PERSPECTIVE

Emerging roles of the neural adaptor FE65 in neurite outgrowth

The brain is the third largest organ in the human body and consists of over 80 billion neurons (Herculano-Houzel, 2009). Neurons are interconnected by neurite to form a complex neural network that allows the communication of neurons to regulate different body functions and activities. Neurites, including axons and dendrites, are the projections of a neuron from the cell body. Dynamic neurite outgrowth is a fundamental neural process for the establishment and maintenance of the functional nervous system. Unfortunately, neurite damage is often observed after brain injuries and in the early stages of many neurodegenerative diseases and in case of age-related neural degeneration. However, stimulation of neurite regeneration has been a major challenge for brain regenerative medicine. For instance, the axon growth of neurons in the central nervous system (CNS) is suppressed by inhibitory molecules released from the neighboring injured cells such as myelin-associated glycoprotein, neurite outgrowth inhibitor-A, oligodendrocyte-myelin glycoprotein, and chondroitin sulfate proteoglycans. Unlike the neurons of the peripheral nervous system, which are capable of regenerating new growth cones (a specialized structure at the tip of the neurite for initiating neurite elongation) shortly after injuries, retraction bulbs are formed in the axons of injured CNS neurons that restrict the formation of proper connections between these neurons. Additionally, the regenerated axons need to grow across a considerable distance to bypass the areas of injury and consequently achieve reconnections. However, many processes that stimulate axon growth are switched off after brain development. Recent findings suggest that these developmental processes can be "re-activated." Activating the intrinsic pathways for neurite outgrowth could potentially lead to axon regeneration in CNS neurons (He and Jin, 2016). Therefore, understanding the regulatory mechanisms of neurite outgrowth would not only advance our knowledge in brain development but also provide insights into methods of inducing neurite re-outgrowth after brain injuries and in the aftermath of neurodegenerative diseases.

Ras-related C3 botulinum toxin substrate 1 (Rac1) in neurite outgrowth: Neurite outgrowth is driven by the dynamic behavior of the growth cones. Growth cones advance through the cyclical extension of filopodia and lamellipodia, which is largely determined by the organization of the actin and microtubule cytoskeleton. Rac1 is a member of the Rho family of GTPases involved in regulating cytoskeleton remodeling. Akin to other small GTPases, the activity of Rac1 is determined by guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs) that allow the cycling of Rac1 between the GTP-bound active and GDP-bound inactive forms respectively. Increasing evidence suggests that Rac1 is a key molecule in neuronal survival and regeneration. It is reported that Rac1 promotes neuronal survival of cerebellar granule neurons through the suppression of c-Jun-mediated apoptosis. Moreover, a study shows that the selective activation of Rac1 could enhance neuronal survival and axonal regeneration while preventing dendrite degeneration in retinal ganglion cells after crush injuries. Additionally, activation of Rac1 is found to enhance neurite outgrowth within the inhibitory environment both *in vitro* and *in vivo*. Together, these findings underscore the significance of Rac1 activation in neural regeneration (Stankiewicz and Linseman, 2014).

FE65 functions as an engulfment and cell motility 1 (ELMO1) activator: FE65, also known as the amyloid-β precursor protein binding family B member 1 (APBB1), is a brain-enriched adaptor with several protein-binding domains, including a tryptophan-tryptophan (WW) domain and two C-terminal phosphotyrosine binding (PTB) domains. Hence, a function of FE65 is to recruit interactors to form functional complexes in different processes. Previously, our group had uncovered that FE65 promotes Rac1-mediated neurite outgrowth (Cheung et al., 2014). However, the precise mechanism(s) by which FE65 stimulates Rac1 remains unclear as it does not contain any enzymatic activity (Chow et al., 2015a). As a cellular adaptor, it is very likely that FE65 recruits protein(s) for stimulating Rac1, and thereby neurite outgrowth. Despite several FE65 interactors having being identified in the past decades, none of them have been observed to possess any GEF activity. Recently, we have shown that FE65 interacts with the ELMO autoregulatory domain (EAD) of ELMO1, a subunit of the ELMO1-dedicator of cytokinesis 180 (DOCK180) GEF complex (Li et al., 2018). DOCK180 is an atypical Rac1 GEF that carries a DOCK homology region-2 GEF domain for catalyzing GDP-GTP exchange. Interestingly, the association of ELMO1 is required for the proper functioning of the GEF as their interaction dramatically enhances DOCK180 GEF activity, membrane localization, and protein stability. Therefore, it is suggested that DOCK180 and ELMO1 function together in Rac1 signaling as a bipartite GEF complex in which ELMO1 serves as a regulatory subunit (Patel et al., 2011).

