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PERSPECTIVE

Role of GSK3 in peripheral nerve regeneration

In recent years, GSK3 has emerged as a key regulatory kinase in the nervous system, which is involved in diverse processes ranging from neural development to mood stabilization to neurodegeneration. In addition, it has been described as a regulator of several aspects of neural morphology such as polarization, axon growth and branching (Seira and Del Rio, 2014). GSK3 exists as two isoforms (GSK3α and GSK3α), which are both generally expressed throughout the cell body, dendrites and axons of post-mitotic neurons and active in resting, unstimulated cells. Nevertheless, neuronal GSK3 activity can be differentially regulated in distinct cellular compartments as it is controlled by complex mechanisms (*i.e.*, distribution, protein-protein interactions or posttranslational phosphorylation) (Beurel et al., 2015). This regulation depends on diverse signaling pathways including protein kinase B (AKT), p38 mitogen-activated protein kinase (MAPK), and protein tyrosine phosphatase (PTPase) (Doble and Woodgett, 2003). Phosphorylation of an amino-terminal serine residue (Ser 21 in GSK3α, Ser 9 in GSK3α) by AKT is one of the most prominent mechanisms of GSK3 inactivation (Seira and Del Rio, 2014). GSK3 itself acts on multiple and diverse targets, thereby regulating gene transcription, axonal transport and cytoskeletal dynamics (**Figure 1**). Therefore, GSK3 appears to be a nodal point for the integration of diverse extra- and intracellular cues affecting axonal growth.

Although GSK3 is indisputably involved in the modulation of regenerative processes upon axonal injury, its role in this context appears at first glance confusing and is still controversially discussed. Some groups suggested that GSK3 inhibition promotes axonal growth/regeneration whereas others reported the opposite. The reasons for these apparent discrepancies might be manifold. For one, they could be founded on the specific experimental conditions employed in various studies, like the use of different GSK3 inhibitors or variation in GSK3 expression levels. Moreover, the role of GSK3 in axon growth/regeneration could depend on physiological parameters, such as the growth environment (central nervous system (CNS) *vs.* peripheral nervous system (PNS)) as well as the animal model and age.

In previous studies, several distinct pharmacological GSK3 inhibitors have been used that differ in their mechanism of action (*i.e.*, ATP competition, inhibitory phosphorylation or protein interactions) and specificity profile (*i.e.*, off-targets effects). For example, lithium as the first 'natural' and widely used GSK3 inhibitor activates AKT and blocks protein phosphatases, thus increasing inhibitory phosphorylation on Ser 21/9 (Eldar-Finkelman and Martinez, 2011). On the other hand, SB-216763 inhibits kinase activity independent of serine phosphorylation *via* inhibition of ATP binding, while 6-bromoindirubin-3′-oxime (6-BIO) is able to act as a Wnt mimetic, modifying processes based on GSK3 protein interactions (Eldar-Finkelman and Martinez, 2011). Thus, different inhibitors potentially elicit inconsistent responses. Moreover, inhibitor dosage is another important aspect to consider as the extent of GSK3 inhibition seems to influence the experimental outcome (Kim et al., 2006; Hur and Zhou, 2010) and inappropriate concentrations likely target other related kinases. For instance, 6-BIO is only ~16-fold more selective for GSK3 relative to cyclin-dependent kinases (Eldar-Finkelman and Martinez, 2011). Another aspect is the timing and localization of inhibitor application. As neurons are particularly polarized cells, the experimental outcome might be different depending on whether GSK3 is inhibited in the cell body, the neurite or the axonal growth cone (**Figure 1**). For example, GSK3 inhibition affecting gene transcription (soma) could affect the intrinsic regenerative state of a neuron without significantly influencing microtubule assembly in axons. Examplary, specific GSK3 inactivation at the distal axon induced efficient axon elongation opposed to growth inhibition upon global inhibition (Conde and Caceres, 2009).

