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REVIEW

New insights into the control of neurotrophic growth factor receptor signaling: Implications for nervous system development and repair

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Abstract

Neurotrophic growth factors control neuronal development by activating specific receptor tyrosine kinase positive signaling pathways, such as Ras-MAPK and PI3K-Akt cascades. Once activated, neurotrophic factor receptors also trigger a cascade of molecular events, named negative receptor signaling, that restricts the intensity of the positive signals and modulates cellular behavior. Thus, to avoid signaling errors that ultimately could lead to aberrant neuronal physiology and disease, negative signaling mechanisms have evolved to ensure that suitable thresholds of neuronal stimulation are achieved and maintained during right periods of time. Recent findings have revealed that neurotrophic factor receptor signaling is tightly modulated through the coordinated action of many different protein regulators that limit or potentiate signal propagation in spatially and temporally controlled manners, acting at specific points after receptor engagement. In this review, we discuss progress in this field, highlighting the importance of these modulators in axonal growth, guidance, neural connectivity, and nervous system regeneration.

Keywords: GDNF-Family Ligands, nervous system regeneration, neuronal development, neurotrophins, RTK signaling modulators.

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Neurotrophic factors are secreted molecules that play a crucial role in the development, function, maintenance, and plasticity of the nervous system. Two of the major families of neurotrophic factors are the neurotrophin family and the glial cell line-derived neurotrophic factor (GDNF) family of ligands (GFLs) (Reichardt 2006; Paratcha and Ledda 2008). Furthermore, there is a group of soluble growth factors, such as epidermal growth factor (EGF), fibroblast growth factor (FGF), and hepatocyte growth factor (HGF), which also influence nervous system development by modulating neural cell proliferation and migration. Because of their trophic actions on the nervous system, they can also be considered neurotrophic growth factors.

The neurotrophins constitute a structurally related family of proteins represented by nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin 3, and neurotrophin 4. The neurotrophins support the differentiation, axonal growth, and survival of specific populations of sensory, sympathetic, and CNS neurons via the activation of their cell-surface receptor tyrosine kinase TrkA, TrkB, and TrkC (Reichardt 2006). Furthermore, pro-neurotrophins, the

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Abbreviations used: AD, Alzheimer's disease; BDNF, brain-derived neurotrophic factor; DA, dopamine; DRG, dorsal root ganglion; EGF, epidermal growth factor; EGFR, EGF receptor; FGF, fibroblast growth factor; GDNF, glial cell line-derived neurotrophic factor; GFLs, GDNF family of ligands; HGF, hepatocyte growth factor; Lingo1, LRR and Ig domain-containing Nogo-receptor-interacting protein 1; NGF, nerve growth factor; PTEN, phosphatase and tensin homolog protein; RTK, receptor tyrosine kinase; Spry, sprouty.

uncleaved neurotrophin precursors, can be secreted and can only bind to a $p75^{NTR}$-sortilin receptor complex to induce neuronal cell death during development and in pathological conditions such as Alzheimer's disease (AD) and spinal cord injury (Friedman 2010; Teng *et al.* 2010).

Glial cell line-derived neurotrophic factor family of ligands are another relatively new family of neurotrophic factors that is composed of GDNF, Neurturin, Artemin, and Persephin. GDNF was originally discovered by its ability to induce the survival of ventral midbrain dopaminergic neurons. GFLs also control growth and survival of specific subpopulations of motor neurons and many peripheral neurons, including sympathetic and sensory neurons. GFLs promote these trophic effects by acting through two types of receptor subunits, one specialized in ligand binding, represented by the glycosyl-phosphatidyl inositol-anchored coreceptor, GFRα, and another involved in transmembrane signaling, represented by the receptor tyrosine kinase, Ret (Rearranged in transformation) (Paratcha and Ledda 2008).

Neurotrophic factor receptors are expressed in subpopulations of peripheral and central neurons, where they contribute to the establishment of neuronal connections (Garces *et al.* 2000; Patel *et al.* 2003; Kramer *et al.* 2006; Wickramasinghe *et al.* 2008). However, they alone cannot account for the specificity of the patterns of axonal connectivity and target innervation. For instance, Ret receptors are expressed in almost all the spinal cord motor neurons, but only appear to be required for targeting axons of a select group of motor neurons toward the dorsal muscles of the hindlimb. In this system, in addition to GDNF, the ephrinA/EphA4 guidance cue was demonstrated to cooperate with GDNF/Ret signaling during motor-axon pathway selection into the hindlimb (Kramer *et al.* 2006). More recently, a cell-specific regulator of Ret signaling has been reported to contribute to the growth of Ret-positive motor axons into their target tissue (Mandai *et al.* 2009). Therefore, in addition to guidance cues, there are cell-type-specific modulators of neurotrophic factor receptor signaling that support specificity during circuit formation.

