Biochemical and Biophysical Research Communications 421 (2012) 286–290

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Polyubiquitination of the neurotrophin receptor p75 directs neuronal cell survival

Thangiah Geetha [a,1], Chen Zheng [a,1], Bradford Unroe [a], Marina Sycheva [a], Heidi Kluess [b], Jeganathan Ramesh Babu [a,*]

[a] Department of Nutrition, Dietetics, and Hospitality Management, Auburn University, Auburn, AL 36849, USA
[b] Department of Kinesiology, Auburn University, Auburn, AL 36849, USA

ARTICLE INFO

Article history:
Received 19 March 2012
Available online 7 April 2012

Keywords:
Neurotrophin receptor p75
NGF
Tyrosine phosphorylation
Ubiquitination
TRAF6
Cell survival

ABSTRACT

Specific binding of nerve growth factor (NGF) to p75 neurotrophin receptor ($p75^{NTR}$) leads to $p75^{NTR}$ polyubiquitination and its subsequent interaction with TRAF6 resulting in neuronal cell survival. However, when the binding of NGF to $p75^{NTR}$ was blocked with p75 antiserum, $p75^{NTR}$ polyubiquitination and neuronal cell survival were impaired. Results showed that tyrosine phosphorylation of $p75^{NTR}$ increased the polyubiquitination of $p75^{NTR}$ and contributed to the observed apparent neuroprotective effects. Similar to $p75^{NTR}$ polyubiquitination, interaction of TRAF6 with $p75^{NTR}$ was NGF/tyrosine phosphorylation dependent suggesting that TRAF6 might function as an E3 ubiquitin ligase. In sum, the results show that specific binding of NGF to $p75^{NTR}$ mediates neuronal cell survival.

Published by Elsevier Inc.

1. Introduction

Nerve growth factor (NGF) is a neurotrophin that regulates survival, differentiation, and maintenance of sensory and sympathetic neurons [1]. Neurotrophins can bind to two classes of cell-surface receptors, a high-affinity tyrosine kinase Trk receptor and a low-affinity $p75^{NTR}$ [2,3]. $p75^{NTR}$ is a member of the tumor necrosis factor (TNF) super family of receptors [4–6], and is involved in cellular apoptosis, cell survival, differentiation, neurite outgrowth [7–9], Schwann cell myelination, and cell development [5,10]. $p75^{NTR}$ contains four ligand-binding pockets in a cysteine-rich extracellular domain and its intracellular region possesses the chopper domain and TNFR-like death domain [11]. $p75^{NTR}$ has no intrinsic enzymatic activity; however, NGF leads to tyrosine phosphorylation of $p75^{NTR}$ and activates the MAPK pathway [12]. The tyrosine phosphorylation of $p75^{NTR}$ can also be induced by the phosphatase inhibitor pervanadate [13].

$p75^{NTR}$ regulates both neuronal cell survival and apoptosis [5,14,15], and can bind to several interacting proteins involved in its functions. The neurotrophin receptor interacting factor (NRIF), $p75^{NTR}$-associated death executor (NADE), and neurotrophin receptor interacting MAGE homologue (NRAGE) proteins bind to different regions of the $p75^{NTR}$ cytoplasmic domain and are involved in the induction of apoptosis [16–18]. $p75^{NTR}$ promotes the activation of sphingomyelinase and ceramide production that functions to activate the JNK kinase [19,20]. Interaction of TRAF6 with $p75^{NTR}$ enhances the cell survival [14,21], but the mechanism for this is unknown.

