● PERSPECTIVE

Novel function of the chemorepellent draxin as a regulator for hippocampal neurogenesis

Hippocampal neurogenesis as a therapeutic target for neurological disorders: Mature granule cells are continuously differentiated from neural stem and progenitor cells and integrated into the pre-existing neural system in the subgranular zone of the hippocampal dentate gyrus throughout life. Accumulating evidence indicates that these newborn granule cells are essential for the physiological functions of the hippocampus, such as memory formation, learning, regulation of emotions, and stress response. Alterations in hippocampal neurogenesis are observed in patients with cognitive and psychological diseases, such as epilepsy, ischemia, and traumatic brain injury (Yu et al., 2016). This suggests that hippocampal neurogenesis is a potential therapeutic target for the treatment of neurological disorders. To date, various secretory molecules have been identified as regulators of neurogenic processes, including neuronal proliferation, differentiation, and survival, such as morphogens, neurotrophic factors, neurotransmitters, transcription factors, and epigenetic modulators Canonical Wnts, which are a type of morphogen, are well-studied regulators of hippocampal neurogenesis. Canonical Wnt signals are transduced *via* the LRP (low-density lipoprotein receptor-related protein) 5/6 and Frizzled receptor complex, followed by the intracellular mediator, β-catenin (MacDonald et al., 2009). Alterations in the Wnt/β-catenin signaling leads to impairments in hippocampal development, and the dysregulation of neuronal proliferation and differentiation of dentate granule cell precursors.

Discovery of a novel chemorepellent draxin: Novel axon repellents have not been identified since discovery of the midline repellent, Slit, in 1999. However, some exciting new data came from Prof. Hideaki Tanaka's research group in 2009. They conducted the Signal-Sequence-Trap to clone genes encoding secretory or membrane-bound proteins, which are potential mediators of the intercellular signaling, and consequently identified a novel chemorepellent named draxin (Islam et al., 2009). Draxin is composed of 349 amino acid residues, corresponding to a molecular mass of 39 kDa, and has no conserved amino acid sequence motifs. One distinctive feature of draxin is that only one copy of the gene has been identified in vertebrates, while the families of other axon guidance molecules, including semaphorin, netrin, slit, and ephrin, consist of several genes that were evolutionally generated by genome-wide gene duplications.

To investigate the functions of draxin in the developing brain, draxin-null mice were generated using a gene targeting strategy (Islam et al., 2009). Phenotypic analyses in *draxin* knockout (KO) mice demonstrated that it is indispensable for the proper formation of the commissural tracts, including the corpus callosum, anterior commissure, hippocampal commissure, spinal commissure, and the thalamocortical tract (Islam et al., 2009; Shinmyo et al., 2015). Further studies have demonstrated that draxin directs the migration of different neurons and their extending axons in the developing brain and spinal cord. It is hypothesized that the axon repulsive signals of draxin are transduced by known netrin receptors. Draxin can interact with netrin receptors, including deleted in colorectal cancer (DCC), neogenin, and Unc5s. However, only DCC and neogenin have been shown to act as functional draxin receptors in thalamocortical projections (Shinmyo et al., 2015). Conversely, other studies report that draxin does not interact with DCC or neogenin; however, it does interact with their ligand, netrin-1. Therefore, there is conflicting evidence about the ability of DCC and neogenin to transduce draxin-mediated axon repulsive signals *via* a direct molecular interaction. Further studies should focus on the elucidation of the molecular mechanisms underlying draxin-mediated axon repulsion. Do DCC and neogenin transduce axon repulsive signals of draxin directly or are additional receptor components necessary? Does draxin interact with netrin-1? Does the heteromerization of draxin and netrin-1 effect axon repulsion *via* DCC and neogenin? The identification of amino acid sequences in draxin responsible for the binding of DCC, neogenin, and netrin-1, are necessary to verify their molecular interactions.

