PERSPECTIVE

Emerging roles for insulin-like growth factor binding protein like protein 1

Insulin-like growth factors (IGFs) mediate diverse cellular processes in various tissues, including the central nervous system (CNS) and thus require robust and delicate regulatory mechanisms. It is now known that IGF signaling is regulated by a superfamily of IGF binding proteins (IGFBPs) which share significant sequence homology and are secreted to the extracellular environment to bind IGF (Nguyen et al., 2013). Currently, there are 6 known IGFBPs and 10 IGFBP related proteins. A new member of the IGFBP superfamily, IGFBP like protein 1 (IGFBPL1), was then identified and shown to have tumor suppressor like properties (Smith et al., 2007). Since then, little has been reported about the physiological roles of IGFBPL1. Recently, IGFBPL1 was found to be critically involved in mediating IGF-1 signaling to control CNS axon growth and regeneration (Guo et al., 2018). The study has uncovered a new signaling loop in the regulation of the pleiotropic functions of IGF-1 and presents a possible novel pharmacological manipulation for promoting nerve regeneration and repair.

Regulation of axon regeneration: CNS axons of adult mammals do not normally regenerate after injury, which presents a major challenge in neuroscience. The optic nerve, formed by axons of retinal ganglion cells (RGCs), is a part of the CNS which is responsible for conveying visual information from the eye to the brain. It is widely recognized that both extrinsic and intrinsic factors contribute to the limited regenerative potential of RGC axons: while the adult CNS presents an inhibitory environment for axonal growth, mature RGCs also exhibit limited intrinsic capability to re-enter an active axon growth status. Myelin-associated glycoproteins, reactive glial scarring and extracellular matrix are thought to contribute to axon regrowth inhibition in the CNS environment (Davies et al., 1997). These inhibitory effects can be partially overcome by boosting the intrinsic axon growth machinery, including mammalian target of rapamycin (mTOR) and cyclic adenosine monophosphate (Cai et al., 1999), or suppressing transcriptional inhibitors of axon growth, such as Kruppel-like factor 4 and suppressor of cytokine signaling 3 (Moore et al., 2009; Smith et al., 2009). To date, the molecular signals that drive functional regeneration of RGC axons after injury remain to be determined.

IGF-1 is among the factors that are required for supporting the intrinsic axon growth status of CNS neurons. Its absence severely impairs CNS axon growth (Dupraz et al., 2013). Importantly, IGF-1 mediates not only axon elongation, but also other biological processes of developing neurons, including proliferation, survival, and synaptogenesis. Since these cellular processes do not occur simultaneously but are segregated into different developmental stages, the questions arise how IGF-1 selectively activates axon growth machinery without inducing proliferation or synaptogenesis in a certain period, and *vice versa*. Recent studies of IGFBPL1 begin to uncover this mystery by showing that IGFBPL1, when bound to IGF-1, selectively activates the axon growth cascades (Guo et al., 2018). The study points to a new therapeutic target for promoting axon regeneration and reversing vision loss.

A role for IGFBPL1 in RGC axon growth *in vitro* and *in vivo*: Mouse RGCs are known to lose their intrinsic axon growth capacity after embryonic day 18 (E18). As shown by Guo et al. (2018), expression of IGFBPL1 in RGCs correlates precisely with their axon growth ability during development, which reaches the peak at E16 and decreased sharply thereafter until becoming barely detectable in the adult. Acute knockdown of IGFBPL1 in purified primary RGC cultures largely reduced their axon growth and the percentage of RGCs bearing axons when compared with scrambled shRNA. In contrast, addition of IGFBPL1 significantly enhanced RGC axon growth and the number of cells bearing axons. Similar results were observed in both PC12 and hippocampal neuron cultures, supporting the general involvement of IGFBPL1 in the regulation of axon growth. Moreover, in the absence of IGFBPL1, *Igfbpl1*$^{-/-}$ mice exhibited reduced numbers of axons in the optic nerve or their RGCs displayed largely diminished ability to extend axons compared with wild-type mice. Addition of IGFBPL1 to RGCs isolated from *Igfbpl1*$^{-/-}$ mice completely rescued their axonal growth defects. Together, these findings for the first time demonstrate a role for IGFBPL1 in the regulation of RGC axon growth.