Ectopic expression of wild-type and genetically modified GSK3 isoforms provides further pitfalls for the investigation of the role of GSK3 in axon regeneration. First of all, additional gene copies might raise GSK3 expression and activity to unphysiological levels with potential adverse effects. In addition, most overexpression studies focus on GSK3β and rather neglect GSK3α, although most inhibitors affect both isoforms and both isoforms share substantial substrate specificity (Eldar-Finkelman and Martinez, 2011; Beurel et al., 2015). As a consequence, manipulation of GSK3β alone might only partially affect a given target and potentially even elicit compensatory responses of GSKα (and *vice versa*). More generally, the role of GSK3 might differ with respect to physiological parameters, such as the developmental age of neurons (embryonic, postnatal, adult), the specific cell type (*e.g.*, retinal ganglion cells, cortical, hippocampal or dorsal root ganglion neurons) and the axonal environment (CNS, PNS) (**Figure 1**), which impedes direct comparison of distinct experimental studies. For instance, SB216763 inhibits axon growth in postnatal and embryonic DRG neurons, but induces the formation of long axons in hippocampal, cerebellar granular and adult DRG neurons (Eldar-Finkelman and Martinez, 2011). Moreover, *in vitro* culture conditions, such as the coating of culture dishes with inhibitory substrates like myelin or the conditioning of neurons, most certainly affect the outcome of an *in vitro* assay. Considering these aspects, GSK3 might not just have one specific function, but rather adopt multiple diverse roles in axon growth and regeneration depending on the respective experimental, physiological and cellular context.

To avoid above mentioned experimental complications, we took advantage of $GSK3\alpha^{S21A}/GSK3\beta^{S9A}$ double knockin (DKI) mice to investigate the global role of AKT-modulated GSK3 activity in axon regeneration of the adult PNS (Gobrecht et al., 2014). These mice are genetically modified to render serine 21/9 phosphorylation of both GSK3 isoforms impossible. Thus, AKT-dependent GSK3 inhibition upon axotomy is prevented without altering GSK3 expression levels or using pharmacological inhibitors. Unexpectedly, this prevention of inhibitory GSK3 serine 21/9 phosphorylation markedly facilitated axon growth of adult cultured DRG neurons. Moreover, *in vivo* axon regeneration upon sciatic nerve injury was strikingly accelerated, leading to improved functional recovery. At first glance, these results appear to conflict with another study reporting normal axon regeneration in these transgenic mice (Zhang et al., 2014). However, axonal growth was differentially quantified in these two studies. Whereas Zhang et al. (2014) manually measured solely the longest axon per cultured neuron, we analyzed growth by automated evaluation of all neurites, which includes branched axons. Moreover, Zhang et al. (2014) investigated *in vivo* sciatic nerve regeneration by electroporation of DRGs with EGFP, which involves plasmid injection into and placement of electrodes around the respective DRG. However, this technique allows visualization of only very few regenerating sensory axons and excludes motor axons. In addition, the manipulation might have induced a regenerative program similar to a pre-conditioning lesion, which could have masked a potential growth-promoting effect of $GSK3^{S/A}$. In comparison, our analysis was based on immunochemical detection of all regenerating axons in transverse and longitudinal sections of the sciatic nerve as well as reforming neuro-muscular junctions in leg muscles. Moreover, improved axon regeneration was accompanied by faster recovery of sensory and motor functions (Gobrecht et al., 2014). Therefore, our results strongly suggest that translational approaches aiming to maintain global GSK3 activity in injured DRG neurons at a level similar to uninjured ones may be a useful strategy to promote nerve regeneration in the adult PNS.