Ubiquitin is a 76 amino acid polypeptide; it covalently binds proteins through an isopeptide bond formed between its C-terminal glycine and the lysine residues of the substrate proteins. Three enzymes are required for ubiquitination. First, E1 (ubiquitin-activating enzyme) forms a high-energy thioester bond with the C-terminus of ubiquitin in an ATP-dependent reaction. Second, the activated ubiquitin is transferred to the cysteine residue in one of 22 known E2 (ubiquitin-conjugating enzyme) enzymes. Third, E3 (ubiquitin ligase) mediates the formation of an isopeptide bond with the lysine residue in the substrate proteins [22]. Substrates can be mono-ubiquitinated [23–25] or polyubiquitinated, [26–28] linked through each of the seven lysine residues present in ubiquitin (Lys^6, Lys^{11}, Lys^{27}, Lys^{29}, Lys^{33}, Lys^{48} and Lys^{63}) [29]. The function of Lys^{48}-linked ubiquitin chains is to target proteins for proteasomal degradation, whereas Lys^{63}-linked ubiquitin chains play a role in endocytosis, protein sorting, and receptor trafficking [30]. Previously, it was shown that $p75^{NTR}$ interacts with TRAF6 [14]. TRAF6 is an E3 RING ubiquitin ligase [31] that directs the synthesis of Lys^{63}-linked polyubiquitin chains [32] and is itself activated by Lys^{63}-linked polyubiquitination [32]. Moreover, $p75^{NTR}$ signaling is deficient in traf6−/− mice [15], suggesting that $p75^{NTR}$/TRAF6 may play a role in the regulation of $p75^{NTR}$ signaling.

Abbreviations: $p75^{NTR}$, p75 neurotrophin receptor; NGF, nerve growth factor; Ub, ubiquitination; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; TRAF6, tumor necrosis factor receptor-associated factor 6; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum.

* Corresponding author. Fax: +1 334 844 3268.
E-mail address: jeganrb@auburn.edu (J.R. Babu).

[1] These authors contributed equally to this work.

0006-291X/$ - see front matter Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.bbrc.2012.04.001

This prompted us to examine whether NGF binding to $p75^{NTR}$ and tyrosine phosphorylation of $p75^{NTR}$ could stimulate $p75^{NTR}$ polyubiquitination and lead to neuroprotection.

2. Materials and methods

2.1. Materials

Rabbit $p75^{NTR}$ and ubiquitin antibodies were purchased from Santa Cruz Biotechnology (La Jolla, CA). $p75^{NTR}$ antibody was purchased from Millipore (Billerica, MA) and rabbit TRAF6 antibody for Western blotting was obtained from Abcam (Cambridge, MA). Anti-phosphotyrosine (PY20) was purchased from BD Transduction Laboratories (San Diego, CA). NGF (2.5S) was from Bioproducts for Science (Indianapolis, IN). $p75^{NTR}$ antiserum (antibody 9561) directed against the third and fourth cysteine-rich repeats of the extracellular domain of mouse $p75^{NTR}$ was generously provided by Dr. Moses Chao, New York University School of Medicine.

2.2. Cell culture

The mouse hippocampal cell line HT-22 was a generous gift from Dr. David Schubert (The Salk Institute, La Jolla, CA) [33]. Cells were grown in Dulbecco's modified Eagle's medium (DMEM, Sigma, St. Louis, MO) supplied with 10% fetal bovine serum (FBS, Gibco, Grand Island, NY) as previously described [34]. Cells were incubated in a 5% CO_2 atmosphere at 37 °C. The cells were lysed with Triton lysis buffer to detect protein–protein interactions (50 mM Tris–HCl [pH 7.5], 150 mM NaCl, 10 mM NaF, 0.5% Triton X-100, 1 mM Na_3VO_4, 1 mM phenylmethylsulfonyl fluoride, and 2 μg/ml leupeptin and aprotinin) or SDS lysis buffer to detect covalent interaction of ubiquitin and $p75^{NTR}$ (Triton lysis buffer containing 1% SDS) [35]. Protein was estimated using the Bradford procedure (Bio-Rad) and with bovine serum albumin (BSA) as a standard for all samples except those that contained SDS. Protein was estimated in those samples using the DC assay (Bio-Rad).

2.3. Immunoprecipitation and Western blotting analysis

Cell lysates (1 mg) were diluted in lysis buffer and incubated with 4 μg of primary antibody at 4 °C for 3 h. The immunoprecipitates were collected with agarose-coupled secondary antibody for 2 h at 4 °C and then were washed three times with lysis buffer. Samples were boiled in sodium dodecyl-sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) sample buffer and resolved on 7.5–12% SDS–PAGE, transferred onto nitrocellulose membranes, and analyzed by Western blotting with the appropriate antibodies.