Requirement of IGF-1 signaling in IGFBPL1-mediated functions: IGF-1 mediates multiple biological processes in neurons, such as axonal growth, cell proliferation, survival, and synaptogenesis. IGF-1 or IGF-1 receptor (IGF-1R) knockout mice displayed overall growth retardation, smaller brain size and reduced axon number. These biological processes occur independently during defined periods of neuron development without much overlapping. For instance, RGCs that actively extend axons do not proliferate nor form synapses; the process of synaptogenesis begins long after RGCs cease axon growth. This raises the question as to how IGF-1 selectively activates axon growth machinery in developing RGCs without simultaneously inducing cell proliferation or synaptogenesis, etc. A tempting hypothesis is that IGF-1 binds to different IGFBPs which enable the activation of selective signaling events and initiation of defined cellular processes in neurons.

IGFBPL1 contains an IGF binding domain at its N-terminal. Taken into the consideration the correlated expression of IGFBPL1 with RGC axon elongation, it was hypothesized that IGFBPL1 acts as a co-factor of IGF-1 to enable the activation of axon growth cascades downstream of IGF-1 to specifically stimulate axon growth in developing neurons. This hypothesis was supported by the observations that: (1) in co-immunoprecipitation experiments, IGFBPL1 was shown to physically interact with IGF-1; (2) interruption of IGFBPL1 and IGF-1 binding completely abolished the axon growth-promoting effect of IGFBPL1 or IGF-1; (3) only in the presence of IGFBPL1, is IGF-1 capable of inducing Ca^{2+} signaling that largely increases the phosphorylation and activation of phosphoinositide 3-kinase/mammalian target of rapamycin (PI3K/mTOR) pathways. Thus, IGFBPL1 is an essential co-factor of IGF-1 to the control of axon growth during development (Guo et al., 2018).

It is reported that the elevation of intracellular calcium ($[Ca^{2+}]_i$) is an essential component of the signaling cascades required for the initiation of RGC axon growth (Jiao et al., 2005). Localized, transient elevation of intracellular Ca^{2+} after neural injury has been identified to contribute to growth cone formation and neuritogenesis. The elevation of intracellular Ca^{2+} in turn triggers CREB and extracellular signal-regulated kinase signaling pathways to induce neurite outgrowth and axon regeneration (Jiao et al., 2005). Although the addition of IGFBPL1 or IGF-1 alone didn't induce significant changes in $[Ca^{2+}]_i$ in mature (P10) RGCs cultures, co-administration of IGFBPL1 and IGF-1 induced robust elevation of $[Ca^{2+}]_i$, which was shown to be responsible for the axon growth-promoting activities of IGF-1 or IGFBPL1. Blockage of $[Ca^{2+}]_i$ with Ca^{2+} chelators abolished the axonal growth-promoting effect of IGF-1 or IGFBPL1, and addition of Ca^{2+} activator alone was sufficient to activate PI3K/mTOR signaling and induce axon growth (Guo et al., 2018). Therefore, neither IGF-1 nor IGFBPL1 alone, but the combination of the two, activates PI3K/mTOR signaling and axon growth cascades (**Figure 1**). These data support the notion that IGF-1 requires the presence of IGFBPL1 to initiate axon growth activity and suggest a novel signaling loop in the regulation of IGF-1's pleiotropic functions.

