

REVIEW ARTICLE

Exploring Optic Nerve Axon Regeneration

Hong-Jiang Li, Zhao-Liang Sun, Xi-Tao Yang, Liang Zhu and Dong-Fu Feng*

Department of Neurosurgery, No.9 People's Hospital, Shanghai Jiaotong University, School of Medicine, Shanghai, 201999, China

Abstract: Background: Traumatic optic nerve injury is a leading cause of irreversible blindness across the world and causes progressive visual impairment attributed to the dysfunction and death of retinal ganglion cells (RGCs). To date, neither pharmacological nor surgical interventions are sufficient to halt or reverse the progress of visual loss. Axon regeneration is critical for functional recovery of vision following optic nerve injury. After optic nerve injury, RGC axons usually fail to regrow and die, leading to the death of the RGCs and subsequently inducing the functional loss of vision. However, the detailed molecular mechanisms underlying axon regeneration after optic nerve injury remain poorly understood.

Methods: Research content related to the detailed molecular mechanisms underlying axon regeneration after optic nerve injury have been reviewed.

Results: The present review provides an overview of regarding potential strategies for axonal regeneration of RGCs and optic nerve repair, focusing on the role of cytokines and their downstream signaling pathways involved in intrinsic growth program and the inhibitory environment together with axon guidance cues for correct axon guidance. A more complete understanding of the factors limiting axonal regeneration will provide a rational basis, which contributes to develop improved treatments for optic nerve regeneration. These findings are encouraging and open the possibility that clinically meaningful regeneration may become achievable in the future.

Conclusion: Combination of treatments towards overcoming growth-inhibitory molecules and enhancing intrinsic growth capacity combined with correct guidance using axon guidance cues is crucial for developing promising therapies to promote axon regeneration and functional recovery after ON injury.

Keywords: Optic nerve, axon regeneration, factors, intrinsic ability, extracellular environment, guidance cues.

1. INTRODUCTION

In contrast to the peripheral nervous system (PNS), which shows a remarkable capacity for regenerative processes, where severed axons often will heal and successfully navigate back to their original targets, the adult mammalian central nervous system (CNS) is extremely limited [1]. One major difference between the CNS and PNS is the glial environment. Whereas peripheral nerve schwann cells are permissive substrate for axon regenerative outgrowth due to their secretion of neurotrophins and lack of associated inhibitory proteins, oligodendrocytes and reactive astrocytes in CNS are inhibitory to axon regeneration, expressing many inhibitory factors [2]. Moreover, the elimination capacity of axon fragments and myelin debris in the PNS after injury is much more efficient than that in the CNS, probably explained by the involvement of different cellular molecules as well as differential access to the immune system [3].

Retinal ganglion cells (RGCs) are a population of CNS neurons located in the innermost layer of the retina and

convey visual signals from the retina along their axons to the brain. Following axotomy, massive retinal ganglion cell (RGC) loss (85%-90%) occurs within the first 2-3 weeks after optic nerve (ON) injury, effectively severing neuronal connections with the contralateral lateral geniculate nucleus and superior colliculus [4-6]. Axonal injury leads to the functional loss of RGCs and subsequently induces death of the neurons. Axon growth is essential for the restoration of neuronal connectivity as well as for the reestablishment of a functional visual system after ON injury. Achieving axonal regeneration with the recovery of function would truly be an extraordinary achievement. Similar to other CNS axons, the ON has a very limited regenerative capacity. To date, no therapeutic treatment has been effective in substantially stimulating long-distance axonal regeneration and functionally repairing axonal connections in the visual pathway. However, studies during the past two decades have shown that under certain circumstances, mature RGCs can transform into an active regenerative state, enabling these neurons to survive and to regenerate axons over long distances in the injured ON and into their correct target zones, resulting in partial recovery of vision. Important signalling pathways and molecules either limiting or facilitating axon regeneration have been identified, potentially providing novel therapeutic targets. It has become clear that stronger regeneration can be

*Address correspondence to this author at the Department of Neurosurgery, No.9 People's Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, 201999, China; Tel: +86 021-56691101; Fax: +86 021-56693614; E-mail: feng_df@yahoo.com

achieved through combinatorial treatments, in which the inhibitory environment of the glial scar and of the myelin in the ON is overcome, the intrinsic growth program is activated, and correct guidance is provided by axon guidance cues, than through each single treatment alone. In this review, we aim to provide an updated overview of the cellular and molecular mechanisms limiting axon regeneration in the mammalian visual system after ON injury.

2. OPTIC NERVE INJURY MODEL FOR STUDYING AXON REGENERATION

The optic nerve crush (ONC) model, a widely used experimental model for traumatic ON injury, has the strongest resemblance to the conditions produced by ON trauma. In this model, ONs are clipped 2 mm posterior to the globe for 9 s [7], 10 s [8], 30 s [9], or 60 s [10], with different clamping forces according to different methods. In addition to the ONC model, the ON transection or axotomy model is another widely used injury model. These models make the visual system ideal for studying regenerative failure because partial or complete damage of the ON blocks the connection between RGCs and their central targets and partially or completely severs all axons into proximal and distal segments [11]. Regenerating axons can be traced by anterogradely transported molecules, such as cholera toxin B after intravitreal injection [12-14]. Surviving RGCs after ONC can be evaluated by retrogradely transported Fluorogold, which is delivered into the region of the superior colliculus [15, 16]. Moreover, damaged axons can be visualized by staining with antibodies against growth-associated protein 43 (GAP-43), which is highly expressed during axon regeneration after injury. Potential axon growth-promoting compounds can either be injected into the vitreous chamber or subretinal space or be directly applied at the lesion of the ON [17]. These different routes of application can also be exploited to test factors for potential effects on the somata or the growth cones of RGCs *in vivo*. Another beneficial characteristic of the visual system as a model in axon regeneration is the opportunity to transduce RGCs specifically and efficiently by using adeno-associated viruses and to study the effect of specific proteins or signalling pathways on axon regeneration. Furthermore, viral expression of Cre recombinase in RGCs can be applied to knockout specific genes [12, 13]. The favourable characteristics of the visual system have made the ON a popular and established paradigm for exploring the mechanisms underlying regenerative failure, and several encouraging findings with general relevance have been made in this model system. In addition, ON injury model is a classical model for neurodegeneration. It induces RGC death and regenerative failure, a process also occurring in glaucoma and other optic neurodegeneration. Moreover, ON injury model is also a good paradigm in which to explore the mechanisms underlying regenerative failure after brain injuries.

3. SPATIAL AND TEMPORAL CHARACTERISTICS OF REGENERATION OF AXONS AFTER OPTIC NERVE INJURY

RGCs are normally unable to regenerate axons after ON injury [18]. However, with adequate stimulation, some mature RGCs can transform into a robust regenerative state,

enabling RGCs to survive and to regenerate axons over long distances in the injured ON [1]. In response to ON injury, the damage-associated signals are retrogradely transported to the RGC body, and only <10% of RGCs become hypertrophic [19]. The hypertrophic ganglion cells initiate a molecular event and motivate a growth program resulting in regeneration [19]. The successful ON regeneration process in non-mammalian species, such as fish, takes more than 4 months after injury and comprises four stages. The first stage in fish is the neurite-sprouting period (1-4 days), which is thought to consist of the degeneration of distal ON axons and the new synthesis of RNAs and proteins for neurite sprouting. The second stage is the axonal elongation period (5-30 days), which is considered to include elongation of the axon towards the tectum and the formation of synaptic connections between regrowing axons and tectorial neurons. The third stage is the synaptic refinement period (35-80 days), which is considered to include synaptic refinement of ON axons with tectal neurons, relying on attractive or repulsive guidance cues for retino-tectal connections. The last stage is the functional recovery period (100-120 days), which includes the complete restoration of visual function and obviously requires reinnervation of the relevant targets with sufficient numbers of the appropriate types of retinal axons [19-22].

Similar to non-mammalian species, ON axon regeneration in mammals roughly comprises four analogous stages; however, the regeneration process is more complicated, more difficult and more time consuming and, worse, is unsatisfactory. In response to ON injury in mice, massive axonal sprouting forms a dense plexus of neurites at the inner surface of the retina [23]. Axons are observed to undergo transient growth responses to injury, so-called 'abortive regeneration'. Sprouting of injured axons occurs as early as 14 h post-lesion with an average growth rate of 20 $\mu\text{m}/\text{day}$, continuing until 10 days post-injury at the lesion site in the adult *albino mouse*. Then, the overall growth rate declines over time, presumably once regenerated sprouts begin to degenerate. Without any treatment, the few axons that spontaneously extend short distances beyond the lesion site are mostly unbranched and occasionally form U-turns. Ideal regeneration needs to regenerate axons over long distances to reinnervate their proper target areas, form synapses in a topographically organized fashion, and restore function. However, the aberrant sprouting in the retina is noneffective regeneration. Many aberrant sprouting fibers return toward the lesion site in the optic nerve, even towards the retina [24]. Some fibers were even found to turn at the optic chiasm and project into the contralateral optic nerve, growing toward the contralateral retina [25]. To convert such abortive local events to sustained axon extension, a set of injury signals generated locally must be retrogradely transported to the cell body and must initiate injury responses. However, not all axons show this abortive response; unfasciculated axons continue in a random growth pattern until at least 100 days post-lesion but do not show long-range growth [26]. After 1 month post-injury in mouse, fewer than 120 axons per ON reach the optic chiasm, which represents only approximately 0.2% of all axons based on the high number of regrowing axons in the ON (assuming a total of 60,000 RGCs) [25]. Regenerating RGC axons are located in the optic chiasm and on both sides of the optic

tract. Small but nearly equal numbers of axons are observed at short distances beyond the chiasm ipsi- and contralaterally after 6 weeks [27], and regenerating fibres cross the midline of the chiasm within 10 weeks of ON injury [28], indicating that in response to guidance signals, many of the ipsilaterally projecting axons that enter early are subsequently withdrawn. Then, the vast majority of the axons crossing the chiasm innervate the ipsilateral side of the hypothalamus, including ectopic regions, instead of bilaterally, and they largely avoid the core region of the suprachiasmatic nucleus [23, 25]. At approximately 2 months post-injury, regenerating axons converge onto suprachiasmatic nucleus neurons that connect with existing brain circuitry and reform glutamatergic excitatory synapses [29], and the growth pattern of the regenerating axons changes continuously for up to 4 months after injury [23]. Functional recovery of vision requires that damaged axons regenerate into the lateral geniculate nucleus and the superior colliculus, a distance of more than 8 mm from the ON lesion [24]. However, during the process of axonal projection, regenerating axons project into the brain ectopically, and many regenerating axons undergo one or several sharp U-turns along their course. In this case, the main impediments to axonal growth seem to be axonal misguidance and the weak intrinsic growth capacity of adult RGCs [30]. For successful regeneration, the cell bodies of injured RGCs must acquire accurate and timely information regarding the site and extent of the axonal damage and then initiate a transcription program to enhance their intrinsic growth ability [31].

As mentioned above, axon regeneration after ONC is a complex process that exhibits spatial and temporal characteristics. It differs depending on the zone and severity of the injury as well as during the different phases of the response. The achievable development of strategies for ON regeneration requires the consideration of these factors.

4. MECHANISMS OF RGC AXON REGENERATION

RGCs of adult mammals normally fail to switch into an active regenerative state after injury, and only a few dissociated neurons can extend axons or regrow into the growth-permissive environment of sciatic nerve grafts transplanted adjacent to the proximal end of the ON [32-34].