2.4. Cell survival assay

Cell survival was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Briefly, HT-22 cells were seeded in six-well plates in DMEM containing 10% FBS. The next day, cells were serum-starved and treated according to the experimental design. The cells were incubated in the presence of MTT, a substrate that is converted to a water-soluble formazan by a dehydrogenase enzyme present in metabolically active cells. The quantity of formazan product was determined by spectrophotometry at 490 nm. The results are shown as the percentage of live cells remaining under experimental conditions relative to that of controls (untreated) specified in the experiment.

3. Results and discussion

3.1. NGF Induces the ubiquitination of $p75^{NTR}$

We sought to explore whether NGF might induce $p75^{NTR}$ ubiquitination. HT-22 cells were treated with 100 ng/ml NGF for different times. The $p75^{NTR}$ ubiquitination was determined by immunoprecipitation with $p75^{NTR}$ antibody and Western blotting with anti-ubiquitin and anti-$p75^{NTR}$. NGF treatment for 10 and 20 min resulted in maximum $p75^{NTR}$ polyubiquitination (Fig. 1A), however, polyubiquitination was sustained above basal levels in the presence of NGF. Antiserum (antibody 9561) directed against the third and fourth cysteine-rich repeats of the extracellular domain of mouse $p75^{NTR}$ inhibits NGF neurotrophic activity by interfering with the NGF-$p75^{NTR}$ interaction [36]. HT-22 cells were treated with $p75^{NTR}$ antiserum and control non-immune serum (final dilutions of 1:100) in the presence or absence of NGF for 10 min. Results showed that $p75^{NTR}$ antiserum abrogated the $p75^{NTR}$ polyubiquitination (Fig. 1B). These suggest that $p75^{NTR}$ is ubiquitinated through a pathway that is activated by the binding of NGF to $p75^{NTR}$.

3.2. $p75^{NTR}$ blocking antibody impairs the interaction of $p75^{NTR}$ with TRAF6

TRAF6 functions as ubiquitin ligase and catalyzes the formation of noncanonical K63-linked polyubiquitin chains [32]. Because TRAF6 has been shown to interact with $p75^{NTR}$ [14,37], we sought to determine if that interaction could be recapitulated *in vivo* in the HT-22 cell line. Cells were stimulated with NGF followed by immunoprecipitation of $p75^{NTR}$ and Western blotting to detect $p75^{NTR}$ and TRAF6. Lysates were also Western blotted for $p75^{NTR}$ and TRAF6. As shown in Fig. 2A, p75 and TRAF6 showed maximum transient co-interaction at 10 and 20 min of NGF stimulation. Interestingly, we observed that this co-interaction (complex) of $p75^{NTR}$ and TRAF6 existed at the same time that $p75^{NTR}$ ubiquitination

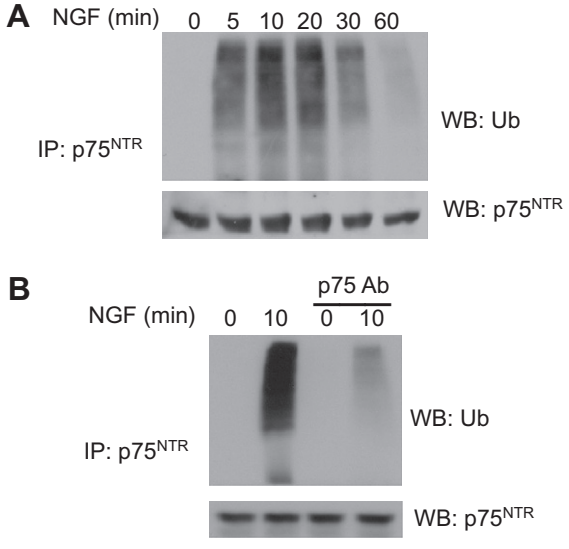

Fig. 1. NGF stimulates $p75^{NTR}$ polyubiquitination. (A) HT-22 cells were treated with NGF (100 ng/ml) for 0, 5, 10, 20, 30, and 60 min at 37 °C. Cells were lysed and ubiquitination was examined by immunoprecipitation (IP) with anti-$p75^{NTR}$ followed by Western blot (WB) analysis with anti-ubiquitin (Ub) and anti-$p75^{NTR}$. (B) HT-22 cells were treated with $p75^{NTR}$ antiserum ($p75^{NTR}$ blocking antibody) directed against the extracellular domain of $p75^{NTR}$ (1:100 final dilution) along with or without NGF (100 ng/ml) for 10 min. Ubiquitination was examined by immunoprecipitation (IP) with anti-$p75^{NTR}$ followed by Western blot (WB) analysis with anti-ubiquitin and anti-$p75^{NTR}$.

