

# We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

**4,800**

Open access books available

**122,000**

International authors and editors

**135M**

Downloads

Our authors are among the

**154**

Countries delivered to

**TOP 1%**

most cited scientists

**12.2%**

Contributors from top 500 universities



**WEB OF SCIENCE™**

Selection of our books indexed in the Book Citation Index  
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?  
Contact [book.department@intechopen.com](mailto:book.department@intechopen.com)

Numbers displayed above are based on latest data collected.

For more information visit [www.intechopen.com](http://www.intechopen.com)



# Selectivity of Cell Signaling in the Neuronal Response Based on NGF Mutations and Peptidomimetics in the Treatment of Alzheimers Disease

Kenneth E. Neet<sup>1</sup>, Sidharth Mahapatra<sup>2</sup>, Hrishikesh M. Mehta<sup>3</sup>

<sup>1</sup>*Department of Biochemistry and Molecular Biology, The Chicago Medical School, Rosalind Franklin University of Medicine and Science, North Chicago*

<sup>2</sup>*Department of Pediatrics, School of Medicine, Stanford University, Palo Alto*

<sup>3</sup>*Department of Pediatrics, Division of Hematology/Oncology/Stem Cell Transplantation Children's Memorial Hospital, Northwestern Feinberg School of Medicine, Lurie Comprehensive Cancer Center, Chicago Northwestern University, Chicago USA*

## 1. Introduction

Neurotrophins are maintained at low concentrations by target tissues. They form highly selective interactions with their respective cognate receptors and maintain the viability of neurons in the central and peripheral nervous systems. Overlap in receptor specificities for neurotrophins, in the tissue distribution of the specific receptors, and in the expression of the high affinity receptors enable growth- and survival-enhancing signals to be transduced with great efficiency and specificity over a wide variety of neuronal cells. Early studies suggested that alterations in neurotrophin levels might underlie the pathogenesis of Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD) and other neurodegenerative disorders (Apfel et al., 1991; Emilien et al., 2000; Hefti, 1983) [reviewed in (Lad et al., 2003a)]. Today, a strong link between such a neurodegenerative condition and an imbalance in neurotrophin and/or receptor levels has been supported with nerve growth factor (NGF) and AD. A disruption or reduction in critical neurotrophin levels thus leads to widespread neurodegeneration. Conversely, administration of NGF and/or its related family of neurotrophins can potentially play a role in treatment of AD or other degenerative neurological disorders. NGF and its peptidomimetics have been proposed and tested in animal studies and clinical trials for AD with complex responses observed in some patients. Intracellular signaling from the NGF receptors is complex, giving rise to neuronal responses that include differentiation, survival, and apoptosis. This review will focus on novel approaches to eliciting selectivity of a cellular response, based on alterations in the NGF molecule or in the peptidomimetic, that may lead to more effective treatments of AD with NGF-related therapeutics. A conceptual comparison to selectivity in other growth factor receptor systems, such as epidermal growth factor (EGF) and insulin, will also be made.

## 2. Neurotrophins, their receptors, and neurodegenerative disease

### 2.1 Neurotrophin receptor interactions

Neurotrophins (NTs) are a family of closely related proteins that have diverse functions ranging from neuronal development, differentiation, and survival to regulation of axonal and dendritic outgrowth, activity-dependent synaptic formation and regulation, cell migration, and cellular proliferation (Diamond et al., 1992; Katz et al., 1990; Levi-Montalcini, 1987; Lindsay, 1988; Segal, 2003). The family of neurotrophins includes nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin 3 (NT-3), and neurotrophin 4 (NT-4) (Ibanez, 1994; Lad et al., 2003a). They possess high sequence homology (ca. ~50%) and adopt similar tertiary structures (Fig. 1A) (Bibel & Barde, 2000; Korsching, 1993). The same neurotrophin can affect a variety of neuronal populations and, conversely, the same tissue is capable of receiving stimulatory inputs from multiple neurotrophins. For example, cholinergic basal forebrain (CBF) neurons are attuned to NGF for their maintenance but have been shown to respond to BDNF and NT-3 (Bibel & Barde, 2000; Korsching, 1993; Lad et al., 2003a). The viability of dopaminergic neurons of the substantia nigra has been linked to the actions of BDNF and NT-4 (Hyman et al., 1991; Lad et al., 2003a; Parain et al., 1999). Also, BDNF can act on the entorhinal cortex, the substantia nigra, and the striatum to affect AD, PD, and PD/HD, respectively (Nagahara & Tuszynski, 2011). Each of these neurotrophins binds selectively to a 140-kDa tropomyosin-receptor-kinase, i.e. NGF to TrkA, BDNF and NT-4 to TrkB, and NT-3 to TrkC (Cordon-Cardo et al., 1991; Kaplan et al., 1991; Soppet et al., 1991). Binding to Trk enables the transduction of positive signals, i.e. survival and differentiation through its intracellular tyrosine kinase domain. In contrast, all neurotrophins can bind a 75-kDa common neurotrophin receptor, p75 (or p75<sup>NTR</sup>), involved in the transduction of negative signals, i.e. growth arrest or apoptosis, when expressed exclusively, or positive signals when co-expressed with Trk receptors (Lad et al., 2003a). The central and peripheral nervous systems possess multiple neurotrophin-dependent cells and tissues that demonstrate co-expression of TrkA and p75.

### 2.2 Importance of neurotrophins in neurodegenerative disorders

Numerous studies have now demonstrated an imbalance between neurotrophins and their receptors in AD, appearing even in early cognitive defects (Mufson et al., 2007; 2008; Lad, et al., 2003a). In cholinergic basal forebrain (CBF) neurons in AD, TrkA and p75 are decreased at both the mRNA and proteins levels and NGF mRNA is not changed (Mufson et al., 1995; Salehi et al., 2000; S. A. Scott et al., 1995). NGF protein is down in CBF neurons with accumulation of NGF in the cortex, suggesting that retrograde transport to provide the neurotrophic factor to the basal forebrain is impaired (Schindowski et al., 2008). These changes begin to appear in early, pre-clinical stages of cognitive impairment (Ginsberg et al., 2006; Mufson et al., 1995; Mufson et al., 2000; Mufson et al., 2002; Schulte-Herbruggen et al., 2008). In addition, the pro-apoptotic proNGF is elevated, suggesting destruction of neurons containing p75 (Cuello et al., 2010; Fahnstock et al., 2001). A relationship of neurotrophins and their receptors to A $\beta$  toxicity and/or deposition is also becoming apparent (Calissano et al., 2010a; Peng et al., 2004). Finally, BDNF, and possibly its precursor form, is implicated in neuronal survival imbalance in AD (Garzon & Fahnstock, 2007; Peng et al., 2005), particularly in the entorhinal cortex region of the brain (Nagahara & Tuszynski, 2011). Consequently, various treatment strategies have been suggested to slow or reverse cognitive symptoms, utilizing administration of neurotrophins (Mufson et al., 2008; Nagahara & Tuszynski, 2011; Nilsson et al., 2010; Schulte-Herbruggen et al., 2008).

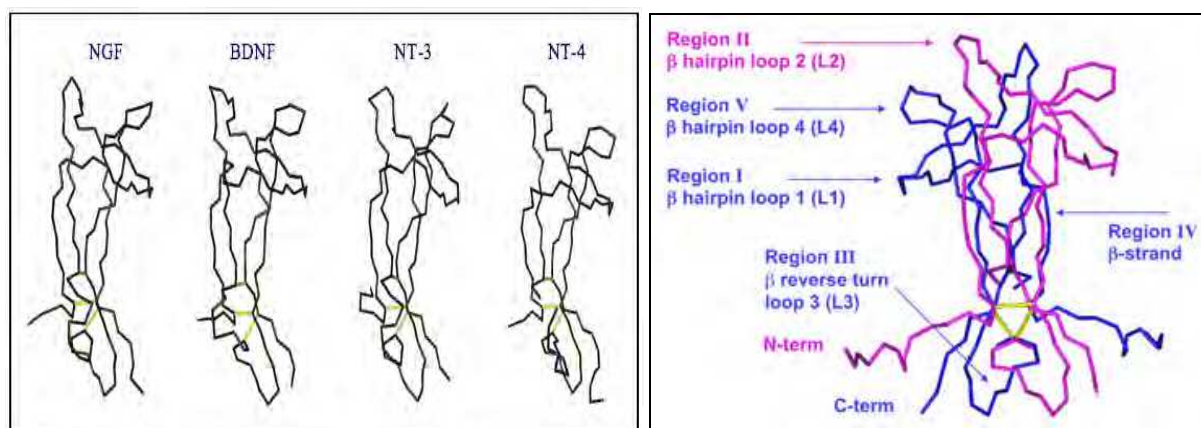


Fig. 1. A (left). Folding of the protomer of the four neurotrophins. B (right). Structure of dimeric NGF showing the loop regions that interact with the receptors. The yellow bonds represent the cystine knot motif. From PDB 1WWW. Note: the variable regions I, II and V defined by sequence homology (Ibanez et al., 1991; Ibanez et al., 1993; Ilag et al., 1994) are equivalent to loops 1, 2, and 4 (L1, L2, L4) defined in the structure (Wiesmann et al., 1999).

### 2.3 Alzheimer's disease – Treatment strategies

The pathogenesis of Alzheimer's disease is related to loss of NGF-mediated survival signals, receptor imbalances at the cell surface, and a shift to proprotein forms capable of inducing apoptosis. Without these positive signals, the progression of intracellular events that ultimately lead to deposition of tangles and plaques occurs uninterrupted. As a result, neurodegeneration in the cholinergic basal forebrain leads to cognitive and memory deficits demonstrable in AD patients. In the current treatment of Alzheimer's disease, the use of cholinesterase therapies to re-establish critical levels of acetylcholine in the inflicted areas are unable to deter the loss of neuronal populations from the CBF and hence to slow the progression of the disease (Bierer et al., 1995; Lad et al., 2003a). Based on these findings, an appropriate way to reduce or prevent neurodegeneration and maintain viability in Alzheimer's disease is to re-establish NGF signaling within the CBF. Additionally, the administered protein must be well-tolerated and stably released over a long period of time to achieve its trophic effects (Apfel, 2002). However, the direct administration of NGF to the CNS via the circulation is precluded by its inability to cross the blood brain barrier (BBB) (Lad et al., 2003a). Furthermore, subcutaneous delivery of NGF elicits acutely painful phenomena due to the presence of TrkA receptors at free nerve endings of pain fibers (Apfel et al., 1998; Lad et al., 2003a). Finally, gastric secretions would rapidly degrade the protein, making oral preparations ineffective.

Other methods have been considered for delivery of growth factors or protein drugs to the brain [for a broad review see references (Alam et al., 2010; Cattaneo et al., 2008)]. Intranasal inhalation has been demonstrated to be a promising mode of non-invasive, facile delivery of biologics to the brain (Benedict et al., 2011). Monocytes can be loaded to secrete NGF and, in proof of principle, shown to migrate across an *in vitro* BBB model consisting of a brain capillary endothelial cell system (Bottger et al., 2010). Encapsulated cell biodelivery is a modified gene therapy approach which encapsulates cells in a immunoprotective semi-permeable hollow fiber synthetic membrane to secrete the protein factor of interest into the desired region of the brain (Fjord-Larsen et al., 2010); NGF and glial derived neurotrophic



factor (GDNF) are currently undergoing clinical trials with this method in Sweden. Transcranial focused ultrasound (FUS) has been shown to transiently-enhance the permeability of the BBB and permit antibodies, and probably other macromolecules into the intrathecal space (Jordão et al., 2010). Early interest in conjugation of NGF to transferrin or transferrin antibodies as a means of transport across the BBB (Friden et al., 1993; Liao et al., 2001) seems to have waned and shifted toward using this means of cellular uptake for anti-cancer or diabetic drugs.

Gene therapy presents the most-advanced, current means of delivering NGF in a cerebral region-specific manner (Lad et al., 2003a; Nagahara & Tuszynski, 2011). The two main forms of gene therapy that have been used in the delivery of NGF include an *in vivo* approach and an *ex vivo* approach. The former requires the injection of a viral vector containing the gene of interest directly into the region of interest. The latter can be done by transfecting cells with the gene of interest *in vitro* and subsequently transplanting the transfected cells into the region of interest. These techniques overcome the problems initially encountered with NGF delivery by circumventing the BBB, but require surgical manipulation. Additionally, by using viral vectors as the mode of NGF gene delivery into a specific target within the brain, these methods provide a stable, renewable system of NGF synthesis, which can help re-establish receptor levels and counter apoptosis driven by proNGF. However, these forms of therapy provide the greatest benefit during early stages of AD before cholinergic deficits set in.

Initial positive results in rat and primate animal models supported studies of NGF in clinical trials of AD (Bishop et al., 2008; Eriksdotter Jonhagen et al., 1998; Tuszynski et al., 2005). The early clinical trials met with a lack of success due to lack of efficacy, toxicity or both. Common problems in these clinical studies, as well as corresponding animal studies, include undesirable side effects (e.g. hyperinnervation, sprouting, sympathetic stimulation, cachexia, hyperalgesia) (Apfel, 2002; Thoenen & Sendtner, 2002; Winkler et al., 1997) [reviewed in (Nagahara & Tuszynski, 2011)]. Intriguingly, a phase I trial of *ex vivo* NGF gene delivery in mild Alzheimer's disease has shown some promise (Tuszynski et al., 2005). Briefly, eight individuals with early-stage, probable AD were administered primary autologous fibroblasts, which had been genetically modified to synthesize and secrete NGF, via stereotaxic injections to the CBF. This approach was shown to survive grafting into the brain and provided stable NGF secretion for up to 18 months in animal studies with improvement of cholinergic function and memory (Emerich et al., 1994; Tuszynski et al., 2002; Tuszynski et al., 2005). At 22 months of follow up, no adverse side effects were reported in the clinical trial. Mini-Mental State Exam scores demonstrated a reduction in the rate of cognitive decline and 18-fluorodeoxyglucose PET scans demonstrated significantly greater cortical glucose uptake (Tuszynski et al., 2005). Although these observations were encouraging, results must be considered with caution because of the small data set used and the absence of placebo groups. Additionally, NGF demonstrated a large trophic response in the CBF of some of these patients. While this response is necessary for target innervation during development of the nervous system, an adult brain where the axonal infrastructure is well-established might suffer from new and incomplete neuritogenesis in an area receiving dense axonal support. Hence, the generation of a more refined therapeutic molecule is desirable. Since this *ex vivo* trial, the focus of the scientific community has largely shifted to *in vivo* delivery with adeno-associated virus (AAV) (Table 1 and (Nagahara & Tuszynski, 2011; Nilsson et al., 2010).