Notably, ELMO1 activation is a prerequisite for the functioning of the bipartite GEF. At its basal state, ELMO1 auto-inactivates itself by adopting an autoinhibitory configuration through an intramolecular interaction between its EAD and ELMO inhibitory domain (EID). Hence, EAD or EID interactors have long been proposed as activating ELMO1 by disrupting the intramolecular interaction (Patel et al., 2011). In fact, our recent finding provides the first source of evidence for the aforementioned proposal as the interaction of FE65 and ELMO1 EAD relieves the ELMO1 autoinhibitory conformation and stimulates Rac1 and neurite outgrowth in primary rat cortical neurons (Li et al., 2018) (**Figure 1**).

FE65 links up molecules for Rac1-mediated neurite outgrowth: We also found that plasma membrane targeting of ELMO1 is reduced substantially in the absence of FE65. Interestingly, we have demonstrated that ADP ribosylation factor 6 (ARF6), a small GTPase that participates in endocytic trafficking, is also an FE65 interactor (Cheung et al., 2014). Although activation of ARF6 has been reported to stimulate Rac1 *via* ELMO1/DOCK180 (Santy et al., 2005), the link between these molecules is yet to be identified. In this scenario, FE65 may serve as an adaptor for bridging up ARF6 and ELMO1/DOCK180 for their plasma membrane targeting. Further investigation in FE65 knockout models may help to figure out if FE65 is the "missing link" of ARF6-ELMO1/DOCK180-mediated Rac1 signaling and neurite outgrowth (**Figure 1**).

As stated above, FE65 aids the plasma membrane targeting of ELMO1/DOCK180. Several binding partners of ELMO1, including ADP-ribosylation factor-like 4A, Gαi2, Gβ, and Ras homology growth-related, have been shown to assist the tethering of the GEF to the plasma membrane for the activation of Rac1 there. It must be noted that several FE65 interactors are membrane-bound molecules. As mentioned earlier, FE65 is also known as APBB1 as it is initially found to interact with the cytoplasmic tail of the transmembrane amyloid-β precursor protein (APP) *via* its PTB2 domain to promote the cleavage of APP. Similar to FE65, APP is highly expressed in the brain and is found enriched in the growth cones. Although the exact mechanism remains elusive, APP is found to be essential for neurite elongation (Sosa et al., 2017). For instance, exogenous addition of the secreted extracellular domain of APP (sAPP) has been shown to enhance neurite outgrowth in cultured neurons (Araki et al., 1991). Therefore, FE65 may stimulate neurite extension by promoting the generation of sAPP *via* APP processing. Notably, a report demonstrated that APP serves as a "dock" for retaining FE65 on membrane structures (Minopoli et al., 2001). Therefore, in addition to the above ELMO1 interactors, FE65 may also participate in ELMO1/DOCK180 complex plasma membrane tethering by docking to APP. Further investigation using an APP shedding-deficient mutant and APP knockout models could help unravel the role of APP in connection with FE65 in Rac1 signaling (**Figure 1**).

Neurite outgrowth and other FE65 interactors: In addition to the above, some other FE65 interactors have been implicated in neurite outgrowth. For example, activation of tyrosine kinase c-Abl, an FE65 WW domain interactor, modulates F-actin cytoskeleton dynamics in a Rac1-independent manner. Interestingly, c-Abl can phosphorylate the actin-binding protein Mena, which is also an FE65 WW domain ligand. Apart from ARF6, several FE65 PTB1 ligands are also reported to contribute to neurite extension, including megalin that has been found to be crucial for transthyretin-mediated neurite outgrowth in primary neurons. Likewise, the LDL receptor-related protein 1 (LRP1) agonist increases neurite outgrowth in dorsal root ganglion neurons. Moreover, the microtubule-associated protein tau is essential for growth cone motility in chick sensory neurons. Thus, FE65 could also participate in neurite outgrowth *via* stimulating pathways other than the Rac1 signaling through the mentioned interactors. Apparently, additional work, such as using binding-deficient mutants in neurite outgrowth analyses, is required to determine if FE65 plays any roles in those pathways. Moreover, super-resolution microscopy and electron tomography could help determine how FE65 orchestrates these interactors in a proper spatial and temporal manner to modulate neurite development.