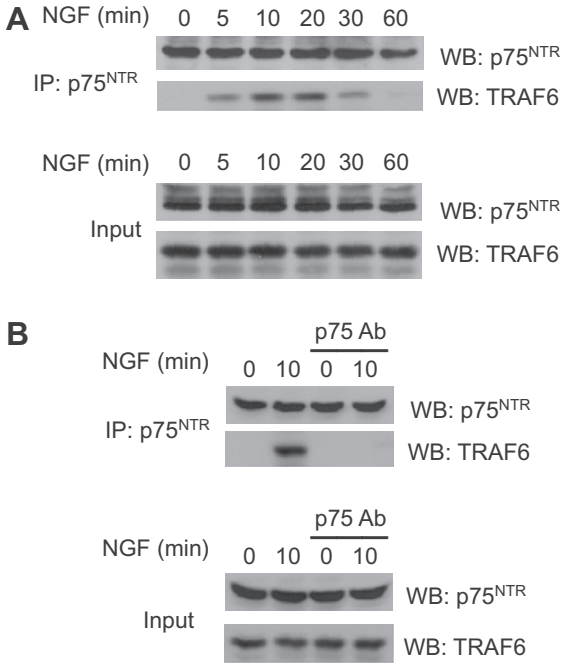

Fig. 2. p75^{NTR} interacts with TRAF6 upon NGF stimulation. (A) HT-22 cells were stimulated with NGF (100 ng/ml) for 0, 5, 10, 20, 30 and 60 min. Cells were lysed and p75 was examined by immunoprecipitation (IP) with anti-p75 and Western blot (WB) analysis with anti-p75^{NTR} and anti-TRAF6. (B) HT-22 cells were treated with or without p75^{NTR} blocking antibody along with NGF (100 ng/ml) for 0 and 10 min. The cell lysate was immunoprecipitated (IP) with anti- p75^{NTR} and Western blotted (WB) with anti-p75^{NTR} and anti-TRAF6. The cell lysate was also blotted with the same antibodies to verify the expression.

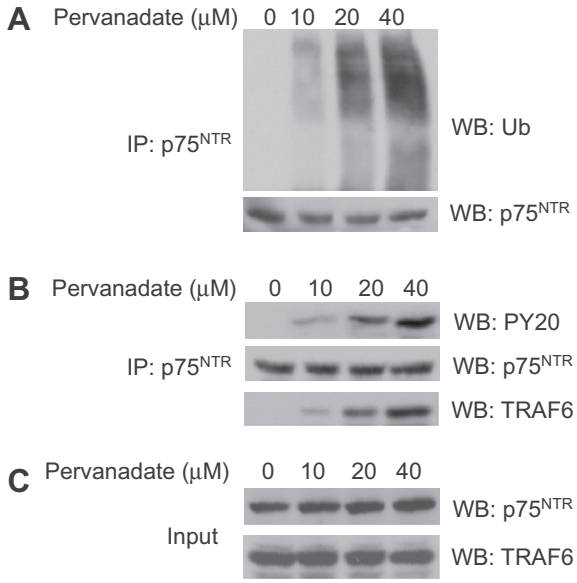

Fig. 3. Phosphorylation of p75^{NTR} enhances the p75^{NTR} polyubiquitination. (A) HT-22 cells were treated with increasing concentrations of pervanadate, lysed, and immunoprecipitated (IP) with anti-p75^{NTR}, followed by Western blot (WB) analysis with anti-ubiquitin (Ub) and anti-p75^{NTR}. (B) Pervanadate treated HT-22 cells were immunoprecipitated (IP) with anti-p75^{NTR} and Western blotted (WB) with PY20, p75^{NTR} and TRAF6 antibody. (C) To examine the input, the cell lysate was also examined by Western blot analysis with p75^{NTR} antibody and TRAF6 antibody.