Interestingly, monoclonal antibodies to NGF were so effective that a Phase III clinical trial for treatment of osteoarthritis of the knee was discontinued because of joint failure due to excessive wear and tear in the absence of the pain sensation (Lane et al., 2010; Wood, 2010).

Disorder	Phase	#	Delivery*	Status	Sponsor
AD	I	8	ex vivo NGF	end 2005 some cognitive improvement (see text)	UCSD
AD	II	50	ICV AAV-NGF Cere-110	Ongoing Recruiting	Ceregene
AD	Ib	6	Encapsulated cell biodelivery NGF	Ongoing	NsGene (Sweden)
HIV associated sensory neuropathy	II	270	SQ NGF	end 2005	NIAID
Osteo-arthritis of knee	III	(a)697 (b)848	IV mAb to NGF	Completed Discontinued (see text)	Pfizer

Table 1. Selected clinical trials of NGF for AD or pain. From clinicaltrials.gov, April 2011.

\*Abbreviations: #, number of patients; ICV, intracerebroventricular; AAV, adeno-associated virus; SQ, subcutaneous; IV, intravenous.

### 3. Signal transduction by neurotrophins

#### 3.1 TrkA signaling

Neurotrophin-mediated signal transduction via the Trk receptors leads to an activation of downstream signaling pathways for survival and differentiation (Bibel & Barde, 2000; Ibanez, 1994; Kaplan & Miller, 2000; Levi-Montalcini, 1987). NGF binding induces TrkA receptor dimerization, transautophosphorylation, and tyrosine kinase ICD activation (Bibel & Barde, 2000; Cunningham & Greene, 1998; Cunningham et al., 1997; Friedman & Greene, 1999). The three major signaling pathways are the phosphatidylinositol-3-kinase (PI3 kinase)/Akt pathway, the Ras/mitogen-activated protein kinase (MAPK) pathway, and the phospholipase C gamma (PLC $\gamma$ ) pathway (Bibel & Barde, 2000; Kaplan & Miller, 2000; Lad et al., 2003a) (See Fig. 2). The PI3 kinase pathway through Akt mediates survival via the suppression of apoptotic proteins and accounts for over 80% of neurotrophin-mediated survival in neurons (Bartlett et al., 1997; Crowder & Freeman, 1998; Kaplan & Miller, 2000). The MAPK pathway, has been implicated in both survival and differentiation, although it has also been implicated in such diverse functions as synaptic plasticity and long-term potentiation (Kaplan & Miller, 2000; Lad et al., 2003a). Depending on the upstream activators and downstream effectors of MAPK, survival, differentiation or both signals can be transduced. For the specific induction of differentiation, a sustained MAPK activation is necessary. The third signal transduction pathway is the PLC $\gamma$  pathway, involved primarily in Ca<sup>2+</sup> regulation of calcium regulated enzymes, neurotrophin secretion, and synaptic

plasticity (Bibel & Barde, 2000; Canossa et al., 1997). These conclusions about distinct pathways have been reached by mutagenesis of the Tyr residues of the Trk-ICD and demonstrate that these signaling pathways can be individually dissected by manipulation of the intracellular initiation of each pathway. TrkB and TrkC have similar signaling pathways but with some individual differences because of the different neuronal environment of these receptors. The division of TrkA signaling into three discrete, but overlapping signal pathways, suggests that pharmaceutical approaches for design of signal selective neurotrophic therapeutics (see Section 5.2) may be able to discriminate among selected paths toward cellular outcomes.

<u>TrkA</u>	<u>Outcome</u>
p-Y490 → Ras → PI3K → Akt →	survival <sup>1</sup>
p-Y490 → Shc → MAPK → pMEK → Bcl2 →	survival <sup>2</sup>
p-Y490 → FRS2 → MAPK → p-Elk1, pCREB →	differentiation <sup>2</sup>
p-Y785* → PLC $\gamma$ → PI → DAG, IP3 → Ca <sup>++</sup> →	synaptic plasticity <sup>3</sup>

Fig. 2. Signaling pathways, PI3K, MAPK, PLC $\gamma$ , from TrkA phospho-tyrosines to cell outcomes. \*Phosphorylation of Y785 is dependent on phosphorylation of Y670, Y674, Y675. <sup>1</sup>Bartlett et al., 1997; Crowder & Freeman, 1998; Rodriguez-Viciano et al., 1994; Holgado-Madruga et al., 1997. <sup>2</sup>Kao et al., 2001; Loeb et al., 1994; Gomez & Cohen, 1991; Qian et al., 1998; <sup>3</sup>Loeb et al., 1994; Canossa et al., 1997; Cunningham et al., 1997.

Phosphorylation of a specific set of tyrosines ensues within the activation loop of the kinase domain. These include two tyrosines located in the juxtamembrane domain (Y490) and C-terminus (Y785) of Trk that serve as docking sites for adaptor proteins and three tyrosines (Y670, Y674, and Y675) within the activation loop that interact with nearby basic residues to stabilize a fully functional, active catalytic core and potentiate transduction of downstream signals (Bibel & Barde, 2000; Cunningham & Greene, 1998; Friedman & Greene, 1999; Grewal et al., 1999; Huang & Reichardt, 2003; Kaplan & Miller, 2000; Lad et al., 2003a).

### 3.2 p75 signaling

Classically, p75 has been thought to be involved in the induction of negative signals like apoptosis and growth arrest in oligodendrocytes, Schwann cells, sympathetic neurons, motor neurons, and sensory neurons (Bamji et al., 1998; Barrett & Bartlett, 1994; Casaccia-Bonofil et al., 1996; Kaplan & Miller, 2000) in a Trk-independent fashion, (Davey & Davies, 1998; Kaplan & Miller, 2000; Soilu-Hanninen et al., 1999). The co-presence of Trk on a neuron silences pro-apoptotic pathways by activation of the pro-survival transcription factor, NF- $\kappa$ B, which subsequently activates Akt (Bibel & Barde, 2000; Kaplan & Miller, 2000; Khursigara et al., 1999). Sympathetic neurons and basal forebrain neurons that were p75-deficient in p75<sup>-/-</sup> mice underwent robust axonal sprouting, hypertrophy, or target innervation with naturally-occurring developmental apoptosis being significantly delayed (Kaplan & Miller, 2000; Walsh et al., 1999; Yeo et al., 1997; Bamji et al., 1998). Without intrinsic catalytic activity, the p75 ICD appears to rely on docking of adaptor

proteins. The complexities of the p75 signaling are outlined by representative examples shown in Fig. 3.

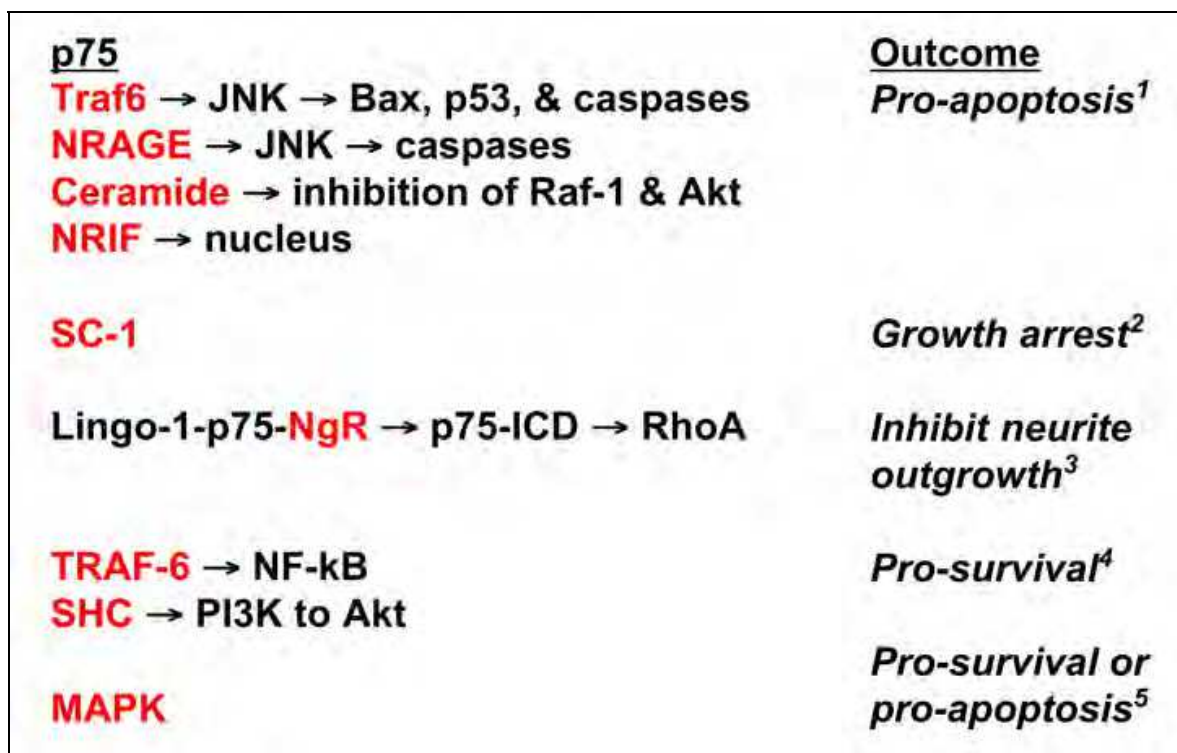


Fig. 3. Some signaling pathways from p75 to cell outcomes. Adaptor proteins interact with p75 to generate most cellular effects of a diverse nature. Uncommon abbreviations: NRAGE, Neurotrophin receptor-interacting MAGE homolog; NRIF, neurotrophin receptor interacting factor, Zn finger transcriptional factor; SC-1, Schwann cell factor 1; NgR, Nogo receptor. RhoA, Ras homolog A. (For reviews see Kaplan & Miller, 2000; Bibbel & Barde, 2000; Schecterson & Bothwell, 2010). <sup>1</sup>Aloyz et al., 1998; Salehi, et al., 2002; Muller, et al., 1998; de Chaves et al., 1997; Casademunt, et al., 1999; <sup>2</sup>Chittka & Chao, 1999; <sup>3</sup>Domeniconi et al., 2005; <sup>4</sup>Khursigara et al., 1999; <sup>5</sup>Susen, et al., 1999; Lad & Neet, 2003.

The self-interaction of p75 also influences cellular outcome. Biophysical and biochemical methods have suggested that homomeric p75 plays a pro-apoptotic role while formation of oligomers abolishes this effect and may even serve an anti-apoptotic role (Kaplan & Miller, 2000; Lad et al., 2003a; Wang et al., 2000). Recently, evidence has been provided to support a model in which the formation of an inter-protomer disulfide bond in p75 allows a scissoring (or snail-tong) action upon NGF binding that promotes binding of intracellular adaptor proteins (Simi & Ibanez, 2010; Vilar et al., 2009).

As mentioned above (Section 2.2), proneurotrophins may play significant role in AD, due to their ability to stimulate p75 in the absence of positive Trk signals thereby promoting apoptosis and death of neurons or glia (Cuello et al., 2010; Fahnstock et al., 2001; Teng, et al. 2010). These effects are thought to be mediated by the same pathways (Fig. 3) as presented for mature NGF (or other neurotrophins). In addition, soluble oligomers of  $\beta$ -amyloid may also interact with p75 to cause apoptotic signals in neuronal tissue of AD or pre-AD patients (Dechant & Barde, 2002; Diarra et al. 2009; Coulson, et al., 2009; Calissano, et al. 2010a; 2010b; Peng, et al., 2004; Susen & Blochl, 2005).



These studies demonstrate that the interaction of neurotrophins or proneurotrophins with p75 can lead to diverse effects that are determined by the developmental state of the organism, the particular cell type within which it is expressed, the additional expression of co-receptors, receptor oligomerization states, and the binding of intracellular adaptors or effectors. The complexity of the p75 pathways suggests that developing signal selective muteins for this receptor would be much more difficult than those outlined for the Trk receptor (Section 5.2) with its more clearly initiated signaling. Since the response of cells is, basically, apoptosis or not, then there would be less advantage at this point in time in generating such reagents for p75.

## **4. Molecular analysis of neurotrophin structure and rationale of mutations**

### **4.1 Conserved and variable regions defined from sequence and 3-D crystallographic structures**

Functional data gathered from systematic mutagenesis studies and through the construction of inter-neurotrophin chimeras coupled with structural data from crystallographic studies have implicated several spatially distinct patches of residues in bestowing receptor specificity to each neurotrophin (Casademunt et al., 1999; de Chaves et al., 1997; Grimes et al., 1997; Ilag et al., 1994; Lad et al., 2003a; Ninkina et al., 1997; Rabizadeh et al., 1993; J. Scott et al., 1983; Walsh et al., 1999; Wiesmann et al., 1999; Yeo et al., 1997). Sequence alignments of NGF, BDNF, and NT-3 (Ibanez et al., 1992; Ibanez et al., 1991; Ibanez et al., 1993; Ilag et al., 1994; Kullander & Ebendal, 1994; Kullander et al., 1997) demonstrate that each neurotrophin possesses seven variable regions that include the same residue positions. These are the N-terminus (residues 1-9), variable region I ( $\beta$ -hairpin loop 1, residues 23-35), variable region II ( $\beta$ -hairpin loop 2, residues 40-49), variable region III ( $\beta$ -reverse turn loop 3, residues 59-66), variable region IV ( $\beta$ -strand, residues 79-88), variable region V ( $\beta$ -hairpin loop 4, residues 95-99), and the C-terminus (residues 111-118) (Fig. 1B). This homology in clustering translates structurally to variable regions occupying complementary sites on each neurotrophin molecule, which are surface-exposed, enabling these regions to function as sites of receptor contacts (Fig. 4). These regions also define amino acid residues to target for redesign of molecules to alter signaling (see Section 5.2).

#### **4.1.1 The specificity patch**

Chimeric molecules retaining the amino terminus, carboxyl terminus, and variable region II of NGF possessed the ability to differentiate sympathetic neurons and activate the TrkA receptor (Ibanez et al., 1991). However, a chimeric molecule that additionally replaced residues 3-9 of the N-terminus reduced TrkA binding, activation, and biological activity to <1% (Ibanez et al., 1993). Other substitution and deletion studies confirmed the importance of the N-terminus in specificity (Kullander et al., 1997; Woo et al., 1995; Kahle et al., 1992; Shih et al., 1994). Data from the crystal structure of human recombinant NGF complexed with the TrkA-d5 domains showed that the amino terminal residues 6-9, which were not well-defined in the original NGF crystal structure, adopted a helical conformation upon complex formation with their side chains almost completely buried in the interface (McDonald et al., 1991; Wiesmann et al., 1999) (Fig. 4). Within the same region, H4, I6, and E11 participated in strong interactions with residues in the ABED TrkA  $\beta$ -sheet. The authors labeled this region the specificity patch suggesting that the most important Trk receptor-

binding determinants lie within the amino terminus (Wiesmann et al., 1999). Residues spanning the specificity patch, i.e. H4, P5, I6, F7, E11, and F12, are clearly important for neurotrophin-Trk interactions and activation.