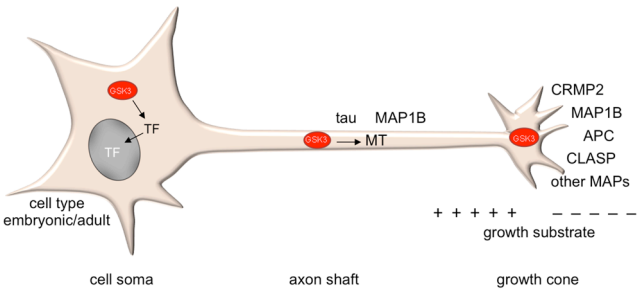

Figure 1 Physiological parameters affecting the role of GSK3 in axon regeneration.
The developmental age of neurons (embryonic versus adult) as well as the specific cell type (*e.g.*, retinal ganglion cells, cortical or hippocampal neurons) might influence the role of GSK3 (red) in axon regeneration. As neurons are particularly polarized cells, an experimental outcome might also depend on the subcellular localization of GSK3 (cell soma, axon shaft, growth cone) and/or its temporal and spatial regulation. In addition, the axonal environment (including growth-permissive (+++) or -inhibitory (– – –) substrates) might determine whether GSK3 modulation is beneficial or compromising for axon regeneration. GSK3 itself regulates diverse regenerative processes such as gene transcription, axonal transport and cytoskeletal dynamics by acting on multiple and distinct targets. In the cell soma, GSK3 is known to influence various transcription factors (TF), such as SMAD, CREB and TCF/LEF, to modulate a neuron's growth state. In neurites, GSK3 is particularly envisaged in the reorganization of microtubules (MT), as many of the microtubule-binding proteins, including APC, CLASP, Tau, CRMP2 and MAP1B, are validated GSK3 substrates.

GSK3 has a plethora of substrates and could hence be involved in several diverse regenerative processes. In this regard, global GSK3 activity might concurrently induce beneficial and adverse effects in a neuron, with growth-promotion prevailing in sciatic nerve axons. A more refined approach of GSK3 manipulation with respect to neuronal compartment and/or affected substrate(s) might therefore induce even stronger axon regeneration. For instance, many of the microtubule-binding proteins, such as APC, CLASP, Tau, CRMP2 and MAP1B (**Figure 1**), are validated GSK3 substrates that differently affect microtubule assembly and therefore axon growth. Most substrates, such as CRMP2 and APC, require priming by a distinct kinase (*e.g.* CK1, CDK5, DYRK) prior to phosphorylation by GSK3 (Seira and Del Rio, 2014), which adds a further activity control level. CRMP2 reportedly promotes microtubule polymerization, while CLASP and APC support microtubule stability in growth cones (Seira and Del Rio, 2014). The functions of these substrates are inhibited by GSK3 phosphorylation, raising the expectation that sustained GSK3 activity would rather compromise axon growth. MAP1B, on the other hand, is activated by GSK3-mediated phosphorylation without the need of prior priming and promotes axon growth by increasing microtubule dynamics (Goold et al., 1999; Gonzalez-Billault et al., 2002). Consistent with increased GSK3 activity, MAP1B phosphorylation levels were significantly higher in regenerating axons of GSK3 DKI mice compared to wild-type animals. Thus, increased microtubule dynamics may underlie the improved sciatic nerve regeneration in adult GSK3 DKI mice, which would have to be confirmed in future experiments. In addition, it is currently unknown whether the activities of CRMP2, APC or other GSK3 substrates co-existing in the growth cone are compromised upon sciatic nerve injury in GSK3 DKI mice. Interestingly, the level of CRMP2 phosphorylation in DRG cell bodies is similar in wild-type and GSK3 DKI mice (Zhang et al., 2014), suggesting regulation by the priming kinase or an alternative mechanism. Nevertheless, CRMP2 might be differentially regulated in the growth cone versus the cell body or other GSK3 substrates might be adversely affected by global GSK3 activation. In this scenario, it might be advantageous to regulate GSK3 activity differentially towards distinct substrates. For example, even more efficient axon regeneration might be achieved with simultaneously increased MAP1B (active GSK3) and CRMP2 (inactive GSK3) activities. In this regard, the potential differential substrate specificity of the two GSK3 isoforms might prove conducive for the development of tools that would allow the manipulation of GSK3 action only towards selected targets or signaling pathways (Beurel et al., 2015). However, it remains to be investigated whether for example MAP1B and CRMP2 might preferentially be phosphorylated by either GSK3α or GSK3β.

