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ORIGINAL ARTICLE

Mechanisms of Axonal Damage and Repair after Central Nervous System Injury

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Abstract Central nervous system (CNS) injury initiates spatial and temporal neurodegeneration. Under pathologic conditions, damaged glial cells cannot supply sufficient metabolites to neurons, leading to energy deficiency for neuronal axons. The widespread disruption of cellular membranes causes disturbed intracellular signaling via dysregulated ionic gradients in neurons. Although several deleterious cascades are activated during the acute phase of CNS injury, some compensatory responses may tend to promote axonal repair during the chronic/remodeling phase. Because it may not be easy to block all multifactorial neurodegenerative pathways after CNS injury, supporting or boosting endogenous regenerative mechanisms would be an important therapeutic approach for CNS diseases. In this mini-review, we briefly but broadly introduce basic mechanisms that trigger axonal degeneration and then discuss potential targets for promoting axonal regeneration after CNS injury.

Keywords Axonal repair · Axonal regeneration · CNS injury

Introduction

Axonal damage is one of the major common hallmarks in many types of central nervous system (CNS) injuries. The extent of axonal damage during the acute phase and the degree of axonal regeneration in the chronic phase are important determinants of the ability to recover from CNS injury. However, it is difficult to predict how axons would eventually be damaged in the affected region because the mechanism of axon degeneration is quite complicated. Axonal dysfunction after injury varies with the injury phase, the types of cells involved, and the severity of injury. Therefore, to configure a therapeutic approach to repair damaged axons, we first need to understand the triggers that induce axonal degeneration after injury.

Although several deleterious cascades are activated during the acute phase of CNS injury, some compensatory responses may tend to promote axonal repair in the chronic/remodeling phase. However, those endogenous repairing system may not always suffice to overcome the deleterious cascades. Therefore, administration of drugs that can boost/promote the compensatory mechanisms can be a promising approach to repair damaged axons in CNS diseases (Fig. 1). In a broad sense, therapeutic targets for axon regeneration may include (i) the regulation of intra-axonal signaling, (ii) the transcriptional enhancement of regeneration-associated gene expression via epigenetic regulation, and (iii) the recruitment of specific cells to the injured area for systematic repair and remodeling. This mini-review paper highlights the basic mechanisms that trigger axonal degeneration during the acute phase of CNS injury and discusses the possibilities for therapeutic strategies that promote axon regeneration during the chronic phase.

Triggers of Axonal Degeneration and Neuronal Death in CNS Injury

After the onset of axonal damage in CNS injuries such as ischemia and trauma, the catastrophic disruption of cellular

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Fig. 1 Basic concept to promote axon regeneration after CNS injury: After CNS injury such as stroke or trauma, several deleterious cascades are activated to cause axonal damage. Although CNS tissues show some compensatory responses to repair damaged axons under the chronic phase, these endogenous repairing systems may not suffice to overcome the deleterious cascades. Because it may not be easy to block all the multifactorial deleterious cascades during the acute phase of CNS injury, administration of drugs that can promote compensatory mechanisms (or block the inhibitory mechanisms) may be an effective strategy in repairing damaged CNS tissues
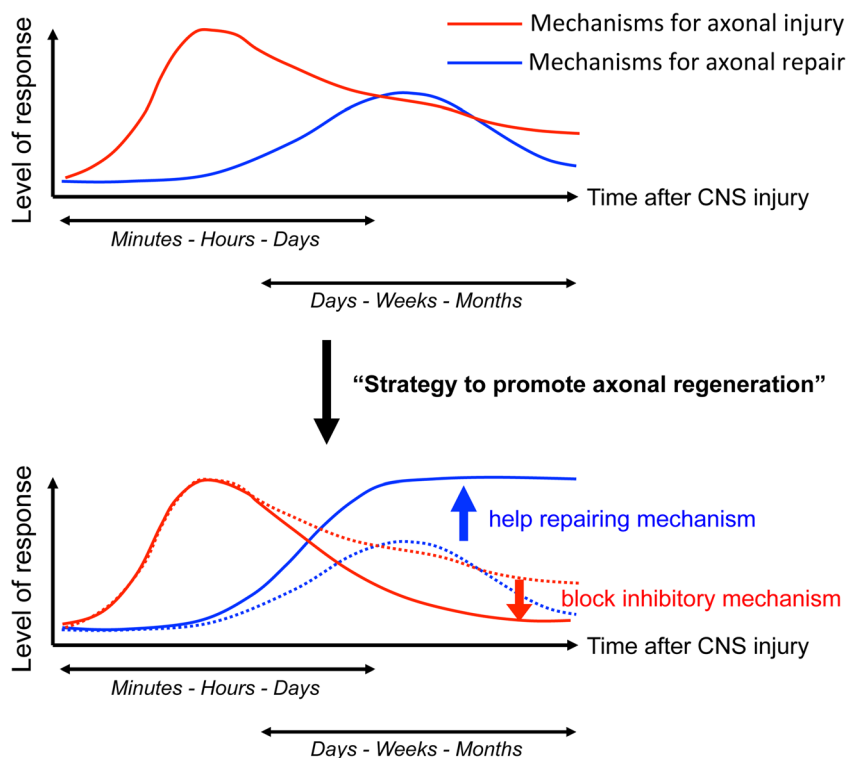

