Neurotrophins – players in the regulation of neuronal survival and apoptosis

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Neurotrophins are a family of trophic factors that has been established as pivotal players in the regulation of neuronal survival and differentiation throughout development and adulthood. The prototypic neurotrophin, nerve-growth factor (NGF), was isolated and purified more than 50 years ago as a target-derived trophic factor for developing neurons. Since then, not only were more members added to the family, but as our understanding of the neurotrophins advances, it became clear that the neurotrophins are involved in almost all aspects of neuronal development. Here we will summarize the critical roles of neurotrophins in the regulation of neuronal survival and apoptosis.

Keywords: Neurotrophins, Trk receptors, p75, neuronal survival, receptor tyrosine kinase.

Introduction

NEURON, the building block of the nervous system, is probably one of the most unique cell types in multicellular organisms. Unlike other cells, neurons are incapable of continual cell division (mitosis) once they are differentiated, and hence neurons are often referred to as post-mitotic cells. Although the adult brain is equipped with a small amount of neural stem cells that will potentially enable replacement of damaged neurons, the rate of neuron generation is far slower compared to that observed in fetal stage. The crucial role of neurons in the functioning of the nervous system, together with the post-mitotic nature of these cells have therefore rendered the regulation of their survival a key event throughout the life time of an individual. Indeed, selective survival or apoptosis of certain neuronal populations both have serious implications on the functioning of the nervous system throughout development and adulthood. While selective preservation of certain neuronal populations during development is essential for the fine-tuning and consolidation of neural connections, loss of neurons induced by neurodegenerative diseases underlies some of the most devastating symptoms of neurological disorders. Deciphering the mechanisms by which neuronal survival is controlled has thus enthralled scientists for decades. Our understanding on the regulation of neuronal survival was revolutionized by the discovery of neurotrophins, a family of neurotrophic factors initially identified to function as target-derived trophic factors for developing neurons. The identification of neurotrophins led to the notion that secreted, diffusible factors could control neuronal development over long distances. Here we will discuss the essential role of neurotrophins in the regulation of neuronal survival and apoptosis during development and neurodegenerative diseases.

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Identification of neurotrophins as essential survival signals for developing neuron

The reliance of developing neurons on the presence of survival signals was first suggested by Victor Hamburger and Rita Levi-Montalcini in the late forties[1]. They found that the size of sympathetic ganglia is correlated with the size of the target innervated by these sympathetic neurons, where removal of target enhances neuronal death. Based on these findings, they hypothesized that neuronal death observed was due to the lack or low levels of biological mediators from the target cells. This hypothesis lays the foundation of what is known to us as the neurotrophic hypothesis today, which states that the number of surviving neurons is determined by the size of the neuronal target during development. This biological mediator crucial for the maintenance of sympathetic neuronal survival during development was later isolated and purified with the help of Stanley Cohen, and it was demonstrated that this factor released by target cells acts retrogradely on the sympathetic neurons to maintain their survival[2–4]. This soluble factor was named nerve-growth factor (NGF), which is the founding member of the neurotrophins[5].

The neurotrophins

While the abundance of NGF in the mouse salivary gland allows its purification and the subsequent characterization of its biological actions, for a long time it was regarded as a one-of-a-kind retrograde soluble peptide essential for supporting the survival of several subpopulations of sympathetic ganglia. Four decades of research went by before the second member of the neurotrophin family, brain-derived neurotrophic factor (BDNF), named for its abun-

dance in pig brain, was added to the family[6]. Advance in cloning technique soon led to the identification of two additional neurotrophins in mammals: neurotrophin-3 (NT-3)[7] and neurotrophin-4/5 (NT-4/5)[8,9]. Recently, neurotrophin-6/7 (NT-6/7) was also identified as a member of the neurotrophin family in lower vertebrates[10,11].

Members of the neurotrophin family are highly homologous, sharing about 50% amino acid identity. In particular, six cysteine residues are conserved in the relative positions of all neurotrophins that enable the formation of disulfide bridges, known as the 'cysteine knot' motif[12]. Despite the structural similarity of the neurotrophins, they are expressed in different regions of the brain and peripheral tissues, affecting different neuronal subpopulations. Northern blot analysis revealed that transcripts of NGF, BDNF and NT3 are all abundantly detected in the brain, while NT-4/5 is most prominently expressed in peripheral tissues. NT-6/7, on the other hand, is expressed in the brain and peripheral tissues such as heart and skin of lower vertebrates[13]. In addition to being expressed in different tissues, the expression of neurotrophins is also developmentally regulated. While expression of NGF remains fairly constant throughout development in the brain, expression of BDNF increases starting from embryonic stage, with maximal expression detected in the adult brain. NT-3, on the other hand, is prominently expressed throughout embryonic and postnatal stages, but expression tapers off in adulthood[14].

Invaluable insights on the neuronal subpopulations that respond to these neurotrophins are gained by delineating the extent and sites of neuronal death in transgenic mice lacking each of the neurotrophins. While NGF-null mice exhibit severe loss of superior cervical ganglia and lumbar dorsal root ganglia[15], disruption of the BDNF gene results in severe loss of vestibular neurons, in addition to varying degrees of neuronal death in dorsal root ganglia, nodose-petrosal and geniculate ganglia[16,17]. Similar effects on nodose-petrosal and geniculate ganglionic neurons are also observed in NT-4/5-null mice, although dorsal root ganglia are not affected[18,19]. NT-3-null mice, on the other hand, exhibit severe death of all the peripheral neurons examined, with the cochlear neurons most severely affected[20,21].

All neurotrophins are synthesized as precursor proteins known as pre-proneurotrophins where mature, biologically active neurotrophins are generated by proteolytic processing of these precursor proteins. Proteolytic processing of pre-proneurotrophins begins with the removal of the hydrophobic signal peptide at the N-terminal, resulting in the generation of proneurotrophins. Proneurotrophins are then often modified by N-glycosylation, and subsequently cleaved intracellularly by furin or proconvertases at a conserved dibasic cleavage site to generate mature neurotrophins. Mature neurotrophins are then secreted as non-covalently bound homodimers, where they have been shown to act as a retrograde survival signal, or in a paracrine and/or autocrine fashion[22,23].

While it has long been suggested that only the mature forms of neurotrophins are secreted, recent studies indicate that proneurotrophins are also secreted by neurons. How some of the proneurotrophins evade intracellular cleavage remains unclear, but the pro-region of proneurotrophins is recently proposed to take part in more important roles than the regulation of protein folding and neurotrophin secretion. Recent studies suggest that proneurotorphins, in addition to the mature form of neurotrophins, can both function as biologically active ligands to regulate neuronal survival. Indeed, it was recently observed that both the pro- and mature forms of neurotrophins are present in large quantity in the brain[22,24]. This new finding will likely challenge the existing view on the role and biological actions of neurotrophin signaling in the nervous system.