Binding of neurotrophic factors to their cell surface receptors triggers different signaling cascades, including the Ras-MAPK, phosphatidylinositol-3 kinase (PI3K)-Akt, and the PLCγ pathway (positive signaling cascades) (Airaksinen and Saarma 2002; Reichardt 2006). Unlike these positive signaling pathways, which are relatively well understood, the molecular mechanisms that restrict neurotrophic factor receptor signaling are currently under intensive study. During the last decade, biochemical and genomic techniques as well as genetic analyses of developmental processes have led to the identification and characterization of new neurotrophic growth factor signaling inhibitors (Table 1). These studies have also revealed the importance of negative-feedback control of receptor tyrosine kinase (RTK) function as a mechanism that guarantees signaling thresholds compatible with the induction of a physiological response (Dikic and Giordano 2003; Ledda and Paratcha 2007). A common feature of negative-feedback loops is the transcriptional induction of molecular inhibitors by the same pathways that are eventually inhibited (late inhibitors). Even when many transcriptionally induced negative regulators have been identified, only few positive-feedback loop molecules have been characterized. Interestingly, negative- as well as positive-feedback loops are mechanisms that may explain how a single neurotrophic receptor-induced signaling pathway can control many biological events, increasing the repertoire of biological functions controlled by neurotrophic factors. In Fig. 1, we illustrate this concept describing different negative- and positive-feedback loop modulators of neurotrophic growth factor receptor signaling. These modulatory molecules act at different points after receptor engagement to restrict ligand binding, attenuate receptor activation, control specific downstream signaling pathways, and regulate receptor degradation.

Another mechanism, known as receptor down-regulation, has evolved to attenuate RTK signaling independently of transcription (early inhibitors). This molecular machinery exists before receptor activation, thereby limiting signal propagation through the induction of receptor ubiquitination, endocytosis, and degradation (Dikic and Giordano 2003; Thien and Langdon 2005; Arevalo *et al.* 2006). These mechanisms can abrogate RTK signaling irreversibly. Interestingly, definitive degradation of neurotrophic receptors fulfills a dual purpose; it terminates signal by removing activated proteins, and it generates a refractory period in the cells before the next signal can be induced again. On the other hand, reversible inhibition can be achieved by specific protein tyrosine phosphatases, which are present before RTK activation, and do not require *de novo* transcription of their mRNAs (Lu *et al.* 1999; Gensler *et al.* 2004; Kim and Dressler 2007; Ledda and Paratcha 2007; Song *et al.* 2010).

Several reports have shown that reduced neurotrophic support contributes to the pathogenesis of neurodegenerative disorders such as, AD, Huntington's disease, Parkinson's disease, and amyotrophic lateral sclerosis (Dawbarn and Allen 2003). In particular, target deletion of the GDNF receptor, Ret, in midbrain dopamine (DA) neurons causes progressive and late loss of DA neurons in the substantia nigra (SN), degeneration of DA nerve terminals in the striatum and reduced levels of evoked dopamine release (Kramer *et al.* 2007). These observations indicate that conditional Ret mutant mice could be considered as an interesting model to study early Parkinson's disease-related pathologies.

Further work established that ablation of BDNF expression in cortical pyramidal cells causes age-associated dendrite degeneration, followed by loss of gabaergic medium-sized spiny neurons, a feature that mimics Huntington's disease striatal degeneration (Baquet *et al.* 2004).