was demonstrated. These results indicate that TRAF6 interaction may lead to p75^{NTR} ubiquitination. Next, we evaluated the contribution of NGF involved in the interaction of p75^{NTR} and TRAF6. p75^{NTR} antiserum that blocks the binding of NGF to the p75^{NTR} was treated to HT-22 cells in the presence or absence of NGF for 10 min. This was followed by p75^{NTR} immunoprecipitation and Western blotting with anti- p75^{NTR} and anti-TRAF6 (Fig. 2B). In control cells, p75^{NTR} interacts with TRAF6 upon NGF treatment. When the cells were treated with p75^{NTR} blocking antibody along with NGF, the co-interaction of p75^{NTR} with TRAF6 was impaired as shown in Fig. 2B. The results parallel the impairment of p75^{NTR} ubiquitination seen in the presence of the p75^{NTR} blocking antibody (Fig. 1B). These findings further support a model whereby TRAF6 interaction is necessary for p75^{NTR} ubiquitination.

3.3. Tyrosine phosphorylation of p75^{NTR} leads to p75^{NTR} ubiquitination and interaction with TRAF6.

Next, we analyzed whether p75^{NTR} tyrosine phosphorylation would lead to p75^{NTR} ubiquitination. Tyrosine phosphorylation of p75^{NTR} was induced by the phosphatase inhibitor pervanadate [13]. HT-22 cells were treated with increasing concentrations of pervanadate for 10 min, and the cell lysates were immunoprecipitated with anti- p75^{NTR} and Western blotted with anti-ubiquitin and anti- p75^{NTR}. Pervanadate (20 and 40 μM) treatment increased the p75^{NTR} ubiquitination as shown in Fig. 3A. In addition, we also determined whether an increase in tyrosine phosphorylation of p75^{NTR} would enhance its own interaction with TRAF6. The pervanadate treated cells were immunoprecipitated with p75^{NTR} antibody and analyzed for the co-interaction of p75^{NTR} with TRAF6 by Western blotting. As the pervanadate concentration increased, the tyrosine phosphorylation of p75^{NTR} increased as shown by Western blotting with phosphotyrosine (PY20) antibody. In addition, as the phosphorylation of p75^{NTR} increased, the co-interaction of TRAF6 with p75^{NTR} increased (Fig. 3B). The cell extracts were also Western blotted with anti- p75^{NTR} and anti-TRAF6 to check the expression of these proteins (Fig. 3C). The results suggested that p75^{NTR} ubiquitination and the subsequent interaction with TRAF6 depend upon the tyrosine phosphorylation of p75^{NTR}.

3.4. p75^{NTR} ubiquitination is a neuronal cell survival factor

p75^{NTR} can lead to neuronal cell survival or cell death [8,11]. To investigate the role of p75^{NTR} ubiquitination and its interaction with TRAF6, we determined whether NGF binding to p75^{NTR} is involved in neuroprotection. Under normal conditions, HT-22 cells are grown in DMEM containing 10% FBS, but serum starvation for 24 h induces cell death. However, we found that NGF treatment protected serum-starved HT-22 cells from death, perhaps by exerting a neuroprotective effect (Fig. 4A). When NGF binding to p75^{NTR} was blocked specifically by adding p75^{NTR} antiserum, the neuroprotective effect of NGF was abolished as shown in Fig. 4A. Additionally, an increase in the tyrosine phosphorylation of p75^{NTR} enhanced the p75^{NTR} ubiquitination and TRAF6 interaction (Fig. 3). Based on these observations, we set out to investigate whether tyrosine phosphorylation of p75^{NTR} also enhances the neuroprotective activity. Tyrosine phosphorylation of p75^{NTR} was induced by the addition of increasing concentrations of pervanadate to the serum-withdrawal media and cell death was monitored biochemically using the MTT assay (Fig. 4B). We discovered that the addition of pervanadate protected serum-starved HT-22 cells from cell death. In addition, as the concentration of pervanadate increased, it enhanced the ability of the cells to survive in parallel with the degree of p75^{NTR} tyrosine phosphorylation. Collectively, these results demonstrate that NGF and pervanadate enhance p75^{NTR} ubiquitination and the subsequent interaction with TRAF6 while also providing a neuroprotective effect.