Regenerative failure has been mainly attributed to the growth-inhibitory environment of the ON and to the insufficient intrinsic ability of mature RGCs to regrow axons. Recent studies have also shown axon navigation defects in the ON and at the optic chiasm under conditions of strong growth stimulation. Counteracting or removing the external inhibitory molecules results in incomplete axon regeneration *in vivo*, emphasizing the necessity of better understanding the cell-intrinsic mechanisms regulating axon growth after injury [35]. Inactivation of the external inhibitory molecules is insufficient for long-distance axon regeneration, as demonstrated in studies on functional interference with chondroitin sulfate proteoglycans (CSPGs) or myelin-based inhibitors [36]. In addition, the optic nerve has shown axon misguidance even accompanied by misrouting at the optic chiasm and by aberrant sprouting in the retina, which thoroughly blocks axon regeneration. Thus, successful regeneration of the ON requires simultaneously overcoming inhibitory signalling and activating the intrinsic ability of mature RGCs to regrow axons with correct axon guidance.

4.1. Intrinsic Ability of Mature RGCs to Regrow Axons

Evidence from multiple studies is accumulating to support the viewpoint that the weak intrinsic regenerative ability of mature neurons is a major impediment to axon regeneration (Fig. 1). Enhancing the intrinsic growth capacity of adult RGCs to reactivate a growth program after ON injury is proposed to be an important strategy for axonal regrowth.

4.1.1. Intrinsic Regulatory Factors for Promoting Axon Regeneration

4.1.1.1. mTOR

After ON injury, elevated expression levels of ciliary neurotrophic factor (CNTF) and leukemia inhibitory factor (LIF) in RGCs activate the phosphoinositide-3-kinase/activated protein kinase (PI3K/Akt) signalling pathway. A downstream signalling target of Akt is mammalian target of rapamycin (mTOR), which controls protein translation *via* E4-binding protein 1 (E4-BP1) and can be specifically blocked by rapamycin [36]. mTOR is rapidly and persistently suppressed in injured RGCs in wild-type mice, and its

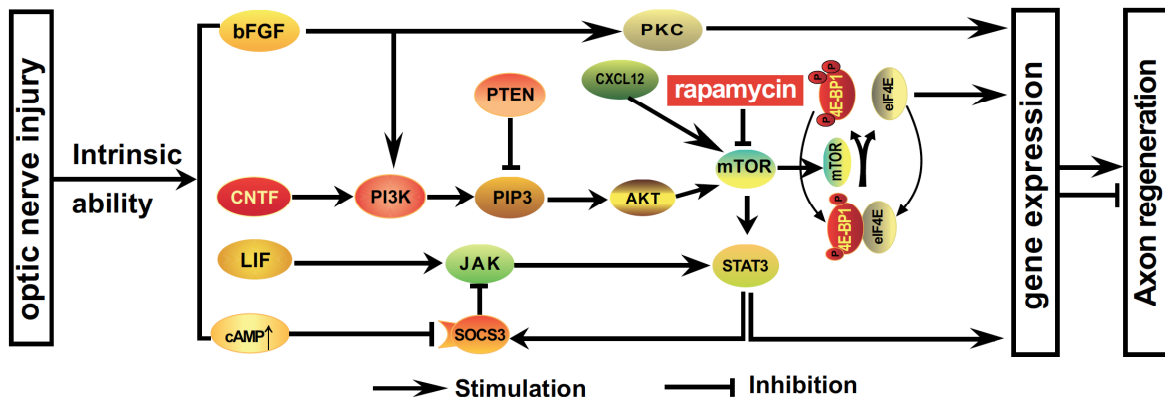


Fig. (1). Intrinsic signaling pathways for axon regeneration towards optic nerve injury. Schematic drawing depicting regulatory factors and their downstream signaling pathways involved in intrinsic growth program in the axon regeneration following optic nerve injury.

expression level is highly correlated with the extent and time course of ON regeneration. Activation of the mTOR pathway seems to potently enhance neuroprotection and axon regeneration [12]. Activation of mTOR signalling in axotomized retinal ganglion cells promotes axonal growth over several millimetres, with some axons reaching the optic chiasm, the midpoint of the visual pathway [24]. However, the effects of mTOR on CNTF and inflammatory stimulation (IS)-mediated axon regeneration of RGCs are more complex. Although CNTF treatment and IS block the downregulation of mTOR activity in RGCs in a PI3K-dependent manner, inhibiting mTOR activity by rapamycin does not prevent the outgrowth of CNTF-induced neurites in culture, the transformation of RGCs into a regenerative state, or the neuroprotective effects of IS *in vivo*. Rapamycin treatment generates mainly long-distance rather than short-distance axon regeneration in the ON following IS [37], suggesting that mTOR activity is significant for maintaining RGCs in the regenerative state. Moreover, mTOR activity is involved in mediating disinhibitory effects, as has been previously shown for the PI3K/AKT axis [38]. Neurite outgrowth of cultured RGCs on a growth-permissive substrate is not reduced by rapamycin, but mTOR inhibition markedly reduces growth on inhibitory substrates containing myelin or CSPGs, indicating that mTOR is downstream of specific inhibitory receptors and may affect a common converging point [37]. Similarly, upregulation of mTOR pathway is sufficient to promote axon regeneration in the adult CNS after brain injuries [12]. The mTOR pathway contributes to the protective effects of ischemic postconditioning against stroke [39].

4.1.1.2. bFGF

Basic fibroblast growth factor (bFGF, also termed FGF-2) has been isolated in relatively high concentrations from the brain and retina [40] and, in the visual system, has been implicated as a trophic factor that protects against injury and stimulates regeneration [41-43]. It has been demonstrated that, in embryonic *Xenopus*, elevating the level of bFGF in the optic tract stimulates RGC axonal elongation [44, 45], and the mechanisms underlying this effect appear to involve the diacylglycerol lipase and Ca²⁺/calmodulin kinase II pathway [46]. The lower levels of bFGF in the tectum may signal the growth cones, *via* the FGF receptor tyrosine kinase, to slow down and enter their target region [44]. bFGF accelerates the growth of early regenerating axons in peripheral nerves [47]. Therefore, one possible explanation for this phenomenon is that the application of bFGF to the cut ON stump accelerates axonal growth. More rapid growth through the distal stump allows the axons to reinnervate their targets sooner and to regain their supply of target-derived neurotrophic factors, thus reducing cell death. After axotomy, bFGF mRNA levels increase sevenfold, the level of GAP-43 protein significantly increases, and the upregulation of GAP-43 is sustained through the period during which retinal axons reconnect with their targets in the tectum. The application of bFGF to the injured nerve but not to the eyeball increases GAP-43 mRNA levels in the retina but decreases both GAP-43 protein levels and the number of immunopositive cell bodies [43]. In the tectum, bFGF application to the axotomized ON increases GAP-43 protein in regenerating retinal

projections [43]. These results suggest that bFGF upregulates the synthesis and alters the distribution of the axonal growth-promoting protein GAP-43, indicating that bFGF may promote axon regeneration.

4.1.1.3. CXCL12/SDF-1

CXC chemokine ligand-12 (CXCL12), also called stromal-derived factor 1 (SDF-1), was first defined as a stimulatory factor for B-lymphocyte precursor cells [48]. It has recently been demonstrated that CXCL12 is a moderate neurite growth-promoting factor for mature RGCs, exerts disinhibitory effects towards myelin and facilitates axon regeneration in the ON [8, 49]. Furthermore, the neurite growth-promoting and disinhibitory effects of CXCL12 are blocked by a specific antagonist of its receptor, CXCR4 and by inhibition of the PI3K/AKT/mTOR signalling pathway but not the Janus kinase/Signal transducer and activator of transcription (JAK/STAT3) pathway [12]. Intravitreal application of CXCL12 sustains mTOR activity in RGCs upon ON injury and moderately stimulates axon regeneration in the ON without affecting RGC survival [37]. In addition, intravitreal application of CXCL12 significantly promotes IS-triggered axon regeneration *in vivo*. Co-treatment of RGCs with CXCL12 promotes CNTF-stimulated neurite growth of RGCs on myelin but not on neurocan. The promotion of neurite growth by CXCL12 is moderate compared to that induced by CNTF but is significantly stronger on myelin [8], suggesting that the disinhibitory effect of CXCL12 towards myelin may facilitate ON regeneration. Promoting role of CXCL12 on axon regeneration is ahead and more comprehensively explored by using spinal cord injury (SCI) model. Several studies have demonstrated that CXCL12 is an interesting compound for regenerative medicine for its ability to attract different kinds of stem/progenitor cells and promote axon growth after SCI [50]. The CXCL12:CXCR4 pathway regulates homing of engrafted stem cells to sites of tissue damage within the spinal cord [51], which improved recovery processes in SCI. Local infusion of SDF-1 into the injured spinal cord resulted in enhanced sprouting of the CST axons [52, 53], possibly through counteracting myelin inhibitors, suggesting that the ability of SDF-1 to neutralize myelin inhibition is highly desirable for SCI.

4.1.2. Intrinsic Regulatory Factors for Inhibiting Axon Regeneration

4.1.2.1. SOCS3

Suppressor of cytokine signalling 3 (SOCS3) has been studied as a critical negative regulatory factor in regulating intrinsic growth capacity of RGC neurons. Pharmacological inhibition of JAK by AG490 blocks CNTF-induced neurite outgrowth from mature RGCs in culture and significantly reduces the regenerative response of RGCs to IS *in vivo*, suggesting that the JAK/STAT3 pathway plays significant roles in initiating axonal growth [32, 54]. SOCS3, whose upregulation is detected in mature axotomized RGCs after IS [55], acts as a critical feedback suppressor of the JAK/STAT3 signalling pathway through binding to JAK and/or specific phosphotyrosine residues on cytokine receptors and is crucial for tight control of the strength and duration of signalling in this pathway [56, 57]. Thus, elevated expression of SOCS3 blocks the JAK/STAT3 pathway and thereby

limits some of the physiological consequences of STAT3-mediated signalling [58, 59]. Consistently, viral over-expression of SOCS3 in RGCs markedly compromises the promotion of axon growth by intravitreally applied recombinant CNTF [60, 61]. In addition, conditional knockout of SOCS3 in RGCs allows regenerated axons to cross the lesion site of injured optic nerves and enhances the effects elicited by intravitreal injection of recombinant CNTF, indicating that SOCS3 functions as an intrinsic inhibitor for CNTF-mediated regeneration [13, 62]. In SOCS3-deleted mice, the additional knockout of gp130 significantly slows axon regeneration, suggesting that axon regeneration induced by SOCS3 deletion is largely dependent on gp130-mediated signalling [13]. SOCS3 expression is suppressed by cAMP elevation [63], which may account for the ability of cAMP-elevating drugs to enhance CNTF- and IS-induced axon regeneration [27, 32, 64]. SOCS3 also negatively regulates JAK/STAT3 pathway [65] and is regarded as a common target for neuronal protection and axon regeneration after SCI [66]. Although SOCS3 was earlier verified as a key negative regulator of reactive astrocytes in the healing process for SCI [67], researches about the effect of SOCS3 in understanding CNS axon regeneration by SCI model is behind in time compared to ONI.

