Ildikó Molnár

Nervous, Immune, Endocrine Regulatory Systems and Diseases Associated with Nerve Growth Factor Co-Secretion

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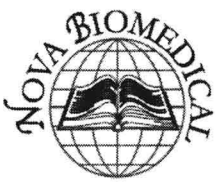

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ABBREVIATIONS

$A_{2A}AR$, $A_{2B}AR$: receptors for adenosine;
AC: adenylate cyclase;
ACTH: adrenocorticotropic hormone;
Akt: serine/threonine kinase;
Apaf-1: adapter protein;
APS: autoimmune polyglandular syndrome;
AR: adrenergic receptor;
Arrestin: regulatory protein;
ATP: adenosine triphosphate;
BDNF: brain-derived neurotrophic factor;
Bad, BAX, Bid: proapoptotic protein;
Bcl2, BclXI: antiapoptotic receptor;
cAMP: cyclic adenosine monophosphate;
C3a, C5: complement split products;
CCR_3: eotaxin receptor;
CD4+: helper CD4+ T lymphocyte;
CD8+: cytotoxic CD8+ T lymphocyte;
CD 27: tumor necrosis factor (TNF) receptor;
CD28, B7: co-stimulatory molecules;
CD30: cell membrane protein of the tumor necrosis factor (TNF) receptor family;
CD40: co-stimulatory protein on antigen-presenting cells;
CD95: Fas receptor;
CD178: Fas ligand;
CGRP: calcitonin gene-related peptide;

CNS:: central nervous system;
COS-7: African Green monkey SV40 transformed kidney fibroblast cells;
COX: cyclooxygenase;
CREB: cyclic AMP response element binding protein;
CRH: corticotropin-releasing hormone;
CRHR: corticotropin-releasing hormone receptor;
CTLA: cytotoxic T-lymphocyte antigen;
Cyt C: cytochrome C;
DAG: diacylglycerol;
DC: dendritic cells;
Deiodinase: enzyme for thyroid hormone conversion;
E: epinephrine;
EGF: epidermal growth factor;
ELAM: endothelial cell leukocyte adhesion molecule;
Eotaxin: chemoattractant;
ER: endoplasmic reticulum;
ERK: extracellular signal-regulated kinase;
FAK: focal adhesion kinase;
Fas: apoptosis antigen;
Fcε: IgE receptor;
FGF: fibrosis growth factor;
Forskolin: cAMP generation stimulating substance;
Foxp3: forkhead box P3;
Gab-1: adaptor molecule;
GITR: glucocorticoid-induced TNF receptor-related molecule;
GH: growth hormone;
GPCR: G protein-coupled receptor;
GR: glucocorticoid receptor;
Grb2: growth factor receptor-binding protein;
GRK: G protein receptor kinase;
GDP: guanosine diphosphate;
GTP: guanosine triphosphate;
HPA axis: hypothalamic-pituitary-adrenal axis;
HR: histamine receptor;
5-HT: 5-hydroxytryptamin;
ICAM: intercellular adhesion molecule;
IFNγ: interferron γ;
IgE: immunoglobulin E;

IGF-1: insulin-like growth factor-1;
IP_3: inositol trisphosphate;
IRS: insulin receptor substrate;
Htg: human thyroglobulin;
JNK: c-jun NH_2 terminal kinase;
LC-NA: locus ceruleus-noradrenergic system;
LH: luteinizing hormone;
LPA_2: phospholipase A_2;
LPS: lipopolysaccharide;
LTB_4: leukotriene B_4;
LTC4: leukotriene C4;
MAPK: mitogen-activated protein kinase;
MEK: MAPK or ERK kinase;
MHC: major histocompatibility complex;
MMP-9: metalloproteinase-9;
NE: norepinephrine;
NGF: nerve growth factor;
NKA: neurokinin A;
NFκB: nuclear factor kappa B;
NT-3: neurotrophin-3;
NT-4/5: neurotrophin-4/5;
NPY: neuropeptide Y;
$p75^{NTR}$: low-affinity neurotrophin receptor;
PAF: platelet-activating factor;
PC12 cells: pheochromocytoma cells;
PDGF: platelet-derived growth factor;
PGD_2, PGE_2, PGI_2: prostanoids;
PI-3K: phosphatidylinositol-3 kinase;
PIP_3: phosphatidylinositol 3,4,5-trisphosphate;
PKA: protein kinase A;
PKB: protein kinase B;
PKC: protein kinase C;
PLCβ: phospholipase Cβ;
PLCγ: phospholipase Cγ;
PLD: phospholipase D;
PPARγ: peroxisome proliferator-activated receptor γ;
PRL: prolactin;
RAF, RAS: oncogenes;