Neurotrophin receptors

Since the isolation of NGF, major effort has been devoted to the identification of NGF receptors. The continued quest for NGF receptor was rewarded by the identification of two receptors that associate with NGF: low-affinity neurotrophin receptor, commonly referred to as p75 (or $p75^{NTR}$) nowadays, and tyrosine receptor kinase Trk (renamed to TrkA to distinguish it from other Trk family members). p75 was initially identified as a NGF receptor[25–27] in 1986. Nonetheless, the absence of intrinsic kinase activity and the low affinity with which NGF associates with p75 all argue against a role of p75 as the main transducer of the biological actions of NGF[28–30]. The disappointment and confusion was soon resolved by the identification of TrkA as NGF receptor[33,34] in 1989. The identification of TrkA as a novel receptor tyrosine kinase, in addition to its mediation of PLCγ phosphorylation following NGF stimulation strongly suggests that TrkA is the cognate receptor for NGF[33,34]. This observation was corroborated by the findings that TrkB and TrkC, members of the same family, were identified as cognate receptors for other neurotrophins including BDNF and NT-3 respectively[35–37].

Trk receptors

Three Trk receptors, namely TrkA, B and C were identified based on sequence homology and structural similarity. Similar to other receptor tyrosine kinases, Trk receptor contains an extracellular ligand binding region, a transmembrane region and a cytosolic region characterized by the presence of a tyrosine kinase domain (Figure 1). Among these regions, the extracellular domain is indispensable for ligand recognition and binding. In particular, the C2-type immunoglobulin-like domain proximal to the

membrane (domain 5) was suggested to mediate ligand binding[38–40]. The extracellular region of Trk also contains consensus sites for N-glycosylation. It was recently observed that inhibition of glycosylation induces constitutive activation of the Trk tyrosine kinase in the absence of ligand binding, in addition to hindering Trk localization to the cell surface[41]. These observations indicate that the extracellular domain may also control receptor dimerization and Trk localization through modulating Trk glycosylation. On the other hand, tyrosine residues located in the intracellular region of Trk receptor are crucial for the initiation of downstream signaling cascade following ligand stimulation. They are autophosphorylated upon ligand binding and serve as docking sites for recruitment of signaling molecules[5].

Despite the structural similarity of TrkA, B and C, they exhibit different expression profiles. TrkA is widely distributed with expression detectable in the CNS, PNS and the immune system. TrkA expression is detected in the basal forebrain cholinergic neurons, and several subtypes of ganglia including the sensory cranial and spinal ganglia, the sympathetic ganglia and dorsal root ganglia[42–45]. TrkB, on the other hand, is abundantly expressed in the nervous system, although expression in the lung, muscle and ovaries is also detected[46,47]. In particular, expression of TrkB in the brain was observed in the cerebral cortex, hippocampus, dentate gyrus, striatum, brainstem, spinal cord, and the spinal and cranial ganglia, and paravertebral trunk of the sympathetic nervous system. TrkB is also expressed in neuroepithelium and neural crest cells during early embryogenesis[46,47]. TrkC is preferentially expressed in the brain, particularly in the hippocampus, cerebral cortex, granule cell layer of the cerebellum, spinal cord motorneurons and various ganglia[37,48,49].

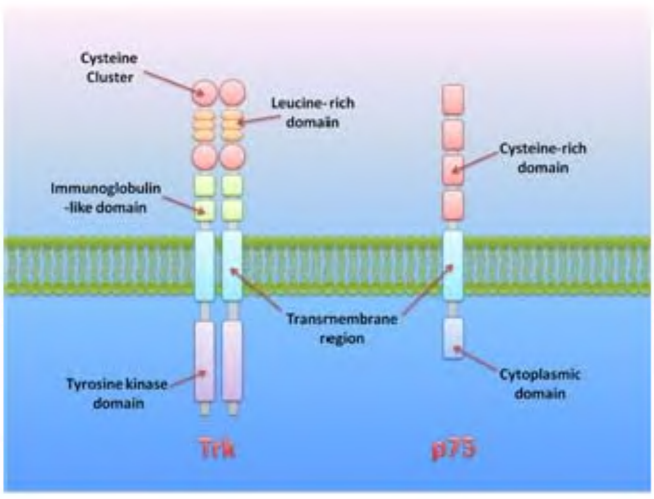

Figure 1. Structure of Trk and p75 receptors. Trks comprise an extracellular ligand binding region, a single transmembrane region and a cytosolic region containing a tyrosine kinase domain. Extracellular domain is characterized by the presence of two cysteine-rich domains, which sandwich a three tandem leucine-rich motif, followed by two C2-type immunoglobulin-like domains. Adjacent to the extracellular domain is the hydrophobic transmembrane domain serving as an anchorage for the receptor to the plasma membrane. The intracellular region, on the other hand, comprises a juxtamembrane region, a tyrosine kinase domain followed by a short carboxy-terminal tail. p75, on the other hand, comprises an extracellular region with four cysteine-rich domains, a transmembrane region, and a cytoplasmic region containing an internal death domain.

Aside from full length Trk receptors, isoforms of Trk receptors have also been identified. Interestingly, their expression profile differs from that of their full length counterparts, suggesting that these isoforms may serve distinct functions. For TrkA, three additional splicing variants were identified in addition to the full length TrkA. The first isoform containing a 6-amino acids insertion between the second Ig-like domain and the transmembrane region was designated as TrkAII[50,51]. Two additional TrkA isoforms that are expressed almost exclusively in the thymus are characterized by the presence of only one or no leucine-rich region[52].

On the other hand, seven isoforms of TrkB have been identified. Two truncated isoforms of TrkB, named TrkB-T1 and TrkB-T2, are distinguished from the full length by the replacement of the intracellular domain with two distinct short C-terminal sequences for TrkB-T1 and TrkB-T2 (ref. 53). Interestingly, while full length TrkB and TrkB-T2 are expressed mostly in neuronal cells, TrkB-T1 expression is detected in both neuronal and non-neuronal tissues such as astrocytes, oligodendrocytes and Schwann cells. Four additional TrkB isoforms were characterized by the presence of only one (L1) or complete absence (L0) of leucine-rich regions in the extracellular domain of TrkB-FL and TrkB-T1 receptors. The L1 and L0 variants do not bind TrkB ligands including BDNF, NT-3 and NT-4/5, and cannot induce survival and morphological transformation in fibroblasts[54].