4.1.2.2. PTEN

Phosphatase and tensin homologue (PTEN) functions as a negative regulator of cellular growth, as evidenced by its designation as a tumour suppressor gene [68, 69]. PTEN counteracts the activation of AKT by catalysing the conversion of PIP3 to PIP2 and thereby negatively regulates the PI3K/AKT pathway. Studies have demonstrated that PTEN, a key downstream target of Nedd4, plays an important role in regulating RGC terminal arborization *in vivo* [70]. Additionally, the genetic deletion of PTEN is reportedly neuroprotective and potently promotes RGC axon regeneration, and the stimulatory effects of PTEN deletion on axon growth are blocked by inhibiting mTOR [12, 62]. In PTEN-knockout mice, numerous sprouts grow out of the interrupted ON fibres and elongate to variable distances in a range of 0.5-4 mm in the ON; furthermore, the co-deletion of SOCS3 and PTEN has synergistic effects on the number and length of regenerating axons [12, 62]. Combining PTEN gene deletion with the application of zymosan, which induces inflammatory responses, and of cAMP permits ON fibres to regrow to their full length to innervate visual brain areas, including the dorsal lateral geniculate nucleus and superior colliculus. Consistently, PTEN depletion in mice in combination with IS allowed some axons to regenerate over approximately 5 mm through the whole ON and to reach their central targets in the brain [27]. Inflammatory stimulation, cAMP elevation and PTEN knockout have a synergistic effect on the promotion of axon regeneration, which is the optimal method for promoting long-distance regeneration of the ON [27].

4.2. Inhibition of Axonal Regeneration by Extracellular Environment

In addition to the intrinsic insufficiency of RGCs to regrow axons, the tissue microenvironment mainly composed

of the glial scar and myelin in the ON is inhibitory to axonal growth, particularly at the injury site, to which the axonal tips are exposed, representing another obstacle for axonal regeneration (Fig. 2).

4.2.1. Inflammatory Stimulation

After ON injury, intraocular inflammation activates retinal astrocytes and Müller cells, stimulating them to secrete multiple factors [71], including the neurotrophins nerve growth factor, brain-derived neurotrophic factor, neurotrophin 4/5, and neurotrophin 3 and cytokines such as IL-6, CNTF, and LIF [72]. IS can transform RGCs into a regenerative state, which is characterized by altered gene expression [73], enabling these neurons to survive after ON injury and to regenerate lengthy axons into the injured ON [1, 74]. IS can be induced by intravitreal application of toll-like receptor 2 agonists such as Pam3Cys [14, 75], which induce retinal astrocytes and Müller cells to express and release neuroprotective and axon growth-promoting cytokines such as CNTF, LIF and IL-6 [32, 76]. The key role of CNTF and LIF in promoting the beneficial effects of IS upon injury has been demonstrated in experimental studies of knockout mice [1].

IS increases the expression of neuroprotective CNTF and LIF in RGCs and activates their downstream signalling pathways, namely the JAK/STAT3 and PI3K/AKT pathways, in RGCs [32, 76]. IS-mediated neuroprotective and axon growth-promoting effects are significantly reduced by intravitreal injections of CNTF antibodies to neutralize the CNTF released from retinal glia or by the genetic knockout of CNTF, and all the beneficial effects of IS are absent in mice deficient for CNTF and LIF [76], underlining the essential role of these two cytokines in this context. *In vitro*, CNTF and LIF potently enhance the neurite outgrowth of mature RGCs in a concentration-dependent manner [34, 77]. These effects require activation of the JAK/STAT3 and PI3K/AKT signalling pathways [54, 78]. Moreover, the elevation of cAMP potentiates the beneficial effects of CNTF *in vitro* and *in vivo* [32, 34, 54, 64]. However, due to its short half-life, the neuroprotective and axon growth-promoting effects of intravitreally applied recombinant CNTF are less pronounced than those observed after IS or when CNTF is continuously provided to RGCs after viral expression in the retina [32, 54, 79, 80]. Moreover, the expression of Apolipoprotein E (ApoE), which potentiates the biological activity of CNTF [81], is reportedly upregulated in Müller cells after IS, and the IS-induced neuroprotective and axon growth-promoting effects are significantly reduced in ApoE-deficient mice [82].

4.2.2. Glial Scarring

Glial cells play a prominent paradoxical role in axon regeneration after ON injury. Activated glial cells in the retina and ON generate multiple pro-regenerative neurotrophic factors [32, 72], such as CNTF and LIF, contributing to axon regeneration. Glial cell activation also potentially contributes to the upregulation of SDF-1 α after ONI in rats [49]. Additionally, a glial scar, primarily consisting of reactive astrocytes and microglia, represents a significant barrier for successful axon regeneration [83]. Inhibitory

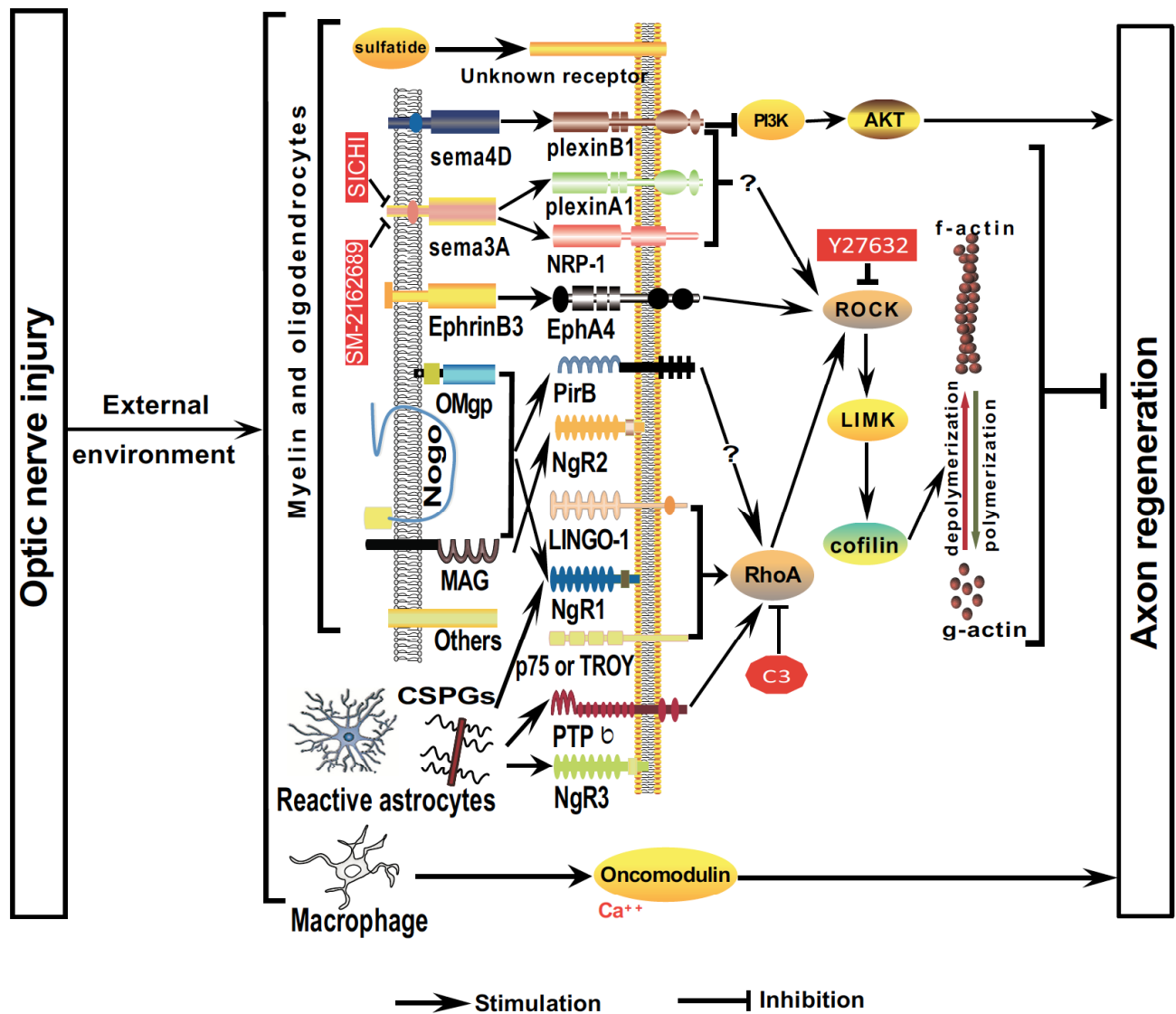


Fig. (2). Extrinsic signaling pathways for axon regeneration towards optic nerve injury. Schematic drawing depicting regulatory factors and their downstream signaling pathways involved in extrinsic growth program in the axon regeneration following optic nerve injury.

extracellular matrix molecules identified in the glial scar include Slit-1, Tenascin-R, semaphorin 3A (sema3A), and, most importantly, chondroitin sulfate proteoglycans (CSPGs), which are the main component of the glial scar [84-90]. CSPGs are extracellular matrix proteoglycans that consist of a protein core with covalently attached glycosaminoglycan (GAG) side chains [91, 92]. After ON injury, CSPGs, which are secreted by astrocytes, neurons and oligodendrocytes, are strongly enriched at the glial scar, where they inhibit axon regenerative growth and restrict plasticity [83, 93, 94]. The release of CSPGs is triggered by the signalling downstream of various cytokines and growth factors associated with the process of scar formation [95]. For example, transforming growth factor- β , which is released into traumatic wounds by platelets and macrophages, induces the deposition of CSPGs from reactive astrocytes and fibrotic scarring [96-98]. Treatment with the bacterial enzyme chondroitinase ABC degrades the sulfated glycosaminoglycan side chains and can block this inhibition and enhance axon regeneration [99]. In

addition to actively inhibiting axon growth, CSPGs can also transform growth-attracting proteins such as semaphorin 5A into repulsive cues [100] or mask growth-promoting proteins such as laminin [101]. Recent studies have reported that the transmembrane protein tyrosine phosphatase σ (PTP σ) is a functional receptor for CSPGs. Accordingly, neurons from PTP σ knockout mice displayed reduced sensitivity towards CSPGs in culture [102] and enhanced axonal regeneration beyond the glial scar in the ON [103]. Moreover, the Nogo receptors NgR1 and NgR3 have been proposed as additional receptors for CSPGs [104].

4.2.3. Myelin

Apart from triggering glial scar-derived inhibitors, damage to the ON environment also leads to the exposure of transected axons to inhibitory myelin molecules, which bind to their specific receptors on the axon, subsequently leading to destabilization of the actin cytoskeleton in filopodia and lamellipodia of the growth cone and thereby blocking axon

regeneration [11, 105]. Inhibitory proteins of the ON myelin include molecules such as Nogo, myelin-associated glycoprotein (MAG), and oligodendrocyte-myelin glycoprotein (OMgp) [106, 107], which can bind with similar affinity to NgR despite being structurally heterogeneous [108-110]. NgR1, NgR2, and NgR3 are three isoforms of NgR that are present in neurons [111, 112]. NgR1 forms a functional ternary complex with the newly characterized leucine-rich repeat transmembrane protein LINGO-1 and members of the tumour necrosis factor receptor family, either p75 neurotrophin receptor or TNF receptor orphan Y (TROY) [113-116], because NgR lacks an intracellular domain and its downstream signalling depends on co-receptors. Recent studies have identified the paired immunoglobulin-like receptor B (PirB), a member of the leukocyte immunoglobulin receptor subfamily that is expressed in the ON and retina, as another novel functional receptor for Nogo, MAG, and OMgp [117, 118]. Furthermore, PirB and NgR1 are functionally linked and collaborate in signalling to inhibit neurite outgrowth [117]. The knockdown of NgR or the overexpression of a dominant-negative form of the receptor lacking the binding site to its co-receptors markedly enhances axon regeneration in the ON *in vivo* when RGCs are transformed into an active regenerative state after injury [119-121]. In contrast, expression of the dominant-negative form alone produces almost no improvement of axonal regeneration, implying that overcoming myelin inhibition alone is not sufficient to yield significant regeneration of the ON. However, other myelin-associated proteins may also contribute to axon growth inhibition in the injured ON. For example, oligodendrocytes and astrocytes express repellents [24], such as Ephrin-B3 [122, 123], sema5A and sema4D [124, 125], and netrin-1 [126], which prevent axons from targeting inappropriate areas during development and are upregulated in the adult ON. More recently, sulfatide, a major constituent of CNS myelin, has been proposed as another myelin-associated inhibitor of neurite outgrowth. Mice that are unable to produce sulfatide exhibit a small but significant enhancement of the extent of IS-induced regeneration of the ON [127].