membranes results in the depletion of metabolites and energy for neurons. Dysregulated ion gradients disturb several intracellular signaling pathways and destabilize the microtube cytoskeleton and eventually lead to axon damage. While damaged tissues including brain attempt to repair damaged neuronal axons, substances derived from damaged glial cells would decrease axonal regeneration. Here, we categorize the key mechanisms associated with axonal degeneration after CNS injury, based on the following triggering events: (i) deficiency of energy and metabolites, (ii) calcium (Ca^{2+})-mediated cell apoptosis and degeneration, and (iii) myelin-associated

inhibitors of axonal regeneration after injury (Fig. 2). We briefly overview these mechanisms in this section.

Deficiency of Energy and Metabolites from Glial Cells

Neurons require a great deal of energy and metabolic support from glial cells. However, after injury, damaged or stressed glial cells may no longer support neurons, and neurons will be depleted of energy and metabolites. Among glial-derived nutrients for neurons, lactate may play an important role in
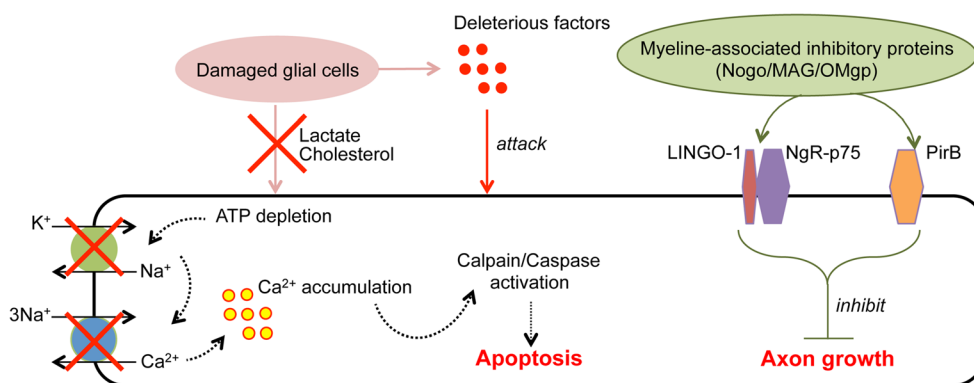
Fig. 2 Triggers that cause axonal degeneration and neuronal death during the acute phase of CNS injury: Multifactorial deleterious mechanisms are involved in axonal damage during the acute phase of CNS injury. Those mechanisms include (i) deficiency of energy and metabolites, (ii) calcium (Ca^{2+})-mediated cell apoptosis and degeneration, and (iii) myelin-associated inhibitors of axonal regeneration after injury. Please see "Triggers of Axonal Degeneration and Neuronal Death in CNS Injury" section for details of these pathological mechanisms

