PERSPECTIVE

Glycogen synthase kinase 3: a crucial regulator of axotomy-induced axon regeneration

Following nerve injury, axonal disconnection in neurons usually results in persistent functional deficits, such as paralysis. However, axons in the adult mammalian central nervous system (CNS) have very limited regenerative ability. Understanding the molecular mechanism of controlling axon regeneration can provide idea for the design of effective therapeutic interventions for CNS injury, such as spinal cord injuries. Efficient axonal regeneration is achieved via gene expression in the neuronal soma, axonal transport of raw materials along the shaft, and membrane and cytoskeleton assembly at the nerve growth cone. Each process is delicately regulated by spatial-temporal controlled signaling pathways that target distinct effectors. Gene expression in the neuronal soma, especially of transcription factors, is often activated immediately following nerve injury. Injury signals at distal axons are interpreted and transmitted back to the soma, initiating a stream of gene expression events which positively regulate subsequent axonal regeneration. Over the past few decades, extensive studies have identified many regeneration-associated genes, including CREB, nuclear factor of activated T-cells, protein 53, Sprr1a, c-Jun, Smad1, activating transcription factor 3, signal transducer and activator of transcription 3, SRF, Sox11, and Kruppel-like factors. However, we know far less about how the coordinated expression of these regeneration-associated genes is regulated during axonal regeneration. Indeed, it is possible that they are regulated by a single common upstream regulator. If so, identification of this upstream regulator will provide us with an invaluable target for the development of more effective treatments for traumatic nerve injuries. Adult dorsal root ganglion (DRG) neurons represent a favorable medium in which to study the molecular mechanisms controlling intrinsic neuronal axon growth ability. Axotomy of the peripheral branch of a DRG neuron, known as a "conditioning lesion", has been well-documented to greatly accelerate axonal growth both *in vivo* and *in vitro*, by enhancing the neuronal intrinsic growth potential. Enhancement of the growth state is thought to be mediated by a transcription-dependent axonal growth system that controls the expression of a number of regeneration-associated genes. Indeed, after peripheral axotomy, well-known regeneration-associated genes such as gap43, c-jun, transcription factor 3, gadd45a, and sprr1a, are all markedly up-regulated in adult DRG neurons. However, signaling pathways specifically triggered by peripheral axotomy that lead to activation of intrinsic axonal growth ability and subsequent axonal regeneration remain poorly understood. In our latest study (Saijilafu et al., 2013), using adult DRG neurons, we demonstrated that peripheral nerve injury activates the phosphatidylinositol 3-kinase (PI3K) pathway, and inhibition of PI3K using pharmacological agents markedly suppressed axonal growth. Downstream of PI3K, we found that peripheral nerve injury could increase the phosphorylation of glycogen synthase kinase 3 (GSK3), which is regulated by PI3K signaling during axonal regeneration. We also confirmed that PI3K-GSK3β signaling plays an essential part in enhancing intrinsic growth ability, by inducing expression of the Smad1 transcription factor. Most importantly, it has been shown that activation of Smad1 promotes axonal regeneration after a spinal cord injury. These findings indicate that GSK3 plays a key regulator effect in controlling the axonal growth potential of adult neurons. GSK3, a serine/threonine kinase, was first identified and purified as a key kinase in glycogen metabolism in the 1980s. GSK3 has been found to be ubiquitously expressed in various tissue types, and to play crucial roles in numerous signaling cascades governing physiological or pathological processes, such as inflammation, tumorigenesis, and neurite outgrowth. Interestingly, numerous studies have shown that many of these regeneration-associated transcription factors are well-established GSK3 substrates. For example, one study demonstrated that GSK3-induced serine-129 phosporylation facilitates and fully activates CREB, which is necessary for CREB functions (Fiol et al., 1994). Inactivation of GSK3 would therefore stabilize Smad1 by preventing its phosphorylation and subsequent recognition by Smurf1. Several early studies also suggest that phosphorylation of the C-terminus of C-Jun by GSK3 tags it for degradation (Wei et al., 2005). Although some transcription factors have not been confirmed directly as GSK3 substrates, such as SRF, Sox11, Id2, and Kruppel-like factors, they contain multiple and well-conserved GSK3β sites. Epigenetic regulation is another way to control gene activity without changes in DNA sequences. Usually, epigenetic regulation uses DNA methylation, histone modification, and non-coding RNAs. Micro-RNAs (miRNAs) are non-coding RNAs, which regulate a variety of biological functions. There is increasing evidence to suggest that axonal regeneration processes are also regulated by miRNA pathways. Our latest study, for example, has shown that endogenous miR-26a is a physiological regulator of mammalian axonal regeneration both *in vitro* and *in vivo*, and that this regulatory effect is mediated by GSK3β (Jiang et al., 2015). Additionally, miR-133b regulates axon growth via PI3K/Akt signaling in PCNs pathway by suppressing RhoA (Lu et al., 2015). Taken together, all of this evidence suggests that GSK3 is potentially a key regulator of intrinsic axonal growth ability during axonal regeneration.