Finally, five TrkC isoforms with truncation or insertion in the intracellular domain have been identified in addition to the full length TrkC. Similar to TrkB, two TrkC isoforms are characterized by truncated kinase domain (TrkC-T1 and TrkC-T2), where the intracellular domain is replaced by distinct short C-terminal sequences. Three other isoforms are characterized by different length of insertions (TrkC-14; refs 25, 39) in the intracellular domain, which interfere with the major autophosphorylation site of the kinase domain, thereby inhibiting mediation of downstream biological response. Interestingly, only the truncated isoforms are expressed in peripheral nerve and non-neural tissues such as astrocytes[55–57].

p75

Although p75 also binds to neurotrophins and functions as an indispensable component of neurotrophin signaling, its structure and biological functions are fairly different compared to Trks. p75 is a 75 kDa glycoprotein that

belongs to the tumour necrosis factor (TNF) family receptor[25,26]. Similar to other TNF members, it contains four cysteine-rich domains in the extracellular region that are essential for binding to neurotrophins, a transmembrane domain and an internal death domain lacking any kinase domain[25]. The intracellular juxtamembrane domain within the internal death domain is also known as the Chopper domain for its ability to induce cell death in dorsal root ganglion cells[58]. Furthermore, the cytoplasmic region is important for the activation of NFkB and apoptosis induction[59] (Figure 1).

Expression of p75 is detected abundantly in various neuronal populations, including the striatal neurons and spinal cord motor neuron, as well as the sympathetic and sensory ganglia. Furthermore, p75 is highly expressed during embryonic and postnatal stages, but expression level tapers off in the adult stage[60–62]. In addition to the full length p75, it was recently demonstrated that there exists a truncated isoform of p75, named s-p75^{NTR} (for short p75^{NTR})[63]. s-p75^{NTR} is characterized by the absence of three of the four cysteine-rich repeats in the extracellular domain, thus rendering it incapable of binding to NGF. Similar to full length p75, s-p75^{NTR} is expressed in the brain and spinal cord, although the expression level is markedly lower than that of full length p75 (ref. 63).

Ligand specificity of neurotrophin receptors

Although all neurotrophins are similar structurally, they exhibit rather remarkable selectivity for various neurotrophin receptors. While NGF and NT-6/7 bind predominantly to TrkA, BDNF and NT4/5 associate with TrkB with high affinity. NT-3 binds strongly to TrkC, but also weakly to TrkA and TrkB. On the other hand, all neurotrophins associate with p75 receptor with low affinity[5]. Recently, p75 has also been observed to exhibit high affinity binding to pro-forms of neurotrophins, suggesting that proneurotrophins may instead be the primary ligand for p75 (Figure 2). However, rather than promoting trophic support, proneurotrophins induce p75-dependent apoptosis in sympathetic neurons and oligodendrocytes[24,64]. The relative importance of proneurotrophins and neurotrophins in mediating p75 signaling will remain an interesting area of further investigation.

Signaling downstream of Trk receptors

Binding of neurotrophins to Trk receptors triggers a cascade of downstream signaling that functions to mediate the biological response of neurotrophins. Similar to other receptor tyrosine kinases, Trk receptors dimerize and trigger transactivation of the tyrosine kinase domain in response to ligand binding. The activated kinase domain then induces the phosphorylation of several tyrosine residues present in the cytoplasmic domains of the receptors. Take TrkA as an example, NGF-binding induces phosphorylation of Y490, Y670, Y674, Y675 and Y785 (Figure 3)[13,65]. Among these residues, Y670, Y674 and Y675 are located in the activation loop of the kinase domain, while Y490 and Y785 are located outside the kinase domain. Phosphorylation of these tyrosine residues not only trigger full transactivation of the Trk receptors, but these residues also function as docking sites for recruitment of adaptor proteins to initiates Trk downstream signaling[13,65].

Direct recruitment of signaling cascade

Initiation of signaling downstream of Trk activation can occur via several mechanisms. Direct recruitment of signaling pathways probably represents one of the most important routes through which downstream effects of Trk receptors are mediated. Phospho-tyrosine residues located in the intracellular domain of Trk receptors function as docking sites for various adaptor molecules. Upon association, these adaptor proteins are usually phosphorylated by Trk itself. The signaling pathways that are activated in this fashion include the Ras-MAPK, PI3-kinase and PLC-γ1 pathways.

Activation of the Ras-MAPK pathway involves a complex network of adaptor proteins and signaling molecules that eventually converge on the activation of small GTPase Ras. Ras can be activated by the recruitment of several adaptor molecules. Recruitment of Shc and Frs-2 to Y490, or rAPS and SH2B to the activation loop tyrosine, all result in the subsequent recruitment of a complex consisted of Grb2 and SOS (son of sevenless)[66–68]. Recruitment of SOS, an exchange factor for Ras, triggers

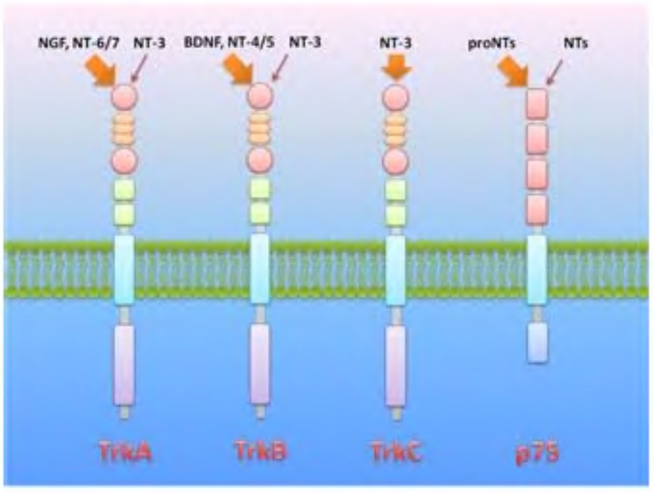

Figure 2. Ligand specificity of neurotrophin receptors. Neurotrophins display selectivity towards different Trk receptors. While NGF and NT-6/7 interact with TrkA, BDNF and NT-4/5 interact with TrkB with high affinity (thick arrow). NT-3 binds strongly to TrkC, but also interacts with TrkA and B with lower affinity (thin arrow). Most neurotrophins (NTs) bind to p75 with low affinity. Recently, proneurotrophins (ProNTs) have also been observed to associate with p75 with high affinity.

Ras activation[69]. It should be noted, however, that Grb2 can also bind directly to activated Trk via the tyrosines residues in the activation loop, leading to Ras-MAPK activation (Figure 3). Activation of Ras results in the sequential activation of the MAPK superfamily, the MAPK kinase kinase Raf-1 and B-Raf, MAPK kinase MEK1/2 and MAPK ERK1/2 (refs 70, 71). Activated ERK1/2 then translocates to the nucleus to activate several transcription factors including Egr-1 and Elk-1, which plays crucial role in NGF-stimulated differentiation and neurite outgrowth in a neuron-like cell line PC12 cells[72–75]. Recently, NGF was found to induce activation of another transcription factor, STAT3, which was partially required for NGF-induced gene transcription in PC12 cells. Inhibition of MEK/ERK pathway attenuated NGF-triggered STAT3 activation, suggesting that STAT3 may also mediate NGF-induced gene transcription downstream of the Ras-MAPK pathway[75].