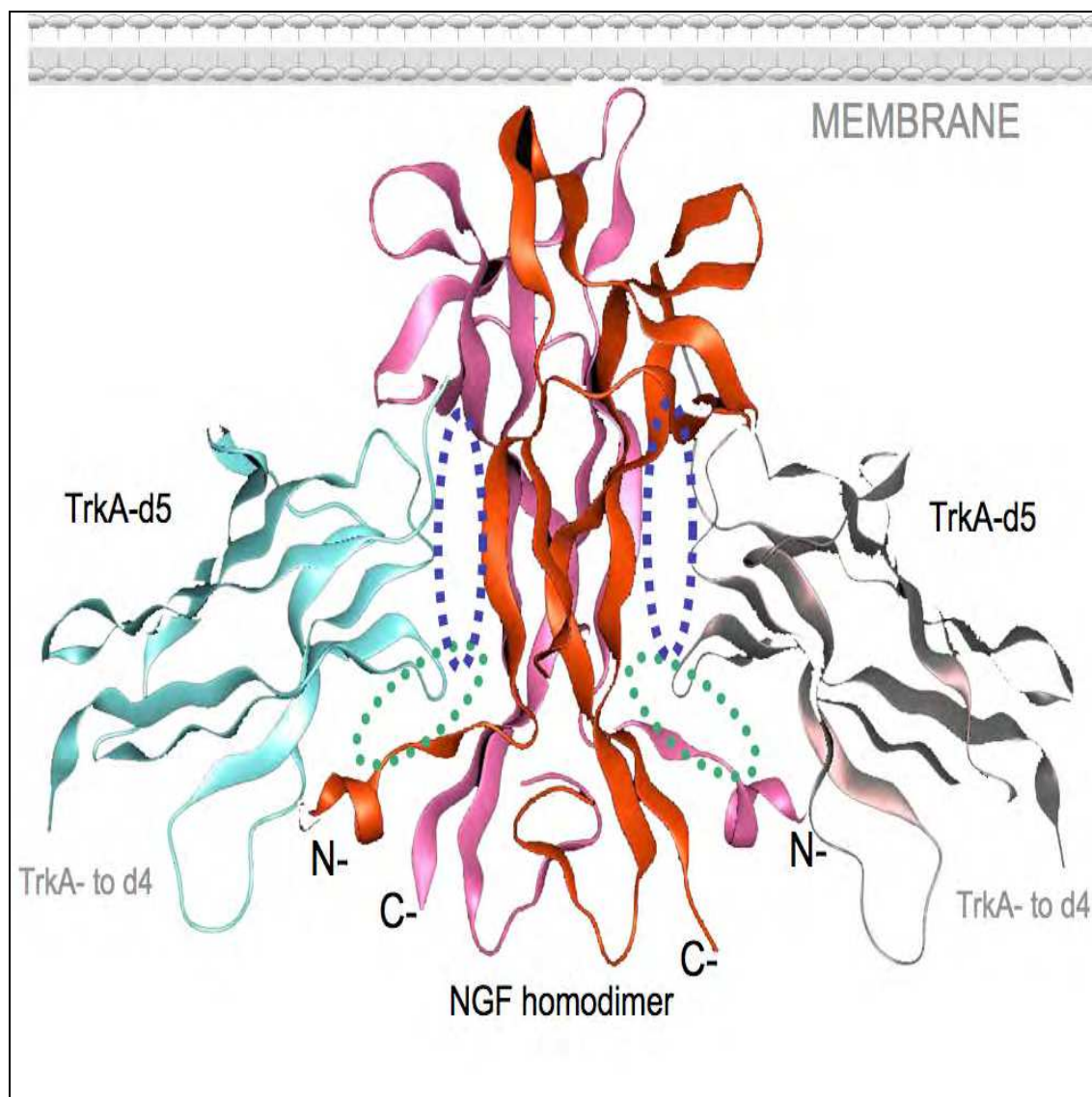


Fig. 4. Schematic representation of dimeric nerve growth factor complexed with two TrkA-d5 domains. NGF and TrkA (PDB 1WWW) form highly specific interactions at the specificity (green dotted oval) and the conserved patch (blue dotted oval). Additional contacts are provided by variable loops I, II, and V (loop 4) that form a continuous surface of binding. Each TrkA-d5 binding site is derived from contributions from loops in both protomers in the NGF dimer. NGF protomers, pink and orange. 1st TrkA-d5; cyan; 2nd TrkA-d5; grey.

#### 4.1.2 The variable regions

The variable regions (Fig. 1B), in general, correspond to loops and surface residues in NGF and the other neurotrophins. Generation of NGF-BDNF chimeras identified variable region II as holding important receptor binding determinants (Ibanez et al., 1991; Ibanez et al., 1993; Ilag et al., 1994; Kullander & Ebendal, 1994; Kullander et al., 1997). The variable region was

further dissected into IIa (residues 40-44) and IIb (residues 45-49) (Ibanez et al., 1993). Region IIb was shown to play a more pivotal role than IIa when replacement of only the latter led to a dramatic reduction in receptor activation and biological activity (Ibanez et al., 1993). The variable region II, with help from variable region V (loop 4), was also shown to confer specificity between NT-3 and NGF (Ilag et al., 1994; Kullander & Ebendal, 1994; Kullander et al., 1997). The C-terminal linker region of TrkA could serve as a possible binding partner of this region (Wiesmann et al., 1999) (Fig. 4). Therefore, residues within variable regions IIa and IIb are considered important for neurotrophin activity, i.e. E41, N43, I44, N45, V48, and F49.

#### 4.1.3 The conserved patch

Crystallographic data of dimeric NGF complexed with TrkA-d5 demonstrated that 5 of 10 residues in variable region IV (Y79, T81, T83, H84, and F86) actually form part of the conserved patch that interacts intimately with the TrkA-d5 domains (Wiesmann & de Vos, 2001) (Fig. 4). This 'conserved' patch was so named because 14 of 23 NGF residues are occupied by homologous amino acids in BDNF and NT-3, while 8 of 15 TrkA residues are conserved in TrkB and TrkC (Wiesmann et al., 1999). The conserved patch forms a continuous binding surface for TrkA-d5 and retains high sequence homology across species (Wiesmann et al., 1999) (Fig. 4). Variable region IV, a  $\beta$ -strand, was specifically shown to retain ~90% homology across species. Within this region, T81 and H84 of NGF are replaced by non-homologous amino acids in the other neurotrophins, suggesting that variable region IV does form specific contacts with TrkA (Ibanez et al., 1993) (Fig. 4). Alanine point mutagenesis of H84 and H75 indicated that these residues participated in receptor binding and activation based on a decline in neurite outgrowth in PC12 cells and the ability to compete for binding sites with radioactively-labeled wild-type NGF (Woo & Neet, 1996). Similarly, R100 and R103 showed trends akin to H84 with respect to sequence alignment analysis, mutagenesis studies, and crystallographic data. R100 and R103 are not only strictly conserved across species but also within the family of neurotrophins. Crystallographic data suggested that R103 was the most important binding determinant in the conserved patch (Wiesmann et al., 1999) (Fig. 4). [Also see Section 5.1.] Residues V22, I31, F53, and F54 within the conserved patch are also highly conserved across species and between neurotrophin family members. Alanine substitution of these four residues significantly affect TrkA binding by competition assays, intracellular receptor phosphorylation studies, and biological activity in PC12 cells (Ibanez et al., 1990; Ibanez et al., 1992; Ibanez et al., 1993; Guo et al., 1996). Hence, the conserved patch represents a binding epitope wherein residues help form critical interactions between neurotrophins and Trk receptors.

#### 4.1.4 Summary of important regions and residues of the neurotrophin family

Sixteen residues of NGF may be identified as most important for function (Table 2) and can be designated as the specificity patch (H4, P5, I6, F7, E11, and F12), the variable loops (I31, E41, N43, I44, N45, V48, and F49), or the conserved patch (I31, F54, H84, and R103). Additional residues, V22, F53, H75, and R100, may not occupy the conserved patch, *per se*, but are as highly conserved as residues residing within or near the patch. Similar considerations have been made for BDNF and NT-3 (Ibanez et al., 1992; Ibanez et al., 1991; Ibanez et al., 1993; Ilag et al., 1994; Kullander & Ebendal, 1994; Kullander et al., 1997; Suter et al., 1992; Urfer et al., 1997). These regions of the NGF molecule were targeted to obtain the signal selective muteins discussed in Section 5.2.

<b>Specificity Patch</b> – induced N-terminal $\alpha$ -helix; mainly hydrophobic and some H-bond interactions to $\beta$ -strands ABED of TrkA-d5.	<b>NGF:</b> H4, P5, I6, F7, E11, F12 <b>BDNF:</b> S4, D5, P6, A7, E11, L12
<b>Conserved Patch</b> – part of hydrophobic core of NGF; interacts with loop EF and C-terminus of TrkA-d5	<b>NGF:</b> V22, I31*, F53, F54, H75, H84*, R103 <b>BDNF:</b> V22, M31, F53, Y54, H75, Q84, R103
Specificity for NGF vs NT-3: <b>Loop II-</b>  <b>N-terminus-</b>	<b>NGF:</b> E41, N43, I44, N45, V48, F49 <b>BDNF:</b> K41, P43, V44, S45 - - <b>NT-3:</b> E41, K43, T44, G45, P48, V49
	<b>NGF:</b> P5, F7 <b>NT-3:</b> H5, S7

Table 2. Important regions of Trk receptor interaction in human NGF and BDNF. Based on crystal structure and mutagenesis analysis of NGF (Mahapatra, 2008; Ibanez et al., 1992; Ibanez et al., 1991; Ibanez et al., 1993; Ilag et al., 1994; Kullander & Ebendal, 1994; Kullander et al., 1997; Wiesmann & de Vos, 2001) and the corresponding regions in BDNF and NT-3. Note: I31 and H84 (with \*) also participate in Loop 1 and Loop 4, respectively, as well as the conserved patch.

## 5. Development of neurotrophin muteins that have potentially improved therapeutic properties

### 5.1 Receptor selective muteins

Two interesting, complementary, and useful muteins have helped elucidate the role of the two receptors in neurotrophin action. The first discovery was that mutations in the 32-35, loop I area would greatly reduce or eliminate binding to the p75 receptor and leave TrkA binding unimpaired (Ibanez et al 1992; 1993). The triNGF (or KKE) mutein contained the three mutations, K32A/K34A/E35A, and was used to demonstrate the functional interaction between p75 and TrkA in the developing embryo (Ryden, et al., 1997). The mutations in the N-terminus of NGF were shown to be important for TrkA binding in several laboratories (Section 4.1.1). One notable mutein was the  $\Delta 9/13$  mutein that bound p75 but not TrkA (Woo et al., 1995), and helped distinguish between differentiation, cell cycle regulation, and apoptosis in PC12 cells (Hughes et al., 2001). Utilization of this  $\Delta 9/13$  mutein also later indicated a unique role for the 'high affinity' binding complex of p75 and TrkA in PC12 cells (Lad, et al., 2003b). Indeed, the combination of these two receptor muteins, KKE/ $\Delta 9/13$  has been studied, briefly, and shown to have no observable activity, as expected (Mahapatra, et al., 2009). Both muteins, KKE and  $\Delta 9/13$ , have been used in a rat glaucoma model to interpret the roles of TrkA (neuroprotective) and p75 (neurotoxic) in retinal degeneration (Bai, et al., 2010). KKE, but not wild type NGF, has recently been reported to partially improve learning in a transgenic APP mouse model (overexpression of Swedish and Indiana mutations) with a reduction of soluble cortical  $\beta$ -amyloid levels, but with no improvement in long-term memory (Aboukassim, et al., 2011). These two receptor selective muteins have potential as an improved therapeutic for disorders in which either TrkA or p75 can be expected, with improved targeting in that case.

In the opposite direction, a neurotrophin mutein has been made that is less restrictive, rather than more restrictive, in binding to Trk receptors. The pan-neurotrophin, PNT, incorporated



elements of variable and conserved regions to produce a mutein that binds to all three receptors, TrkA, TrkB, TrkC (Ibanez, et al., 1993; Ilag, et al., 1995). To the authors' knowledge, a pan-neurotrophin has not been made that was promiscuous for Trk receptors but restricted so as to not bind p75. Such pan-neurotrophins might have utility in the treatment of some neurodegenerative disorders in which multiple receptors indiscriminately needed extra support (Funakoshi, et al., 1998).

The observation of a mutation in NGF that affects the pain response suggests that changes in signaling from mutations in the ligand have already occurred in nature. A nociceptive response is mediated by NGF (Pezet & McMahon, 2006), partly through the TrkA receptor and partly through p75 (Einarsdottir et al., 2004; Indo, 2002; Nicol & Vasko, 2007). In a large family from northern Sweden, an R100W mutation was uncovered within the coding region of NGF. Individuals from this family suffered from insensitivity to both temperature and deep pain perception, while retaining normal mental abilities, a disorder termed hereditary sensory and autonomic neuropathy type V (HSANV) (Einarsdottir et al., 2004). The R100W mutation selectively disrupts binding of NGF to the p75 receptor, while the affinity for TrkA receptor is less affected (Covaceuszach et al., 2010). Also, the consequence of the R100W mutation on p75 receptor binding is greater for mature NGF rather than proNGF. Signaling pathways reflect this selectivity (Capsoni et al., 2011), suggesting that these findings might lead to a 'painless' NGF protein with therapeutic potential. This NGF mutation is an example of a naturally-occurring, receptor-selective change in the neurotrophin.

## 5.2 Development of signal selective muteins

To alleviate the potentially undesirable effects of inappropriate over extension of neurites during clinical trials (Section 2.3), designer neurotrophins have been developed (Mahapatra et al., 2009). Two survival-specific (or signal-selective, SS) recombinant NGF muteins with decreased differentiation potential were designed from a set of rationally selected mutations within the specificity, conserved, and variable regions of NGF that may play a discriminatory role in signal transduction. One of these muteins is the F7A/H84A/R103A NGF triple mutein, also called SS-1 or 7-84-103 for short. The other signal-selective hextuple mutein is the K32A/K34A/E35A/F7A/H84A/R103A NGF mutein, or called SS-2 or KKE/7-84-103 (Mahapatra et al., 2009). The main difference between the two SS muteins is that the KKE addition to the F7A/H84A/R103A mutation simply makes the hextuple mutein, SS2, incapable of binding p75 (see Section 5.1).

