenetic Protein 4. Cell 86:599-606.