4.2.4. Macrophages

In an *in vitro* model of transected adult rat ON, macrophages have been suggested to change the nonpermissive environment to a permissive state supportive of regeneration [128]. The implantation of macrophages activated by incubation with peripheral nerve segments into the injured ON has been shown to stimulate axon regeneration and tissue repair [129]. Researchers have found that macrophages are recruited upon lens injury and elicit an 8-fold increase in RGC survival and a 100-fold increase in regeneration of RGC axons past the site of an ON injury [14, 74, 130]. Oncomodulin (Ocm), a low-molecular-weight (11.7 kDa) calcium-binding protein mainly secreted by activated macrophages [131], has been demonstrated to bind to a high-affinity receptor on RGCs in a cAMP-dependent fashion and to stimulate extensive axon outgrowth in culture and *in vivo* [132]. When released from a slow-release polymer with a cAMP analogue, Ocm yields nearly as much regeneration as does intraocular IS; conversely, blocking the activity of Ocm immunologically or with an anti-Ocm neutralizing antibody blocks the effect of intravitreal inflammation on axon

regeneration, although not the effect on RGC survival. The increase in Ocm levels that occurs shortly after inducing intraocular inflammation appears to be due to the influx of both neutrophils and macrophages into the eye [27]. When intraocular inflammation is combined with treatments that enhance regeneration by other mechanisms, many RGCs can regenerate axons through the glial scar and myelin at the injury site, with some axons extending as far as the thalamus [55, 119]. P1, a small peptide that competes with Ocm for receptor occupancy, has been shown to eliminate most of the regeneration induced by intraocular inflammation but cannot diminish the effects of PTEN deletion [131]. Some studies argue that CNTF mediates these effects [54], but this seems highly unlikely considering the loss of regeneration caused by blocking Ocm and considering the many studies indicating that CNTF exerts relatively weak axon-promoting effects on RGCs *in vivo* in the absence of intraocular inflammation [4, 13, 130, 133]. In addition, as protamine has been reported to effectively deliver small interfering RNA (siRNA) into cells, researchers constructed an Ocm and truncated protamine (tp) fusion protein (Ocm/tp) expression vector, which has been used as a vehicle for the delivery of NgR siRNA into RGCs for gene therapy. Ocm/tp-NgR siRNA dramatically promotes axonal growth of RGCs compared with the application of Ocm/tp recombinant protein or NgR siRNA alone, implying that the combination of Ocm/tp and NgR siRNA promotes axonal growth in RGCs. In addition, Ocm/tp-NgR siRNA strongly elevates intracellular cAMP levels and inhibits activation of the Ras homologue gene family, member A (RhoA) [134].

4.2.5. Neutrophils: The Immune Response

Neutrophils are the primary responders of the innate immune system and are activated by injury [135]. Neural injury activates an inflammatory response that can profoundly influence the neurological outcome [136]. RGCs are normally unable to regenerate their axons following ON injury but become able to do so after the induction of an inflammatory reaction in the eye [130, 137]. Inflammation leads to a dramatic increase in the expression of Ocm, which plays a key role in inflammation-induced regeneration, as described above [27, 131, 132]. Numerous neutrophils are recruited into the eye by 12 h after zymosan injection, and they express high levels of Ocm mRNA and protein. The immunodepletion of neutrophils decreases Ocm levels in the retina and, most importantly, suppresses axon regeneration. The sharp reduction in regeneration observed after neutrophil depletion suggests that other cell types cannot induce extensive regeneration by themselves. It is possible, however, that neutrophils normally stimulate other cells to release relevant growth factors or that the loss of neutrophils affects the subsequent inflammatory chain of events [138].

4.2.6. Signalling Pathways Involved in the Extracellular Environment: The RhoA/ROCK Pathway

Intracellular signalling pathways downstream of myelin inhibitors as well as molecules associated with the inhibitory glial scar converge on the activation of the RhoA/rho-associated protein kinase (RhoA/ROCK)-pathway, leading to actin depolymerization *via* LIM kinase and cofilin stimulation, thereby ultimately inducing immobility and/or

collapse of the growth cone [139-141]. It might, therefore, be more efficient to target the axon growth inhibitory signalling cascade at the level of RhoA and/or ROCK rather than to directly neutralize molecules or receptors. ADP-ribosyltransferase C3 is a bacterial protein that efficiently and irreversibly inactivates RhoA. Treatment of primary retinal neurons with C3 protein reverses the inhibitory effects of myelin and MAG [142]. Application of C3 at the injury site of the ON or intravitreal injection of a cell-permeable version of C3 leads to RGC axons crossing the lesion site and growing into the distal nerve segment [142, 143]. Furthermore, viral expression of C3 in RGCs enables axons to regenerate in the distal part of the injured ON and further enhances the extent of axon regeneration after lens injury [55]. Similarly, treatment of RGCs with specific inhibitors for ROCK overcomes myelin and CSPG inhibition *in vitro* and allows axons to regenerate beyond the lesion site of the ON *in vivo* [141, 144].

4.2.7. Guidance Cues

Numerous axon misguidance events block RGC axons from extending into the brain and eventually to their corresponding targets during ON regeneration after injury. Some fibres have even been observed in the contralateral ON, making multiple turns, growing towards the contralateral retina [25], or blocking complete axonal regeneration in the visual system of adult mice, indicating that axon guidance cues affect ON axon regeneration [24]. Consistently, regenerative axonal growth induced by overexpressing STAT3 or CNTF is characterized by irregular axonal trajectories, such as kinks, which are frequent, and by approximately 40% of the axons showing U-turns, in which the growing axon extends back towards the lesion site [23, 30]. Mice with the co-deletion of PTEN and SOCS3 mice have produced similar observations: more than half of the few regenerating axons crossing the optic chiasm join the ipsilateral optic tract and terminate in the ventral part of the hypothalamus [24, 25]. Studies have demonstrated two unexpected observations for regenerating fibres in the injured ON axon in 3D by light sheet microscopy: the return of many regrowing fibres towards the lesion site/retina in the ON and pronounced axonal misrouting at the optic chiasm [24, 25, 30]. These results suggest that the role of guidance cues for correct crossing is significant for ON axon regeneration and must be recognized as such.

Several members of the guidance molecule families, including netrins, slits, semaphorins, and ephrins, are expressed throughout adulthood in the CNS, and their roles in ON regeneration are only beginning to be illuminated [145, 146]. Guidance cues are not only involved in the axonal response but have also been shown to affect the responses of glial cells and the immune system after ON injury [145, 147]. While most classes of guidance cues can act bimodally, mediating attraction or repulsion dependent upon downstream signalling mechanisms, the response of injured axons to ephrins and semaphorins has been largely one of repulsion after injury [145, 146].

Sema3A, whose receptors are neuropilin 1 (NRP-1) and plexinA1 [148], is a strong axonal chemorepellent that induces growth cone collapse and inhibits axonal regeneration after

ON injury [149]. Semaphorin induced chemorepulsion inhibitor (SICHI), a stable N-alkylglycine peptoid, specifically interferes with sema3A binding to the NRP-1/plexinA1 receptor complex and blocks sema3A-induced biological functions by blocking GSK-3 activation [150] in both the developing and the adult brain, and SICHI application enhances the regeneration of lesioned axons [149]. Moreover, the biological activity of SICHI is specific to sema3A because this compound does not affect chemorepulsion induced by sema3F or netrin-1. Like SICHI, SM-2162689 (xanthofulvin) appears to be fairly specific for sema3A signalling; it inhibits binding of sema3A to NRP-1 and plexinA1 and promotes axonal regeneration [89, 149, 151].

The transmembrane semaphorin sema4D, whose receptor is plexinB1, is expressed selectively by oligodendrocytes and myelin in the adult CNS and is upregulated in oligodendrocytes after ON injury. Sema4D/plexinB1 signalling inactivates PI3K and dephosphorylates Akt and GSK-3 β through R-Ras GAP activity, triggering growth cone collapse and blocking axonal regeneration [152, 153]. EphB3, a transmembrane member of the EphB axon guidance molecule subfamily, reappears in the adult ON after injury, coincident with RGC axon sprouting and remodeling. *In vitro* assays demonstrate that adult RGC axon growth is stimulated by EphB3, and RGC axons in animals with reduced or absent EphB3 function have dramatically decreased axonal regeneration after ON injury [154]. EphrinB3/EphA4, a single guidance system, is required for the guidance of both ascending and descending axon tracts [155]. The effect of EphrinB3/EphA4 on ON regeneration remains unclear and merits further exploration. Sema5A, found on neuroepithelial cells surrounding the retinal axons, has been shown to induce growth cone collapse, and the application of antibodies against sema5A induces retinal axons to escape from the ON bundle [156].

It is currently unclear whether and to what extent these guidance cues present in the injured ON contribute to aberrant axon trajectories shown in the adult ON. In addition, ROCK is an important mediator of many repulsive molecules, including ephrins [157, 158]. Inhibiting ROCK with a pharmacological blocker (Y27632) leads to longer regenerating axons, straighter fibre morphology, and a large reduction in the number of U-turns [30]. Ideally, controlling the enhancement of intrinsic growth ability and moderately overcoming the extrinsic inhibitory environment, in combination with correct guidance by axon guidance cues, will be necessary to ensure successful long-distance regeneration of adult ON axons.

Target finding and circuit formation for sprouting and regenerating fibers may be different in the optic nerve from other parts of the CNS [24]. In the spinal cord, for example, growing fibers have access to neuronal circuits in their immediate vicinity in the grey matter. Connections of regenerating axons to spinal circuits below a lesion have been shown to form even if the path of regeneration is anatomically abnormal. In addition, detour pathways can be formed, for example, *via* propriospinal neurons [159, 160]. Anatomical and biochemical plasticity, increased levels of neurotrophic factors [161] and decreased inflammation are positive features of spinal cord [162], which is not in line

with optic nerve. Such features could explain why functional recoveries have been frequently and reproducibly observed with several different regeneration enhancing treatments in partial spinal cord lesion paradigms, whereas they are much more difficult to obtain in the visual system, where regeneration over long distances and reconnection to specific targets are required.