Table 1 Classification of neurotrophic growth factor receptor signaling modulators according to their mechanism of action

Modulator	Type of modulator	Target	Mechanism of action	References
BDP1 phosphatase	Early/reversible	EGFR/ErbB2R	Reduction of receptor autophosphorylation	Gensler *et al.* 2004;
SAP (Slam-Associated Protein)	Early/reversible	TrkA, TrkB and TrkC	Reduction of Trk receptor autophosphorylation	Lo *et al.* 2005
PTEN	Early/reversible	EGFR, Ret, Trks	Inhibition of PI3K-Akt pathway	Lu *et al.* 1999; Kim and Dressler 2007; Song *et al.* 2010;
c-Cbl	Early/irreversible	Several RTKs	Receptor ubiquitination and degradation	Thien and Langdon 2005;
Nedd proteins	Early/irreversible	TrkA	Receptor ubiquitination and down-regulation	Arevalo *et al.* 2006;
Mig6/Ralt/Gene33	Late/reversible	EGFR/ErbB2R and Met	Inhibition of EGFR/ErbB2R phosphorylation and Met-Rho GTPase pathway	Zhang *et al.* 2007; Hackel *et al.* 2001; Pante *et al.* 2005;
Sef	Late/reversible	FGFR	Inhibition of FGFR-Ras-MAPK pathway	Tsang and Dawid (2004); Torii *et al.* 2004;
Sprouty family members	Late/reversible	FGFR, Met, TrkA, TrkB and Ret	Inhibition of Ca^{2+} release, Ras-MAPK and Rac1 GTPase pathways	Sasaki *et al.* 2001; Ishida *et al.* 2007;
		EGFR	Inhibition of Cbl-mediated EGFR degradation	Gross *et al.* 2007; Panagiotaki *et al.* 2010; Alsina *et al.* 2012; Haglund *et al.* 2005;.
Linx	Late/reversible	TrkA, Ret	Potentiation of Ras-MAPK Pathway	Mandai *et al.* 2009;.
Lrig1	Late/irreversible	EGFR/ErbB, Met	Receptor ubiquitination and degradation	Gur *et al.* 2004; Laederich *et al.* 2004; Ledda *et al.*
	Late/reversible	Ret	Inhibition of GDNF-binding, receptor activation.	2008;.
Lrig2	ND/reversible	EGFR	Stabilization of EGFR	Wang *et al.* 2009;
Lingo1	ND/reversible	EGFR	Inhibition of EGFR-Akt pathway	Inoue *et al.* 2007

ND, Not determined.

A molecular link correlating NGF deprivation with AD symptoms was provided using a transgenic mouse model, where anti-NGF antibodies, neutralizing NGF, are expressed both peripherally and within the CNS (Ruberti *et al.* 2000). Interestingly, this mouse model displays AD-like pathology including β-amyloid peptide (Aβ) plaques, loss of basal forebrain cholinergic neurons, hyperphosphorylated tau tangles, and hippocampal-dependent memory deficits (Cattaneo *et al.* 2008).

With regards to regeneration, several studies indicate that axotomy promotes the secretion of neurotrophic factors and/or their coreceptors in the lesion sites after peripheral nerve injury. In further support, growing evidence reveals the importance of injury-induced neurotrophic factors in axon regeneration of peripheral neurons (Shen *et al.* 1999; Tanabe *et al.* 2003; Paratcha and Ledda 2008; Gordon 2009). However, despite numerous efforts, delivery of neurotrophic factors has only limited effects on promoting regeneration of certain types of CNS axons (Gordon 2009). On the other hand, accumulating evidence supports the existence of neuronal intrinsic barriers preventing axon regeneration in adult CNS neurons (Sun and He 2010). Recent studies indicate that targeted deletion of negative regulators of neurotrophic factor receptor signaling allow neurons to gain regenerative potential after nerve injury (Mi *et al.* 2007; Liu *et al.* 2010). Hence, strategies combining neurotrophic factor administration together with inhibition of their endogenous negative regulators may represent a promising approach to enhance survival, axon regeneration, and functional recovery after nerve injury.

Neurotrophic factor receptors coordinate a wide range of biological effects and are therefore subjected to multiple control levels. In this review, we discuss recent findings in the control of neurotrophic growth factor-activated RTKs, summarizing their relevance for nervous system development, disease, and regeneration.