Conclusion and future perspective: It is demonstrated that IGFBPL1 enables IGF-1 to activate intracellular Ca^{2+} signaling and PI3K/mTOR pathways and stimulate axonal growth. The finding unveiled a new regulatory mechanism of IGF-1 and possible pharmacological manipulations for promoting axon regeneration after injury (Cho and Chen, 2008). Besides PI3K/mTOR pathways, several intracellular mechanisms, including cytoskeletal dynamics, axonal transport, transcriptional regulation of the growth program, and epigenetic modifications, are also known to participate in axon

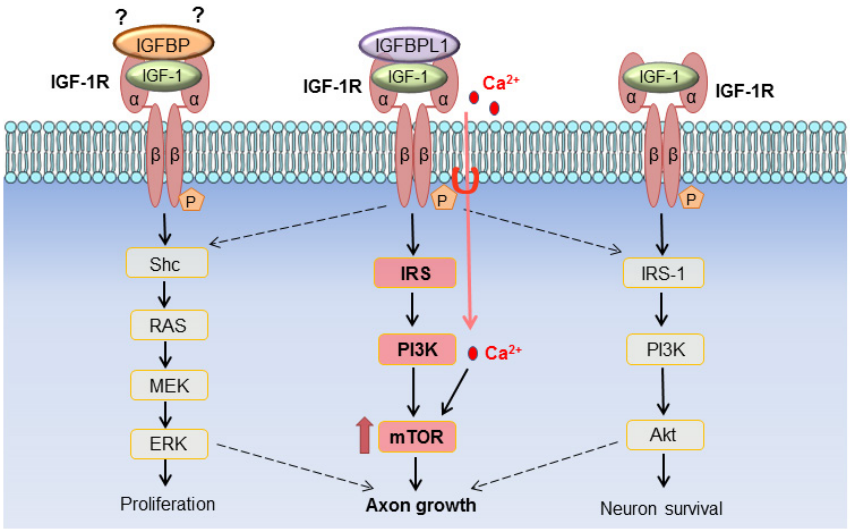

Figure 1 Schematic of IGF-1 and IGFBPL1 signaling.
IGF-1, in the presence of IGFBPL1, acts *via* IGF-1R to trigger Ca^{2+} influx and the phosphorylation of PI3K and mTOR. Elevation of intracellular Ca^{2+} further boost mTOR activation to induce axon growth activity from RGCs. Dotted lines indicate potential involvement of the other pathways. IGF-1, when acts alone, supports RGC survival in the adult without causing axon growth. It is tempting to hypothesize that IGF-1 binds different IGFBPs to enable the activation of selective signaling events and initiation of define cellular processes in neurons. IGF-1: Insulin-like growth factor-1; IGFBPL1: insulin-like growth factor binding protein like protein 1; IGF-1R: IGF-1 receptor; PI3K: phosphoinositide 3-kinase; mTOR: mammalian target of rapamycin; RGCs: retinal ganglion cells; MEK:mitogen-activated extracellular signal-regulated kinase; ERK: extracellular signal-regulated kinase; IRS: insulin receptor substrate; Akt: protein kinase B.

regeneration regulation (Curcio and Bradke, 2018). IGF-1 is reported to mediate most of these pathways. As IGF-1R is localized on growth cones, this enables direct local effect of IGF-1 on driving the growth, such as by stimulating axon membrane expansion and actin cytoskeleton and tubulin polymerization (Laurino et al., 2005). Glycogen synthase kinase 3 and Rho-family GTPase Cdc42 are known downstream targets of IGF-1 to mediate microtubule polymerization and stabilization. The question has always been how IGF-1 selectively activates these pathways during only certain period of neural development. Currently, much remains to be done to fully uncover the functional roles and signaling events underlying IGF-1 and IGFBPL1 interactions. As IGFBPL1 is widely expressed in the developing mouse retina and brain, with peak levels during axon elongation and undetectable in the postnatal stage (Gonda et al., 2007), it suggests a much broader involvement of IGFBPL1 in other CNS neurons.

Since IGF-1 also acts on many cell types, it should be of interest to further elucidate the physiological function of IGFBPL1 in other cell types during development and/or in disease or injury. Notwithstanding these results, additional studies are required to validate and fully characterize the roles of IGFBPL1 and its interactions with IGF-1 and IGF-1R in the normal retina and brain and its involvement under neurodegenerative conditions. These studies may shed light on developing approaches to promote neuron regeneration and repair after injury or disease.

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