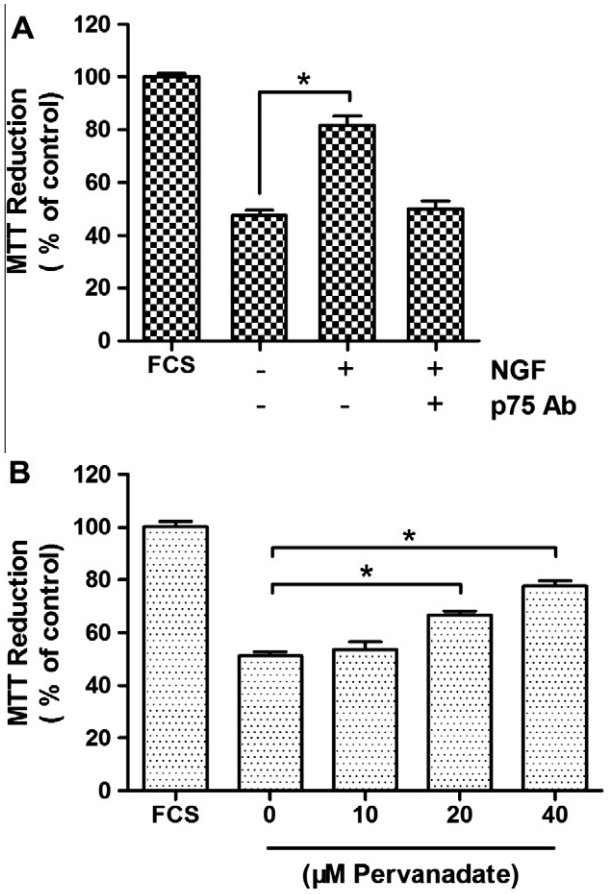

Fig. 4. NGF and pervanadate enhance cell survival. (A) Neuronal HT22 cells were serum-starved and incubated with NGF (50 ng/ml) for 24 h in the presence or absence of p75 blocking antibody. (B) HT22 cells were incubated with increasing concentrations of pervanadate in serum-starved media for 24 h. Cell death was assessed biochemically using the MTT assay. The mean and standard deviation of three experiments is shown ($^*P < 0.05$).

In the present study, we provide evidence that NGF induces $p75^{NTR}$ polyubiquitination and the subsequent $p75^{NTR}$ interaction with TRAF6 to regulate neuronal cell survival. NGF is known to bind both Trk receptor and $p75^{NTR}$ [35,38]. To confirm that NGF binding specifically to $p75^{NTR}$ can lead to $p75^{NTR}$ polyubiquitination and neuroprotection, we blocked the binding of NGF to $p75^{NTR}$ with $p75^{NTR}$ antiserum, which does not block NGF binding to the Trk receptor [36]. Interaction of TRAF6 with $p75^{NTR}$ is NGF dependent, as is $p75^{NTR}$ polyubiquitination, suggesting that TRAF6 might function as E3 ubiquitin ligase. Our results parallel those of Powell et al. [10], who suggested that $p75^{NTR}$ is a substrate for TRAF6-mediated ubiquitination. We also showed that tyrosine phosphorylation of $p75^{NTR}$ increases the polyubiquitination of $p75^{NTR}$ and subsequent interaction with TRAF6 and also contributes a neuroprotective effect. These observations indicate the signaling cascade can be represented as follows: NGF → $p75^{NTR}$ stimulation → tyrosine phosphorylation/$p75^{NTR}$ polyubiquitination/TRAF6 interaction → neuronal cell survival.

Acknowledgments

This work was supported by the Auburn University Intramural Grants Program (AU-IGP) to JRB. We thank Dr. Moses Chao, New York University School of Medicine for providing us the p75 antiserum (antibody 9561) and Dr. Catherine Wernette at Auburn University for editing this manuscript.