Survival in several cell lines and differentiation in PC12 cells were studied with these recombinant NGF triple and hextuple muteins and compared to wild type NGF. Each of these two muteins induced slightly lower levels of survival in MG139 and PC12 cells with greatly reduced neuritogenesis in PC12 cells. Neuritogenesis data indicated that residues Phe7, His84, and Arg103 played a critical role in biological activity, in agreement with earlier data in the literature (see Section 4). The observed neuritogenic potential of each recombinant triple mutein suggested that His84 (conserved patch) played a discriminatory role in neurite outgrowth. When His84 was mutated to alanine, to make 7-84-103 and KKE/7-84-103, the maximum neuritic response was almost 40% lower than when His84 was left unaltered. With 7-84-103 and KKE/7-84-103, a synergistic effect also occurred when combining mutations to these three residues. These data suggested that mutations within the specificity and conserved patches were more than additive and led to a reduction of neuritogenesis. In 7-84-103 and KKE/7-84-103, the two point mutations were made in close

proximity within the same spatial region, *i.e.* His84 and Arg103, within the conserved patch. Combining mutations in close proximity may have a cumulative effect on ligand-receptor interactions and ultimately reduce the efficiency of some aspects of intracellular signaling. When independent single mutations were incorporated in close proximity within critical regions at the ligand-receptor interface, survival and differentiation were both affected, with a more pronounced effect on neuritogenesis. The greater decrease in differentiation suggests the possibility of multiple binding epitopes for the mediation of survival, while suggesting more limited patches needed for the induction of differentiation. Comparison with binding to immobilized TrkA by surface plasmon resonance suggested that differentiation more closely follows affinity than does survival. Furthermore, similarity in measurements between the recombinant NGF triple mutein and its KKE hexuple counterpart suggested that p75 binding was inconsequential to the induction of survival or differentiation in these cell lines. Studies with ectopically expressed receptors in fibroblast cell lines, MG139 (TrkA+, p75-) and PCNA (TrkA-, p75+), also supported the interpretation that the distinction in signaling properties was due to signaling from the TrkA tyrosine kinase receptor.

The 7-84-103 mutein supported better activation of the PI 3-kinase/Akt survival pathway than the Ras/MAPK pathway, from which differentiation results. These differences in signaling pathways (Akt vs MAPK, see Section 3) indicate that qualitative differences result from a different mode of binding to the receptor, or re-orientation of the receptor upon binding (see Section 6), and are not simply due to an affinity change that affects all signaling pathways equally. In other words, the level of TrkA phosphorylation with 7-84-103 is more efficiently transferred to MAPK and Akt activation for survival than it is for differentiation.

The discrimination ratio, or ratio of relative (to wtNGF) EC50 values, was 30-fold based on the relative EC50 values of 100 and 3.3 for differentiation and survival, respectively (Mahapatra et al., 2009). A 30-fold difference in effective EC50 values, in the pathophysiological context of an early cognitively diseased brain, might be sufficient to greatly reduce unwanted neuritic response while still maintaining neuronal viability. Whether the design of these two signal-selective muteins is optimal at this stage, or whether further improvement in discrimination between survival and differentiation pathways is possible, needs to be examined.

### **5.3 How can a transmembrane receptor be stimulated from the outside of a cell and initiate different kinds of intracellular signals?**

The intricacies of intracellular signaling from tyrosine kinase ICDs are just now being elucidated and are more complicated than might be expected from the simple requirement of a receptor dimer to transautophosphorylate tyrosine residues on its ICD domain. Activation subsequent to ligand binding to the ECD typically requires phosphorylation of a tyrosine in a loop in the active site in order to auto-activate the ICD kinase by moving the loop out of its inhibitory position in the active site (Bae & Schlessinger, 2010; Lemmon & Schlessinger, 2010). Then the kinase continues to phosphorylate tyrosine residues in the C-terminal tail of the adjacent ICD of the receptor tyrosine kinase that provide a docking site for SH2 domain adaptor proteins or enzymes (Bae & Schlessinger, 2010; Jura et al., 2011; Lemmon & Schlessinger, 2010). Three distinct molecular mechanisms have been detected: (i) The activation loop interacts directly with the active site of the kinase and blocks access to

substrates. (ii) The juxtamembrane region interacts with elements within the active site of the kinase to stabilize an inactive conformation. (iii) The C-terminal tail interacts with the active site of the tyrosine kinase domain to stabilize an inactive conformation. In each case, phosphorylation of the appropriate tyrosine relieves the kinase of its inactivation.

Evidence from crystallographic studies of the EGF receptor ICD has suggested an allosteric mechanism of initiating the signaling cascade (X. Zhang et al., 2006). Two distinct EGFR ICD interactions were described: one being a symmetric interaction and the other an asymmetric interaction that is capable of explaining how the EGF signal is transduced across the cell membrane (X. Zhang et al., 2006). Direct contacts occur between the C-lobe of one ICD, acting as an activator, and the N-lobe of another ICD, acting as a receiver. The activator kinase ICD destabilizes autoinhibitory interactions that involve the activation loop of the receiver ICD. Interactions between the docking proteins, Gab1 and Shc, and the EGFR-ICD generate kinetic discrimination between the unliganded and EGF-liganded states (Fan et al., 2004). The formation of the signal transduction 'signalosome' could well depend upon the intricate relationship of the ICDs with docking proteins and, thus, be susceptible to the manner in which the ligand (neurotrophin mutein or peptidomimetic) brought together two Trk ECDs and their connected ICDs with docking partners. In such a scenario, a larger protein ligand, such as EGF or NGF, would seem to be more capable of having multiple ways to force intracellular interactions in the signalosome than a much smaller peptidomimetic with limited 'hot spots' for interaction.

Thus, to explain the NGF mutein data discussed in Section 5.2 (Mahapatra et al., 2009), the wild-type NGF might orient the two ICDs in a signalosome that generates a "normal" ratio of MAPK to Akt activation. In the survival-selective muteins, 7-84-103 and KKE/7-84-103, the orientation of the ICDs or juxtamembrane regions would have been altered such that the output ratio has been shifted in favor of Akt over MAPK signaling and, hence, survival over neurogenesis.

## 6. Neurotrophin peptidomimetics and cell signaling

The use of small molecules is often preferable over the use large proteins as pharmacological agents due to their stability, lower manufacturing costs, ability to pass tissue barriers and good pharmacokinetic profiles. The small molecules can be rationally designed to mimic a region of the ligand that is involved in binding to the receptor or involved in activation of the receptor, thus the term 'peptidomimetics'. Binding of a peptidomimetic to the target receptor may not necessarily result in activation and thus these mimetics may act as antagonists or agonists, depending on their ability to induce a functional response. An inability to induce a functional response would mean that a small molecule with good affinity would be a good inhibitor/antagonist. Two terms that are useful in the design of mimetics are pharmacophore and 'hot spots'. The term 'pharmacophore' relates to the steric and electronic interactions of the mimetic that are necessary to bind to the target receptor, either as an agonist or as an antagonist. The term 'hot spot' refers to the regions on the target protein that specifically interact with a ligand to induce a biological response. Hence, the rational design of mimetics utilizes the information from both the pharmacophore and the hot spots to create libraries of compounds that have subtle structural differences, but significant functional differences (Peleshok & Saragovi, 2006).

### 6.1 Early neurotrophomimetics

Early studies with cyclized monomeric peptides representing the four loop regions (see Fig. 1B) of NGF (LeSauter et al., 1995) produced antagonists that competed with NGF for binding and inhibited its activity. The most potent antagonist was derived using amino acids from loop 4 (amino acids 92-97); the peptides designed around loop 2 (amino acids 43-48) or loop 1 (amino acids 30-35) were less effective. As expected, potency was also effected by length and amino acid substitution, with linear peptides showing no activity. Formation of the  $\beta$ -turn is not simply defined by the amino acid sequence and the activity requires mimicking of the 3-dimensional conformation (LeSauter et al., 1995). Interestingly, in cells expressing both TrkA and p75, the addition of an anti-p75 monoclonal antibody (MC192) that synergizes with NGF caused the antagonistic loop 4 mimetics to behave as agonists with phosphorylation of TrkA and induction of neurites (Maliartchouk et al., 2000). Peptides that formed type I or type  $\gamma$ L- $\alpha$ R turns showed greater activity in presence of MC192, suggesting that binding between TrkA and the loop 4 region may require an 'induced fit' mechanism (Beglova et al., 2000).

### 6.2 Small molecule Trk agonists and antagonists

Other studies, however, reported that cyclized monomeric loop 4 peptide mimetics had to be presented as dimers of cyclized peptides to show agonistic activity (Xie et al., 2000). Their dimeric cyclized peptide, involving amino acids 92-96, showed NGF like activity and induced neurite outgrowth and Akt activation in a TrkA- and ERK- dependent manner. Thus, the dimeric peptide was able to induce an NGF-like signal, by binding to and activating TrkA. Modifications in the peptide sequence abolished the agonistic activity, suggesting specificity conferred by the amino acid sequence (Xie et al., 2000). On the other hand, dimerization of a peptidomimetic does not always behave as expected. Conversion of a monovalent peptidomimetic into a divalent mimetic by chemical coupling resulted, surprisingly, in an antagonist (Brahimi et al., 2010).

Loop 1 peptidomimetics show very unique activity. The loop 1 region (amino acids 30-35) has not only been implicated in activation of TrkA, but amino acids K32, K34 and E35 are extremely important for p75 binding (Ibanez et al., 1992; Ibanez et al., 1993) (see Section 5.1). Therefore, this region influences both TrkA and p75 binding. Cyclized monomeric peptidomimetics designed using the  $\beta$ -turn of NT-3 are good TrkC agonists, however some of the peptides showed partial agonistic response towards TrkA. Interestingly, some of the mimetics were able to induce a neurite outgrowth response through TrkA or TrkC, but failed to induce a survival response on TrkA-only expressing cells (Zaccaro et al., 2005). Neurite outgrowth studies were done using PC12 cells which express both TrkA and p75, whereas survival studies were done using TrkA-only expressing 3T3 cells. The authors, therefore, argued that this peptidomimetic binds to a special 'hot spot' on TrkA that is accessible only when p75 is co-expressed. The co-expression of p75 with TrkA could result in formation of the TrkA-p75 heteroreceptor complex and binding of p75 to TrkA, perhaps, results in conformational changes in the receptor, making the 'hot spot' accessible (Zaccaro et al., 2005). The D3 mimetic in this series was also tested in cognitively impaired aged rats and showed rescue of the cholinergic phenotype in the cortex and nucleus basalis (Bruno, et al., 2004) and to improve learning and reduce  $\beta$ -amyloid in APP mice (Aboukassim, et al., 2011).



### 6.3 Small molecule p75 agonists and antagonists

In contrast, Longo's group made loop 1 mimetics (LM11A-24 and LM11A-31) that targeted p75 and were able to induce a survival response through p75 in hippocampal neurons (Massa et al., 2006). Hippocampal neurons express TrkB and p75, but not TrkA; therefore, this survival induced by loop 1 mimetics was suggested to result via p75. They further showed that the survival response was lost in hippocampal neurons from p75<sup>-/-</sup> mice and that these peptides showed competitive binding to p75 but not TrkA, supporting the interpretation that the activity was through p75. The survival response observed was mediated by NF- $\kappa$ B and the PI3K-Akt pathway (Massa et al., 2006). They further demonstrated that the mimetics were able to rescue the neurons from proNGF-induced (Massa et al., 2006) or A $\beta$ -induced (Yang et al., 2008) cell death, which have implications for Alzheimer's pathogenesis. NGF, even though it can bind p75, was not able to prevent the A $\beta$ -induced neuritic dystrophy. Differences between the signaling by the mimetics vs NGF via p75 were also observed. Both NGF and the mimetics were able to inhibit the ability of A $\beta$  to down regulate Akt signaling; in contrast, the peptidomimetics demonstrated p75-dependent inhibition of A $\beta$ -induced GSK3b and A $\beta$ -induced JNK activation, whereas NGF could not affect these latter two activities. Thus, these loop 1 peptidomimetics induced additional survival promoting cell signaling via p75 in presence of A $\beta$ , not observed with NGF via p75 (Yang et al., 2008). Hence, the selectivity progressed from receptor to signaling pathway. From another laboratory, a different loop1-loop4 mimetic was shown to have some ability to reduce neuropathic pain in a rat model (Colangelo, et al., 2008).

### 6.4 The prognosis for signal selective mimetics

These studies with NGF peptidomimetics illustrate the advances that have been made as well as outlining the complexities involved. Whether a monovalent or a divalent (dimeric) NGF peptidomimetic is functional and most appropriate for clinical use is, as yet, unresolved. Good NGF mimetics at this stage appear to be D3 or its derivatives and LM11A-24 or LM11A-31, although each may potentially be useful for a distinct purpose. Also being developed are TrkB (O'Leary & Hughes, 2003; Fletcher, et al., 2008; Fletcher & Hughes, 2009) and TrkC (Brahimi, et al., 2009; Chen, et al., 2009; Liu, et al., 2010) antagonists/agonists. BDNF mimetics have made progress toward a useful therapeutic reagent with efficacy studies in rodents (Massa et al., 2010). Some of these neurotrophin reagents show receptor selectivity, as well as selective cellular response, i.e. survival vs differentiation (Saragovi et al., 2009). Whether a small peptidomimetic or a modified, full-length NGF protein will ultimately prove to be the most effective clinical reagent for treatment of AD is an open question at present. The pharmacokinetic advantages of a small molecule mimetic may eventually outweigh the limited information content, relative to the full length protein, provided by such a peptide. In other words, will the chemists be able to design sufficient specificity and selectivity into the peptidomimetics to compete with the potential specificity of a 26 kDa neurotrophin protein with its myriad interaction sites? The answer in some cases for selective signaling appears to be yes (Zaccaro et al., 2005; Longo, et al., 2007; Yang et al., 2008). Conversely, will the means of delivery (intracerebroventricular injection, intranasal inhalation, encapsulation) of a full-length neurotrophin mutein be developed sufficiently to make easy administration to the AD brain competitive with a, perhaps, less effective small molecule that is easily delivered? In either case, exquisite signal selectivity to allow ultra-focus may be most effective and/or desirable.