LIGs as modulators of neurotrophic factor receptor signaling

Research over the last 10 years has led to the identification of a leucine-rich repeat and immunoglobulin (LIG) family of transmembrane proteins that physically interact with

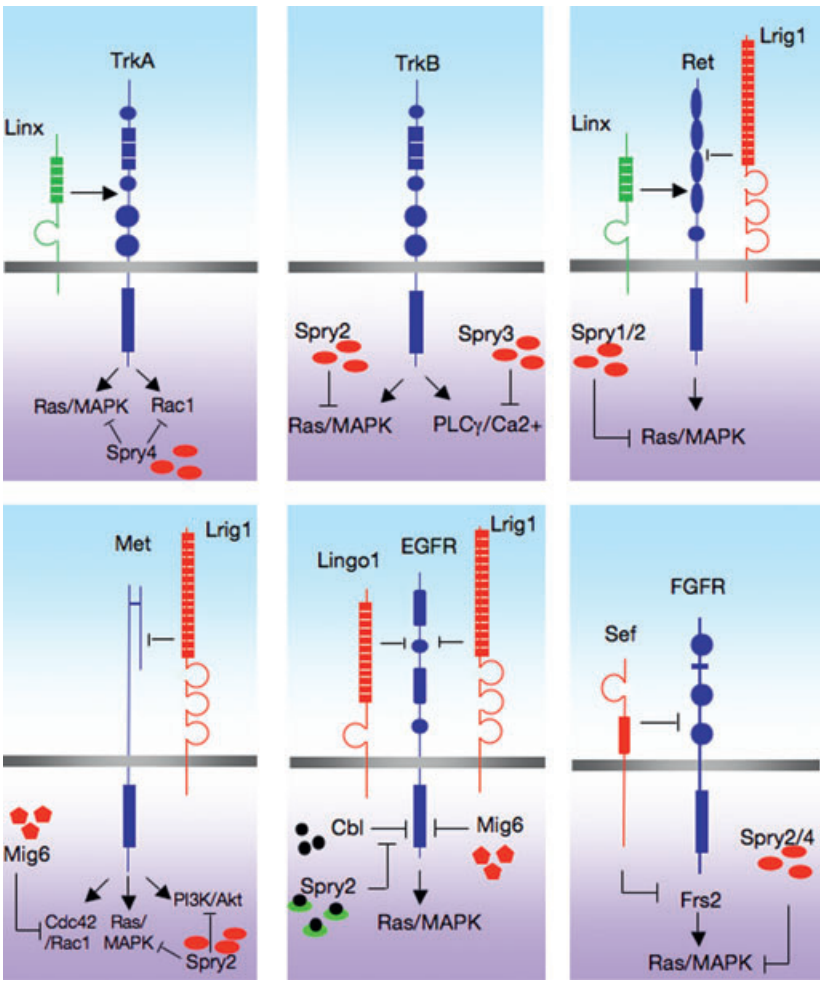

Fig. 1 Mechanisms of negative- and positive-feedback loop modulation of neurotrophic growth factor receptor signaling. Negative modulators are shown in red, and positive modulators of receptor tyrosine kinase signaling are presented in green. The figure illustrates different modulatory molecules acting at different points after receptor engagement to restrict ligand binding, attenuate receptor activation, control specific downstream signaling pathways, and regulate receptor degradation.

neurotrophic growth factor receptors to regulate their activation in different cell types (Chen *et al.* 2006; Mandai *et al.* 2009).

The structural similarity of the LIG family member, Lrig1 with Kekkon1, an EGF receptor (EGFR) inhibitor previously described in *Drosophila* (Ghiglione *et al.* 1999, 2003), led to the prediction that Lrig1 could interact and attenuate EGF signaling in mammalian cells (Nilsson *et al.* 2001; Hedman *et al.* 2002). On the basis of this evidence, two research groups reported that Lrig1 can act as a negative feedback regulator of the mammalian ErbB/EGF receptors (Gur *et al.* 2004; Laederich *et al.* 2004). Although both Lrig1 and Kekkon1 interact with the EGFR, their mechanisms of action differ substantially. The physical interaction of Kekkon1 with ErbB receptors interferes with ligand binding and receptor activation (Ghiglione *et al.* 1999, 2003). On the other hand, Lrig1 appears to restrict mammalian ErbB/EGF receptor signaling by enhancing Cbl (Casitas B-lineage lymphoma)-mediated receptor ubiquitination and degradation (Gur *et al.* 2004; Laederich *et al.* 2004). The N-terminal region of the E3 ligase Cbl directly binds to the juxtamembrane domain of Lrig1. Thus, by recruitment of Cbl to the proximity of ErbB receptors, both Lrig1 and the receptor undergo ubiquitination and subsequent degradation. In addition, it has also been demonstrated that Lrig1 can interact and destabilize the RTK Met in a Cbl-independent manner, regardless of its activation status (Shattuck *et al.* 2007). Although the precise mechanism by which Lrig1 destabilizes Met receptor is still unknown, it has been proposed that Lrig1 likely acts to facilitate the association of Met with the protein degradation machinery.