CONCLUSION

Axonal injury in the mammalian ON causes irreversible damage and functional loss of vision due to the limited capacity for axon regeneration, and to date, no clinical treatment has been markedly effective for patients. Recent studies on ON regeneration have shed light on a significant and, as of yet, neglected hurdle of axon regeneration: axon guidance. As summarized in this review, barriers to axon regeneration in the injured ON are attributable to various factors involved in intrinsic insufficiency for axonal growth, inhibitory signalling in the axonal growth cone induced by molecules associated with myelin and glial scarring, and guidance cues for correct axon navigation. Combinatorial treatments have been shown to yield stronger regeneration than individual treatments, supporting the possibility that in order to further optimize treatments to enhance the number and length of regenerating axons, strategies must be developed to guide and reconnect axons to their former targets in a topographically correct manner. Greater insight into the combination of treatments geared towards overcoming growth-inhibitory molecules and enhancing intrinsic growth capacity combined with correct guidance using axon guidance cues is crucial for developing promising therapies to promote axon regeneration and functional recovery after ON injury.

LIST OF ABBREVIATIONS

| | | |
|--------------|---|--|
| Akt | = | Activated protein kinase |
| ApoE | = | Apolipoprotein E |
| bFGF | = | Basic fibroblast growth factor |
| CNTF | = | Ciliary neurotrophic factor |
| CSPGs | = | Chondroitin sulfate proteoglycans |
| CXCL12 | = | CXC chemokine ligand-12 |
| JAK | = | Janus kinase |
| LIF | = | Leukemia inhibitory factor |
| LIMK | = | LIM kinase |
| MAG | = | Myelin-associated glycoprotein |
| mTOR | = | Mammalian target of rapamycin |
| NRP-1 | = | Neuropilin 1 |
| OMgp | = | Oligodendrocyte-myelin glycoprotein |
| PI3K | = | Phosphoinositide-3-kinase |
| PirB | = | Paired immunoglobulin-like receptor B |
| PTEN | = | Phosphatase and tensin homologue |
| PTP σ | = | Receptor protein tyrosine phosphatase σ |

| | | |
|--------|---|--|
| RhoA | = | Ras homologue gene family, member A |
| ROCK | = | Rho-associated protein kinase |
| SDF-1 | = | Stromal-derived factor 1 |
| sema3A | = | Semaphorin 3A |
| SICHI | = | Semaphorin induced chemorepulsion inhibitor |
| SOCS3 | = | Suppressor of cytokine signalling 3 |
| STAT3 | = | Signal transducer and activator of transcription |
| TROY | = | TNF receptor orphan Y |

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

This work was supported by the National Natural Science Foundation of China (81401587, 81171796, 81372047).

REFERENCES

- [1] Fischer, D. Stimulating axonal regeneration of mature retinal ganglion cells and overcoming inhibitory signaling. *Cell Tissue Res.*, **2012**, *349*, 79-85.
- [2] Moore, D.L.; Goldberg, J.L. Four steps to optic nerve regeneration. *J. Neuroophthalmol.*, **2010**, *30*, 347-360.
- [3] Case, L.C.; Tessier-Lavigne, M. Regeneration of the adult central nervous system. *Curr. Biol.*, **2005**, *15*, R749-R753.
- [4] Leaver, S.G.; Cui, Q.; Plant, G.W. AAV-mediated expression of CNTF promotes long-term survival and regeneration of adult rat retinal ganglion cells. *Gene Ther.*, **2006**, *13*, 1328-1341.
- [5] Bahr, M. Live or let die - retinal ganglion cell death and survival during development and in the lesioned adult CNS. *Trends Neurosci.*, **2000**, *23*, 483-490. [https://doi.org/10.1016/S0166-2236(00)01637-4]
- [6] Isenmann, S.; Wahl, C.; Krajewski, S.; Reed, J.C.; Bahr, M. Up-regulation of Bax protein in degenerating retinal ganglion cells precedes apoptotic cell death after optic nerve lesion in the rat. *Eur. J. Neurosci.*, **1997**, *9*, 1763-1772. [https://doi.org/10.1111/j.1460-9568.1997.tb01534.x]
- [7] Feng, D.F.; Chen, E.T.; Li, X.Y.; Liu, Y.; Wang, Y. Standardizing optic nerve crushes with an aneurysm clip. *Neurol. Res.*, **2010**, *32*, 476-481. [https://doi.org/10.1179/016164110X12556180206158]
- [8] Heskamp, A.; Leibinger, M.; Andreadaki, A.; Gobrecht, P.; Diekmann, H.; Fischer, D. CXCL12/SDF-1 facilitates optic nerve regeneration. *Neurobiol. Dis.*, **2013**, *55*, 76-86. [https://doi.org/10.1016/j.nbd.2013.04.001]
- [9] Sarikcioglu, L.; Demir, N.; Demirtop, A. A standardized method to create optic nerve crush: Yasargil aneurysm clip. *Exp. Eye Res.*, **2007**, *84*, 373-377. [https://doi.org/10.1016/j.exer.2006.10.013]
- [10] Zhang, R.; Zhang, H.; Xu, L.; Ma, K.; Wallrapp, C.; Jonas, J.B. Neuroprotective effect of intravitreal cell-based glucagon-like peptide-1 production in the optic nerve crush model. *Acta Ophthalmol.*, **2011**, *89*, e320-e326. [https://doi.org/10.1111/j.1755-3768.2010.02044.x]
- [11] Berry, M.; Ahmed, Z.; Lorber, B.; Douglas, M.; Logan, A. Regeneration of axons in the visual system. *Restor. Neurol. Neurosci.*, **2008**, *26*, 147-174.
- [12] Park, K.K.; Liu, K.; Hu, Y. Promoting axon regeneration in the adult CNS by modulation of the PTEN/mTOR pathway. *Science*, **2008**, *322*, 963-966. [https://doi.org/10.1126/science.1161566]
- [13] Smith, P.D.; Sun, F.; Park, K.K. SOCS3 deletion promotes optic nerve regeneration *in vivo*. *Neuron*, **2009**, *64*, 617-623. [https://doi.org/10.1016/j.neuron.2009.11.021]

- [14] Fischer, D.; Heiduschka, P.; Thanos, S. Lens-injury-stimulated axonal regeneration throughout the optic pathway of adult rats. *Exp. Neurol.*, **2001**, *172*, 257-272. [https://doi.org/10.1006/exnr.2001.7822]
- [15] Xie, J.; Jiang, L.; Zhang, T.; Jin, Y.; Yang, D.; Chen, F. Neuroprotective effects of Epigallocatechin-3-gallate (EGCG) in optic nerve crush model in rats. *Neurosci. Lett.*, **2010**, *479*, 26-30. [https://doi.org/10.1016/j.neulet.2010.05.020]
- [16] Salinas-Navarro, M.; Mayor-Torroglosa, S.; Jimenez-Lopez, M. A computerized analysis of the entire retinal ganglion cell population and its spatial distribution in adult rats. *Vision Res.*, **2009**, *49*, 115-126. [https://doi.org/10.1016/j.visres.2008.09.029]
- [17] Sengottuvel, V.; Fischer, D. Facilitating axon regeneration in the injured CNS by microtubules stabilization. *Commun. Integr. Biol.*, **2011**, *4*, 391-393. [https://doi.org/10.4161/cib.15552]
- [18] Benowitz, L.I.; Yin, Y. Optic nerve regeneration. *Arch. Ophthalmol.*, **2010**, *128*, 1059-1064. [https://doi.org/10.1001/archophthalmol.2010.152]
- [19] Kato, S.; Matsukawa, T.; Koriyama, Y.; Sugitani, K.; Ogai, K. A molecular mechanism of optic nerve regeneration in fish: the retinoid signaling pathway. *Prog. Retin. Eye Res.*, **2013**, *37*, 13-30. [https://doi.org/10.1016/j.preteyeres.2013.07.004]
- [20] Lanners, H.N.; Grafstein, B. Early stages of axonal regeneration in the goldfish optic tract: an electron microscopic study. *J. Neurocytol.*, **1980**, *9*, 733-751. [https://doi.org/10.1007/BF01205016]
- [21] Meyer, R.L. Mapping the normal and regenerating retinotectal projection of goldfish with autoradiographic methods. *J. Comp. Neurol.*, **1980**, *189*, 273-289. [https://doi.org/10.1002/cne.901890205]
- [22] Kato, S.; Devadas, M.; Okada, K. Fast and slow recovery phases of goldfish behavior after transection of the optic nerve revealed by a computer image processing system. *Neuroscience*, **1999**, *93*, 907-914. [https://doi.org/10.1016/S0306-4522(99)00202-X]
- [23] Pernet, V.; Joly, S.; Dalkara, D. Long-distance axonal regeneration induced by CNTF gene transfer is impaired by axonal misguidance in the injured adult optic nerve. *Neurobiol. Dis.*, **2013**, *51*, 202-213. [https://doi.org/10.1016/j.nbd.2012.11.011]
- [24] Pernet, V.; Schwab, M.E. Lost in the jungle: new hurdles for optic nerve axon regeneration. *Trends Neurosci.*, **2014**, *37*, 381-387. [https://doi.org/10.1016/j.tins.2014.05.002]
- [25] Luo, X.; Salgueiro, Y.; Beckerman, S.R.; Lemmon, V.P.; Tsoulfas, P.; Park, K.K. Three-dimensional evaluation of retinal ganglion cell axon regeneration and pathfinding in whole mouse tissue after injury. *Exp. Neurol.*, **2013**, *247*, 653-662.
- [26] McConnell, P.; Berry, M. Regeneration of axons in the mouse retina after injury. *Bibl. Anat.*, **1982**, *23*, 26-37.
- [27] Kurimoto, T.; Yin, Y.; Omura, K. Long-distance axon regeneration in the mature optic nerve: contributions of oncomodulin, cAMP, and pten gene deletion. *J. Neurosci.*, **2010**, *30*, 15654-15663.
- [28] de Lima, S.; Koriyama, Y.; Kurimoto, T. Full-length axon regeneration in the adult mouse optic nerve and partial recovery of simple visual behaviors. *Proc. Natl. Acad. Sci. USA*, **2012**, *109*, 9149-9154.
- [29] Li, S.; He, Q.; Wang, H. Injured adult retinal axons with Pten and Soc3 co-deletion reform active synapses with suprachiasmatic neurons. *Neurobiol. Dis.*, **2015**, *73*, 366-376.
- [30] Pernet, V.; Joly, S.; Jordi, N. Misguidance and modulation of axonal regeneration by Stat3 and Rho/ROCK signaling in the transparent optic nerve. *Cell Death Dis.*, **2013**, *4*, e734.
- [31] Yang, P.; Yang, Z. Enhancing intrinsic growth capacity promotes adult CNS regeneration. *J. Neurol. Sci.*, **2012**, *312*, 1-6.
- [32] Muller, A.; Hauk, T.G.; Fischer, D. Astrocyte-derived CNTF switches mature RGCs to a regenerative state following inflammatory stimulation. *Brain*, **2007**, *130*, 3308-3320.
- [33] Grozdanov, V.; Muller, A.; Sengottuvel, V.; Leibinger, M.; Fischer, D. A method for preparing primary retinal cell cultures for evaluating the neuroprotective and neuritogenic effect of factors on axotomized mature CNS neurons. *Curr. Protoc. Neurosci.*, **2010**, *53*, 3.22.1-3.22.10.
- [34] Yin, Y.; Cui, Q.; Li, Y. Macrophage-derived factors stimulate optic nerve regeneration. *J. Neurosci.*, **2003**, *23*, 2284-2293.
- [35] Liu, K.; Tedeschi, A.; Park, K.K.; He, Z. Neuronal intrinsic mechanisms of axon regeneration. *Annu. Rev. Neurosci.*, **2011**, *34*, 131-152.
- [36] Fischer, D.; Leibinger, M. Promoting optic nerve regeneration. *Prog. Retin. Eye Res.*, **2012**, *31*, 688-701.
- [37] Leibinger, M.; Andreadaki, A.; Fischer, D. Role of mTOR in neuroprotection and axon regeneration after inflammatory stimulation. *Neurobiol. Dis.*, **2012**, *46*, 314-324.
- [38] Perdigoto, A.L.; Chaudhry, N.; Barnes, G.N.; Filbin, M.T.; Carter, B.D. A novel role for PTEIN in the inhibition of neurite outgrowth by myelin-associated glycoprotein in cortical neurons. *Mol. Cell. Neurosci.*, **2011**, *46*, 235-244.
- [39] Xie, R.; Wang, P.; Cheng, M.; Sapolsky, R.; Ji, X.; Zhao, H. Mammalian target of rapamycin cell signaling pathway contributes to the protective effects of ischemic postconditioning against stroke. *Stroke*, **2014**, *45*, 2769-2776.
- [40] Gonzalez, A.M.; Berry, M.; Maher, P.A.; Logan, A.; Baird, A. A comprehensive analysis of the distribution of FGF-2 and FGFR1 in the rat brain. *Brain Res.*, **1995**, *701*, 201-226.
- [41] Hicks, D. Characterization and possible roles of fibroblast growth factors in retinal photoreceptor cells. *Keio J. Med.*, **1996**, *45*, 140-154.
- [42] Viollet, C.; Doherty, P. CAMs and the FGF receptor: an interacting role in axonal growth. *Cell Tissue Res.*, **1997**, *290*, 451-455. [https://doi.org/10.1007/s004410050952]
- [43] Soto, I.; Marie, B.; Baro, D.J.; Blanco, R.E. FGF-2 modulates expression and distribution of GAP-43 in frog retinal ganglion cells after optic nerve injury. *J. Neurosci. Res.*, **2003**, *73*, 507-517. [https://doi.org/10.1002/jnr.10673]
- [44] McFarlane, S.; Cornel, E.; Amaya, E.; Holt, C.E. Inhibition of FGF receptor activity in retinal ganglion cell axons causes errors in target recognition. *Neuron*, **1996**, *17*, 245-254.
- [45] McFarlane, S.; McNeill, L.; Holt, C.E. FGF signaling and target recognition in the developing *Xenopus* visual system. *Neuron*, **1995**, *15*, 1017-1028.
- [46] Lom, B.; Hopker, V.; McFarlane, S.; Bixby, J.L.; Holt, C.E. Fibroblast growth factor receptor signaling in *Xenopus* retinal axon extension. *J. Neurobiol.*, **1998**, *37*, 633-641.
- [47] Fujimoto, E.; Mizoguchi, A.; Hanada, K.; Yajima, M.; Ide, C. Basic fibroblast growth factor promotes extension of regenerating axons of peripheral nerve. *In vivo* experiments using a Schwann cell basal lamina tube model. *J. Neurocytol.*, **1997**, *26*, 511-528.
- [48] Pan, J.; Mestas, J.; Burdick, M.D. Stromal derived factor-1 (SDF-1/CXCL12) and CXCR4 in renal cell carcinoma metastasis. *Mol. Cancer*, **2006**, *5*, 56.
- [49] Yang, X.T.; Pan, D.C.; Chen, E.T.; Bi, Y.Y.; Feng, D.F. Glial cells activation potentially contributes to the upregulation of stromal cell-derived factor-1alpha after optic nerve crush in rats. *Neurochem. Res.*, **2013**, *38*, 1996-2008.
- [50] Jaerve, A.; Bosse, F.; Muller, H.W. SDF-1/CXCL12: its role in spinal cord injury. *Int. J. Biochem. Cell Biol.*, **2012**, *44*, 452-456.
- [51] Takeuchi, H.; Natsume, A.; Wakabayashi, T. Intravenously transplanted human neural stem cells migrate to the injured spinal cord in adult mice in an SDF-1- and HGF-dependent manner. *Neurosci. Lett.*, **2007**, *426*, 69-74.
- [52] Jaerve, A.; Schiwy, N.; Schmitz, C.; Mueller, H.W. Differential effect of aging on axon sprouting and regenerative growth in spinal cord injury. *Exp. Neurol.*, **2011**, *231*, 284-294.
- [53] Opatz, J.; Kury, P.; Schiwy, N. SDF-1 stimulates neurite growth on inhibitory CNS myelin. *Mol. Cell. Neurosci.*, **2009**, *40*, 293-300.
- [54] Muller, A.; Hauk, T.G.; Leibinger, M.; Marienfeld, R.; Fischer, D. Exogenous CNTF stimulates axon regeneration of retinal ganglion cells partially via endogenous CNTF. *Mol. Cell. Neurosci.*, **2009**, *41*, 233-246.
- [55] Fischer, D.; Petkova, V.; Thanos, S.; Benowitz, L.I. Switching mature retinal ganglion cells to a robust growth state *in vivo*: gene expression and synergy with RhoA inactivation. *J. Neurosci.*, **2004**, *24*, 8726-8740.
- [56] Croker, B.A.; Kiu, H.; Nicholson, S.E. SOCS regulation of the JAK/STAT signalling pathway. *Semin. Cell Dev. Biol.*, **2008**, *19*, 414-422. [https://doi.org/10.1016/j.semdb.2008.07.010]
- [57] Nicholson, S.E.; De Souza, D.; Fabri, L.J. Suppressor of cytokine signaling-3 preferentially binds to the SHP-2-binding site on the shared cytokine receptor subunit gp130. *Proc. Natl. Acad. Sci. USA*, **2000**, *97*, 6493-6498. [https://doi.org/10.1073/pnas.100135197]
- [58] Shouda, T.; Yoshida, T.; Hanada, T. Induction of the cytokine signal regulator SOCS3/CIS3 as a therapeutic strategy for treating inflammatory arthritis. *J. Clin. Invest.*, **2001**, *108*, 1781-1788. [https://doi.org/10.1172/JCI13568]