Another pathway activated downstream of Trk activation via the recruitment of adaptor molecule is the PI3-kinase pathway. There exists at least two mechanisms by which PI3-kinase is activated in response to Trk activation. In response to the recruitment of Grb2 to Trk receptors, association of Grb2 with Gab1 (Grb2-associated binder-1) and Gab2 (Grb2-associated binder-2) leads to their phosphorylation and association with the regulatory subunit of PI3-kinase, p85, to activate PI3-kinase. Alternatively, the catalytic subunit of PI3-kinase may directly bind Ras to initiate PI3-kinase activation. Activated PI3-kinase then activates PDK-1 (phosphatidylionoside-dependent protein kinase), and subsequently Akt[76–78]. Upon activation, Akt phosphorylates and modulates the activity of several proteins to promote neuronal survival[79]. For example, phosphorylation of caspase-9 by Akt inhibits the activity of caspase-9, thereby hindering the progress of the apoptotic pathway[80]. Akt also suppresses the expression of several pro-apoptotic genes through phosphorylating the Forkhead family of transcription factors (Figure 3)[81–83]. Akt activation hence represents a key mechanism through which cell survival is promoted by the PI3-kinase pathway. In addition to the maintenance of neuronal survival, accumulating evidence indicates that PI3-kinase may also regulate neuronal architecture. PI3-kinase was shown to mediate the activation of Rho GTPases such as Cdc-42, Rac1 and RhoA, which are important regulators of actin polymerization[84]. Localized activation of Ras and PI3-kinase following Trk activation also promotes growth cone steering and cell motility[85–88]. These observations suggest that PI3-kinase signaling may mediate other functions of Trks other than promoting neuronal survival.

A third pathway that is activated downstream of Trk activation is PLC-γ1 pathway. Direct association of PLC-γ1 with the phosphorylated Y785 of activated TrkA triggers activation of PLC-γ1 signaling (Figure 3)[89]. Activated PLC-γ1 then induces the hydrolysis of phosphatidylinositol 4,5-P2 to produce inositol 1,4,5-trisphosphate (IP3) and diacylglycerol (DAG). IP3 triggers activation of Ca^{2+}-regulated enzymes such as CaM kinases and Ca^{2+}-regulated isoform of PKC through inducing Ca^{2+} release from intracellular stores. On the other hand, DAG stimulates DAG-regulated protein kinase C isoforms[90]. Recent studies suggest that Trk-induced activation of PLC-γ1 may regulate electrical activity in neuronal cells, indicating that Trk-mediated PLC-γ1 signaling pathway may be critical for neurotrophin-evoked electrical activity[91].

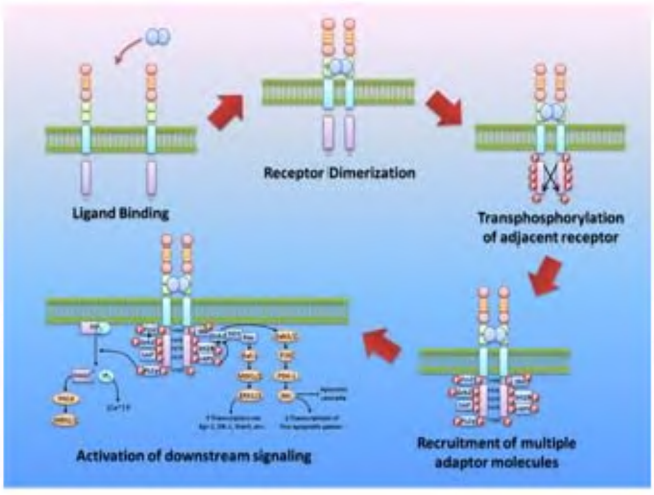

Figure 3. Activation and signaling of the Trk receptors. Binding of neurotrophins to Trk receptor initiates dimerization of two Trk receptors. This triggers the activation of the kinase domain, thereby resulting in *trans*-phosphorylation of tyrosine residues on the neighboring receptor. Positional information for the tyrosine residues listed in the figure corresponds to the tyrosines phosphorylated in activated TrkA. The phosphotyrosines then provide docking sites for various adaptor proteins of signaling molecules including Shc, PLCγ-1, Frs2, SH2B, rAPS, SAP, etc. to activate MAPK, P13K and PLCγ signaling pathways.

Crosstalk with other pathways

In addition to direct recruitment of signaling molecules, the biological actions of neurotrophin signaling may also occur via crosstalks with other pathways. Earlier studies revealed that neurotrophin receptor signaling exhibit certain extent of crosstalk with neuropoietic cytokine-initiated signaling. The neuropoietic cytokine is another family of trophic factors whose members include leukemia inhibitory factor (LIF), ciliary neurotrophic factor (CNTF) and interleukin-6 (IL-6)[14]. Collaboration between neurotrophins and neuropoietic cytokines has been demonstrated to regulate neuronal differentiation and developmental apoptosis[92]. For example, concurrent p75 signaling is required for the induction of apoptosis by LIF in cultured sympathetic neurons[93]. Differentiation of sensory neurons, on the other hand, demands a combination of LIF and NGF[94]. Furthermore, neurotrophins and neuropoietic cytokines have been demonstrated to work together to

modulate neuronal phenotypes. For example, NGF, LIF and CNTF are implicated in the regulation of cholinergic phenotype expression under a variety of physiological and pathological conditions[95]. These findings collectively suggest that neurotrophins and neuropoietic cytokines synergistically affect neuronal differentiation.

Another pathway that has been demonstrated to exhibit cross-talk with the neurotrophins is the G-protein-coupled receptor (GPCR) pathway. An earlier study demonstrated that GPCR signaling initiates activation of Trk, PLC-γ1 and PI3-kinase pathways in the absence of neurotrophin binding[96,97]. In contrast to neurotrophin-mediated activation, Trk transactivation via GPCR signaling results in prolonged Akt activation to promote neuronal survival after trophic factor withdrawal[98]. These observations demonstrate that Trk activation induced by neurotrophins and GPCR pathways occurs via different kinetics, suggesting that neurotrophins and GPCR pathways may serve as parallel pathways for Trk activation, but may mediate different biological response through fine-tuning of the kinetics of activation.

Recently, TrkB was observed to crosstalk with a serine/threonine kinase known as cyclin-dependent kinase 5 (Cdk5). Cdk5 is a member of the Cdk family that is activated through association with its neural-specific activators p35 and p39. Unlike its family members whose roles are predominantly in the control of cell cycle progression, Cdk5 is implicated in a myriad of biological processes, including the regulation of neuronal migration, neuronal survival and synaptic functions[99,100]. Interestingly, TrkB was recently identified as a Cdk5 substrate. Activation of TrkB through BDNF stimulation triggers recruitment of Cdk5 to TrkB, which is phosphorylated by TrkB at Tyr15 to enhance Cdk5 activity. Cdk5 in turn phosphorylates TrkB at Ser478 at the intracellular juxtamembrane region of TrkB. Inhibition of this phosphorylation markedly attenuates BDNF-induced Cdc42 activation and dendrite growth in hippocampal neurons, thus revealing that phosphorylation of TrkB by Cdk5 is required for BDNF-induced Cdc42 activation and dendritic growth[101].