Another neurotrophic receptor tyrosine kinase regulated by Lrig1 is the GFL receptor Ret (Ledda *et al.* 2008). In this case, Lrig1 restricts ligand-induced Ret activation working through a negative feedback loop. Here, physical interaction between newly synthesized Lrig1 and Ret inhibits GDNF binding, recruitment of Ret to raft domains and neurite outgrowth of motor and sympathetic neurons in response to GDNF. Together, these findings reveal Lrig1 to be a remarkably versatile molecule with the capacity to inhibit many RTKs acting through different mechanisms of action.

In contrast, the Lrig2 subfamily member has opposite functions to those described for Lrig1. Down-regulation of Lrig2 expression in glioma cells results in a rapid EGF-mediated loss of EGFR, less activation of EGFR signaling and decreased cell proliferation (Wang *et al.* 2009).

Research over recent years has demonstrated that the Lrig1 ectodomain can be proteolytically shed to function as a non-cell autonomous regulator of growth factor receptor signaling (Yi *et al.* 2011). Thus, soluble Lrig1 ectodomain could have therapeutic potential for the treatment of growth factor-dependent cancers. Taking this into account, it will be of interest to determine whether Lrig1 as well as other LIG-family members could be proteolytically released from neural tissue to control neurotrophic factor receptor signaling *in trans*. Undoubtedly, future studies will reveal whether non-cell autonomous modulation of neurotrophic factor receptor signaling by LIGs represents a novel mechanism of intercellular communications relevant for nervous system development and regeneration.

In the central nervous system, the leucine-rich repeat transmembrane protein LRR and Ig domain-containing Nogo-receptor-interacting protein 1 (Lingo1) is a key negative regulator of myelination (Mi *et al.* 2005, 2007). Several studies revealed detrimental roles of Lingo1 during nervous system development (Mi *et al.* 2009). In one of these studies, Inoue *et al.* demonstrated that Lingo1 antagonists improve dopamine neuron survival, growth, and function using *in vivo* models of Parkinson's disease. This neuroprotective effect involves the activation of the EGFR/Akt signaling pathway through a direct inhibition of Lingo-1′s binding to the EGFR (Inoue *et al.* 2007).

Slitrk is another subfamily of structurally related transmembrane proteins belonging to the LIG superfamily (Aruga and Mikoshiba 2003). Sequence analysis revealed that the extracellular domain of Slitrks is similar to the repulsive factor, Slit. In addition, some regions in their intracellular domain present a high degree of homology with Trk receptors; this being the reason these proteins were named Slitrks. There are six Slitrk members (Slitrk1-6) and all of them are highly expressed in neural tissue (Aruga and Mikoshiba 2003; Proenca *et al.* 2011). Previous studies indicated that Slitrks function by modulating neurotrophin signaling. In particular, ectopic expression of each Slitrk member in PC12 cells treated with NGF develop a reduced number of neurites compared with control ones, indicating that Slitrk represents a negative regulator of TrkA signaling (Aruga and Mikoshiba 2003). Additional evidence supporting a modulatory role of Slitrk on neurotrophic signaling, is the fact that *BDNF* and neurotrophin 3 mRNA levels, as well as their cognate receptors TrkB and TrkC were down-regulated in the inner ear of Slitrk6-knockout mice, indicating that Slitrk acts as positive regulators of TrkB and TrkC (Katayama *et al.* 2009). These findings suggest that the role of Slitrks on the control of neurotrophin signaling is still unclear, because of their ability to regulate Trk receptor signaling in a negative or positive manner. It is possible that the modulatory effect of Slitrk on Trk signaling depends on the Trk receptor or of the cellular context. Future studies will reveal how each Slitrk member modulates neurotrophin actions as well as the physiological relevance of this control for nervous system development.

Using genome-wide microarray screens to isolate novel modulators of neurotrophic receptor systems involved in the control of axonal projection of dorsal root ganglion (DRG) sensory neurons, Mandai and colleagues identified the LIG transmembrane protein Linx (Leucine-rich repeat and immunoglobulin domain-containing axon extension protein) (Mandai *et al.* 2009). Linx is expressed in specific populations of DRG sensory and spinal cord motor neurons and physically associates with both Trk and Ret receptors to increase neurotrophin and GDNF signaling, respectively. Axonal projection defects in *Linx*-deficient mice resemble those in mice lacking *Ngf, TrkA, and Ret*, revealing the physiological significance of Linx to modulate specific stages of sensory and motor neuron axonal growth, guidance, and target field innervation in response to neurotrophic factors (Mandai *et al.* 2009). The mechanism through which Linx modulates ligand-induced TrkA and Ret signaling on developing neurons remains elusive.