Targeting FE65 and/or ELMO1 phosphorylation as a therapeutic approach for promoting neurite outgrowth: We have shown that FE65-ELMO1 association is important for Rac1-mediated neurite outgrowth, and FE65 plays a significant role in activating ELMO1. One important question that remains unanswered is "What is the upstream signal(s) that regulates the interaction?" Phosphorylation is a common mechanism that regulates many cellular processes, including protein–protein interaction. In fact, we and others have revealed that the interaction between FE65 and its interactors can be altered by phosphorylation (Chow et al., 2015b). Although the mechanism remains unknown, ELMO1 tyrosine 724 phosphorylation is associated with Rac1 activation (Makino et al., 2015). As transient kinase activation has been proven to be beneficial in certain disease conditions, it will be important to

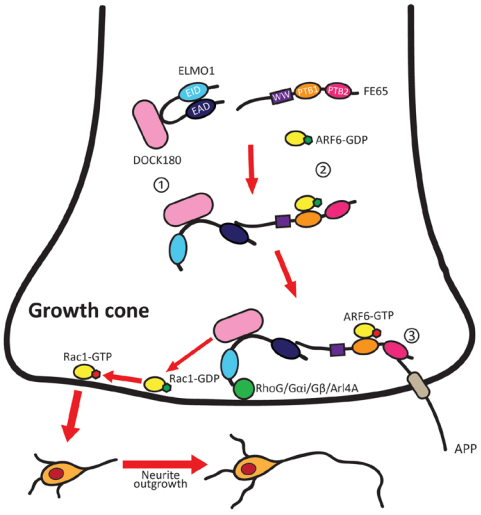

Figure 1 A schematic diagram illustrates the roles/potential roles of FE65 in Ras-related C3 botulinum toxin substrate 1 (Rac1)-mediated neurite outgrowth.
In the growth cone, ① FE65 N-terminal region interacts with engulfment and cell motility 1 (ELMO1)- dedicator of cytokinesis 180 (DOCK180) bipartite Rac1 guanine nucleotide exchange factor (GEF) complex. Such binding disrupts the ELMO1 autoregulatory domain (EAD)-ELMO inhibitory domain (EID) intramolecular interaction and thereby activates ELMO1. ② Meanwhile, FE65 phosphotyrosine binding (PTB)1 domain could also recruit ADP ribosylation factor 6 (ARF6) for the trafficking of FE65-ELMO1-DOCK180 complex to the plasma membrane. Once arrived, the complex could bind to membrane situated ELMO1 interactors, such as ADP-ribosylation factor-like 4A (Arl4A), Gαi2, Gβ or Ras homology growth-related (RhoG), in order to retain on the plasma membrane where the GEF stimulates Rac1. ③ FE65 could also participate in this membrane tethering process by interacting with transmembrane amyloid-β precursor protein (APP) molecule using its PTB2 domain.

determine if FE65-ELMO1 interaction is modulated by phosphorylation and identify the kinase(s) responsible. Similarly, phosphorylation could also be important for regulating the interactions of other FE65 ligands, that participate in neurite outgrowth, towards FE65. Future FE65 phosphorylation and interaction studies will provide insights into the development of therapeutic strategies for stimulating neurite outgrowth.

Conclusion: In summary, our recent finding reveals a novel role of the neural adaptor FE65 in Rac1-mediated neurite outgrowth through the recruitment and activation of ELMO1 in the ELMO1-DOCK180 bipartite Rac1 GEF. Apart from that, it is likely that FE65 has other roles in this Rac1 signaling pathway by recruiting further molecules, such as ARF6, to facilitate the process. Although it remains to be elucidated, FE65 could also function as a hub for other neurite outgrowth stimulating pathways, since some of its reported interactors are associated with neurite extension. Therefore, FE65 opens a novel avenue for neurite outgrowth research. As promoting neurite outgrowth in CNS neurons is an important step for regeneration, targeting FE65 interaction with other molecules may serve as a therapeutic approach for promoting neurite outgrowth in injured neurons (**Figure 2**).

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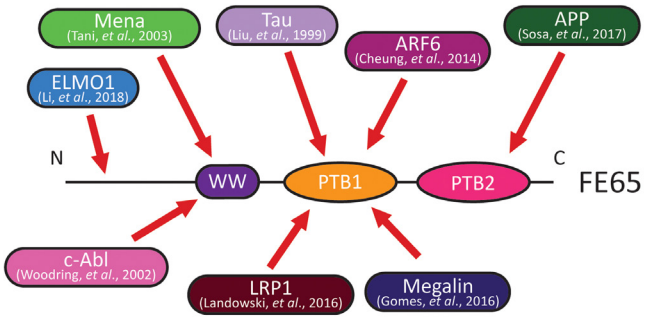

Figure 2 A summary of FE65 interactors that are implicated in neurite outgrowth.
WW: Tryptophan-tryptophan; PTB: phosphotyrosine binding; Mena: mammalian enabled; ARF6: ADP ribosylation factor 6; APP: amyloid-β precursor protein; LRP1: LDL receptor-related protein 1.

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