- [59] Jo, D.; Liu, D.; Yao, S.; Collins, R.D.; Hawiger, J. Intracellular protein therapy with SOCS3 inhibits inflammation and apoptosis. *Nat. Med.*, **2005**, *11*, 892-898.
- [60] Hellstrom, M.; Harvey, A.R. Retinal ganglion cell gene therapy and visual system repair. *Curr. Gene Ther.*, **2011**, *11*, 116-131.
- [61] Hellstrom, M.; Muhling, J.; Ehler, E.M. Negative impact of rAAV2 mediated expression of SOCS3 on the regeneration of adult retinal ganglion cell axons. *Mol. Cell. Neurosci.*, **2011**, *46*, 507-515.
- [62] Sun, F.; Park, K.K.; Belin, S. Sustained axon regeneration induced by co-deletion of PTEN and SOCS3. *Nature*, **2011**, *480*, 372-375.
- [63] Park, K.K.; Hu, Y.; Muhling, J. Cytokine-induced SOCS expression is inhibited by cAMP analogue: impact on regeneration in injured retina. *Mol. Cell. Neurosci.*, **2009**, *41*, 313-324.
- [64] Cui, Q.; Yip, H.K.; Zhao, R.C.; So, K.F.; Harvey, A.R. Intraocular elevation of cyclic AMP potentiates ciliary neurotrophic factor-induced regeneration of adult rat retinal ganglion cell axons. *Mol. Cell. Neurosci.*, **2003**, *22*, 49-61.
- [65] Park, K.W.; Lin, C.Y.; Benveniste, E.N.; Lee, Y.S. Mitochondrial STAT3 is negatively regulated by SOCS3 and upregulated after spinal cord injury. *Exp. Neurol.*, **2016**, *284*, 98-105.
- [66] Liu, X.; Williams, P.R.; He, Z. SOCS3: a common target for neuronal protection and axon regeneration after spinal cord injury. *Exp. Neurol.*, **2015**, *263*, 364-367.
- [67] Okada, S.; Nakamura, M.; Katoh, H. Conditional ablation of Stat3 or Socs3 discloses a dual role for reactive astrocytes after spinal cord injury. *Nat. Med.*, **2006**, *12*, 829-834.
- [68] Cantrup, R.; Dixit, R.; Palmesino, E. Cell-type specific roles for PTEN in establishing a functional retinal architecture. *PLoS One*, **2012**, *7*, e32795. [https://doi.org/10.1371/journal.pone.0032795]
- [69] Marino, S.; Krimpenfort, P.; Leung, C. PTEN is essential for cell migration but not for fate determination and tumorigenesis in the cerebellum. *Development*, **2002**, *129*, 3513-3522.
- [70] Drinjakovic, J.; Jung, H.; Campbell, D.S.; Strohlic, L.; Dwivedy, A.; Holt, C.E. E3 ligase Nedd4 promotes axon branching by downregulating PTEN. *Neuron*, **2010**, *65*, 341-357. [https://doi.org/10.1016/j.neuron.2010.01.017]
- [71] Lorber, B.; Berry, M.; Logan, A. Different factors promote axonal regeneration of adult rat retinal ganglion cells after lens injury and intravitreal peripheral nerve grafting. *J. Neurosci. Res.*, **2008**, *86*, 894-903. [https://doi.org/10.1002/jnr.21545]
- [72] Ahmed, Z.; Aslam, M.; Lorber, B.; Suggate, E.L.; Berry, M.; Logan, A. Optic nerve and vitreal inflammation are both RGC neuroprotective but only the latter is RGC axogenic. *Neurobiol. Dis.*, **2010**, *37*, 441-454. [https://doi.org/10.1016/j.nbd.2009.10.024]
- [73] McCurley, A.T.; Callard, G.V. Time course analysis of gene expression patterns in zebrafish eye during optic nerve regeneration. *J. Exp. Neurosci.*, **2010**, *2010*, 17-33.
- [74] Fischer, D.; Pavlidis, M.; Thanos, S. Cataractogenic lens injury prevents traumatic ganglion cell death and promotes axonal regeneration both *in vivo* and in culture. *Invest. Ophthalmol. Vis. Sci.*, **2000**, *41*, 3943-3954.
- [75] Hauk, T.G.; Leibinger, M.; Muller, A.; Andreadaki, A.; Knippschild, U.; Fischer, D. Stimulation of axon regeneration in the mature optic nerve by intravitreal application of the toll-like receptor 2 agonist Pam3Cys. *Invest. Ophthalmol. Vis. Sci.*, **2010**, *51*, 459-464. [https://doi.org/10.1167/iovs.09-4203]
- [76] Leibinger, M.; Muller, A.; Andreadaki, A.; Hauk, T.G.; Kirsch, M.; Fischer, D. Neuroprotective and axon growth-promoting effects following inflammatory stimulation on mature retinal ganglion cells in mice depend on ciliary neurotrophic factor and leukemia inhibitory factor. *J. Neurosci.*, **2009**, *29*, 14334-14341. [https://doi.org/10.1523/JNEUROSCI.2770-09.2009]
- [77] Sengottuvel, V.; Leibinger, M.; Pfreimer, M.; Andreadaki, A.; Fischer, D. Taxol facilitates axon regeneration in the mature CNS. *J. Neurosci.*, **2011**, *31*, 2688-2699. [https://doi.org/10.1523/JNEUROSCI.4885-10.2011]
- [78] Lingor, P.; Tonges, L.; Pieper, N. ROCK inhibition and CNTF interact on intrinsic signalling pathways and differentially regulate survival and regeneration in retinal ganglion cells. *Brain*, **2008**, *131*, 250-263. [https://doi.org/10.1093/brain/awm284]
- [79] Leaver, S.G.; Cui, Q.; Bernard, O.; Harvey, A.R. Cooperative effects of bcl-2 and AAV-mediated expression of CNTF on retinal ganglion cell survival and axonal regeneration in adult transgenic mice. *Eur. J. Neurosci.*, **2006**, *24*, 3323-3332. [https://doi.org/10.1111/j.1460-9568.2006.05230.x]
- [80] Bischof, J.; Muller, A.; Fander, M.; Knippschild, U.; Fischer, D. Neurite outgrowth of mature retinal ganglion cells and PC12 cells requires activity of CK1delta and CK1epsilon. *PLoS One*, **2011**, *6*, e20857. [https://doi.org/10.1371/journal.pone.0020857]
- [81] Gutman, C.R.; Strittmatter, W.J.; Weisgraber, K.H.; Mathew, W.D. Apolipoprotein E binds to and potentiates the biological activity of ciliary neurotrophic factor. *J. Neurosci.*, **1997**, *17*, 6114-6121.
- [82] Lorber, B.; Berry, M.; Douglas, M.R.; Nakazawa, T.; Logan, A. Activated retinal glia promote neurite outgrowth of retinal ganglion cells via apolipoprotein E. *J. Neurosci. Res.*, **2009**, *87*, 2645-2652. [https://doi.org/10.1002/jnr.22095]
- [83] Silver, J.; Miller, J.H. Regeneration beyond the glial scar. *Nat. Rev. Neurosci.*, **2004**, *5*, 146-156. [https://doi.org/10.1038/nrn1326]
- [84] McKeon, R.J.; Schreiber, R.C.; Rudge, J.S.; Silver, J. Reduction of neurite outgrowth in a model of glial scarring following CNS injury is correlated with the expression of inhibitory molecules on reactive astrocytes. *J. Neurosci.*, **1991**, *11*, 3398-3411.
- [85] Niederost, B.P.; Zimmermann, D.R.; Schwab, M.E.; Bandtlow, C.E. Bovine CNS myelin contains neurite growth-inhibitory activity associated with chondroitin sulfate proteoglycans. *J. Neurosci.*, **1999**, *19*, 8979-8989.
- [86] Becker, T.; Anliker, B.; Becker, C.G. Tenascin-R inhibits regrowth of optic fibers *in vitro* and persists in the optic nerve of mice after injury. *Glia*, **2000**, *29*, 330-346.
- [87] Hagino, S.; Iseki, K.; Mori, T. Slit and glypican-1 mRNAs are coexpressed in the reactive astrocytes of the injured adult brain. *Glia*, **2003**, *42*, 130-138.
- [88] Tang, B.L. Inhibitors of neuronal regeneration: mediators and signaling mechanisms. *Neurochem. Int.*, **2003**, *42*, 189-203.
- [89] Kaneko, S.; Iwanami, A.; Nakamura, M. A selective Sema3A inhibitor enhances regenerative responses and functional recovery of the injured spinal cord. *Nat. Med.*, **2006**, *12*, 1380-1389.
- [90] Soleman, S.; Filippov, M.A.; Dityatev, A.; Fawcett, J.W. Targeting the neural extracellular matrix in neurological disorders. *Neuroscience*, **2013**, *253*, 194-213.
- [91] Properzi, F.; Asher, R.A.; Fawcett, J.W. Chondroitin sulphate proteoglycans in the central nervous system: changes and synthesis after injury. *Biochem. Soc. Trans.*, **2003**, *31*, 335-336.
- [92] Silver, J.; Schwab, M.E.; Popovich, P.G. Central nervous system regenerative failure: role of oligodendrocytes, astrocytes, and microglia. *Cold Spring Harb. Perspect. Biol.*, **2015**, *7*, a20602.
- [93] Bradbury, E.J.; Moon, L.D.; Popat, R.J. Chondroitinase ABC promotes functional recovery after spinal cord injury. *Nature*, **2002**, *416*, 636-640.
- [94] Morgenstern, D.A.; Asher, R.A.; Fawcett, J.W. Chondroitin sulphate proteoglycans in the CNS injury response. *Prog. Brain Res.*, **2002**, *137*, 313-332.
- [95] Logan, A.; Berry, M. Cellular and molecular determinants of glial scar formation. *Adv. Exp. Med. Biol.*, **2002**, *513*, 115-158. [https://doi.org/10.1007/978-1-4615-0123-7_4]
- [96] Logan, A.; Berry, M.; Gonzalez, A.M.; Frautschi, S.A.; Sporn, M.B.; Baird, A. Effects of transforming growth factor beta 1 on scar production in the injured central nervous system of the rat. *Eur. J. Neurosci.*, **1994**, *6*, 355-363. [https://doi.org/10.1111/j.1460-9568.1994.tb00278.x]
- [97] Logan, A.; Green, J.; Hunter, A.; Jackson, R.; Berry, M. Inhibition of glial scarring in the injured rat brain by a recombinant human monoclonal antibody to transforming growth factor-beta2. *Eur. J. Neurosci.*, **1999**, *11*, 2367-2374. [https://doi.org/10.1046/j.1460-9568.1999.00654.x]
- [98] Lagord, C.; Berry, M.; Logan, A. Expression of TGFbeta2 but not TGFbeta1 correlates with the deposition of scar tissue in the lesioned spinal cord. *Mol. Cell. Neurosci.*, **2002**, *20*, 69-92. [https://doi.org/10.1006/mcne.2002.1121]
- [99] Rhodes, K.E.; Fawcett, J.W. Chondroitin sulphate proteoglycans: preventing plasticity or protecting the CNS? *J. Anat.*, **2004**, *204*, 33-48. [https://doi.org/10.1111/j.1469-7580.2004.00261.x]
- [100] Kantor, D.B.; Chivatakarn, O.; Peer, K.L. Semaphorin 5A is a bifunctional axon guidance cue regulated by heparan and chondroitin sulfate proteoglycans. *Neuron*, **2004**, *44*, 961-975.