regulating axonal function. Lactate is necessary for production of adenosine triphosphate (ATP), which is a major energy source for maintaining neuronal function. Monocarboxylate transporters (MCTs) in glial cells (astrocytes and oligodendrocytes) mediate the transport of lactate from glial cells to neurons (reviews by Morrison et al. and Simpson et al. [1, 2]). Hence, under diseased conditions, energy loss in neurons occurs due to disruption of glial MCTs, which eventually lead to axonal damage and neuronal loss [3]. Another example of nutrition from glial cells to neurons is that of cholesterol, which is necessary for the structural mobility and axonal plasticity of neurons. Cholesterol is mainly synthesized in glial cells and secreted to neurons in the form of apolipoprotein E (ApoE)-containing lipoprotein particles (review by Camargo et al. [4]). However, once again, damaged glial cells cannot produce sufficient cholesterol for neurons [5]. Taken together, glial cells may no longer support neurons under diseased conditions, which would be one of the major causes for axonal dysfunction after CNS injury. Importantly, beside the mechanism of the loss of glial support to neurons, glial cells may secrete deleterious factors, such as inflammatory cytokines, that could damage neurons. Additionally, reactive astrocytes form glial scars that physically block neurite extension. Nevertheless, it should be noted that those reactive astrocytes may in turn contribute to axonal regeneration under some conditions of CNS diseases. In fact, attenuated astrocytic reactivity by a genetic approach impaired/delayed neurological recovery by reducing corticospinal tract axonal remodeling in mice [6], suggesting beneficial effects of reactive astrocytes for axonal remodeling/repairing. Therefore, glial cells play multiple roles in regulating the extracellular microenvironment for axonal damage and repair after CNS injury.

Calcium-Mediated Cell Apoptosis and Degeneration

A physiological level of intracellular Ca^{2+} is necessary for maintaining neuronal function. However, after CNS injury, intracellular Ca^{2+} levels in neurons would increase and the excessive levels of Ca^{2+} cause axonal damage through (i) the activation of Ca^{2+}-dependent proteases (e.g., calpain) and phospholipase, (ii) the disruption of mitochondrial oxidative phosphorylation, and (iii) the formation of free radicals and dysregulated Ca^{2+}-mediated signaling cascade. Under pathological conditions, the absence of an adequate energy supply causes Na^+-K^+ ATPase failure, which leads to persistent Na^+ entry and K^+ efflux. These changes in neurons eventually induce the reversal of the Na^+/Ca^{2+} exchanger and the resultant accumulation of intra-axonal Ca^{2+}. In addition, in the setting of severe injuries such as ischemia and trauma, proper ionic gradients cannot be re-established due to the breach of axonal membranes, and massive Ca^{2+} release occurs from intracellular Ca^{2+} stores. This massive wave of Ca^{2+} increase triggers catastrophic apoptotic events in the core region of

CNS injury. Nevertheless, a moderate level of intra-neuronal Ca^{2+} concentration may be necessary for axon regeneration. After axonal damage, the injury-induced retrograde Ca^{2+} wave (i.e., from the injury site to the cell body) induces nuclear export of histone deacetylase 5 (HDAC5) via protein kinase C activation, which promotes axonal regeneration [7]. Therefore, regulating intra-neuronal Ca^{2+} level would be important to reduce axonal damage as well as to support axonal regeneration. In the "Regulation of Intracellular Signaling for Axon Regeneration" section below, we will discuss the therapeutic potential in regulating Ca^{2+}-dependent signaling cascades for promoting axonal regeneration.