## 7. Other growth factors with cellular signaling selectivity

Neurotrophins are not the only ligand-receptor system in which molecular engineering approaches have been employed to improve the therapeutic potential. Mutants of both insulin and tumor necrosis factor (TNF $\alpha$ ) have also been developed with enhancement of their therapeutic properties. TNF $\alpha$  was re-designed with the intent of improving its death-inducing targeting toward tumors and reducing its systemic side effects (Wajant, et al., 2005; Gerspach, et al., 2009). This attempt is a true challenge with a multifunctional, pleiotropic cytokine such as TNF $\alpha$ ; nevertheless some progress has been made. Similarly, insulin has been engineered to improve its stability as a single chain (Hua, et al., 2008), its stability as a zinc 'stapled' hexamer with even longer lasting attributes (Phillips, et al., 2010), and its selectivity for the insulin receptor over the non-cognate IGF-1 receptor (Zhao, et al., 2009). A reported 3-fold improvement in receptor selectivity has the potential of reducing colorectal cancer risk for diabetic patients using insulin replacement therapy (Zhao, et al., 2009). How well these receptor and/or signal selective mutant cytokines/hormones may work in therapeutic trials is still uncertain.

## 8. Conclusions and the future

The importance of ligand-receptor interactions in AD and other neurodegenerative conditions is well appreciated. Protein levels and mRNA levels for both TrkA and p75 receptors are reduced in early AD even in the presence of stable NGF expression. A shift from mature NGF to proNGF, which binds p75 and induces apoptosis, has also been observed. Thus, the neurodegeneration in the basal forebrain in AD is aggravated by a reduction in signaling from TrkA receptors coupled with apoptotic signaling by proNGF binding to p75 (see Section 2.2). Animal studies and clinical trials of NGF gene delivery show promise in curtailing neuronal apoptosis in Alzheimer's disease or animal models. However, the CBF in some patients demonstrated a trophic response of immature neuritic processes, i.e. neurite outgrowth where it was not needed or useful. Adult brains with AD possessing an established axonal network may be adversely affected by this type of aberrant neuritogenesis. Survival support for existing neurons is preferable without inducing *de novo* connections that might be dysfunctional. A key goal for future intervention of this type is to promote viability of specific neurons without causing neuritic or axonal overgrowth.

The possibility and potential of designer growth factors has been highlighted in this review. Step-wise mutagenesis of NGF led to the generation of survival-selective muteins that are potentially therapeutic lead candidates for Alzheimer Disease or other neurodegenerative disorders. Two mechanism-selective recombinant NGF muteins show a marked difference in the ratio of survival to neuritogenesis in several assays, including signaling through the Akt survival pathway. Thus, in principle one can separate intracellular signaling pathways for a receptor via modifications to its ligand. Successful parallel studies with molecular engineering of insulin and TNF $\alpha$  support this viewpoint. Structural studies of other receptor systems, e.g., EGF, have provided a logical, molecular basis for understanding how these signals may be separately activated. Progress with small molecule mimetics of NGF that are agonists show some of the same receptor- and signaling- selectivity that receptor- or signal-selective muteins of the parent protein do. The future should provide major advances in this exciting area of improving upon nature and developing novel treatments for AD. Coupling of designer neurotrophins with improved delivery to the brain provides great promise.

## 9. Acknowledgements

We wish to acknowledge the generous support of the NINDS, NIH, USPHS, USA for nearly 20 years through the grant NS24380. We are grateful to many interesting and challenging discussions with other members of the Neet lab who indirectly contributed to this review. Finally, we would like to acknowledge the collegiality of the investigators in the neurotrophin field and all of their accomplishments that are summarized in this review.

## 10. Nomenclature

7-84-103, NGF mutein, F7A/H84A/R103A  
 A $\beta$ , beta amyloid  
 AD, Alzheimer's disease  
 APP, amyloid precursor protein  
 Akt, a ser-thr kinase (also PKB)  
 BDNF, brain derived neurotrophic factor  
 BBB, blood brain barrier  
 CBF, cholinergic basal forebrain  
 ECD, extracellular domain  
 EGF, epidermal growth factor  
 EGFR, EGF receptor  
 GSK3b, glycogen synthase kinase 3b  
 ICD, intracellular domain  
 IGF-1, insulin like growth factor 1  
 JNK, c-Jun N-terminal kinase  
 KKE, K32A/K34A/E35A NGF mutein  
 KKE/7-84-103, hextuple mutein, K32A/K34A/E35A/F7A/H84A/R103A NGF mutein  
 MAPK, mitogen-activated protein kinase  
 NF- $\kappa$ B, Nuclear factor kappa-light-chain-enhancer of activated B cells  
 NGF, nerve growth factor ( $\beta$ -subunit)  
 NT-3, neurotrophin 3  
 NT-4, neurotrophin 4  
 p75, common neurotrophin receptor  
 PC12, pheochromocytoma 12 cell line  
 PI3K, phosphoinositide 3-kinase  
 PLC $\gamma$ , phospholipase C gamma  
 proNGF, precursor form of NGF  
 TNF $\alpha$ , tumor necrosis factor alpha  
 Trk, tropomyosin related kinase  
 $\Delta$ 9/13, NGF mutein with residues 9 through 13 deleted

## 11. References

Aboukassim, T., Tong, X.-K., Tse, Y. C., Wong, T.-P., Woo, S. B., Neet, K. E., Brahimi, F., Hamel, E., & Saragovi, H. U. (2011). Ligand-dependent TrkA activity in brain impacts differently on spatial learning and long-term memory. *Molecular Pharmacology*, DOI 10.1124/mol.111.071332 in press.

- Alam, M.I., Beg, S., Samad, A., Baboota, S., Kohli, K., Ali, J., Ahuja, A., & Akbar, M. (2010). Strategy for effective brain drug delivery. *European Journal of Pharmaceutical Sciences*, Vol. 40, No. 5, pp. 385-403.
- Aloyz, R.S., Bamji, S.X., Pozniak, C.D., Toma, J.G., Atwal, J., Kaplan, D.R., & Miller, F.D. (1998). p53 is essential for developmental neuron death as regulated by the TrkA and p75 neurotrophin receptors. *Journal of Cell Biology*, Vol. 143, No. 6, pp. 1691-1703.
- Apfel, S.C. (2002). Nerve growth factor for the treatment of diabetic neuropathy: what went wrong, what went right, and what does the future hold? *International Review of Neurobiology*, Vol. 50, pp. 393-413.
- Apfel, S.C., Kessler, J.A., Adornato, B.T., Litchy, W.J., Sanders, C., & Rask, C.A. (1998). Recombinant human nerve growth factor in the treatment of diabetic polyneuropathy. NGF Study Group. *Neurology*, Vol. 51, No. 3, pp. 695-702.
- Apfel, S.C., Lipton, R.B., Arezzo, J.C., & Kessler, J.A. (1991). Nerve growth factor prevents toxic neuropathy in mice. *Annals of Neurology*, Vol. 29, No. 1, pp. 87-90.
- Bae, J., & Schlessinger, J. (2010). Asymmetric tyrosine kinase arrangements in activation or autophosphorylation of receptor tyrosine kinases. *Molecules and Cells*, Vol. 29, No. 5, pp. 443-448.
- Bai, Y., Dergham, P., Nedev, H., Xu, J., Galan, A., Rivera, J. C., ZhiHua, S., Mehta, H. M., Woo, S. B., Sarunic, M. V., Neet, K. E., & Saragovi, H. U. (2010). Chronic and acute models of retinal neurodegeneration of TrkA activity are neuroprotective whereas p75NTR activity is neurotoxic through a paracrine mechanism. *Journal of Biological Chemistry*, Vol. 285, No. 50, pp. 39392-39400.
- Bamji, S.X., Majdan, M., Pozniak, C.D., Belliveau, D.J., Aloyz, R., Kohn, J., Causing, C.G., & Miller, F.D. (1998). The p75 neurotrophin receptor mediates neuronal apoptosis and is essential for naturally occurring sympathetic neuron death. *J Cell Biol*, Vol. 140, No. 4, pp. 911-923.
- Barrett, G.L., & Bartlett, P.F. (1994). The p75 nerve growth factor receptor mediates survival or death depending on the stage of sensory neuron development. *Proceedings of the National Academy of Sciences, U.S.A.*, Vol. 91, No. 14, pp. 6501-6505.
- Bartlett, S.E., Reynolds, A.J., Weible, M., Heydon, K., & Hendry, I.A. (1997). In sympathetic but not sensory neurones, phosphoinositide-3 kinase is important for NGF-dependent survival and the retrograde transport of 125I-betaNGF. *Brain Research*, Vol. 761, No. 2, pp. 257-262.
- Beglova, N., Maliartchouk, S., Ekiel, I., Zaccaro, M.C., Saragovi, H.U., & Gehring, K. (2000). Design and Solution Structure of Functional Peptide Mimetics of Nerve Growth Factor. *Journal of Medicinal Chemistry*, Vol. 43, No. 19, pp. 3530-3540.
- Benedict, C., Frey, W.H., 2nd, Schioth, H.B., Schultes, B., Born, J., & Hallschmid, M. (2011). Intranasal insulin as a therapeutic option in the treatment of cognitive impairments. *Experimental Gerontology*, Vol. 46, No. 2-3, pp. 112-115.
- Bibel, M., & Barde, Y.A. (2000). Neurotrophins: key regulators of cell fate and cell shape in the vertebrate nervous system. *Genes & Development*, Vol. 14, No. 23, pp. 2919-2937.
- Bierer, L.M., Haroutunian, V., Gabriel, S., Knott, P.J., Carlin, L.S., Purohit, D.P., Perl, D.P., Schmeidler, J., Kanof, P., & Davis, K.L. (1995). Neurochemical correlates of dementia severity in Alzheimer's disease: relative importance of the cholinergic deficits. *Journal of Neurochemistry*, Vol. 64, No. 2, pp. 749-760.



- Bishop, K.M., Hofer, E.K., Mehta, A., Ramirez, A., Sun, L., Tuszynski, M., & Bartus, R.T. (2008). Therapeutic potential of CERE-110 (AAV2-NGF): targeted, stable, and sustained NGF delivery and trophic activity on rodent basal forebrain cholinergic neurons. *Experimental Neurology*, Vol. 211, No. 2, pp. 574-584.
- Bottger, D., Ullrich, C., & Humpel, C. (2010). Monocytes deliver bioactive nerve growth factor through a brain capillary endothelial cell-monolayer in vitro and counteract degeneration of cholinergic neurons. *Brain Research*, Vol. 1312, pp. 108-119.
- Brahimi, F., Liu, J., Malakhov, A., Chowdhury, S., Purisima, E.O., Ivanisevic, L., Caron, A., Burgess, K., & Saragovi, H.U. (2010). A monovalent agonist of TrkA tyrosine kinase receptors can be converted into a bivalent antagonist. *Biochimica et Biophysica Acta (BBA) - General Subjects*, Vol. 1800, No. 9, pp. 1018-1026.
- Brahimi, F., Malakhov, A., Lee, H. B., Pattarawarapan, M., Ivanisevic, L., Burgess, K., & Saragovi, H. U. (2009). A peptidomimetic of NT-3 acts as a TrkC antagonist. *Peptides*, Vol. 30, No. 10, pp. 1833-1839.
- Bruno, M. A., Clarke, P. B., Seltzer, A., Quirion, R., Burgess, K., Cuello, A. C., & Saragovi, H. U. (2004). Long-lasting rescue of age-associated deficits in cognition and the CNS cholinergic phenotype by a partial agonist peptidomimetic ligand of TrkA. *Journal of Neuroscience*, Vol. 24, No. 37, pp. 8009-8018.
- Calissano, P., Amadoro, G., Matrone, C., Ciafrè, S., Marolda, R., Corsetti, V., Ciotti, M. T., Mercanti, D., Di Luzio, A., Severini, C., Provenzano, C., & Canu, N. (2010a) Does the term trophic actually mean anti-amyloidogenic? The case of NGF. *Cell Death & Differentiation* Vol. 17, No. 7, pp. 1126-33.
- Calissano, P., Matrone, C., & Amadoro, G. (2010b). Nerve growth factor as a paradigm of neurotrophins related to Alzheimer's disease. *Developmental Neurobiology*, Vol. 70, No. 5, pp. 372-383.
- Canossa, M., Griesbeck, O., Berninger, B., Campana, G., Kolbeck, R., & Thoenen, H. (1997). Neurotrophin release by neurotrophins: implications for activity-dependent neuronal plasticity. *Proceedings of the National Academy of Sciences, U.S.A.*, Vol. 94, No. 24, pp. 13279-13286.
- Capsoni, S., Covaceuszach, S., Marinelli, S., Ceci, M., Bernardo, A., Minghetti, L., Ugolini, G., Pavone, F., & Cattaneo, A. (2011). Taking Pain Out of NGF: A "Painless" NGF Mutant, Linked to Hereditary Sensory Autonomic Neuropathy Type V, with Full Neurotrophic Activity. *PLoS ONE*, Vol. 6, No. 2, pp. e17321.
- Casaccia-Bonnel, P., Carter, B.D., Dobrowsky, R.T., & Chao, M.V. (1996). Death of oligodendrocytes mediated by the interaction of nerve growth factor with its receptor p75. *Nature*, Vol. 383, No. 6602, pp. 716-719.
- Casademunt, E., Carter, B.D., Benzell, I., Frade, J.M., Dechant, G., & Barde, Y.A. (1999). The zinc finger protein NRIF interacts with the neurotrophin receptor p75(NTR) and participates in programmed cell death. *EMBO Journal*, Vol. 18, No. 21, pp. 6050-6061.
- Cattaneo, A., Capsoni, S., & Paoletti, F. (2008). Towards Non Invasive Nerve Growth Factor Therapies for Alzheimer's Disease. *Journal of Alzheimer's Disease*, Vol. 15, No. 2, pp. 255-283.
- Chen, D., Brahimi, F., Angell, Y., Li, Y. C., Moskowicz, J., Saragovi, H. U., & Burgess, K. (2009). Bivalent peptidomimetic ligands of TrkC are biased agonists and selectively induce neurite outgrowth or potentiate neurotrophin-3 trophic signals. *ACS Chemical Biology*, Vol. 4, No. 9, pp. 769-781.