Retrograde transport

A third mechanism by which the actions of neurotrophins are mediated involves the retrograde transport of neurotrophin–receptor complex. Subsequent to ligand binding and activation of downstream signaling, neurotrophin–receptor complex is internalized through dynamin and clathrin-dependent endosytosis. Internalized Trk receptors are then transported, recycled to the membrane surface for subsequent ligand binding and activation, or degraded in lysosomal and/or proteasomal degradation system[102]. It has been observed that all endocytosed neurotrophin receptors, including TrkA, TrkB, TrkC and the p75 receptor, are retrogradely transported *in vivo*[103–106]. Retrograde transport of Trks, for example, occurs via a dyenin-dependent mechanism[107,108]. In addition, inhibition of PI3-kinase at the nerve terminal inhibits neurotrophin retrograde transport, suggesting that PI3-kinase may play an important role in retrograde transport[109]. It was recently demonstrated that internalized neurotrophin–receptor complex constitutes an active signaling entity in the form of a 'signaling endosome' to mediate biological actions distinct from that initiated by cell surface Trk receptors[102]. For example, initiation of survival signals at the cell body following stimulation with target-derived neurotrophins requires the transport of endocytosed neurotrophin–receptor complex[108]. Stimulation of distal axons of sensory or sympathetic neurons with neurotrophins triggers transport of neurotrophin–receptor complex that induces ERK5 and PI3-kinase signaling in the cell body[110,111]. Translocation of activated ERK5 to the nucleus will then lead to phosphorylation of pro-survival transcription factors CREB and MEF2, thereby enhancing neuronal survival[110].

Negative regulation of Trk-mediated signaling

Concurrent with the initiation of downstream signaling cascade, Trk activation also initiates several feedback mechanisms to limit Trk activation. This is important for maintaining responsiveness to further ligand stimulation, and to prevent over-amplification of downstream signals. As a first line of defense, Trk activation induces internalization of Trk, thereby temporarily shutting down response to excess ligands. Alternatively, activated Trk receptors or downstream signaling molecules may also be tyrosine dephosphorylated to prevent further Trk activation. For example, phosphatases SHP-1 and PTEN (phosphatase and tensin homologue deleted on chromosome 10) have been observed to limit TrkA activation following NGF treatment[112–114]. Up-regulation of MAP kinase phosphatase 1 (MKP-1) by NGF, on the other hand, has been reported to inactivate ERK1/2 by dephosphorylation[115,116]. Recently, a novel interacting protein of Trk receptors, known as SLAM-associated protein (SAP), was found to function as a negative regulator of Trk signaling[117]. Binding of SAP to Trk receptors markedly attenuates tyrosine phosphorylation and activation of Trk receptors. Furthermore, overexpression of SAP attenuates recruitment of SH2B and Shc to activated TrkB, indicating that SAP functions as a negative regulator of Trk activation and downstream signaling[117]. Finally, ligand-induced downregulation of Trk expression also serves as another mechanism for limiting Trk signaling. Downregulation of TrkB protein and mRNA levels was observed after prolonged BDNF treatment (ranging from 30 min to 24 h)[118–120]. All these mechanisms collaborate to prevent over-stimulation of Trk signaling.

Initiation of signaling downstream of p75

Since p75 does not contain a kinase domain, mediation of extracellular signal by p75 requires its association with other cell surface receptors or adaptor proteins in the cytoplasm. To date, a myriad of molecules of dissimilar structure and properties have been identified to associate with p75, but the signaling cascade initiated is barely beginning to be unraveled. Identification of p75 downstream signaling and functions therefore remain a continued challenge and also a subject of intense interest in the field of neurotrophin research.

Recruitment of adaptor molecules

Given the lack of a kinase domain, recruitment of adaptor molecule for signal transduction is unavoidable for p75. Most p75 interacting proteins were identified only in the past decade. While existing knowledge is focused mostly on the structural aspect of the interacting proteins, the functional significance of the interaction and the signaling components implicated is slowly being explicated. Examples of p75 interacting proteins include NRIF (neurotrophin receptor interacting factor), NRAGE (neurotrophin receptor-interacting MAGE homologue) and NADE (p75-NTR-associated cell death executor)[121–123]. NRIF and NRGAE associate with p75 through the juxtamembrane domain of p75 (refs 121, 123), while NADE was observed to interact with p75 via the death domain of p75 (ref. 122). Recruitments of NRIF, NADE and NRGAE have all been associated with a pro-apoptotic role of p75. In particular, activation of JNK and initiation of the apoptotic cascade have been observed following recruitment of NRAGE and NRIF, but the precise signaling cascade implicated subsequent to NADE association remains enigmatic[121–123].

In contrast to a proposed pro-apoptotic role, activation of p75 has also been associated with enhanced neuronal survival via the activation of NFκB activation. Two adaptor proteins recruited to p75, TRAF6 (TNF receptor-associated factor 6) and RIP-2 (serine/threonine kinase receptor interacting protein-2), are implicated in the activation of NFκB[124,125]. Binding of TRAF6 and RIP2 to p75 following NGF treatment both lead to NFκB activation. Finally, p75 also interacts with small GTPase RhoA, which binds to the intracellular domain of p75 (ref. 126). The association between RhoA and p75 is suggested to play an important role in p75-mediated neurite outgrowth (Figure 4).

Coupling with other co-receptors

In addition to the recruitment of intracellular adaptor molecules, p75 has also been observed to recruit transmembrane receptors for the transduction of downstream signals. For example, recruitment of Nogo receptor (Ngr) and Lingo-1 by p75 has been implicated in the modulation of neurite elongation[127–129]. Several proteins present in the CNS myelin, including Nogo-A, oligodnedrocyte-myelin glycoprotein (OMgP) and myelin-associated glycoprotein (MAG), all exhibit inhibitory action on neurite elongation. This is particularly detrimental for the regenerative attempts made by damaged CNS axons. Nogo-A, OMgP and MAG all function as ligand to Ngr, and are therefore capable of activating the p75/Ngr/Lingo-1 tripartite complex. Association of OMgP, MAG or Nogo-A with p75/Ngr/Lingo-1 complex increases RhoA activity, which underlies the ability of p75/Ngr/Lingo-1 complex to inhibit axon elongation[127,130,131]. Interestingly, a recent study revealed that peptides derived from Ngr, Pep4 and NEP1-40, protect against NGF/p75-induced death of cultured motor neurons. Pep4 and NEP1-40 also ameliorate axotomy-induced motor neurons loss, which was demonstrated to require p75 activation[132]. This finding reveals a novel possibility that in addition to forming a receptor complex to regulate neurite growth, Ngr and p75 may also modulate their respective downstream function through direct interaction.