Along with gene transcription, microtubule-dependent local axon assembly in the neuronal growth cone is another determinant for successful axonal regeneration. Microtubule assembly usually includes three steps: microtubule polymerization, stabilization, and the maintenance of dynamicity. During axonal regeneration, microtubule assembly is regulated by many molecules, including microtubule associated proteins (MAPs) and plus-end tracking proteins. MAPs are proteins that interact with cytoskeletal microtubules and bind to tubulin subunits to regulate their dynamics and stabilities in the growth cone. Interestingly, it has been reported that GSK3β directly phosphorylates many well-known MAPs such as collapsin response mediator protein-2, adenomatous polyposis coli, MAP1b, and tau proteins. Recently, a study revealed that GSK3 supports the axon growth by stabilizing microtubules (Inami et al., 2018). Our study also demonstrated that function of cytoplasmic linker protein-associated protein, a microtubule plus-end binding protein, is regulated by GSK3β during axonal regeneration (Hur et al., 2011). Additionally, Rho-ROCK signaling pathway activity is another well-known key regulator of the actin cytoskeleton remodeling during axon regeneration. Moreover, it has been reported that inhibition of GSK-3 can remarkably inhibits RhoA activity (Liu et al., 2013). Thus, it is clear that GSK3β is a principle regulator of local cytoskeleton assembly in the neuronal growth cone as well.

In addition to the pathways described above, GSK3 signaling also plays key roles in regulating raw materials axonal transport during axonal regeneration. The intracellular transport of raw materials such as proteins, lipids, and RNA along the axon shaft from the neuronal soma to the growth cone is essential for axonal regeneration. Typically in such a scenario, two microtubule-based motor protein types, kinesins and dyneins, carry cargos along the axon shaft in the anterograde and retrograde directions, respectively. Knocking down of endogenous dynein by siRNA has been shown to significantly inhibit microtubule protrusion in the neuronal growth cone (Myers et al., 2006). In contrast, knocking down of en-

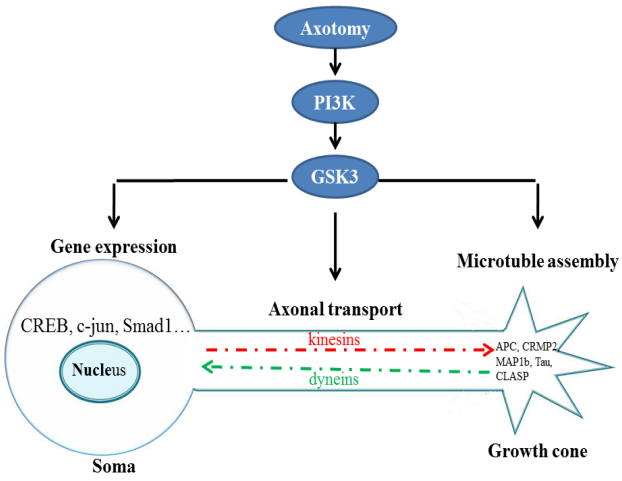

Figure 1 Glycogen synthase kinase 3β controls axonal growth by coordinating gene transcription, axonal transport, and local cytoskeleton assembly.
APC: Adenomatous polyposis colil; CLASP: CLIP-associated protein; CREB: cAMP response element binding protein; CRMP2: collapsin response mediator protein 2; GSK3: glycogen synthase kinase-3; MAP1b: microtubule associated protein 1b; PI3K: phosphatidylinositol-3 kinase.

dogenous kinesin significantly promotes axonal growth. It has been reported that GSK3β can directly phosphorylate cytoplasmic kinesins and dyneins, or their assistant adaptor proteins (Gibbs et al., 2015). For instance, recent data indicate that one of the transportation motor proteins, kinesin, can be negatively regulated by GSK3β (Weaver et al., 2013). Another published literature also suggested that GSK3 regulates motor proteins during axon regeneration via the presenilin loop region (Banerjee et al., 2018). Thus, GSK3β controls axon regeneration by regulation of axonal transport as well.

Axonal regeneration in the injured mammalian nervous system is a very complex process. In order to achieve long distance axonal regeneration, combinational strategies are necessary in spinal cord injury treatment. Fortunately, GSK3 plays several key regulating roles during axon regeneration, from gene expression in the soma and local axon assembly at the growth cone, to axonal transport (**Figure 1**). GSK3 kinase has two closely homologous isoforms, GSK3α and GSK3β. However, interestingly, they have the distinct effect on axon regeneration. GSK3β deletion alone can promote significant axon regeneration; in contrast, the deletion of GSK3α exerts little effect. Thus, better understanding of the precise mechanisms of GSK3 activity on axon regeneration may give us a valuable tool to treat intractable nerve injury, such as spinal cord injuries.

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