Collectively, the studies summarized here demonstrate that LIG family members can potentiate or attenuate receptor tyrosine kinase activity in a temporally controlled manner to provide fine-tuning of neurotrophic growth factor signaling pathways involved in neuronal growth and connectivity.

Several studies established that neurotrophic factor signaling and responses can be modified based on the timing and location of the stimulation (Ginty and Segal 2002; Ascano *et al.* 2012). Therefore, it will be of interest to determine whether LIGs expressed on sensory and motor neurons can modulate retrograde transport of signaling endosomes containing neurotrophic factor-bound receptor complexes. At the same time, these studies will reveal whether distal axon modulation of Trk and Ret receptors by LIG family members could represent a physiological mechanism to control survival, axonal extension, and target innervation.

The Spred/Sprouty family of inhibitory proteins

During the last years, the Sprouty (Spry) family of proteins (Sprouty1–4) has emerged as negative signaling regulators of many neurotrophic growth factors (Cabrita and Christofori 2008). Spry was first described as an inhibitor of FGF-stimulated tracheal branching during *Drosophila* development (Hacohen *et al.* 1998; Kramer *et al.* 1999). Subsequent studies revealed that the mammalian genome contains four *Sprouty* genes, encoding proteins of 32–34 kDa. It has been reported that trophic factors regulate the activity of Spry inducing its expression and promoting the phosphorylation

of Spry proteins on critical tyrosine residues (Mason *et al.* 2006).

The emerging picture from studies on the Spry family members indicates that they specifically inhibit the Ras-Raf-MAPK pathway activated by a broad range of neurotrophic growth factors, including NGF, BDNF, GDNF, HGF, and FGF (Hacohen *et al.* 1998; Impagnatiello *et al.* 2001; Sasaki *et al.* 2001; Gross *et al.* 2007; Ishida *et al.* 2007; Alsina *et al.* 2012). However, they do not affect MAPK activated by EGF (Sasaki *et al.* 2001; Haglund *et al.* 2005). The molecular points at which Spry family members disconnect the MAPK pathway remains unclear, although it may depend on the cellular context or the RTK involved. Interestingly, Sprouty2 was reported to potentiate biological effects induced by EGF, inhibiting epidermal growth factor receptor ubiquitination and down-regulation (Wong *et al.* 2001; Egan *et al.* 2002; Rubin *et al.* 2003). These findings suggest that Spry may also regulate RTK signaling in a positive manner (Fig. 1).

The regulatory molecules that control the rate of neurite growth and the signals that determine when and where the axons and dendrites have to grow are still largely unknown. During the last years, various reports confirmed the physiological role of Spry members in the control of neurite growth and branching induced by neurotrophic growth factors, such as GDNF, BDNF, and NGF. In these studies, the authors have also linked the inhibitory effects of different Spry family members to the Erk/MAPK pathway.

However, work from recent years has revealed that Spry targets are more diverse than originally assumed. In particular, Panagiotaki *et al.* (2010) found that Sprouty3 inhibits axonal morphogenesis *in vivo* and prevents filopodia formation in spinal cord neurons probably inhibiting Ca^{2+} signaling pathways activated by BDNF (Panagiotaki *et al.* 2010). Remarkably, in this study, Sprouty3 acts as a very weak inhibitor of Erk/MAPK pathway downstream of BDNF/TrkB signaling. Consistent with this, Sprouty4 was recently described to regulate neurite outgrowth of DRG sensory neurons, not only reducing the MAPK pathway, but also restricting Rac1 activation in response to NGF (Alsina *et al.* 2012). Therefore, these reports highlight the versatility of Spry family members to regulate diverse neurotrophic factor-induced signaling pathways, implying they are more than general inhibitors of RTK-induced MAPK pathway.