- Chittka, A., & Chao, M.V. (1999). Identification of a zinc finger protein whose subcellular distribution is regulated by serum and nerve growth factor. *Proceedings of the National Academy of Sciences, U.S.A.*, Vol. 96, No. 19, pp. 10705-10710.
- Colangelo, A. M., Bianco, M. R., Vitagliano, L., Cavaliere, C., Cirillo, G., De Gioia, L., Diana, D., Colombo, D., Redaelli, C., Zaccaro, L., Morelli, G., Papa, M., Sarmientos, P., Alberghina, L., & Martegani, E. (2008). A new nerve growth factor-mimetic peptide active on neuropathic pain in rats. *Journal of Neuroscience*, Vol. 28, No. 11, pp. 2698-2709.
- Cordon-Cardo, C., Tapley, P., Jing, S.Q., Nanduri, V., O'Rourke, E., Lamballe, F., Kovary, K., Klein, R., Jones, K.R., Reichardt, L.F., & Barbacid, M. (1991). The trk tyrosine protein kinase mediates the mitogenic properties of nerve growth factor and neurotrophin-3. *Cell*, Vol. 66, No. 1, pp. 173-183.
- Coulson, J., May, L. M., Sykes, A. M., & Hamlin, A. S. (2009). The role of the p75 neurotrophin receptor in cholinergic dysfunction in Alzheimer's disease. *Neuroscientist*, Vol. 15, No. 4, pp. 317-23.
- Covaceuszach, S., Capsoni, S., Marinelli, S., Pavone, F., Ceci, M., Ugolini, G., Vignone, D., Amato, G., Paoletti, F., Lamba, D., & Cattaneo, A. (2010). In vitro receptor binding properties of a "painless" NGF mutein, linked to hereditary sensory autonomic neuropathy type V. *Biochemical and Biophysical Research Communications*, Vol. 391, No. 1, pp. 824-829.
- Crowder, R.J., & Freeman, R.S. (1998). Phosphatidylinositol 3-kinase and Akt protein kinase are necessary and sufficient for the survival of nerve growth factor-dependent sympathetic neurons. *Journal of Neuroscience*, Vol. 18, No. 8, pp. 2933-2943.
- Cuello, A., Bruno, M., Allard, S., Leon, W., & Iulita, M. (2010). Cholinergic Involvement in Alzheimer's Disease. A Link with NGF Maturation and Degradation. *Journal of Molecular Neuroscience*, Vol. 40, No. 1, pp. 230-235.
- Cunningham, M.E., & Greene, L.A. (1998). A function-structure model for NGF-activated TRK. *EMBO Journal*, Vol. 17, No. 24, pp. 7282-7293.
- Cunningham, M.E., Stephens, R.M., Kaplan, D.R., & Greene, L.A. (1997). Autophosphorylation of activation loop tyrosines regulates signaling by the TRK nerve growth factor receptor. *Journal of Biological Chemistry*, Vol. 272, No. 16, pp. 10957-10967.
- Davey, F., & Davies, A.M. (1998). TrkB signalling inhibits p75-mediated apoptosis induced by nerve growth factor in embryonic proprioceptive neurons. *Current Biology*, Vol. 8, No. 16, pp. 915-918.
- de Chaves, E.I., Bussiere, M., Vance, D.E., Campenot, R.B., & Vance, J.E. (1997). Elevation of ceramide within distal neurites inhibits neurite growth in cultured rat sympathetic neurons. *Journal of Biological Chemistry*, Vol. 272, No. 5, pp. 3028-3035.
- Dechant, G., Barde, Y. A. (2002). The neurotrophin receptor p75(NTR): novel functions and implications for diseases of the nervous system. *Nature Neuroscience*, Vol. 5, No. 11, pp. 1131-1136.
- DeKosky, S.T., Ikonomic, M.D., Styren, S.D., Beckett, L., Wisniewski, S., Bennett, D.A., Cochran, E.J., Kordower, J.H., & Mufson, E.J. (2002). Upregulation of choline acetyltransferase activity in hippocampus and frontal cortex of elderly subjects with mild cognitive impairment. *Annals of Neurology*, Vol. 51, No. 2, pp. 145-155.

- Diamond, J., Foerster, A., Holmes, M., & Coughlin, M. (1992). Sensory nerves in adult rats regenerate and restore sensory function to the skin independently of endogenous NGF. *Journal of Neuroscience*, Vol. 12, No. 4, pp. 1467-1476.
- Diarra, A., Geetha, T., Potter, P., & Babu, J. R. (2009). Signaling of the neurotrophin receptor p75 in relation to Alzheimer's disease. *Biochemical and Biophysical Research Communications*, Vol. 390, No. 3, pp. 352-356.
- Domeniconi, M., Zampieri, N., Spencer, T., Hilaire, M., Mellado, W., Chao, M.V., & Filbin, M.T. (2005). MAG induces regulated intramembrane proteolysis of the p75 neurotrophin receptor to inhibit neurite outgrowth. *Neuron*, Vol. 46, No. 6, pp. 849-855.
- Einarsdottir, E., Carlsson, A., Minde, J., Toolanen, G., Svensson, O., Solders, G., Holmgren, G., Holmberg, D., & Holmberg, M. (2004). A mutation in the nerve growth factor beta gene (NGFB) causes loss of pain perception. *Human Molecular Genetics*, Vol. 13, No. 8, pp. 799-805.
- Emerich, D.F., Winn, S.R., Harper, J., Hammang, J.P., Baetge, E.E., & Kordower, J.H. (1994). Implants of polymer-encapsulated human NGF-secreting cells in the nonhuman primate: rescue and sprouting of degenerating cholinergic basal forebrain neurons. *Journal of Computational Neurology*, Vol. 349, No. 1, pp. 148-164.
- Emilien, G., Maloteaux, J.M., Beyreuther, K., & Masters, C.L. (2000). Alzheimer disease: mouse models pave the way for therapeutic opportunities. *Archives of Neurology*, Vol. 57, No. 2, pp. 176-181.
- Eriksdotter Jonhagen, M., Nordberg, A., Amberla, K., Backman, L., Ebendal, T., Meyerson, B., Olson, L., Seiger, Shigeta, M., Theodorsson, E., Viitanen, M., Winblad, B., & Wahlund, L. O. (1998). Intracerebroventricular infusion of nerve growth factor in three patients with Alzheimer's disease. *Dementia and Geriatric Cognitive Disorders*, Vol. 9, No. 5, pp. 246-257.
- Fahnestock, M., Michalski, B., Xu, B., & Coughlin, M.D. (2001). The Precursor Pro-Nerve Growth Factor Is the Predominant Form of Nerve Growth Factor in Brain and Is Increased in Alzheimer's Disease. *Molecular and Cellular Neuroscience*, Vol. 18, No. 2, pp. 210-220.
- Fan, Y.-X., Wong, L., Deb, T.B., & Johnson, G.R. (2004). Ligand Regulates Epidermal Growth Factor Receptor Kinase Specificity. *Journal of Biological Chemistry*, Vol. 279, No. 37, pp. 38143-38150.
- Fjord-Larsen, L., Kusk, P., Tornoe, J., Juliusson, B., Torp, M., Bjarkam, C.R., Nielsen, M.S., Handberg, A., Sorensen, J.C.H., & Wahlberg, L.U. (2010). Long-term Delivery of Nerve Growth Factor by Encapsulated Cell Biodelivery in the Gottingen Minipig Basal Forebrain. *Molecular Therapy*, Vol. 18, No. 12, pp. 2164-2172.
- Fletcher, J. M., Morton, C. J., Zwar, R. A., Murray, S. S., O'Leary, P. D., & Hughes, R. A. (2008). Design of a conformationally defined and proteolytically stable circular mimetic of brain-derived neurotrophic factor. *Journal of Biological Chemistry*, Vol. 283, No 48, pp. 33375-33383.
- Fletcher, J. M. & Hughes, R. A. (2009). Modified low molecular weight cyclic peptides as mimetics of BDNF with improved potency, proteolytic stability and transmembrane passage in vitro. *Bioorganic Medicinal Chemistry*, Vol. 17, No. 7, pp. 2695-2702.

- Friden, P., Walus, L., Watson, P., Doctrow, Kozarich, J., Backman, C., Bergman, H., Hoffer, B., Bloom, F., & Granholm, A. (1993). Blood-brain barrier penetration and in vivo activity of an NGF conjugate. *Science*, Vol. 259, No. 5093, pp. 373-377.
- Friedman, W.J., & Greene, L.A. (1999). Neurotrophin signaling via Trks and p75. *Experimental Cell Research*, Vol. 253, No. 1, pp. 131-142.
- Funakoshi, H., Risling, M., Carlstedt, T., Lendahl, U., Timmusk, T., Metsis, M., Yamamoto, Y., & Ibanez, C. F. (1998). Targeted expression of a multifunctional chimeric neurotrophin in the lesioned sciatic nerve accelerates regeneration of sensory and motor axons. *Proceedings of the National Academy of Sciences, U.S.A.*, Vol. 95, No. 9, pp. 5269-5274.
- Garzon, D.J., & Fahnstock, M. (2007). Oligomeric Amyloid Decreases Basal Levels of Brain-Derived Neurotrophic factor (BDNF) mRNA via Specific Downregulation of BDNF Transcripts IV and V in Differentiated Human Neuroblastoma Cells. *The Journal of Neuroscience*, Vol. 27, No. 10, pp. 2628-2635.
- Gerspach, J., Pfizenmaier, K., & Wajant, H. (2009). Improving TNF as a cancer therapeutic: tailor-made TNF fusion proteins with conserved antitumor activity and reduced systemic side effects. *Biofactors*. 35, No. 4, pp. 364-372.
- Ginsberg, S.D., Che, S., Wu, J., Counts, S.E., & Mufson, E.J. (2006). Down regulation of trk but not p75NTR gene expression in single cholinergic basal forebrain neurons mark the progression of Alzheimer's disease. *Journal of Neurochemistry*, Vol. 97, No. 2, pp. 475-487.
- Gomez, N., & Cohen, P. (1991). Dissection of the protein kinase cascade by which nerve growth factor activates MAP kinases. *Nature*, Vol. 353, No. 6340, pp. 170-173.
- Grewal, S.S., York, R.D., & Stork, P.J. (1999). Extracellular-signal-regulated kinase signalling in neurons. *Current Opinion in Neurobiology*, Vol. 9, No. 5, pp. 544-553.
- Grimes, M.L., Beattie, E., & Mobley, W.C. (1997). A signaling organelle containing the nerve growth factor-activated receptor tyrosine kinase, TrkA. *Proceedings of the National Academy of Sciences, U.S.A.*, Vol. 94, No. 18, pp. 9909-9914.
- Guo, M., Meyer, S.L., Kaur, H., Gao, J.J., & Neet, K.E. (1996). Mutational studies of conserved residues in the dimer interface of nerve growth factor. *Protein Science*, Vol. 5, No. 3, pp. 447-455.
- Hefti, F. (1983). Is Alzheimer disease caused by lack of nerve growth factor? *Annals of Neurology*, Vol. 13, No. 1, pp. 109-110.
- Holgado-Madruga, M., Moscatello, D.K., Emllet, D.R., Dieterich, R., & Wong, A.J. (1997). Grb2-associated binder-1 mediates phosphatidylinositol 3-kinase activation and the promotion of cell survival by nerve growth factor. *Proceedings of the National Academy of Sciences, U.S.A.*, Vol. 94, No. 23, pp. 12419-12424.
- Hua, Q. X., Nakagawa, S. H., Jia, W., Huang, K., Phillips, N. B., Hu, S. Q., & Weiss, M. A. (2008). Design of an active ultrastable single-chain insulin analog: synthesis, structure, and therapeutic implications. *Journal of Biological Chemistry*, Vol. 283, No. 21, pp. 14703-14716.
- Huang, E.J., & Reichardt, L.F. (2003). Trk receptors: roles in neuronal signal transduction. *Annual Review of Biochemistry*, Vol. 72, pp. 609-642.
- Hubbard, S.R., Wei, L., Ellis, L., & Hendrickson, W.A. (1994). Crystal structure of the tyrosine kinase domain of the human insulin receptor. *Nature*, Vol. 372, No. 6508, pp. 746-754.



- Hughes, A. L., Messineo-Jones, D., Lad, S. P., & Neet, K. E. (2001). Distinction between differentiation, cell cycle, and apoptosis signals in PC12 cells by the nerve growth factor mutant delta9/13, which is selective for the p75 neurotrophin receptor. *Journal of Neuroscience Research*, Vol. 63, No. 1, pp. 10-19.
- Hyman, C., Hofer, M., Barde, Y.-A., Juhasz, M., Yancopoulos, G.D., Squinto, S.P., & Lindsay, R.M. (1991). BDNF is a neurotrophic factor for dopaminergic neurons of the substantia nigra. *Nature*, Vol. 350, No. 6315, pp. 230-232.
- Ibanez, C.F., Hallbook, F., Ebendal, T., & Persson, H. (1990). Structure-function studies of nerve growth factor: functional importance of highly conserved amino acid residues. *EMBO Journal*, Vol. 9, No. 5, pp. 1477-1483.
- Ibanez, C.F., Ebendal, T., & Persson, H. (1991). Chimeric molecules with multiple neurotrophic activities reveal structural elements determining the specificities of NGF and BDNF. *EMBO Journal*, Vol. 10, No. 8, pp. 2105-2110.
- Ibanez, C.F., Ebendal, T., Barbany, G., Murray-Rust, J., Blundell, T.L., & Persson, H. (1992). Disruption of the low affinity receptor-binding site in NGF allows neuronal survival and differentiation by binding to the trk gene product. *Cell*, Vol. 69, No. 2, pp. 329-341.
- Ibanez, C.F., Ilag, L.L., Murray-Rust, J., & Persson, H. (1993). An extended surface of binding to Trk tyrosine kinase receptors in NGF and BDNF allows the engineering of a multifunctional pan-neurotrophin. *EMBO Journal*, Vol. 12, No. 6, pp. 2281-2293.
- Ibanez, C.F. (1994). Structure-function relationships in the neurotrophin family. *Journal of Neurobiology*, Vol. 25, No. 11, pp. 1349-1361.
- Ilag, L.L., Lonnerberg, P., Persson, H., & Ibanez, C.F. (1994). Role of variable beta-hairpin loop in determining biological specificities in neurotrophin family. *Journal of Biological Chemistry*, Vol. 269, No. 31, pp. 19941-19946.
- Ilag, L. L., Curtis, R., Glass, D., Funakoshi, H., Tobkes, N. J., Ryan, T. E., Acheson, A., Lindsay, R. M., Persson, H., Yancopoulos, G. D., DiStefano, P. S. & Ibanez, C. F. (1995). Pan-neurotrophin 1: a genetically engineered neurotrophic factor displaying multiple specificities in peripheral neurons in vitro and in vivo. *Proceedings of the National Academy of Sciences, U.S.A.*, Vol. 92, No. 2, pp. 607-611.
- Indo, Y. (2002). Genetics of congenital insensitivity to pain with anhidrosis (CIPA) or hereditary sensory and autonomic neuropathy type IV. Clinical, biological and molecular aspects of mutations in TRKA(NTRK1) gene encoding the receptor tyrosine kinase for nerve growth factor. *Clinical Autonomic Research*, Vol. 12, Suppl 1, pp. I20-32.
- Jordão, J.F., Ayala-Grosso, C.A., Markham, K., Huang, Y., Chopra, R., McLaurin, J., Hynynen, K., & Aubert, I. (2010). Antibodies Targeted to the Brain with Image-Guided Focused Ultrasound Reduces Amyloid- $\beta$  Plaque Load in the TgCRND8 Mouse Model of Alzheimer's Disease. *PLoS ONE*, Vol. 5, No. 5, pp. e10549.
- Jura, N., Zhang, X., Endres, Nicholas F., Seeliger, Markus A., Schindler, T., & Kuriyan, J. (2011). Catalytic Control in the EGF Receptor and Its Connection to General Kinase Regulatory Mechanisms. *Molecular Cell*, Vol. 42, No. 1, pp. 9-22.
- Kahle, P., Burton, L.E., Schmelzer, C.H., & Hertel, C. (1992). The amino terminus of nerve growth factor is involved in the interaction with the receptor tyrosine kinase p140trkA. *Journal of Biological Chemistry*, Vol. 267, No. 32, pp. 22707-22710.
- Kao, S., Jaiswal, R.K., Kolch, W., & Landreth, G.E. (2001). Identification of the mechanisms regulating the differential activation of the mapk cascade by epidermal growth