Myelin-Associated Inhibitory Proteins of Axonal Regeneration After CNS Injury

Although myelinated oligodendrocytes generally support axonal function, some proteins derived from myelin have been shown to inhibit axonal regeneration. These inhibitory proteins include Nogo, myelin-associated glycoprotein (MAG), and oligodendrocyte myelin glycoprotein (OMgp) (review by Filbin [8]). They all bind the Nogo receptor (NgR)-p75NTR complex, and activation of NgR-p75NTR complex initiates Rho signaling pathways to inhibit neuron outgrowth through LIM kinase (LIMK) [9]. Among the Nogo family, Nogo-A proteins are enriched in oligodendrocytes and are mostly localized in endoplasmic reticulum [10, 11], and Nogo-66, which is one domain of Nogo proteins, is situated outside of cells and binds the NgR-p75NTR complex. Nogo is a major endogenous inhibitory protein for neuronal extension, and in fact, Nogo-A/B-deficient myelin showed reduced inhibitory activity in a neurite outgrowth assay in vitro [12]. However, after dorsal hemisection of the spinal cord in vivo, the Nogo-deficient mutant did not increase regeneration or sprouting of corticospinal tract fibers [12], suggesting that elimination of Nogo alone may not be sufficient to promote axon regeneration under pathological conditions in vivo. The other two, MAG and OMgp, are considered to play the similar roles in axon regeneration as Nogo, through binding the NgR-p75NTR complex. MAG is a member of the immunoglobulin super family and binds sialo-glycoprotein and sialo-glycolipids (gangliosides) [13, 14]. OMgp contains a leucine-rich repeat (LRR) domain and is expressed not only in the CNS but also in the peripheral nervous system (PNS) [15, 16]. Although all these inhibitory proteins negatively impact neurite extension, the triple-mutant mice (e.g., Nogo-/MAG-/OMgp-deficient mice) failed to exhibit enhanced regeneration of axonal tract after spinal cord injury in vivo [17], indicating that blocking those pathways may not be sufficient to restore meaningful behavior after axonal damage. The receptor NgR is the most studied receptor for these inhibitory proteins. However, another candidate receptor, paired immunoglobulin-like receptor B (PirB), has recently been

identified [18]. Activation of PirB leads to inhibition of neural and axonal regeneration, but again, genetic deletion of PirB did not promote axonal plasticity and functional recovery in a mouse model of traumatic brain injury [19]. Taken together, those myelin-derived inhibitory molecules (Nogo/MAG/OMgp) and their receptors (NgR and PirB) negatively impact neurite extension, and therefore, those signaling pathways could be potential targets for axonal repairing and remodeling for CNS diseases. However, as mentioned above, blocking one or even multiple pathways from those inhibitory proteins may not suffice for recovering neurological and behavioral deficits. Translation from basic research to clinical application is difficult, especially in stroke recovery studies (review by Jolkkonen and Kwakkel [20]). Therefore, we will need to achieve a greater understanding of the function of these inhibitory proteins under pathological conditions, in order to target them more effectively in efforts to promote axonal repair.

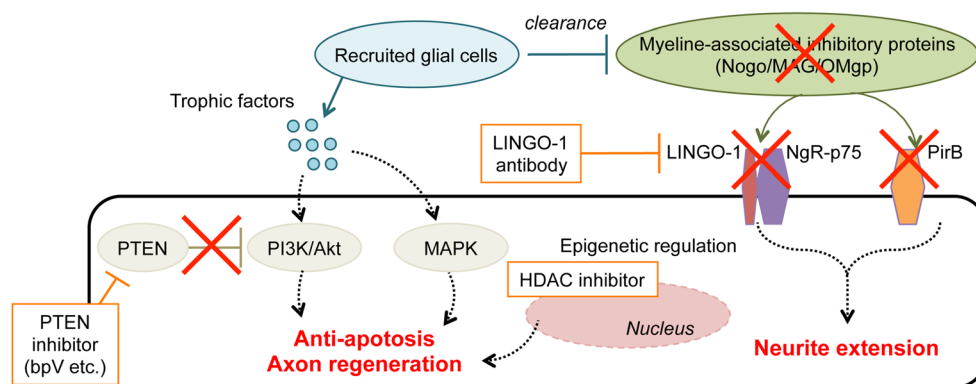## Strategies to Promote Axonal Regeneration in CNS

The intrinsic properties of neurons depend on the actions of supportive glial cells. However, as discussed, neighboring glial cells exert inhibitory effects for axonal sprouting by disturbing intra-neuronal signaling cascades, which results in a limited axonal regenerative response in the damaged CNS tissues. Therefore, strategies to regenerate axons and guide them towards a precise reconnection may need to focus on the following aspects of recovery: (i) regulation of intracellular signaling for axon sprouting, (ii) transcriptional enhancement of the intrinsic signaling for axon regeneration, and (iii) clearance or blockage of glia-associated inhibitors of axonal regeneration (Fig. 3). In this section, we discuss the

therapeutic potential of these mechanisms in enhancing axonal regeneration after CNS injury.