The Spry-related protein Spred1 represents another negative regulator of the Ras/MAPK pathway induced by EGF and FGF mitogens (Wakioka *et al.* 2001). Recently, Phoenix and Temple reported that Spred1 is highly enriched in CNS germinal zones during neurogenesis. These authors also determined that while *Spred1* knockdown increases neural stem cell self-renewal, its over-expression causes premature neuronal cell differentiation. Interestingly, these findings reveal that Spred1 is a critical organizer of cerebral cortex development, as it modulates progenitor self-renewal/proliferation and helps to maintain the organization of VZ/SVZ germinal zones (Phoenix and Temple 2010).

Another feedback-induced MAPK antagonist is the protein Sef (Similar expression to FGF). Sef is specifically induced by FGF signaling and antagonizes the MAP kinase pathway acting through a cell-autonomous mechanism (Furthauer *et al.* 2002; Kovalenko *et al.* 2003; Preger *et al.* 2004; Torii *et al.* 2004; Tsang and Dawid 2004), either sequestering activated Erk1/2 in the cytoplasm or by preventing phosphorylation of the FGF receptor (Kovalenko *et al.* 2003; Preger *et al.* 2004; Torii *et al.* 2004). Interestingly, Sef-mutant mice are viable with mid-hindbrain patterning defects apparent only when Spry is also inhibited (Lin *et al.* 2005). Additional studies also revealed that Sef regulation of FGF signaling is sufficient to influence both the development and function of the auditory brainstem (Abraira *et al.* 2007).

Finally, it will also be interesting to consider the Mitogen-inducible gene 6 (Mig6, also known as ERRFI1 and RALT), which was originally isolated as a negative regulator of EGF/ErbB receptor signaling in neuronal cells (Hackel *et al.* 2001; Pante *et al.* 2005; Zhang *et al.* 2007). Similar to Lrig1, and Spry family members, Mig6 protein also belongs to the group of regulators that act through a negative feedback loop. In the case of EGF/ErbB receptors, it was shown that Mig6 blocks mitogen signaling induced by EGF, inhibiting EGFR autophosphorylation by direct binding to its cytoplasmic domain. In agreement with this, *Mig6*-deficient mice develop spontaneous tumors in different organs, supporting Mig6 as a tumor suppressor gene (Hackel *et al.* 2001; Zhang *et al.* 2007).

More recently, Pante and colleagues reported that Mig6 is an endogenous inhibitor of HGF/Met-induced neuronal migration of cortical neurons and neurite outgrowth and branching of primary sympathetic neurons. In this case, Mig6′s mechanism involves the inhibition of Rho-like GTPases Cdc42/Rac1 (Pante *et al.* 2005). So far, there is no evidence linking Mig6 to Trk and Ret receptor signaling. However, this is an important analysis that deserves to be addressed.

Negative receptor signaling and nervous system disease

While overactivation of RTK signaling is associated with cancer, it is also possible that reduced neurotrophic growth factor signaling because of alterations in RTK modulatory molecules might contribute to the pathogenesis of neurological disorders (Ledda and Paratcha 2007). In this regard, it has recently been demonstrated that PTEN (Phosphatase and tensin homolog protein), a known inhibitor of different signaling pathways triggered by neurotrophic factors, such as Akt and mTOR, is part of a novel mechanism of intracellular signaling cross-talk between pro-neurotrophins $p75^{NTR}$-induced apoptotic signaling and Trk-mediated survival

signaling in CNS neurons. In this study, the authors demonstrate that proNGF induces PTEN via $p75^{NTR}$ to suppress Trk-PI3K-Akt-mediated survival signaling triggered by mature neurotrophins in neurons (Song *et al.* 2010). This mechanism may explain the association of pro-neurotrophins/$p75^{NTR}$ with neuronal pathologies such as neurodegeneration in Alzheimer's disease. Thus, the interaction between pro-neurotrophin, $p75^{NTR}$ and PTEN may provide a new target pathway for neuroprotection and therapeutic treatment of neurodegenerative diseases. Interestingly, further work has demonstrated that PTEN deletion enhances the regenerative ability of adult corticospinal neurons in response to spinal cord injury (Liu *et al.* 2010). However, despite the robust regenerative growth of corticospinal axons observed in PTEN-deficient mice, many axons failed to grow through the lesion site. On the other hand, it is known that delivery of neurotrophic factors at the lesion site can promote some degree of axon regeneration in certain types of neurons (McCall *et al.* 2012) and that overexpression of TrkB receptors can induce corticospinal axon regrowth into a BDNF-expressing graft after a subcortical injury (Hollis *et al.* 2009). Therefore, the combination of BDNF administration together with the inhibition of PTEN activity may be the clue to stimulate larger number of injured axon growth and to promote functional recovery.