Another transmembrane receptor that is recently demonstrated to associate with p75 is sortilin. Recruitment of sortilin is required for the mediation of pro-apoptotic signaling by p75 subsequent to proneurotrophins (ProNGF

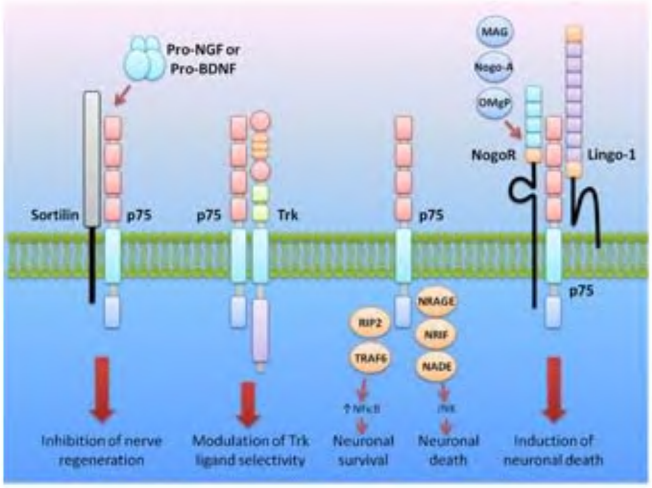

Figure 4. Recruitment of adaptor molecules and co-receptors downstream p75 receptor. p75 receptor has been observed to mediate both neuronal survival and apoptosis depending on the recruitment of different adaptor proteins. While recruitment of NRAGE, NADE and NRIF results in JNK activation and neuronal death, recruitment of TRAF6 and RIP2 leads to NFκB activation and neuronal survival. On the other hand, actions of p75 can also be mediated by the recruitment of co-receptor. Recruitment of Nogo receptor (NgR) and Lingo-1 is important for the p75-mediated inhibition of nerve regeneration. MAG, Nogo-A and OMgP all function as ligand for the p75/Lingo-1/NgR complex. Recruitment of sortilin, on the other hand, is essential for p75-induced neuronal death in response to proneurotrophins (ProNTs). Finally, association of p75 and Trk has been associated with the reciprocal regulation of ligand specificity for mature neurotrophins.

and ProBDNF) stimulation[64,133]. The low affinity with which all mature neurotrophins associate with p75 has left scientists in search of other high affinity ligand for p75. Recently, proneurotrophins are observed to exhibit a much higher affinity towards p75 compared to mature neurotrophins, suggesting that proneurotrophins, instead of mature neurotrophins, may function as the cognate ligand for p75 (refs 24, 64). More importantly, sortilin is recently demonstrated to function as an essential component of the pro-apoptotic signals mediated by proneurotrophin-p75 signaling[133]. These observations collectively suggest that the function of p75 is potentially regulated by the differential expression of co-receptors and whether the processed or mature form of neurotrophins is more abundant.

Finally, Trks and p75 association has long been demonstrated[33]. Although whether p75/Trk complex functions as a transducing receptor remains unknown, the affinity of TrkA for NGF is significantly enhanced in the presence of p75. This is accompanied by a concomitant reduction of NGF's affinity for p75 (refs 134, 135). Furthemore, association of p75 with Trks regulates ligand specificity of Trk receptors. For example, the selectivity of TrkB for BDNF over NT-3 is significantly elevated in the presence of p75 (ref. 134). These observations collectively suggest that in addition to mediating separate downstream signaling, Trks and p75 may also reciprocally regulate the function and signaling of each other through direct association (Figure 4).

Neurotrophins as regulator of neuronal survival and death

Through the generation of neurotrophin and neurotrophin receptor knock-out mice, and the explication of downstream signaling mediated by neurotrophin receptors, it has become increasingly clear that neurotrophins are so much more than survival factors for developing neurons. Rather, neurotrophins are implicated in almost all aspects of neuronal development and physiology. Aside from the regulation of neuronal survival and apoptosis during both development and pathological conditions, neurotrophins are involved in the regulation of neuronal architecture and synaptic plasticity. Recent evidence indicates the neurotrophins may also play essential roles in higher cognitive functions such as learning and memory[5]. While the actions of neurotrophins are diverse, here we focus on the roles of neurotrophins in the life and death decision of neurons throughout their lifetime.

Trk receptor: mediation of survival signals

Regulation of neuronal survival during development: During the initial generation of the nervous system, neural progenitor cells are located in the ventricular zone of the developing CNS, where the number of progenitors increases via mitosis. Upon leaving the ventricular zone, these cells cease to divide, and enter into the mantle (or intermediate) zone where they mature and differentiate into neurons. Newly born neurons then migrate to their final destinations, where they send out axons in an attempt to establish appropriate connections with target cells. It has long been observed that during this period of connectivity, a massive phase of neuronal death occurs that eliminates about 50% of the neurons. While for a long time it remains unclear as to why neuronal death occurs during development, this enigma was later resolved when the extent of developmental cell death was observed to correlate with the number of target cells, suggesting that the number of surviving neurons are dependent on the size of target populations[136]. Indeed, developmental cell death occurs at a time when both the CNS and PNS are laden with excessive number of neurons. Due to the limited number of targets cells, not all neurons are successful in making functional connections. To ensure selective survival of the neurons that have established functional connections, neuronal death is induced for the cells that have failed to wire up with the target cells. This massive phase of cell death was later known as developmental apoptosis or programmed cell death.

Initial observation of the dying cells eliminated at this early stage of development reveals that these cells appear to follow a sequence of controlled events, resulting in predictable appearance of highly characteristic morphological changes. These reproducible changes in morphology led to the term 'programmed cell death', which suggests that an inherent death programme exists in these cells that eventually leads to their own demise[137]. Morphologically, cells dying during this massive elimination phase are characterized by initial shrinkage and condensation of cell body and nucleus, nuclear margination, with apparently intact plasma membrane and functional intracellular organelles. Eventually, blebbing of the plasma membranes results in the formation of small round entities enclosing pieces of intracellular organelles and fragmented nucleus, known as apoptotic bodies. They are rapidly phagocytosed by neighbouring macrophages or microglial cells. Biochemically, apoptosis is characterized by internucleasomal DNA degradation together with the activation of caspases, a family of protease that plays central role in the execution of the apoptotic programme. This characteristic type of cell death was later coined 'apoptosis', which literally means 'falling away' in Greek, because it was believed that this massive phase of cell death was indeed an integral part of normal development, where the removal of these cells is essential for the consolidation of functional connection[137].