Likewise, GSK3 phosphorylation reportedly supports regeneration-promoting gene transcription (*e.g.*, SMAD expression) (Saijilafu et al., 2013). Therefore, opposing GSK3 regulation in different neuronal compartments (*e.g.*, soma *vs.* growth cone) or direct manipulation of downstream GSK3 targets instead of GSK3 itself may further increase the regenerative response in comparison to globally increased GSK3 activity. Finally, it needs to be investigated whether GSK3 DKI mice show improved axon regeneration also in the CNS. We envisage that increased microtubule dynamics might be rather unfavorable for regenerating axons that encounter an inhibitory (thus microtubule-depolymerizing) environment as in the CNS. Therefore, negligible or even opposite effects of $GSK3^{S/A}$ on CNS axonal regeneration are equally expectable and further experiments are required to address these possibilities. Surely, the complexity of GSK3 signaling will provide more interesting features in the future, which could then potentially be developed into novel therapeutic treatment options for nerve injuries.

Heike Diekmann, Dietmar Fischer*

Division of Experimental Neurology, Department of Neurology, Heinrich-Heine-University of Düsseldorf, Düsseldorf, Germany

***Correspondence to:** *Dietmar Fischer, Ph.D., dietmar.fischer@hhu.de.*
***Accepted:** *2015-09-08*
***orcid:** *0000-0003-1315-9064 (Dietmar Fischer)*
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Diekmann H, Fischer D (2015) Role of GSK3 in peripheral nerve regeneration. Neural Regen Res 10(10):1602-1603.

References

Beurel E, Grieco SF, Jope RS (2015) Glycogen synthase kinase-3 (GSK3): regulation, actions, and diseases. Pharmacol Ther 148:114-131.
Conde C, Caceres A (2009) Microtubule assembly, organization and dynamics in axons and dendrites. Nat Rev Neurosci 10:319-332.
Doble BW, Woodgett JR (2003) GSK-3: tricks of the trade for a multi-tasking kinase. J Cell Sci 116:1175-1186.
Eldar-Finkelman H, Martinez A (2011) GSK-3 inhibitors: preclinical and clinical focus on CNS. Front Mol Neurosci 4:32.
Gobrecht P, Leibinger M, Andreadaki A, Fischer D (2014) Sustained GSK3 activity markedly facilitates nerve regeneration. Nat Commun 5:4561.
Gonzalez-Billault C, Owen R, Gordon-Weeks PR, Avila J (2002) Microtubule-associated protein 1B is involved in the initial stages of axonogenesis in peripheral nervous system cultured neurons. Brain Res 943:56-67.
Goold RG, Owen R, Gordon-Weeks PR (1999) Glycogen synthase kinase 3beta phosphorylation of microtubule-associated protein 1B regulates the stability of microtubules in growth cones. J Cell Sci 112:3373-3384.
Hur EM, Zhou FQ (2010) GSK3 signalling in neural development. Nat Rev Neurosci 11:539-551.
Kim WY, Zhou FQ, Zhou J, Yokota Y, Wang YM, Yoshimura T, Kaibuchi K, Woodgett JR, Anton ES, Snider WD (2006) Essential roles for GSK-3s and GSK-3-primed substrates in neurotrophin-induced and hippocampal axon growth. Neuron 52:981-996.
Saijilafu, Hur EM, Liu CM, Jiao Z, Xu WL, Zhou FQ (2013) PI3K-GSK3 signalling regulates mammalian axon regeneration by inducing the expression of Smad1. Nat Commun 4:2690.
Seira O, Del Rio JA (2014) Glycogen synthase kinase 3 beta (GSK3beta) at the tip of neuronal development and regeneration. Mol Neurobiol 49:931-944.
Zhang BY, Saijilafu, Liu CM, Wang RY, Zhu Q, Jiao Z, Zhou FQ (2014) Akt-independent GSK3 inactivation downstream of PI3K signaling regulates mammalian axon regeneration. Biochem Biophys Res Commun 443:743-748.