RGS: regulators for G protein signaling;
SP: substance P;
ROS: reactive oxygen species;
Shc: adapter protein;
SOS: son of sevenless protein;
Sortilin: apoptosis inducing receptor protein;
Src: regulatory protein;
T_3: triiodothyronine;
T_4: thyroxine;
TCR: T cell receptor;
TDZ: thiazolidinedione;
TGFβ: transforming growth factor β;
Th cell: T helper lymphocyte;
TLR: Toll-like receptor;
TNF: tumor necrosis factor;
TPO: thyroid peroxidase;
Tr1 cell: regulatory T lymphocyte releasing IL-10;
Treg cell: regulatory T lymphocyte;
TRH: thyrotropin-releasing hormone;
TRPV1: transient receptor potential V1;
TSH: thyroid-stimulating hormone;
TRAIL: TNF-related apoptosis inducing ligand;
TRAK: antibodies against TSH receptor;
Trk: tyrosine kinase;
VCAM: vascular cell adhesion molecule;
VEGF: vascular endothelial growth factor;
VIP: vasoactive intestinal peptide;

CONTENTS

Chapter 1

NERVE GROWTH FACTOR, ITS RECEPTORS AND THE SIGNALING MECHANISMS

ABSTRACT

Nerve growth factor (NGF) plays an essential role in the cellular interactions as a member of neurotrophins via controlling the balance between the cell survival and apoptosis, as well as in the differentiation of the motor and sensory neurons. The NGF-specific receptor activation via tyrosine kinase A (TrkA) differs from the activations induced by hormones and other growth factors, which exhibit mainly G protein-mediated protein kinase activations. Much data have been reported on a cross-talk or transactivation of the NGF receptors with the adrenergic or G protein-coupled receptors, which could be demonstrated in the receptor signaling mechanisms of the different receptor-mediated activations. The $p75^{NTR}$ receptor is involved in the regulation of the programmed cell death during the neurogenesis and the NGF withdrawal.

Furthermore a transactivation between the adenosine and the TrkA receptor activations have been demonstrated after tissue injury caused by hypoxia.

INTRODUCTION

NGF was discovered 50 years ago as a substance, which is necessary for the survival and differentiation of the sensory and sympathetic neurons. However, the actions of NGF are more diverse considering the receptor signaling cascades, the various tissue origins and the counterregulating responses. There is a cross-talk between the NGF-specific tyrosine kinase and the adrenergic, G protein-coupled receptors. This connection allows us to explain why an increased sympathoadrenal activity leads to a hypertrophy, a vascular abnormality or an abnormal adiposity.

NGF acts as a target-derived growth factor and it is generated from proNGF precursor. NGF exerts its pleiotropic effect after binding to its membrane receptors: to the high-affinity TrkA receptors and to the low-affinity $p75^{NTR}$ receptors. Three main signaling cascades are activated via TrkA receptors, which are involved in the cell survival, cell growth, synaptic plasticity and cell differentiation. $p75^{NTR}$ serves as a pro-apoptotic receptor playing an important role in the prenatal neurogenesis and the postnatal apoptotic events. The transactivations between the different receptor signaling mechanisms explain the neural effects in the cell hypertrophy, the metabolic state of the myocardium, the airway hypersensitivity and the neurogenic pain.