References

[1] R. Levi-Montalcini, The nerve growth factor 35 years later, Science 237 (1987) 1154–1162.
[2] S.O. Meakin, E.M. Shooter, The nerve growth-factor family of receptors, Trends Neurosci. 15 (1992) 323–331.
[3] L.A. Greene, D.R. Kaplan, Early events in neurotrophin signalling via Trk and p75 receptors, Curr. Opin. Neurobiol. 5 (1995) 579–587.
[4] W.J. Friedman, L.A. Greene, Neurotrophin signaling via Trks and p75, Exp. Cell Res. 253 (1999) 131–142.
[5] G. Khursigara, J. Bertin, H. Yano, H. Moffett, P.S. DiStefano, M.V. Chao, A prosurvival function for the p75 receptor death domain mediated via the caspase recruitment domain receptor-interacting protein 2, J. Neurosci. 21 (2001) 5854–5863.
[6] T. Geetha, R.S. Kenchappa, M.W. Wooten, B.D. Carter, TRAF6-mediated ubiquitination regulates nuclear translocation of NRIF, the p75 receptor interactor, EMBO J. 24 (2005) 3859–3868.
[7] Y. Chen, J. Zeng, L. Cen, X. Wang, G. Yao, W. Wang, W. Qi, K. Kong, Multiple roles of the p75 neurotrophin receptor in the nervous system, J. Int. Med. Res. 37 (2009) 281–288.
[8] V. Mamidipudi, M.W. Wooten, Dual role for p75(NTR) signaling in survival and cell death: can intracellular mediators provide an explanation?, J Neurosci. Res. 68 (2002) 373–384.
[9] A.W. Harrington, J.Y. Kim, S.O. Yoon, Activation of Rac GTPase by p75 is necessary for c-jun N-terminal kinase-mediated apoptosis, J. Neurosci. 22 (2002) 156–166.
[10] J.C. Powell, C. Twomey, R. Jain, J.V. McCarthy, Association between Presenilin-1 and TRAF6 modulates regulated intramembrane proteolysis of the p75NTR neurotrophin receptor, J. Neurochem. 108 (2009) 216–230.
[11] A. Diarra, T. Geetha, P. Potter, J.R. Babu, Signaling of the neurotrophin receptor p75 in relation to Alzheimer's disease, Biochem. Biophys. Res. Commun. 390 (2009) 352–356.
[12] K. Susen, R. Heumann, A. Blochl, Nerve growth factor stimulates MAPK via the low affinity receptor p75(LNTR), FEBS Lett. 463 (1999) 231–234.
[13] T. Ohrt, A. Mancini, T. Tamura, R. Niedenthal, C-Cbl binds to tyrosine-phosphorylated neurotrophin receptor p75 and induces its ubiquitination, Cell Signal. 16 (2004) 1291–1298.
[14] G. Khursigara, J.R. Orlinick, M.V. Chao, Association of the p75 neurotrophin receptor with TRAF6, J. Biol. Chem. 274 (1999) 2597–2600.
[15] E.C. Yeiser, N.J. Rutkoski, A. Naito, J. Inoue, B.D. Carter, Neurotrophin signaling through the p75 receptor is deficient in traf6-/- mice, J. Neurosci. 24 (2004) 10521–10529.
[16] A.H. Salehi, P.P. Roux, C.J. Kubu, C. Zeindler, A. Bhakar, L.L. Tannis, J.M. Verdi, P.A. Barker, NRAGE, a novel MAGE protein, interacts with the p75 neurotrophin receptor and facilitates nerve growth factor-dependent apoptosis, Neuron 27 (2000) 279–288.
[17] E. Casademunt, B.D. Carter, I. Benzel, J.M. Frade, G. Dechant, Y.A. Barde, The zinc finger protein NRIF interacts with the neurotrophin receptor p75(NTR) and participates in programmed cell death, EMBO J. 18 (1999) 6050–6061.
[18] J. Mukai, T. Hachiya, S. Shoji-Hoshino, M.T. Kimura, D. Nadano, P. Suvanto, T. Hanaoka, Y. Li, S. Irie, L.A. Greene, T.A. Sato, NADE, a p75NTR-associated cell death executor, is involved in signal transduction mediated by the common neurotrophin receptor p75NTR, J. Biol. Chem. 275 (2000) 17566–17570.
[19] R.T. Dobrowsky, M.H. Werner, A.M. Castellino, M.V. Chao, Y.A. Hannun, Activation of the sphingomyelin cycle through the low-affinity neurotrophin receptor, Science 265 (1994) 1596–1599.
[20] R.T. Dobrowsky, G.M. Jenkins, Y.A. Hannun, Neurotrophins induce sphingomyelin hydrolysis. Modulation by co-expression of p75NTR with Trk receptors, J. Biol. Chem. 270 (1995) 22135–22142.
[21] H. Kong, A.H. Kim, J.R. Orlinick, M.V. Chao, A comparison of the cytoplasmic domains of the Fas receptor and the p75 neurotrophin receptor, Cell Death Differ. 6 (1999) 1133–1142.
[22] J.D. Laney, M. Hochstrasser, Substrate targeting in the ubiquitin system, Cell 97 (1999) 427–430.
[23] J. Terrell, S. Shih, R. Dunn, L. Hicke, A function for monoubiquitination in the internalization of a G protein-coupled receptor, Mol. Cell. 1 (1998) 193–202.
[24] K. Haglund, S. Sigismund, S. Polo, I. Szymkiewicz, P.P. Di Fiore, I. Dikic, Multiple monoubiquitination of RTKs is sufficient for their endocytosis and degradation, Nat. Cell Biol. 5 (2003) 461–466.
[25] Y. Mosesson, K. Shtiegman, M. Katz, Y. Zwang, G. Vereb, J. Szollosi, Y. Yarden, Endocytosis of receptor tyrosine kinases is driven by monoubiquitylation, not polyubiquitylation, J. Biol. Chem. 278 (2003) 21323–21326.
[26] R. Govers, P. van Kerkhof, A.L. Schwartz, G.J. Strous, Linkage of the ubiquitin-conjugating system and the endocytic pathway in ligand-induced internalization of the growth hormone receptor, EMBO J. 16 (1997) 4851–4858.
[27] A.F. Roth, N.G. Davis, Ubiquitination of the yeast a-factor receptor, J. Cell Biol. 134 (1996) 661–674.
[28] R. Kolling, S. Losko, The linker region of the ABC-transporter Ste6 mediates ubiquitination and fast turnover of the protein, EMBO J. 16 (1997) 2251–2261.
[29] J. Peng, D. Schwartz, J.E. Elias, C.C. Thoreen, D. Cheng, G. Marsischky, J. Roelofs, D. Finley, S.P. Gygi, A proteomics approach to understanding protein ubiquitination, Nat. Biotechnol. 21 (2003) 921–926.
[30] K. Madura, The ubiquitin-associated (UBA) domain: on the path from prudence to prurience, Cell Cycle 1 (2002) 235–244.