Since the isolation of NGF, the role of neurotrophins as key survival and differentiation factors for neurons during development has long been established. Recent studies indicate that neurotrophins also promote proliferation

and survival of embryonic stem cells and neural progenitors, in addition to facilitating their differentiation program[138,139]. The importance of neurotrophins in supporting the survival of developing neurons was evident from massive studies demonstrating that neurotrophins stimulation is required to keep the in supporting the survival of developing neurons alive in cell cultures. Withdrawal of neurotrophins lead to apoptosis of these cultured neurons, recapitulating the necessity of neurotrophins in the inhibition of developmental apoptosis[136]. Interestingly, different neuronal subpopulations are responsive to different neurotrophins. For example, while NGF is essential for the differentiation of both sympathetic and sensory neurons, a subpopulation of dorsal root ganglion (DRG) sensory neurons and proprioceptive neurons respond to BDNF and NT3 instead[14]. The dependence of different neuronal subpopulations on different neurotrophins-Trk signaling is further consolidated by examining the surviving neuronal populations in neurotrophin- and Trk-knockout mice, which have strikingly similar phenotypes. For example, complete loss of sympathetic and sensory neurons was evident in both *trkA* and *NGF* mutant mice[15,140]. Mice deficient in NT4/5, another TrkA ligand, also exhibit loss of nodose-petrosal and geniculate ganglia sensory neurons[18,19]. On the other hand, *BDNF* and *trkB*-deficient mice display malfunctions in the vestibular system and neuron loss in the trigeminal, nodose ganglia and DRGs, in addition to the motor neuron loss observed in *trkB* knockout mice[16,141]. Finally, *NT-3* and *trkC* deficient mice are characterized by abnormal movements and postures with a deficiency in proprioceptive neurons[20,142]. These observations reveal that different neurotrophins functions to control the survival of different neuronal populations during development, possibly through their distinctive expression profiles during developmental stage.

In addition to findings from targeted gene disruption analysis and biochemical studies, naturally occurring mutation in TrkA also reveals the crucial involvement of TrkA in neuronal survival. Mutations in *trkA* have been linked to a human syndrome, congenital insensitivity to pain and anhidrosis (CIPA), or hereditary sensory and autonomic neuropathy type-IV[143]. CIPA patients lack sympathetic neurons and small unmyelinated nociceptive sensory neurons that rely on TrkA activation for survival during development. These patients thus exhibit defects in thermoregulation and sensitivity to pain, which results in injuries, self-mutilation and death-causing episodes of hyperpyrexia[143,144]. Collectively, these observations demonstrate unequivocally the crucial role of neurotrophins in the maintenance of neuronal survival during development.

Neuronal death during pathological condition: Can neurotrophin signaling also play a role in pathological conditions? While apoptosis was originally thought to occur only during the elimination of excessive developing neurons, it soon became clear that apoptosis represents a subtype of cell death that can occur throughout the lifetime of a neuron. Indeed, careful examination of the afflicted neurons in various neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease or amyotrophic lateral sclerosis reveals the presence of apoptotic neurons. Neurons dying in an apoptotic manner are also typical in brain regions following traumatic injury or stroke[137]. Although developmental apoptosis and pathological neuronal death may appear to occur through different mechanisms, they both occur as a result of imbalance between survival and death signals. Removal of survival signals, or the sudden upregulation of death-inducing signaling, will both tip the balance in favour of triggering the apoptotic machinery. Nonetheless, while the presence of survival signals is crucial for preservation of neuronal survival in developing neurons, generation of death signals is often the inducer of neuronal death during pathological conditions (Figure 5). Noxious stimuli such as the presence of misfolded protein and abnormal protein aggregates, or elevated generation of reactive oxygen species in neurons have been observed concomitantly with dying neurons in neurodegenerative diseases[137,145].

Since neurotrophins are important sources of survival signals, it is not surprising to observe that nerve-injury induced neuronal loss can be delayed with the supply of exogenous neurotrophic factors. For example, severing of axons induces apoptotic death for the retinal ganglion cells and motor neurons[146,147]. Exogenous application of BDNF has been observed to prevent cell death for several days. Nonetheless, the neuroprotective efficacy soon

Figure 5. Neuronal survival and death is regulated by a balance of survival and death signals. During both physiological and pathological conditions, neuronal survival is maintained if survival signals prevail over death signals. Nonetheless, either reduction in survival signals or enhancement in death signal can tip the balance towards the induction of apoptosis. Loss of retrograde trophic support during development or the presence of misfolded protein and protein aggregates during pathological condition will both result in apoptosis.

dwindles away. The loss of BDNF's neuroprotective effect for retinal ganglion cells has been attributed to the loss of responsiveness of these damaged neurons to BDNF in the absence of electrical activity[148]. Therefore, while addition of neurotrophins can temporarily prevent apoptosis by supplying additional survival signals to tip the balance away from apoptosis, long-term rescue of neurons following injury depends upon other factors, such as the responsiveness of the cells.

p75 receptor: mediator of pro-apoptotic signals?

Contrary to the Trk receptors, the function of p75 has remained a little obscure due to the lack of an intracellular kinase domain. Nonetheless, generation of knockout mice lacking p75 provided some important clues on the function of p75 during development. Two *p75* knockout mice have been generated, the first constructed via targeted disruption of exon 3 of *p75*, which disrupts the expression of the long form of p75 but not the short form; the second one via deletion of exon 4, thereby preventing expression of both the long and short isoforms of p75 (refs 63, 149). Significant phenotypic differences exist between the exon 3 and exon 4 *p75* knockout mice. Mice with *p75* exon 4 deletion are smaller in size compared to the exon 3-deleted counterpart. In addition, exon 4-deleted mice exhibit both serious loss of peripheral sensory neurons and peripheral innervation, and reduced phototoxicity-induced photoreceptor when compared to wildtype[63]. While these observations suggest that p75 may contribute to photoreceptor apoptosis in diseases such as retinitis pigmentosa, the loss of certain neuronal suppopulation indicates that p75 may also play an important role in neuronal survival. Indeed, as we will discuss here, p75 activation has been attributed to both maintenance of neuronal survival and initiation of cell death, in contrast to the survival promoting effect of Trk activation.

With p75 being a member of the TNF receptor family and bearing a death domain, it was not surprising that p75 may function as a death receptor. Indeed, *p75* exon 3 knockout mice exhibit reduced apoptosis in the retina[149]. In addition, overexpression of p75 in primary cortical neurons, PC12 cells or glioma cells leads to activation of JNK and caspase activation[150–152].

The mechanisms through which ligand binding of p75 result in the initiation of apoptotic cascade, however, is far from clear. Recent studies reveal that recruitment of adaptor molecules constitutes one of the major mechanisms. For example, interacting protein such as NRIF and NADE were recruited to the death domain of p75 to mediate the pro-apoptotic property of p75 (refs 121, 122). Deletion of NRIF in NRIF knockout mice rescues sympathetic neurons from apoptosis subsequent to neurotrophin stimulation, suggesting that NRIF is required for p75-induced apoptosis[121,153]. In addition, overexpression of NADE was found to initiate apoptosis and caspase activation when overexpressed together with p75 in 293T cells[122]. Recruitment of another p75 interacting protein NRAGE, on the other hand, also results in robust JNK activation and caspase activation in PC12 cells[154]. Taken together, these observations indicate that recruitment of pro-apoptotic interacting partners serves as one of the mechanisms by which p75 initiates apoptosis. Explicating the circumstances under which p75 prefers one interacting protein over the other will provide essential information on how the function of p75 is regulated.