- factor and nerve growth factor in PC12 cells. *Journal of Biological Chemistry*, Vol. 276, No. 21, pp. 18169-18177.
- Kaplan, D.R., Hempstead, B.L., Martin-Zanca, D., Chao, M.V., & Parada, L.F. (1991). The trk proto-oncogene product: a signal transducing receptor for nerve growth factor. *Science*, Vol. 252, No. 5005, pp. 554-558.
- Kaplan, D.R., & Miller, F.D. (2000). Neurotrophin signal transduction in the nervous system. *Current Opinion in Neurobiology*, Vol. 10, No. 3, pp. 381-391.
- Katz, D.M., Erb, M., Lillis, R., & Neet, K. (1990). Trophic regulation of nodose ganglion cell development: evidence for an expanded role of nerve growth factor during embryogenesis in the rat. *Experimental Neurology*, Vol. 110, No. 1, pp. 1-10.
- Khursigara, G., Orlinick, J.R., & Chao, M.V. (1999). Association of the p75 neurotrophin receptor with TRAF6. *Journal of Biological Chemistry*, Vol. 274, No. 5, pp. 2597-2600.
- Korsching, S. (1993). The neurotrophic factor concept: a reexamination. *Journal of Neuroscience*, Vol. 13, No. 7, pp. 2739-2748.
- Kullander, K., & Ebendal, T. (1994). Neurotrophin-3 acquires NGF-like activity after exchange to five NGF amino acid residues: molecular analysis of the sites in NGF mediating the specific interaction with the NGF high affinity receptor. *Journal of Neuroscience Research*, Vol. 39, No. 2, pp. 195-210.
- Kullander, K., Kaplan, D., & Ebendal, T. (1997). Two restricted sites on the surface of the nerve growth factor molecule independently determine specific TrkA receptor binding and activation. *Journal of Biological Chemistry*, Vol. 272, No. 14, pp. 9300-9307.
- Lad, S. P. & Neet, K. E. (2003). Activation of the mitogen-activated protein kinase pathway through p75NTR: a common mechanism for the neurotrophin family. *Journal of Neuroscience Research*, Vol. 73, No. 5, pp. 614-626.
- Lad, S.P., Neet, K.E., & Mufson, E.J. (2003a). Nerve growth factor: structure, function and therapeutic implications for Alzheimer's disease. *Current Drug Targets- CNS and Neurological Disorders*, Vol. 2, No. 5, pp. 315-334.
- Lad, S.P., Peterson, D.A., Bradshaw, R.A., & Neet, K.E. (2003b). Individual and combined effects of TrkA and p75NTR nerve growth factor receptors. A role for the high affinity receptor site. *Journal of Biological Chemistry*, Vol. 278, No. 27, pp. 24808-24817.
- Lane, N.E., Schnitzer, T.J., Birbara, C.A., Mokhtarani, M., Shelton, D.L., Smith, M.D., & Brown, M.T. (2010). Tanezumab for the Treatment of Pain from Osteoarthritis of the Knee. *New England Journal of Medicine*, Vol. 363, No. 16, pp. 1521-1531.
- Lemmon, M.A., & Schlessinger, J. (2010). Cell Signaling by Receptor Tyrosine Kinases. *Cell*, Vol. 141, No. 7, pp. 1117-1134.
- LeSauter, L., Wei, L., Gibbs, B.F., & Saragovi, H.U. (1995). Small Peptide Mimics of Nerve Growth Factor Bind TrkA Receptors and Affect Biological Responses. *Journal of Biological Chemistry*, Vol. 270, No. 12, pp. 6564-6569.
- Levi-Montalcini, R. (1987). The nerve growth factor 35 years later. *Science*, Vol. 237, No. 4819, pp. 1154-1162.
- Liao, G.S., Li, X.B., Zhang, C.Y., Shu, Y.Y., & Tang, S.X. (2001). Pharmacological actions of nerve growth factor-transferrin conjugate on the central nervous system. *Journal of Natural Toxins*, Vol. 10, No. 4, pp. 291-297.

- Lindsay, R.M. (1988). Nerve growth factors (NGF, BDNF) enhance axonal regeneration but are not required for survival of adult sensory neurons. *Journal of Neuroscience*, Vol. 8, No. 7, pp. 2394-2405.
- Liu, J., Brahimi, F., Saragovi, H. U., & Burgess K. (2010) Bivalent diketopiperazine-based tropomyosin receptor kinase C (TrkC) antagonists. *Journal of Medicinal Chemistry*, Vol. 53, No. 13, pp. 5044-5048.
- Loeb, D.M., Stephens, R.M., Copeland, T., Kaplan, D.R., & Greene, L.A. (1994). A Trk nerve growth factor (NGF) receptor point mutation affecting interaction with phospholipase C-gamma 1 abolishes NGF-promoted peripherin induction but not neurite outgrowth. *Journal of Biological Chemistry*, Vol. 269, No. 12, pp. 8901-8910.
- Longo, F. M., Yang, T., Knowles, J. K., Xie, Y., Moore, L. A., & Massa, S. M. (2007). Small molecule neurotrophin receptor ligands: novel strategies for targeting Alzheimer's disease mechanisms. *Currents in Alzheimer Research*, Vol 4, No. 5, pp. 503-506.
- Mahapatra, S. (2008). *Identification of Critical Residues within the Conserved and Specificity Patches of Nerve Growth Factor Leading to Survival or Differentiation*. Ph.D. Thesis, Rosalind Franklin University of Medicine and Science, North Chicago.
- Mahapatra, S., Mehta, H., Woo, S.B., & Neet, K.E. (2009). Identification of Critical Residues within the Conserved and Specificity Patches of Nerve Growth Factor Leading to Survival or Differentiation. *Journal of Biological Chemistry*, Vol. 284, No. 48, pp. 33600-33613.
- Maliartchouk, S., Debeir, T., Beglova, N., Cuello, A.C., Gehring, K., & Saragovi, H.U. (2000). Genuine Monovalent Ligands of TrkA Nerve Growth Factor Receptors Reveal a Novel Pharmacological Mechanism of Action. *Journal of Biological Chemistry*, Vol. 275, No. 14, pp. 9946-9956.
- Massa, S.M., Xie, Y., Yang, T., Harrington, A.W., Kim, M.L., Yoon, S.O., Kraemer, R., Moore, L.A., Hempstead, B.L., & Longo, F.M. (2006). Small, Nonpeptide p75<sup>NTR</sup> Ligands Induce Survival Signaling and Inhibit proNGF-Induced Death. *The Journal of Neuroscience*, Vol. 26, No. 20, pp. 5288-5300.
- Massa, S.M., Yang, T., Xie, Y., Shi, J., Bilgen, M., Joyce, J.N., Nehama, D., Rajadas, J., & Longo, F.M. (2010). Small molecule BDNF mimetics activate TrkB signaling and prevent neuronal degeneration in rodents. *The Journal of Clinical Investigation*, Vol. 120, No. 5, pp. 1774-1785.
- McDonald, N.Q., Lapatto, R., Murray-Rust, J., Gunning, J., Wlodawer, A., & Blundell, T.L. (1991). New protein fold revealed by a 2.3-Å resolution crystal structure of nerve growth factor. *Nature*, Vol. 354, No. 6352, pp. 411-414.
- Mufson, E.J., Conner, J.M., & Kordower, J.H. (1995). Nerve growth factor in Alzheimer's disease: defective retrograde transport to nucleus basalis. *Neuroreport*, Vol. 6, No. 7, pp. 1063-1066.
- Mufson, E.J., Counts, S.E., Fahnstock, M., & Ginsberg, S.D. (2007). Cholinergic molecular substrates of mild cognitive impairment in the elderly. *Current Alzheimer Research*, Vol. 4, No. 4, pp. 340-350.
- Mufson, E.J., Counts, S.E., Perez, S.E., & Ginsberg, S.D. (2008). Cholinergic system during the progression of Alzheimer's disease: therapeutic implications. *Expert Review of Neurotherapeutics*, Vol. 8, No. 11, pp. 1703-1718.
- Mufson, E.J., Ma, S.Y., Cochran, E.J., Bennett, D.A., Beckett, L.A., Jaffar, S., Saragovi, H.U., & Kordower, J.H. (2000). Loss of nucleus basalis neurons containing trkA

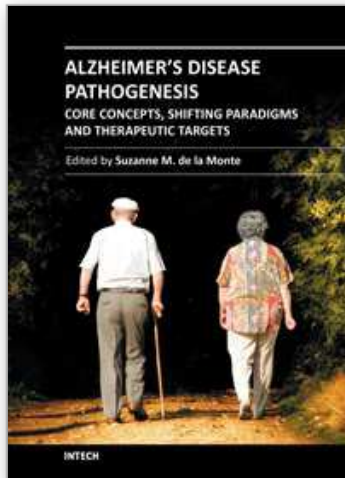
- immunoreactivity in individuals with mild cognitive impairment and early Alzheimer's disease. *Journal of Computational Neurology*, Vol. 427, No. 1, pp. 19-30.
- Mufson, E.J., Ma, S.Y., Dills, J., Cochran, E.J., Leurgans, S., Wu, J., Bennett, D.A., Jaffar, S., Gilmor, M.L., Levey, A.I., & Kordower, J.H. (2002). Loss of basal forebrain p75NTR immunoreactivity in subjects with mild cognitive impairment and Alzheimer's disease. *The Journal of Comparative Neurology*, Vol. 443, No. 2, pp. 136-153.
- Muller, G., Storz, P., Bourteele, S., Doppler, H., Pfizenmaier, K., Mischak, H., Philipp, A., Kaiser, C., & Kolch, W. (1998). Regulation of Raf-1 kinase by TNF via its second messenger ceramide and cross-talk with mitogenic signalling. *EMBO Journal*, Vol. 17, No. 3, pp. 732-742.
- Nagahara, A.H., & Tuszynski, M.H. (2011). Potential therapeutic uses of BDNF in neurological and psychiatric disorders. *Nature Reviews Drug Discovery*, Vol. 10, No. 3, pp. 209-219.
- Nicol, G.D., & Vasko, M.R. (2007). Unraveling the Story of NGF-mediated Sensitization of Nociceptive Sensory Neurons: ON or OFF the Trks? *Molecular Interventions*, Vol. 7, No. 1, pp. 26-41.
- Nilsson, P., Iwata, N., Muramatsu, S.-i., Tjernberg, L.O., Winblad, B., & Saido, T.C. (2010). Gene therapy in Alzheimer's disease - potential for disease modification. *Journal of Cellular and Molecular Medicine*, Vol. 14, No. 4, pp. 741-757.
- Ninkina, N., Grashchuck, M., Buchman, V.L., & Davies, A.M. (1997). TrkB variants with deletions in the leucine-rich motifs of the extracellular domain. *Journal of Biological Chemistry*, Vol. 272, No. 20, pp. 13019-13025.
- O'Leary, P. D. & Hughes, R. A. (2003). Design of Potent Peptide Mimetics of Brain-derived Neurotrophic Factor. *Journal of Biological Chemistry*, Vol. 278, No. 28, pp. 25738-25744.
- Parain, K., Murer, M.G., Yan, Q., Faucheux, B., Agid, Y., Hirsch, E., & Raisman-Vozari, R. (1999). Reduced expression of brain-derived neurotrophic factor protein in Parkinson's disease substantia nigra. *Neuroreport*, Vol. 10, No. 3, pp. 557-561.
- Peleshok, J., & Saragovi, H.U. (2006). Functional mimetics of neurotrophins and their receptors. *Biochemical Society Transactions*, Vol. 34, No. Pt 4, pp. 612-617.
- Peng, S., Wu, J., Mufson, E.J., & Fahnstock, M. (2004). Increased proNGF Levels in Subjects with Mild Cognitive Impairment and Mild Alzheimer Disease. *Journal of Neuropathology & Experimental Neurology*, Vol. 63, No. 6, pp. 641-649.
- Peng, S., Wu, J., Mufson, E.J., & Fahnstock, M. (2005). Precursor form of brain-derived neurotrophic factor and mature brain-derived neurotrophic factor are decreased in the pre-clinical stages of Alzheimer's disease. *Journal of Neurochemistry*, Vol. 93, No. 6, pp. 1412-1421.
- Pezet, S., & McMahon, S.B. (2006). Neurotrophins: Mediators and Modulators of Pain. *Annual Review of Neuroscience*, Vol. 29, pp. 507-538.
- Phillips, N. B., Wan, Z. L., Whittaker, L., Hu, S. Q., Huang, K., Hua, Q. X, Whittaker, J., Ismail-Beigi, F., & Weiss, M. A. (2010). Supramolecular protein engineering: design of zinc-stapled insulin hexamers as a long acting depot. *Journal of Biological Chemistry*, Vol. 285, No. 16, pp. 11755-11759.
- Qian, X., Riccio, A., Zhang, Y., & Ginty, D.D. (1998). Identification and characterization of novel substrates of Trk receptors in developing neurons. *Neuron*, Vol. 21, No. 5, pp. 1017-1029.