As MAPK pathway has been reported to be critical for neurotrophin-induced axonal growth and regeneration in different injury models (Hollis *et al.* 2009), it will be interesting to explore whether targeted deletion of MAPK inhibitors, such as Spry/Spred can also enhance neurotrophic factor-induced axon regeneration.

The physiological roles of LIGs and Spry/Spred family members during development make it possible that they could contribute to certain human pathologies. Indeed, Lrig1 has been implicated as a tumor suppressor gene for several human cancers (Ye *et al.* 2009; Krig *et al.* 2011; Powell *et al.* 2012; Wong *et al.* 2012). In contrast to what has been described for Lrig1, down-regulation of Lrig2 expression by RNA interference inhibits both EGFR activation and glioblastoma cell growth, suggesting the attractiveness of Lrig2 as a target gene for glioma therapy (Holmlund *et al.* 2009; Wang *et al.* 2009).

Interestingly, mutations in the Spry-related gene *Spred1* have been identified in pathologies characterized by macroencephaly and mental retardation (Denayer *et al.* 2008). Thus, further characterization of the mechanisms through which Spry control neuronal physiology may also help to understand the pathogenesis of many neurological syndromes.

Recent human genetic studies and genetic mouse models have led to the identification of Slitrks as candidate genes that might be involved in the development of neuropsychiatric diseases, such as schizophrenia and obsessive compulsive spectrum disorders (Wendland *et al.* 2006; Proenca *et al.* 2011). Despite this, additional studies will be required to clarify the potential role of Slitrks in neuropsychiatric disorders. Delineating the physiology and signaling mechanisms of each Slitrk family member will not only allow us to understand their roles in normal nervous system development, but will also provide solid evidence linking Slitrks to the development of neurological disorders.

Neurotrophic factors have emerged as promising disease-modifying factors for neurodegenerative diseases, and some of them are currently being used in clinical trials (Lindvall and Wahlberg 2008). In particular, the physiological requirement of GDNF/Ret signaling for survival, maintenance, and regeneration of the dopaminergic system supports additional studies toward optimizing the ongoing GDNF clinical trials for Parkinson's disease using activators or blockers of endogenous inhibitors of Ret signaling. Targeting endogenous modulators of GFL signaling (i.e. Spry and Lrig1) might be of clinical interest, maximizing the therapeutic power of this neurotrophic factor on degenerating dopaminergic neurons. At the same time, additional characterization of the binding partners of these endogenous RTK modulators may contribute to understanding the underlying causes of certain brain tumors and many neurological syndromes and neurodegenerative diseases.

Concluding remarks and perspectives

A central question in developmental neurobiology is how axons from specific neuronal populations develop to achieve target specificity during the formation of neuronal connectivity. Cooperation between neurotrophic factors and guidance cues is a mechanism that confers specificity during circuit formation. Recent studies also illustrate that intrinsic cell-type-specific modulation of neurotrophic factor receptor signaling is another key mechanism that controls axonal growth, branching, and target field innervation during nervous system development. One of these studies demonstrated that many LIG modulators are not expressed in overlapping populations of motor and sensory neurons, raising the possibility that different LIG family members encode target specificity during the assembly of neuronal circuits (Mandai *et al.* 2009). Thus, depending on the cellular context, LIG molecules could enhance the multiplicity of signaling events and responses that can be triggered by Trk and Ret receptors in different neuronal populations.

Extensive studies have also demonstrated that negative- and positive-feedback loops are signaling events that evolved to provide an effective control of biological processes induced by neurotrophic growth factors such as survival, neurite outgrowth, axon guidance, and target selection (Torii *et al.* 2004; Tsang and Dawid 2004; Ledda *et al.* 2008; Mandai *et al.* 2009; Alsina *et al.* 2012).

Another concept derived from these studies indicates that neurotrophic growth factor receptors are tightly regulated

through the coordinated action of many negative protein modulators that function at multiple levels of the signaling cascades and at different time points after receptor engagement. Current understanding of the mechanisms used by several of the already identified neurotrophic growth factor receptor signaling regulators is still at an early stage. A more complete understanding of the molecular targets and physiological relevance of these RTK modulators will contribute to the identification of tumor-suppressor markers and to design more efficient therapies for human diseases. This knowledge could be used to maximize the therapeutic power and to reduce side effects of neurotrophic factor administration.

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