Interestingly, despite the presence of an intracellular death domain, p75 has also been suggested to favour neuronal survival. This hypothesis is supported by the absence of peripheral sensory neurons in the *p75* exon 4 knockout mice, suggesting that p75 is crucial for maintaining the survival of this neuron subpopulation[63]. Accumulating evidence indicates that the ability of p75 to enhance neuronal survival is associated with an increase in NFκB activation. For example, NGF was observed to bind to p75 and activates NFκB in TrkA-lacking Schwann cells[155]. Furthermore, neurotrophins trigger p75-dependent NFκB activation in sympathetic neurons. Although the mechanisms by which p75 activation results in NFκB induction have not been completely elucidated, recent evidence suggests that it may involve recruitment of certain p75 interacting proteins. Association of p75 with TRAF6, for example, was shown to mediate the downstream NFκB activation[125]. In addition, binding of RIP-2 also leads to NFκB activation[124]. It thus appears that p75 may promote both apoptosis and survival depending on the identity of the interacting proteins that are recruited.

Prior to the identification of proneurotrophins as ligand for p75, low-level activation of p75 by mature neurotrophins has been proposed to result in developmental neuronal death by contributing pro-apoptotic signals when Trk-mediated pro-survival signaling is absent. Interestingly, a recent study revealed that activation of p75 by proneurotrophin also triggers neuronal apoptosis during development[24,64]. When sortilin, the p75 co-receptor required for binding to proneurotrophins, was knocked-down in transgenic animal, developmental apoptosis was markedly reduced in sortilin$^{-/-}$ retina. This suggest that proneurotrophin-induced activation of p75/sortilin receptor complex may also contribute to developmental apoptosis[156]. On the other hand, increasing reports suggest that upregulation of p75 signaling is also involved in neuronal death observed following traumatic injury and neurological disorders. Expression of p75, proneurotrophins and mature neurotrophins are all elevated in multiple cell types following injury. Consistent with this observation, injury-induced neuronal death is alleviated in *p75* exon 3 knockout mice or when p75 expression is reduced by antisense oligonucleotides[149]. Furthermore, ProNGF and ProBDNF have recently been demonstrated as pathophysiological ligand for the induction of neuronal death

following injury in the CNS[22]. The importance of p75 in mediating the death-promoting effect of neurotrophins was further supported by a recent study demonstrating that neurons isolation from sortilin$^{-/-}$ mice was resistant to proneurotrophin induced cell death[156]. Furthermore, survival of corticospinal motor neurons following axotomy was markedly elevated in sortilin$^{-/-}$ mice, indicating an essential role of p75/sortilin signaling in injury-induced neuronal death[156]. These observations collectively suggest that during pathological condition, enhanced p75 signaling may exacerbate neuronal death.

Neurotrophins: instigator of both pro-survival and pro-apoptotic signaling

While neurotrophins have long been considered to function strictly as essential anti-apoptotic agents, it is now increasingly clear that neurotrophins, when given the right receptors and processing (as in the lack of processing for proneurotrophins), can also be an active player in the induction of neuronal death. In addition, the role of neurotrophins on the regulation of neuronal survival and death is also no longer restricted to the developmental phase of apoptosis. Under physiological condition, association of mature neurotrophins with Trk receptors induces survival signals, which results in neuronal survival by the suppression of the apoptotic machinery, and the inhibition of he pro-apoptotic gene transcription. However, in the absence of Trk receptors, or in the absence of the preferred neurotrophin provided by the target cells, other mature neurotrophins will then associate with p75 to initiate a low level of pro-apoptotic signal, which could be sufficient to induce developmental apoptosis. Proneurotrophins signaling may also contribute to developmental apoptosis, but their contributions remained unknown at this stage. During pathological condition, upregulation of p75 and proneurotrophins expression may contribute to the amplification of apoptotic signaling, thereby exacerbating neuronal death observed following traumatic injury. Addition of mature neurotrophins may alleviate cell death temporarily via the Trk-mediated suppression of apoptotic signaling, but the efficacy of neuroprotection is dependent on other parameters of the cell (Figure 6). This emerging view on neurotrophin signaling suggests that neurotrophins are no longer mere passive guardian angels against other noxious stimuli, but neurotrophins themselves, when combined with the right receptor can also function as active instigator of neuronal apoptosis.

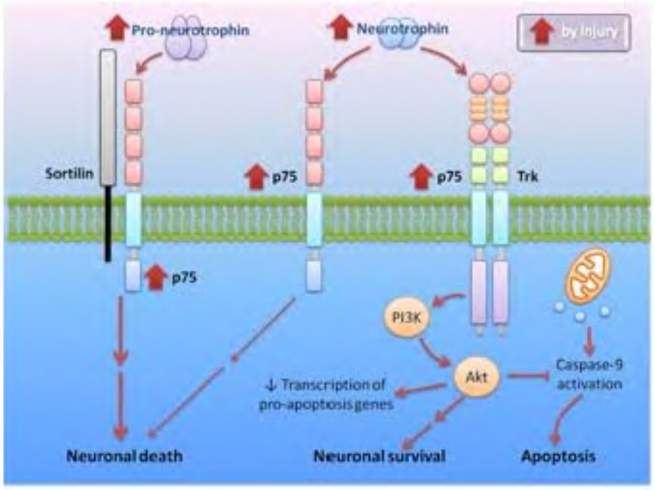

Figure 6. Neurotrophins: instigator of both pro-survival and pro-apoptotic signaling. This emerging view on neurotrophin signaling suggests that neurotrophins can function as active instigator of both pro-survival and pro-apoptotic signaling depending on the relative abundance of Trk and p75, and that of mature neurotrophins and proneurotrophins. In addition, neurotrophins signaling are likely important contributor to the life/death decision of a neuron during both physiological and pathological conditions. Under physiological condition, association of mature neurotrophins with Trk receptors induces survival signals, which results in neuronal survival by the suppression of the apoptotic machinery, and the inhibition of pro-apoptotic gene transcription. However, in the absence of Trk receptors, or in the absence of the preferred neurotrophin provided by the target cells, other mature neurotrophins will then associate with p75 to initiate a low level of pro-apoptotic signal, which could be sufficient for the induction of developmental apoptosis. During pathological condition, upregulation of p75 and proneurotrophins expression may contribute to the amplification of apoptotic signaling, thereby exacerbating neuronal death observed following traumatic injury.

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