Novel function of draxin as a prosurvival ligand for the dependence receptor DCC: Further phenotypic analyses of the *draxin* KO hippocampus led to an additional finding regarding the function of draxin. These analyses revealed an impairment in hippocampal development that could not be explained by the loss of draxin-mediated axon repulsion. *Draxin* deficiency resulted in an increased number of cells that were immunoreactive to apoptotic markers, including single-strand DNA and cleaved caspase-3, in the neurogenic niches of embryonic and postnatal dentate gyrus. Furthermore, a reduction in the neural population of early/late progenitors, neuroblasts and mature granule cells, but not stem cells, was observed in *draxin* KO mice. When the expression pattern of draxin in the dentate gyrus were examined, it was found to be exclusively expressed in the late progenitors and neuroblasts of the dentate granule cell lineage (Tawarayama et al., 2018). While, a draxin receptor DCC is mainly expressed in neuroblasts. DCC is known to be part of the dependence receptor family, which trigger apoptosis in the absence of a ligand (Mehlen and Thibert, 2004). On the other hand, the prosurvival activity of draxin was confirmed using neural stem and progenitor cells derived from the rodent dentate gyrus *in vitro* (Tawarayama et al., 2018): gain- and loss-of-function DCC studies showed an increase and decrease in the apoptosis of neural stem and progenitor cells, respectively. Furthermore, the increase in apoptosis was attenuated when the draxin recombinant proteins were added to the cultures or amino acid mutations were introduced into caspases, which mediate DCC-induced apoptosis, in the intracellular region of DCC. Taken together, it is postulated that draxin attenuates the DCC-induced apoptosis of neuroblasts *via* a direct (or indirect) molecular interaction with the dependence receptor DCC in a cell or non-cell autonomous manner in the subgranular zone of the dentate gyrus.

It is known that excessive neural lineage cells are eliminated in the nervous tissues including the hippocampus during development (Kim and Sun, 2011). We postulate that DCC-induced neuroblast apoptosis is not implicated in the neuronal elimination. Because previous study indicated that DCC increases apoptosis in differentiating hippocampal neural stem/progenitor cells in the absence of draxin, whereas promotes survival of the cells in the presence of draxin. Given that DCC^{+} neuroblasts also express draxin, DCC-induced apoptosis of neuroblasts is inhibited in nature. Then, what is the functions of DCC-induced cell death in hippocampal neurogenesis during development? DCC may play a pivotal role in elimination of aberrant newborn neurons, which innervate inappropriate targets. DCC is expressed not only in neuroblasts but also in a small population of NeuN-immunoreactive cells, probably young neurons (Tawarayama et al., 2018). Therefore, young neurons expressing DCC but not draxin may undergo apoptosis if the DCC-induced cell death is not attenuated by survival-promoting factors, *e.g.*, neurotrophins secreted from projection targets of axons. The speculative function of DCC-induced apoptosis in hippocampal neurogenesis must be verified in future studies.

Another potential role of draxin in hippocampal neurogenesis: Our recent study has revealed the role of draxin in hippocampal neurogenesis (Zhang et al., 2010; Tawarayama et al., 2018). We found that draxin attenuated DCC-induced apoptosis in neuroblasts by acting as a prosurvival ligand for the dependence receptor. This explains the reduced population of neuroblasts observed in the dentate gyrus of *draxin* KO mice. However, the reduced number of cells in the granule cell lineage was found not only in DCC-positive neuroblasts but also earlier differentiation stages of cells such as progenitors, which do not express DCC. This indicates that there are other potential roles of draxin in hippocampal neurogenesis, which have not been identified. One possible role of draxin is the regulation of canonical Wnt/β-catenin signaling. It was previously shown that draxin physically interacts with the receptor component