Regulation of Intracellular Signaling for Axon Regeneration

Regulation of Ca^{2+}-mediated intracellular signaling after CNS injury is essential for neuronal survival. These signaling pathways include (i) Ca^{2+}-dependent caspase/calpain activity, (ii) phosphoinositide 3-kinase (PI3K)/AKT signaling pathway, and (iii) mitogen-activated protein kinase (MAPK) signaling. Caspases and calpains are important regulators for cell death pathways in most types of cells including neurons. Among the caspase family, caspase-9 works as an active initiator in ischemic stroke brains. Neuronal caspase-9 activity induces caspase-6 activation, resulting in the axonal degradation [21]. At the same time, calpains are also activated after stroke, and systematic calpain inhibition partially protects axonal initial segment loss from brain ischemia [22]. The PI3K/AKT signaling pathway, which is a major pro-survival mechanism in cells, also plays an important role in axon regeneration. Mammalian target of rapamycin (mTOR) signaling is a downstream pathway of PI3K/Akt, and it has been reported to participate in neuronal protection [23] as well as axon regeneration after injury [24, 25]. An opposing effect is seen in the activity of phosphatase and tensin homolog (PTEN), which is highly expressed in CNS [26]. After CNS injury, PTEN is upregulated and reduces the PI3K/Akt-mediated axonal regeneration and cellular survival [27]. Therefore, strategies for inhibiting PTEN may enhance axonal outgrowth and providing neuroprotection after CNS injury. In fact, a specific inhibitor of PTEN signaling, bisperoxovanadium (bpV), was shown to promote axonal regeneration in a mouse model of

Strategies to promote axonal regeneration during chronic phase of CNS injury

Fig. 3 Strategies to promote axon regeneration during the chronic phase of CNS injury: Although compensatory mechanisms are activated in damaged CNS tissues in response to the damage after injury, those reparative trials usually fail because of overly activation of deleterious cascades. Therefore, strategies to regenerate axons will need to incorporate a stronger focus on overcoming those deleterious

mechanisms by (i) regulating intracellular signaling for axon sprouting, (ii) transcriptional enhancement of the intrinsic signaling for axon regeneration, and (iii) clearance or blockage of glia-associated inhibitors of axonal regeneration. Please see "Strategies to Promote Axonal Regeneration in CNS" section for details of these therapeutic strategies

stroke [28]. The three main MAPK signaling pathways are the extracellular signal-regulated kinases (Erks), p38 MAPK, and c-Jun N-terminal kinases (JNKs). Among them, the Erk cascade has been considered as a major pro-survival intracellular signaling. In fact, a study of spinal cord injury showed that GDNF promoted neurite outgrowth via downstream Erk1/2 signaling [29]. Instead, activations of p38 MAPK and JNK are known to induce apoptosis. However, under some conditions, both p38 MAPK and JNK may show positive effects for axon regeneration and regrowth. Coordinated activation of p38 and JNK MAPK signaling pathways is required for the axon regeneration in *Caenorhabditis elegans* [30]. Also, JNK and its upstream kinase dual leucine zipper kinase (DLK) are highly expressed at the axon head and function in axon guidance. Axonal outgrowth after axotomy is inhibited by blockage of DLK, indicating that DLK-JNK signaling is essential for axonal regeneration [31, 32]. Since those intracellular pathways introduced above are all related to axonal regeneration either in a positive or negative way, modulating them can be an effective therapeutic approach to promote axonal repair. However, the intracellular molecules of those pathways may interact closely with each other. Therefore, further analysis is needed for a better understanding of the pathological mechanisms as to how those intra-neuronal signaling pathways regulate each other after CNS injury.