- Rabizadeh, S., Oh, J., Zhong, L.T., Yang, J., Bitler, C.M., Butcher, L.L., & Bredesen, D.E. (1993). Induction of apoptosis by the low-affinity NGF receptor. *Science*, Vol. 261, No. 5119, pp. 345-348.
- Rodriguez-Viciano, P., Warne, P.H., Dhand, R., Vanhaesebroeck, B., Gout, I., Fry, M.J., Waterfield, M.D., & Downward, J. (1994). Phosphatidylinositol-3-OH kinase as a direct target of Ras. *Nature*, Vol. 370, No. 6490, pp. 527-532.
- Rydén, M., Hempstead, B., & Ibáñez, C. F. (1997). Differential modulation of neuron survival during development by nerve growth factor binding to the p75 neurotrophin receptor. *Journal of Biological Chemistry*, Vol. 272, No. 26, 16322-16328.
- Salehi, A., Ocampo, M., Verhaagen, J., & Swaab, D.F. (2000). P75 neurotrophin receptor in the nucleus basalis of meynert in relation to age, sex, and Alzheimer's disease. *Experimental Neurology*, Vol. 161, No. 1, pp. 245-258.
- Salehi, A. H., Xanthoudakis, S., & Barker, P. A. (2002). NRAGE, a p75 neurotrophin receptor-interacting protein, induces caspase activation and cell death through a JNK-dependent mitochondrial pathway. *Journal of Biological Chemistry*, Vol. 277, No. 50, pp. 48043-48050.
- Saragovi, H.U., Hamel, E., & Di Polo, A. (2009). A neurotrophic rationale for the therapy of neurodegenerative disorders. *Current Alzheimer Research*, Vol. 6, No. 5, pp. 419-423.
- Schechter, L. C. & Bothwell, M. (2010). Neurotrophin receptors: Old friends with new partners. *Developmental Neurobiology*, Vol. 70, No. 5, pp. 332-338.
- Schindowski, K., Belarbi, K., & Buée, L. (2008). Neurotrophic factors in Alzheimer's disease: role of axonal transport. *Genes, Brain and Behavior*, Vol. 7, pp. 43-56.
- Schulte-Herbruggen, O., Jockers-Scherubl, M.C., & Hellweg, R. (2008). Neurotrophins: from pathophysiology to treatment in Alzheimer's disease. *Current Alzheimer Research*, Vol. 5, No. 1, pp. 38-44.
- Scott, J., Selby, M., Urdea, M., Quiroga, M., Bell, G.I., & Rutter, W.J. (1983). Isolation and nucleotide sequence of a cDNA encoding the precursor of mouse nerve growth factor. *Nature*, Vol. 302, No. 5908, pp. 538-540.
- Scott, S.A., Mufson, E.J., Weingartner, J.A., Skau, K.A., & Crutcher, K.A. (1995). Nerve growth factor in Alzheimer's disease: increased levels throughout the brain coupled with declines in nucleus basalis. *Journal of Neuroscience*, Vol. 15, No. 9, pp. 6213-6221.
- Segal, R.A. (2003). Selectivity in neurotrophin signaling: theme and variations. *Annual Review of Neuroscience*, Vol. 26, pp. 299-330.
- Shih, A., Laramée, G.R., Schmelzer, C.H., Burton, L.E., & Winslow, J.W. (1994). Mutagenesis identifies amino-terminal residues of nerve growth factor necessary for Trk receptor binding and biological activity. *Journal of Biological Chemistry*, Vol. 269, No. 44, pp. 27679-27686.
- Simi, A., & Ibanez, C.F. (2010). Assembly and activation of neurotrophic factor receptor complexes. *Developmental Neurobiology*, Vol. 70, No. 5, pp. 323-331.
- Soilu-Hanninen, M., Ekert, P., Bucci, T., Syroid, D., Bartlett, P.F., & Kilpatrick, T.J. (1999). Nerve growth factor signaling through p75 induces apoptosis in Schwann cells via a Bcl-2-independent pathway. *Journal of Neuroscience*, Vol. 19, No. 12, pp. 4828-4838.
- Soppet, D., Escandon, E., Maragos, J., Middlemas, D.S., Reid, S.W., Blair, J., Burton, L.E., Stanton, B.R., Kaplan, D.R., Hunter, T., Nikolics, K., & Parada L.F. (1991). The

- neurotrophic factors brain-derived neurotrophic factor and neurotrophin-3 are ligands for the trkB tyrosine kinase receptor. *Cell*, Vol. 65, No. 5, pp. 895-903.
- Susén K., Heumann, R., & Blöchl, A. (1999). Nerve growth factor stimulates MAPK via the low affinity receptor p75(LNTR). *FEBS Letters*. Vol. 463, No. 3, pp. 231-234.
- Susén, K. & Blöchl, A. (2005). Low concentrations of aggregated beta-amyloid induce neurite formation via the neurotrophin receptor p75. *Journal of Molecular Medicine*, Vol. 83, No. 9, pp. 720-735.
- Suter, U., Angst, C., Tien, C., Drinkwater, C., Lindsay, R., & Shooter, E. (1992). NGF/BDNF chimeric proteins: analysis of neurotrophin specificity by homolog-scanning mutagenesis. *The Journal of Neuroscience*, Vol. 12, No. 1, pp. 306-318.
- Teng, K. K., Felice, S., Kim, T., & Hempstead, B. L. (2010). Understanding proneurotrophin actions: Recent advances and challenges. *Developmental Neurobiology*, Vol. 70, No. 5, pp. 350-359.
- Thoenen, H., & Sendtner, M. (2002). Neurotrophins: from enthusiastic expectations through sobering experiences to rational therapeutic approaches. *Nature Neuroscience*, Vol. 5, No. 11s, pp. 1046-1050.
- Tuszynski, M.H., Conner, J., Blesch, A., Smith, D., Merrill, D.A., & Vahlsing, H.L. (2002). New strategies in neural repair. *Progress in Brain Research*, Vol. 138, pp. 401-409.
- Tuszynski, M.H., Thal, L., Pay, M., Salmon, D.P., U, H.S., Bakay, R., Patel, P., Blesch, A., Vahlsing, H.L., Ho, G., Tong, G., Potkin, S. G., Fallon, J., Hansen, L., Mufson, E. J., Kordower J. H., Gall, C., & Conner, J. (2005). A phase 1 clinical trial of nerve growth factor gene therapy for Alzheimer disease. *Nature Medicine*, Vol. 11, No. 5, pp. 551-5.
- Urfer, R., Tsoulfas, P., O'Connell, L., & Presta, L.G. (1997). Specificity Determinants in Neurotrophin-3 and Design of Nerve Growth Factor-Based trkC Agonists by Changing Central  $\beta$ -Strand Bundle Residues to Their Neurotrophin-3 Analogs. *Biochemistry*, Vol. 36, No. 16, pp. 4775-4781.
- Vilar, M., Charalampopoulos, I., Kenchappa, R.S., Reversi, A., Klos-Applequist, J.M., Karaca, E., Simi, A., Spuch, C., Choi, S., Friedman, W.J., Ericson, J., Schiavo, G., Carter, B. D., & Ibáñez, C. F. (2009). Ligand-independent signaling by disulfide-crosslinked dimers of the p75 neurotrophin receptor. *Journal of Cell Science*, Vol. 122, No. 18, pp. 3351-3357.
- Wajant, H., Gerspach, J., & Pfizenmaier, K. (2005). Tumor therapeutics by design: targeting and activation of death receptors. *Cytokine Growth Factor Reviews*. Vol. 16, No. 1, pp. 55-76.
- Walsh, G.S., Krol, K.M., Crutcher, K.A., & Kawaja, M.D. (1999). Enhanced neurotrophin-induced axon growth in myelinated portions of the CNS in mice lacking the p75 neurotrophin receptor. *Journal of Neuroscience*, Vol. 19, No. 10, pp. 4155-4168.
- Wang, J.J., Rabizadeh, S., Tasinato, A., Sperandio, S., Ye, X., Green, M., Assa-Munt, N., Spencer, D., & Bredesen, D.E. (2000). Dimerization-dependent block of the proapoptotic effect of p75(NTR). *Journal of Neuroscience Research*, Vol. 60, No. 5, pp. 587-593.
- Wiesmann, C., Ultsch, M.H., Bass, S.H., & de Vos, A.M. (1999). Crystal structure of nerve growth factor in complex with the ligand-binding domain of the TrkA receptor. *Nature*, Vol. 401, No. 6749, pp. 184-188.
- Wiesmann, C., & de Vos, A.M. (2001). Nerve growth factor: structure and function. *Cellular and Molecular Life Sciences*, Vol. 58, No. 5-6, pp. 748-759.

- Winkler, J., Ramirez, G.A., Kuhn, H.G., Peterson, D.A., Day-Lollini, P.A., Stewart, G.R., Tuszynski, M.H., Gage, F.H., & Thal, L.J. (1997). Reversible Schwann cell hyperplasia and sprouting of sensory and sympathetic neurites after intraventricular administration of nerve growth factor. *Annals of Neurology*, Vol. 41, No. 1, pp. 82-93.
- Woo, S.B., Timm, D.E., & Neet, K.E. (1995). Alteration of NH<sub>2</sub>-terminal residues of nerve growth factor affects activity and Trk binding without affecting stability or conformation. *Journal of Biological Chemistry*, Vol. 270, No. 11, pp. 6278-6285.
- Woo, S.B., & Neet, K.E. (1996). Characterization of histidine residues essential for receptor binding and activity of nerve growth factor. *Journal of Biological Chemistry*, Vol. 271, No. 40, pp. 24433-24441.
- Wood, J.N. (2010). Nerve Growth Factor and Pain. *New England Journal of Medicine*, Vol. 363, No. 16, pp. 1572-1573.
- Xie, Y., Tisi, M.A., Yeo, T.T., & Longo, F.M. (2000). Nerve Growth Factor (NGF) Loop 4 Dimeric Mimetics Activate ERK and AKT and Promote NGF-like Neurotrophic Effects. *Journal of Biological Chemistry*, Vol. 275, No. 38, pp. 29868-29874.
- Yang, T., Knowles, J.K., Lu, Q., Zhang, H., Arancio, O., Moore, L.A., Chang, T., Wang, Q., Andreasson, K., Rajadas, J., Fuller, G.G., Xie, Y., Massa, S.M., & Longo, F.M. (2008). Small Molecule, Non-Peptide p75<sup>NTR</sup> Ligands Inhibit A $\beta$ -Induced Neurodegeneration and Synaptic Impairment. *PLoS ONE*, Vol. 3, No. 11, pp. e3604.
- Yeo, T.T., Chua-Couzens, J., Butcher, L.L., Bredesen, D.E., Cooper, J.D., Valletta, J.S., Mobley, W.C., & Longo, F.M. (1997). Absence of p75<sup>NTR</sup> causes increased basal forebrain cholinergic neuron size, choline acetyltransferase activity, and target innervation. *Journal of Neuroscience*, Vol. 17, No. 20, pp. 7594-7605.
- Yoon, S.O., Soltoff, S.P., & Chao, M.V. (1997). A dominant role of the juxtamembrane region of the TrkA nerve growth factor receptor during neuronal cell differentiation. *Journal of Biological Chemistry*, Vol. 272, No. 37, pp. 23231-23238.
- Zaccaro, M.C., Lee, H.B., Pattarawarapan, M., Xia, Z., Caron, A., L'Heureux, P.-J., Bengio, Y., Burgess, K., & Saragovi, H.U. (2005). Selective Small Molecule Peptidomimetic Ligands of TrkC and TrkA Receptors Afford Discrete or Complete Neurotrophic Activities. *Chemistry & Biology*, Vol. 12, No. 9, pp. 1015-1028.
- Zhang, X., Gureasko, J., Shen, K., Cole, P.A., & Kuriyan, J. (2006). An Allosteric Mechanism for Activation of the Kinase Domain of Epidermal Growth Factor Receptor. *Cell*, Vol. 125, No. 6, pp. 1137-1149.
- Zhang, Y., Moheban, D.B., Conway, B.R., Bhattacharyya, A., & Segal, R.A. (2000). Cell surface Trk receptors mediate NGF-induced survival while internalized receptors regulate NGF-induced differentiation. *Journal of Neuroscience*, Vol. 20, No. 15, pp. 5671-5678.
- Zhao, M., Wan, Z. L., Whittaker, L., Xu, B., Phillips, N. B., Katsoyannis, P. G., Ismail-Beigi, F., Whittaker, J., & Weiss, M. A. (2009). Design of an insulin analog with enhanced receptor binding selectivity: rationale, structure, and therapeutic implications. *Journal of Biological Chemistry*, Vol. 284, No. 46, pp. 32178-32187.



## **Alzheimer's Disease Pathogenesis-Core Concepts, Shifting Paradigms and Therapeutic Targets**

Edited by Dr. Suzanne De La Monte

ISBN 978-953-307-690-4

Hard cover, 686 pages

**Publisher** InTech

**Published online** 12, September, 2011

**Published in print edition** September, 2011

Alzheimer's Disease Pathogenesis: Core Concepts, Shifting Paradigms, and Therapeutic Targets, delivers the concepts embodied within its title. This exciting book presents the full array of theories about the causes of Alzheimer's, including fresh concepts that have gained ground among both professionals and the lay public. Acknowledged experts provide highly informative yet critical reviews of the factors that most likely contribute to Alzheimer's, including genetics, metabolic deficiencies, oxidative stress, and possibly environmental exposures. Evidence that Alzheimer's resembles a brain form of diabetes is discussed from different perspectives, ranging from disease mechanisms to therapeutics. This book is further energized by discussions of how neurotransmitter deficits, neuro-inflammation, and oxidative stress impair neuronal plasticity and contribute to Alzheimer's neurodegeneration. The diversity of topics presented in just the right depth will interest clinicians and researchers alike. This book inspires confidence that effective treatments could be developed based upon the expanding list of potential therapeutic targets.

### **How to reference**

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Kenneth E. Neet, Sidharth Mahapatra, Hrishikesh M. Mehta (2011). Selectivity of Cell Signaling in the Neuronal Response Based on NGF Mutations and Peptidomimetics in the Treatment of Alzheimers Disease, Alzheimer's Disease Pathogenesis-Core Concepts, Shifting Paradigms and Therapeutic Targets, Dr. Suzanne De La Monte (Ed.), ISBN: 978-953-307-690-4, InTech, Available from: <http://www.intechopen.com/books/alzheimer-s-disease-pathogenesis-core-concepts-shifting-paradigms-and-therapeutic-targets/selectivity-of-cell-signaling-in-the-neuronal-response-based-on-ngf-mutations-and-peptidomimetics-in>

**INTECH**  
open science | open minds

### **InTech Europe**

University Campus STeP Ri  
Slavka Krautzeka 83/A  
51000 Rijeka, Croatia  
Phone: +385 (51) 770 447  
Fax: +385 (51) 686 166  
[www.intechopen.com](http://www.intechopen.com)

### **InTech China**

Unit 405, Office Block, Hotel Equatorial Shanghai  
No.65, Yan An Road (West), Shanghai, 200040, China  
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元  
Phone: +86-21-62489820  
Fax: +86-21-62489821



© 2011 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the [Creative Commons Attribution-NonCommercial-ShareAlike-3.0 License](https://creativecommons.org/licenses/by-nc-sa/3.0/), which permits use, distribution and reproduction for non-commercial purposes, provided the original is properly cited and derivative works building on this content are distributed under the same license.

IntechOpen

IntechOpen