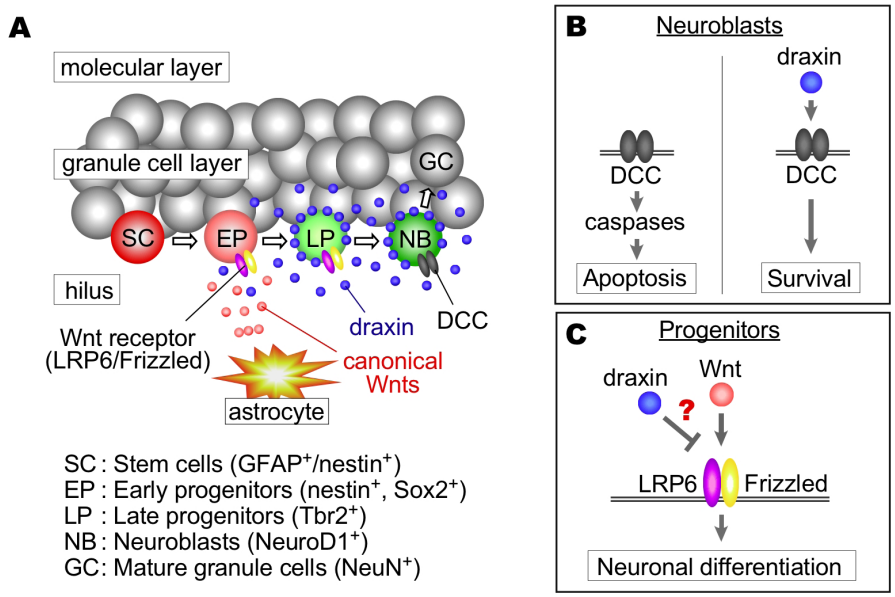

Figure 1 Putative functions of draxin in hippocampal neurogenesis.
(A) Draxin is expressed in Tbr2$^+$ late progenitors and NeuroD1$^+$ neuroblasts, while its receptor deleted in colorectal cancer (DCC), belonging to the dependence receptor family, is principally expressed in neuroblasts. (B) DCC induces apoptosis in neuroblasts in a caspase-dependent manner in the absence of draxin. Further, DCC mediates the ligand-induced promotion of neuronal survival. Therefore, draxin secreted from late progenitors and neuroblasts would prevent neuroblasts from undergoing DCC-induced apoptosis. (C) On the other hand, draxin seems to modulate neuronal differentiation in early and late progenitors, probably by competing with canonical Wnts for binding to their receptor LRP6 expressing on these progenitors.

of the canonical Wnt, LRP6 (Miyake et al., 2009); therefore, *draxin* attenuates canonical Wnt/ß-catenin signaling by competing with Wnt for LRP6. As predicted, the upregulated expression of target genes for canonical Wnt was observed in the *draxin* morphants of zebrafish (Miyake et al., 2012). Similarly, loss of *draxin* would result in the hyperactivation of canonical Wnt/ß-catenin signaling in the dentate gyrus of *draxin* KO mice, leading to the dysregulation of proliferation and/or differentiation of neural stem and progenitor cells. A reduced population of neural stem and progenitor cells might be attributable to this dysregulated proliferation and/or differentiation. Further studies are needed to verify this hypothesis.

Conclusions: Draxin was identified as a chemorepellent that is indispensable for the proper formation of the commissural tracts in the brain and spinal cord in 2009. Since then, studies have been performed to elucidate the function of draxin in the developing nervous system. These studies have been successful not only in creating an overview of the cellular and molecular mechanisms underlying draxin-mediated wiring of neural networks, but also in finding a novel function of draxin in hippocampal neurogenesis as a prosurvival ligand for the dependence receptor DCC (**Figure 1**). Furthermore, our recent study shed a light on another potential function of *draxin* to modulate Wnt-driven progenitor differentiation, probably by competing with canonical Wnts for binding to their receptor LRP6 (**Figure 1**). Taken together, it is thought that draxin is a key molecules to regulate hippocampal neurogenesis.

It is surprising to find that *draxin* transduces its repulsive signals *via* the netrin-1 receptors, DCC and neogenin; however, there is debate regarding whether *draxin* interacts with these molecules directly. On the other hand, it has been shown that draxin can interact with netrin-1, another guidance molecule and dependence ligand for DCC. Considering these findings, the molecular mechanisms of draxin-mediated axon repulsion and apoptotic inhibition still remain unclear. Further studies are necessary to elucidate these mechanisms.

Draxin KO mice are viable and fertile, and display drastic phenotypes in the developing nervous systems, such as axon misrouting and defective neurogenesis. Nevertheless, few reports implicate draxin in human neurological disorders. Recently, genome-wide association studies (GWASs) have been conducted to identify genes responsible for cognitive disorders; therefore, altered expression and/or mutations in the *draxin* gene that are associated with the pathogenesis of neurological disorders may be determined soon.

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