[31] C.A. Joazeiro, A.M. Weissman, RING finger proteins: mediators of ubiquitin ligase activity, Cell 102 (2000) 549–552.
[32] L. Deng, C. Wang, E. Spencer, L. Yang, A. Braun, J. You, C. Slaughter, C. Pickart, Z.J. Chen, Activation of the IkappaB kinase complex by TRAF6 requires a dimeric ubiquitin-conjugating enzyme complex and a unique polyubiquitin chain, Cell 103 (2000) 351–361.
[33] Y. Li, P. Maher, D. Schubert, Requirement for cGMP in nerve cell death caused by glutathione depletion, J. Cell Biol. 139 (1997) 1317–1324.
[34] Z. Suo, M. Wu, B.A. Citron, R.E. Palazzo, B.W. Festoff, Rapid tau aggregation and delayed hippocampal neuronal death induced by persistent thrombin signaling, J. Biol. Chem. 278 (2003) 37681–37689.
[35] T. Geetha, J. Jiang, M.W. Wooten, Lysine 63 polyubiquitination of the nerve growth factor receptor TrkA directs internalization and signaling, Mol. Cell 20 (2005) 301–312.
[36] L.J. Huber, M.V. Chao, Mesenchymal and neuronal cell expression of the p75 neurotrophin receptor gene occur by different mechanisms, Dev. Biol. 167 (1995) 227–238.
[37] M.W. Wooten, M.L. Vandenplas, M.L. Seibenhener, T. Geetha, M.T. Diaz-Meco, Nerve growth factor stimulates multisite tyrosine phosphorylation and activation of the atypical protein kinase C's via a src kinase pathway, Mol. Cell Biol. 21 (2001) 8414–8427.
[38] M.V. Chao, The p75 neurotrophin receptor, J. Neurobiol. 25 (1994) 1373–1385.