- [101] Bovolenta, P.; Feraud-Espinosa, I. Nervous system proteoglycans as modulators of neurite outgrowth. *Prog. Neurobiol.*, **2000**, *61*, 113-132.
- [102] Shen, Y.; Tenney, A.P.; Busch, S.A. PTPsigma is a receptor for chondroitin sulfate proteoglycan, an inhibitor of neural regeneration. *Science*, **2009**, *326*, 592-596.
- [103] Sapiha, P.S.; Duplan, L.; Uetani, N. Receptor protein tyrosine phosphatase sigma inhibits axon regrowth in the adult injured CNS. *Mol. Cell. Neurosci.*, **2005**, *28*, 625-635.
- [104] Dickendesher, T.L.; Baldwin, K.T.; Mironova, Y.A. NgR1 and NgR3 are receptors for chondroitin sulfate proteoglycans. *Nat. Neurosci.*, **2012**, *15*, 703-712.
- [105] Yiu, G.; He, Z. Glial inhibition of CNS axon regeneration. *Nat. Rev. Neurosci.*, **2006**, *7*, 617-627.
- [106] GrandPre, T.; Nakamura, F.; Vartanian, T.; Strittmatter, S.M. Identification of the Nogo inhibitor of axon regeneration as a Reticulon protein. *Nature*, **2000**, *403*, 439-444.
- [107] McKerracher, L.; David, S.; Jackson, D.L.; Kottis, V.; Dunn, R.J.; Braun, P.E. Identification of myelin-associated glycoprotein as a major myelin-derived inhibitor of neurite growth. *Neuron*, **1994**, *13*, 805-811.
- [108] Domeniconi, M.; Cao, Z.; Spencer, T. Myelin-associated glycoprotein interacts with the Nogo66 receptor to inhibit neurite outgrowth. *Neuron*, **2002**, *35*, 283-290.
- [109] Liu, B.P.; Fournier, A.; GrandPre, T.; Strittmatter, S.M. Myelin-associated glycoprotein as a functional ligand for the Nogo-66 receptor. *Science*, **2002**, *297*, 1190-1193.
- [110] Wang, K.C.; Koprivica, V.; Kim, J.A. Oligodendrocyte-myelin glycoprotein is a Nogo receptor ligand that inhibits neurite outgrowth. *Nature*, **2002**, *417*, 941-944.
- [111] Lauren, J.; Airaksinen, M.S.; Saarma, M.; Timmusk, T. Two novel mammalian Nogo receptor homologs differentially expressed in the central and peripheral nervous systems. *Mol. Cell. Neurosci.*, **2003**, *24*, 581-594.
- [112] Pignot, V.; Hein, A.E.; Barske, C. Characterization of two novel proteins, NgRH1 and NgRH2, structurally and biochemically homologous to the Nogo-66 receptor. *J. Neurochem.*, **2003**, *85*, 717-728.
- [113] Wang, K.C.; Kim, J.A.; Sivasankaran, R.; Segal, R.; He, Z. P75 interacts with the Nogo receptor as a co-receptor for Nogo, MAG and OMgp. *Nature*, **2002**, *420*, 74-78.
- [114] Wong, S.T.; Henley, J.R.; Kanning, K.C.; Huang, K.H.; Bothwell, M.; Poo, M.M. p75(NTR) and Nogo receptor complex mediates repulsive signaling by myelin-associated glycoprotein. *Nat. Neurosci.*, **2002**, *5*, 1302-1308.
- [115] Yamashita, T.; Higuchi, H.; Tohyama, M. The p75 receptor transduces the signal from myelin-associated glycoprotein to Rho. *J. Cell Biol.*, **2002**, *157*, 565-570.
- [116] Mi, S.; Lee, X.; Shao, Z. LINGO-1 is a component of the Nogo-66 receptor/p75 signaling complex. *Nat. Neurosci.*, **2004**, *7*, 221-228.
- [117] Atwal, J.K.; Pinkston-Gosse, J.; Syken, J. PirB is a functional receptor for myelin inhibitors of axonal regeneration. *Science*, **2008**, *322*, 967-970.
- [118] Cai, X.; Yuan, R.; Hu, Z. Expression of PirB protein in intact and injured optic nerve and retina of mice. *Neurochem. Res.*, **2012**, *37*, 647-654.
- [119] Fischer, D.; He, Z.; Benowitz, L.I. Counteracting the Nogo receptor enhances optic nerve regeneration if retinal ganglion cells are in an active growth state. *J. Neurosci.*, **2004**, *24*, 1646-1651.
- [120] Chen, C.; Chen, X.; Yin, X.; Yuan, R.; Wang, B.; Ye, J. NgR RNA interference, combined with zymosan intravitreal injection, enhances optic nerve regeneration. *J. Neurochem.*, **2009**, *110*, 1628-1634. [https://doi.org/10.1111/j.1471-4159.2009.06264.x]
- [121] Su, Y.; Wang, F.; Teng, Y.; Zhao, S.G.; Cui, H.; Pan, S.H. Axonal regeneration of optic nerve after crush in Nogo66 receptor knockout mice. *Neurosci. Lett.*, **2009**, *460*, 223-226. [https://doi.org/10.1016/j.neulet.2009.05.072]
- [122] Benson, M.D.; Romero, M.I.; Lush, M.E.; Lu, Q.R.; Henkemeyer, M.; Parada, L.F. Ephrin-B3 is a myelin-based inhibitor of neurite outgrowth. *Proc. Natl. Acad. Sci. USA*, **2005**, *102*, 10694-10699. [https://doi.org/10.1073/pnas.0504021102]
- [123] Duffy, P.; Wang, X.; Siegel, C.S. Myelin-derived ephrinB3 restricts axonal regeneration and recovery after adult CNS injury. *Proc. Natl. Acad. Sci. USA*, **2012**, *109*, 5063-5068. [https://doi.org/10.1073/pnas.1113953109]
- [124] Moreau-Fauvarque, C.; Kumanogoh, A.; Camand, E. The transmembrane semaphorin Sema4D/CD100, an inhibitor of axonal growth, is expressed on oligodendrocytes and upregulated after CNS lesion. *J. Neurosci.*, **2003**, *23*, 9229-9239.
- [125] Goldberg, J.L.; Vargas, M.E.; Wang, J.T. An oligodendrocyte lineage-specific semaphorin, Sema5A, inhibits axon growth by retinal ganglion cells. *J. Neurosci.*, **2004**, *24*, 4989-4999. [https://doi.org/10.1523/JNEUROSCI.4390-03.2004]
- [126] Ellezam, B.; Selles-Navarro, I.; Manitt, C.; Kennedy, T.E.; McKerracher, L. Expression of netrin-1 and its receptors DCC and UNC-5H2 after axotomy and during regeneration of adult rat retinal ganglion cells. *Exp. Neurol.*, **2001**, *168*, 105-115. [https://doi.org/10.1006/exnr.2000.7589]
- [127] Winzler, A.M.; Mandemakers, W.J.; Sun, M.Z.; Stafford, M.; Phillips, C.T.; Barres, B.A. The lipid sulfatide is a novel myelin-associated inhibitor of CNS axon outgrowth. *J. Neurosci.*, **2011**, *31*, 6481-6492. [https://doi.org/10.1523/JNEUROSCI.3004-10.2011]
- [128] David, S.; Bouchard, C.; Tsatas, O.; Giftchristos, N. Macrophages can modify the nonpermissive nature of the adult mammalian central nervous system. *Neuron*, **1990**, *5*, 463-469. [https://doi.org/10.1016/0896-6273(90)90085-T]
- [129] Lazarov-Spiegler, O.; Solomon, A.S.; Zeev-Brann, A.B.; Hirschberg, D.L.; Lavie, V.; Schwartz, M. Transplantation of activated macrophages overcomes central nervous system regrowth failure. *FASEB J.*, **1996**, *10*, 1296-1302.
- [130] Leon, S.; Yin, Y.; Nguyen, J.; Irwin, N.; Benowitz, L.I. Lens injury stimulates axon regeneration in the mature rat optic nerve. *J. Neurosci.*, **2000**, *20*, 4615-4626.
- [131] Yin, Y.; Cui, Q.; Gilbert, H.Y. Oncomodulin links inflammation to optic nerve regeneration. *Proc. Natl. Acad. Sci. USA*, **2009**, *106*, 19587-19592. [https://doi.org/10.1073/pnas.0907085106]
- [132] Yin, Y.; Henzl, M.T.; Lorber, B. Oncomodulin is a macrophage-derived signal for axon regeneration in retinal ganglion cells. *Nat. Neurosci.*, **2006**, *9*, 843-852. [https://doi.org/10.1038/nn1701]
- [133] Weise, J.; Isenmann, S.; Klocker, N. Adenovirus-mediated expression of ciliary neurotrophic factor (CNTF) rescues axotomized rat retinal ganglion cells but does not support axonal regeneration *in vivo*. *Neurobiol. Dis.*, **2000**, *7*, 212-223. [https://doi.org/10.1006/nbdi.2000.0285]
- [134] Cui, Z.; Kang, J.; Hu, D.; Zhou, J.; Wang, Y. Oncomodulin/truncated protamine-mediated Nogo-66 receptor small interference RNA delivery promotes axon regeneration in retinal ganglion cells. *Mol. Cells*, **2014**, *37*, 613-619. [https://doi.org/10.14348/molcells.2014.0155]
- [135] Donnelly, D.J.; Popovich, P.G. Inflammation and its role in neuroprotection, axonal regeneration and functional recovery after spinal cord injury. *Exp. Neurol.*, **2008**, *209*, 378-388. [https://doi.org/10.1016/j.expneurol.2007.06.009]
- [136] Popovich, P.G.; Longbrake, E.E. Can the immune system be harnessed to repair the CNS? *Nat. Rev. Neurosci.*, **2008**, *9*, 481-493. [https://doi.org/10.1038/nrn2398]
- [137] Yin, Y.; Cui, Q.; Li, Y. Macrophage-derived factors stimulate optic nerve regeneration. *J. Neurosci.*, **2003**, *23*, 2284-2293.
- [138] Kurimoto, T.; Yin, Y.; Habboub, G. Neurophils express oncomodulin and promote optic nerve regeneration. *J. Neurosci.*, **2013**, *33*, 14816-14824. [https://doi.org/10.1523/JNEUROSCI.5511-12.2013]
- [139] Mueller, B.K. Growth cone guidance: first steps towards a deeper understanding. *Annu. Rev. Neurosci.*, **1999**, *22*, 351-388. [https://doi.org/10.1146/annurev.neuro.22.1.351]
- [140] Hsieh, S.H.; Ferraro, G.B.; Fournier, A.E. Myelin-associated inhibitors coflin phosphorylation and neuronal inhibition through LIM kinase and Slingshot phosphatase. *J. Neurosci.*, **2006**, *26*, 1006-1015. [https://doi.org/10.1523/JNEUROSCI.2806-05.2006]
- [141] Lingor, P.; Teusch, N.; Schwarz, K. Inhibition of Rho kinase (ROCK) increases neurite outgrowth on chondroitin sulphate proteoglycan *in vitro* and axonal regeneration in the adult optic nerve *in vivo*. *J. Neurochem.*, **2007**, *103*, 181-189. [https://doi.org/10.1111/j.1471-4159.2007.04756.x]
- [142] Lehmann, M.; Fournier, A.; Selles-Navarro, I. Inactivation of Rho signaling pathway promotes CNS axon regeneration. *J. Neurosci.*, **1999**, *19*, 7537-7547.
- [143] Bertrand, J.; Winton, M.J.; Rodriguez-Hernandez, N.; Campenot, R.B.; McKerracher, L. Application of Rho antagonist to neuronal cell bodies promotes neurite growth in compartmented cultures and