Transcriptional Enhancement of the Intrinsic Signaling for Axon Regeneration

Regenerative transcriptional programing is activated after axonal injury in the PNS but much less extensively in the CNS. This may suggest that the accessibility of transcriptional factors to the promoter region of regenerative genes is strictly regulated in the mature CNS. DNA chromatin modifications alter gene expression, a process known as epigenetic regulation. Recent studies have demonstrated that epigenetic regulation could be closely related to development, plasticity of neurogenesis, and axonal outgrowth and regeneration (reviews by Lindner et al. and Hirabayashi et al. [33, 34]). Roughly speaking, epigenetic regulation can be categorized into DNA methylation and histone modification. Combinations of methylated DNA with acetylated/methylated histones are associated with "open" or "closed" epigenetic conditions for gene expression. DNA methylation is mediated by DNA methyltransferase enzymes (DNMTs), which are composed of DNMT1, DNMT3A, and DNMT3B. In general, DNMT1 is necessary for the maintenance of DNA methylation, and DNMT3A/3B are necessary for de novo DNA methylation (review by Wu and Zhang [35]). Although the mechanisms/roles of DNA methylation on axon regrowth are still largely unknown, there are some reports that showed the relationship between DNA methylation and axon growth. Methyl-CpG binding domain protein 2 (MECP2)

binds to methylated DNA sites in the methylated gene promoters and represses the transcription from the gene (review by Jaenisch and Bird [36]). The disruption or mutation of MECP2 causes the X-linked Rett syndrome, a neurological disorder associated with axon growth deficiency and autistic symptoms [37]. However, DNA methylation may also adversely affect neuronal recovery after the CNS injury. DNA methylation is increased after brain ischemia in a DNMT1-activity-dependent manner [38]. Interestingly, mice with heterozygous mutation for DNMT1 are resistant to mild ischemic damage [38], suggesting that an increase in DNA methylation may exacerbate brain damage, including axonal dysfunction, after brain injury.

Besides DNA methylation, histone acetylation may also participate in axon regeneration. The degree of histone acetylation is generally determined by a balance between histone acetyltransferases (HATs) and HDACs, which acetylates or deacetylates lysine residues of histones, respectively. HATs bind to transformation-related protein (TRP53) to form a transcriptional complex, which enhances the promoter accessibility on the promoter of the regeneration-associated gene RAGs, such as RAB13, CORO1B, and growth-associated protein (GAP43). GAP43 is a neurotrophin-dependent membrane bound phospho-protein, which is expressed in axons of plastic regions of CNS, including regenerating tissues after CNS injury. Increasing histone acetylation by HDAC inhibitors induced GAP43 expression as well as improvement of axon regeneration after injury [39–41]. Mammalian HDACs are categorized into four classes based on their domain organization (review by Cho and Cavalli [42]). Among them, as shown in the "Calcium-Mediated Cell Apoptosis and Degeneration" section, HDAC5 from the class IIa HDACs may play an important role in axonal regeneration after injury via regulating microtuble dynamics [7, 43]. In addition, HDAC6 from the class IIb may also contribute to axon regeneration because inhibiting HDAC6 enhanced tubulin acetylation levels to promote axon regeneration of DRG neurons on the presence of myelin-associated glycoprotein (e.g., a condition that mimics inhibitory environments after CNS injury) [44]. Therefore, the epigenetic enhancement of gene expression related to axon regeneration could be a promising approach to enhance axonal regeneration after CNS injury.

Clearance or Blockage of Myelin-Associated Inhibitors

Clearance/blockage of myelin-associated inhibitors of axonal regeneration may promote neuronal regeneration after CNS injury (review by Filbin [8]). One of the promising strategies for the blockade of myelin-associated inhibitors consists of the use of inhibitors for the NgR-p75NTR complex signaling. An example target is the LRR and immunoglobulin-like domain containing protein 1 (LINGO-1), which contain 12 LRR motifs and one Ig domain. LINGO-1 suppresses neuron

outgrowth through a ternary complex with NgR and p75 (review by Mi et al. [45]). LINGO-1 expression is upregulated after CNS injury in diverse animal models and in human CNS diseases [46, 47]. Blocking LINGO-1 by monoclonal antibody enhanced axonal regeneration with reduced RhoA activation and improved hind limb function after spinal cord injury [48]. Therefore, monoclonal antibody to LINGO-1 would be an attractive approach in promoting endogenous compensatory responses for axon regeneration. However, as discussed in the "Myelin-Associated Inhibitory Proteins of Axonal Regeneration After CNS injury" section, the myelin-associated inhibitors (and their receptors) may substitute each other for the roles of inhibition in axon extension in vivo. Therefore, whether blocking LINGO-1 with monoclonal antibody (or its specific inhibitors) restores meaningful behavior after axonal damage may need to be carefully examined in future studies.