- regeneration of retinal ganglion cell axons in the optic nerve of adult rats. *J. Neurosci.*, **2005**, *25*, 1113-1121. [https://doi.org/10.1523/JNEUROSCI.3931-04.2005]
- [144] Ahmed, Z.; Berry, M.; Logan, A. ROCK inhibition promotes adult retinal ganglion cell neurite outgrowth only in the presence of growth promoting factors. *Mol. Cell. Neurosci.*, **2009**, *42*, 128-133. [https://doi.org/10.1016/j.mcn.2009.06.005]
- [145] Giger, R.J.; Hollis, E.N.; Tuszynski, M.H. EphB3: an endogenous mediator of adult axonal plasticity and regrowth after CNS injury. *J. Neurosci.*, **2006**, *26*, 3087-3101. [https://doi.org/10.1523/JNEUROSCI.4797-05.2006]
- [146] Hollis, E.N. Axon guidance molecules and neural circuit remodeling after spinal cord injury. *Neurotherapeutics*, **2016**, *13*, 360-369. [https://doi.org/10.1007/s13311-015-0416-0]
- [147] Bundesen, L.Q.; Scheel, T.A.; Bregman, B.S.; Kromer, L.F. Ephrin-B2 and EphB2 regulation of astrocyte-meningeal fibroblast interactions in response to spinal cord lesions in adult rats. *J. Neurosci.*, **2003**, *23*, 7789-7800.
- [148] Torres-Vazquez, J.; Gitler, A.D.; Fraser, S.D. Semaphorin-plexin signaling guides patterning of the developing vasculature. *Dev. Cell*, **2004**, *7*, 117-123.
- [149] Montolio, M.; Messegue, J.; Masip, I. A semaphorin 3A inhibitor blocks axonal chemorepulsion and enhances axon regeneration. *Chem. Biol.*, **2009**, *16*, 691-701.
- [150] Eickholt, B.J.; Walsh, F.S.; Doherty, P. An inactive pool of GSK-3 at the leading edge of growth cones is implicated in Semaphorin 3A signaling. *J. Cell Biol.*, **2002**, *157*, 211-217.
- [151] Kikuchi, K.; Kishino, A.; Konishi, O. *In vitro* and *in vivo* characterization of a novel semaphorin 3A inhibitor, SM-216289 or xanthofulvin. *J. Biol. Chem.*, **2003**, *278*, 42985-42991.
- [152] Moreau-Fauvarque, C.; Kumanogoh, A.; Camand, E. The transmembrane semaphorin Sema4D/CD100, an inhibitor of axonal growth, is expressed on oligodendrocytes and upregulated after CNS lesion. *J. Neurosci.*, **2003**, *23*, 9229-9239.
- [153] Ito, Y.; Oinuma, I.; Katoh, H.; Kaibuchi, K.; Negishi, M. Sema4D/plexin-B1 activates GSK-3beta through R-Ras GAP activity, inducing growth cone collapse. *EMBO Rep.*, **2006**, *7*, 704-709.
- [154] Liu, X.; Hawkes, E.; Ishimaru, T.; Tran, T.; Sretavan, D.W. EphB3: an endogenous mediator of adult axonal plasticity and regrowth after CNS injury. *J. Neurosci.*, **2006**, *26*, 3087-3101.
- [155] Paixao, S.; Balijepalli, A.; Serradj, N. EphrinB3/EphA4-mediated guidance of ascending and descending spinal tracts. *Neuron*, **2013**, *80*, 1407-1420.
- [156] Oster, S.F.; Bodeker, M.O.; He, F.; Sretavan, D.W. Invariant Sema5A inhibition serves an ensheathing function during optic nerve development. *Development*, **2003**, *130*, 775-784.
- [157] Fu, W.Y.; Chen, Y.; Sahin, M. Cdk5 regulates EphA4-mediated dendritic spine retraction through an ephexin1-dependent mechanism. *Nat. Neurosci.*, **2007**, *10*, 67-76.
- [158] Niederost, B.; Oertle, T.; Fritsche, J.; McKinney, R.A.; Bandtlow, C.E. Nogo-A and myelin-associated glycoprotein mediate neurite growth inhibition by antagonistic regulation of RhoA and Rac1. *J. Neurosci.*, **2002**, *22*, 10368-10376.
- [159] Courtine, G.; Song, B.; Roy, R.R. Recovery of supraspinal control of stepping *via* indirect propriospinal relay connections after spinal cord injury. *Nat. Med.*, **2008**, *14*, 69-74.
- [160] Bareyre, F.M.; Kerschensteiner, M.; Raineteau, O.; Mettenleiter, T.C.; Weinmann, O.; Schwab, M.E. The injured spinal cord spontaneously forms a new intraspinal circuit in adult rats. *Nat. Neurosci.*, **2004**, *7*, 269-277.
- [161] Gomez-Pinilla, F.; Ying, Z.; Roy, R.R.; Molteni, R.; Edgerton, V.R. Voluntary exercise induces a BDNF-mediated mechanism that promotes neuroplasticity. *J. Neurophysiol.*, **2002**, *88*, 2187-2195.
- [162] Sandrow-Feinberg, H.R.; Izzii, J.; Shumsky, J.S.; Zhukareva, V.; Houle, J.D. Forced exercise as a rehabilitation strategy after unilateral cervical spinal cord contusion injury. *J. Neurotrauma*, **2009**, *26*, 721-731.