As an alternative strategy that targets myelin-associated inhibitors may be supplementation with specific types of cells because some kinds of cells clear the myelin (or cell debris) in damaged area after injury. Immune cells and microglia are recruited or polarized to an injured region during the acute phase of CNS injury (review by Benakis et al. [49]). However, their roles within the damaged area are not simple. They produce inflammatory cytokines and reactive oxygen species, which accelerate brain damage. But on the other hand, those actions may be in turn beneficial during the chronic phase. For example, the breakdown of blood-brain barrier by those deleterious factors promotes the infiltration of macrophage from blood to brain parenchyma, which helps to build up favorable environments for axon regeneration by removing myelin and cell debris. Besides the roles in clearing damaged tissues, those recruited glial cells would release trophic factors, which can activate pro-survival PI3K/Akt and/or MAPK signaling pathways in neurons. Neurons may participate in the recruitment of those glial cells and macrophages. After the injury, damaged/dead neurons release damage-associated molecular pattern molecules to attract/activate microglia and astrocytes (review by Iadecola and Anrather [50]). Therefore, drugs that can enhance the recruiting of those glial cells would be an important therapeutic approach to promote axon regeneration. However, as mentioned, those activated glial cells show deleterious effects (e.g., attack neighboring cells by secreting inflammatory factors or form glial scars) depending on the context. Therefore, future studies need to examine how to enhance the beneficial actions but suppress deleterious ones of those cells.

Although myelin produces some proteins that inhibit neurite outgrowth after CNS injury, the myelin sheath and myelinating oligodendrocytes are indeed critical for proper axonal function. Under diseased conditions, damaged oligodendrocytes no longer support sufficient levels of myelin sheath formation. In addition, mature oligodendrocytes do not proliferate to generate new oligodendrocytes. Hence, oligodendrocyte precursor cells (OPCs) may play an essential role for oligodendrogenesis during the chronic phase of CNS diseases by contributing to the repair of damaged axons. In general, OPCs are active during development and generate myelin-producing oligodendrocytes. After adolescence, residual OPCs remain quiescent, but in response to CNS injury, they proliferate and differentiate into oligodendrocytes to ease myelin damage (reviews by Zhang et al. [51] and Maki et al. [52]). A recent study demonstrated that in a mouse model of infraorbital optic nerve crush injury, OPCs may create an environment that is permissive to axonal regeneration through β-catenin-dependent signaling [53]. In addition, OPCs are now proposed as a new cell source for cell-based therapy. Stem cell therapy has demonstrated safety and efficacy in experimental animal models of CNS diseases including stroke (review by Rodríguez-Frutos et al. [54] and Napoli and Borlongan [55]). In fact, several studies reported that transplanted OPCs could improve remyelination in animal models of demyelination [56–60]. Therefore, while oligodendrocyte-derived myelin produces some inhibitory proteins for axon regeneration, promoting oligodendrocyte (re)generation from endogenous (or even transplanted) OPCs may be an important step for axon regeneration during the chronic phase of CNS injury.

Conclusion

Axonal damage is a major common pathology in many types of CNS diseases. As seen above, pathways and cascades that lead to axon damage after CNS injury are complex. Because axon regeneration is critical for functional recovery and blocking the multifactorial deleterious cascades during the acute phase of CNS injury is difficult, the use of drugs that can boost axonal regeneration would be a promising therapeutic approach for CNS diseases. In this mini-review, we briefly but broadly overviewed key signaling pathways for axonal injury under the acute phase of CNS injury, along with the potential targets to promote axonal regeneration in the chronic phase. Basic mechanisms for axonal dysfunction and compensatory axon regenerative responses may be shared in many CNS diseases. However, the exact pathways that cause axonal dysfunction or promote axonal regeneration are likely different between disease types. Therefore, for the purpose of translation of basic research into clinical applications, candidate compounds for those targets will require rigorous investigation using disease-specific animal models before testing them in clinical studies.

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