CONCLUSION

1. The Characteristics of Nerve Growth Factor

NGF is a member of the neurotrophin family as well as the family of the small proteins. NGF regulates the neuronal survival and promotes the neurite outgrowth and branching. It is essential in the differentiation of neurons, initiating its axonal transport in the sympathetic and sensory nervous systems [1]. NGF plays a relevant role in the maintenance of sympathetic neuroplasticity, the neuronal density as well as in the restitution after tissue injury [2,3]. NGF acts as a target-derived growth factor and is secreted from the innervated target tissues.

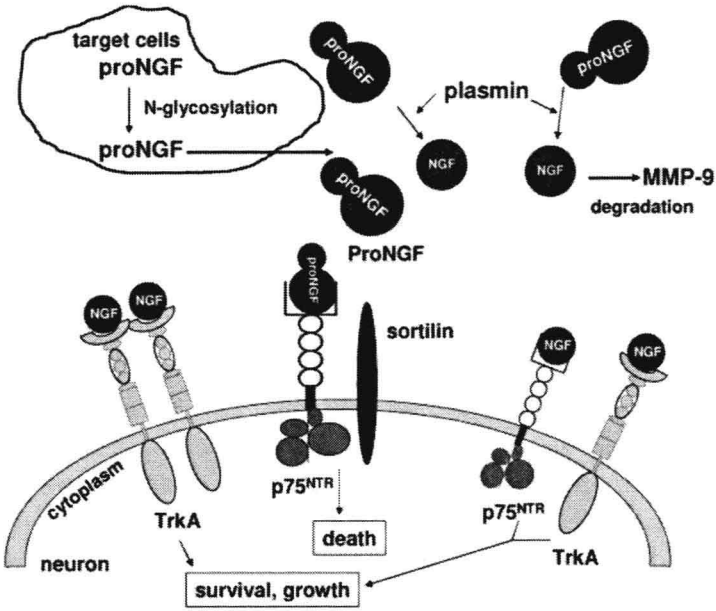

Figure 1. Nerve growth factor maturation and its receptors. Nerve growth factor (NGF) protein arises from proNGF via N-glycosylation and maturates after plasmin cleavage. The nonbinding form of nerve growth factor undergoes degradation via metalloproteinase-9. TrkA: tyrosine kinase receptor A; $p75^{NTR}$: low-affinity tyrosine kinase receptor; sortilin: apoptosis inducing receptor protein; MMP-9: metalloproteinase-9.

The mature form of the NGF is generated from its precursor: proNGF [4,5]. The synthesis of proNGF is derived from two alternative splicing of the preproNGF protein resulting in 25 and 32 kDa isoforms. The glycosylation of these isoforms, leads to the end form, proNGF with the molecular weight of 40 kDa (Figure 1). ProNGF is neurotoxic, so its elevation in the brain associates with neurodegeneration [6]. NGF derives from proNGF after its cleaves possessing a molecular weight of 13 kDa. The release of the proNGF occurs during the nerve stimulation, while the NGF production is caused by plasmin activity generated by proteases in the extracellular spaces [4]. Both forms - proNGF and NGF - can bind to receptors or degrade rapidly by the metalloproteinase-9 (MMP-9). The balance between the neuronal cell death and the growth or survival depends on the receptor signaling mechanisms induced by the binding of proNGF or NGF. Sortilin is an apoptotic receptor protein expressed broadly in the nervous system. Sortilin influences the balance to the direction of cell death through interacting with the $p75^{NTR}$

receptor [7,8]. NGF can exert its pleiotropic effect after bindig to the transmembrane receptors: TrkA and glycoprotein receptor of $p75^{NTR}$.

2. Nerve Growth Factor Receptors

2.1. Tyrosine Kinase (TrkA) Receptor

There are three types of tyrosine kinase receptors (TrkA, TrkB and TrkC) that transmit the cellular signaling processes after binding to neurotrophins, such as NGF: nerve growth factor, BDNF: brain-derived neurotrophic factor, NT3: neurotrophin-3 and NT4/5: neurotrophin-4/5 [9,10]. NGF binds to two different classes of transmembrane receptors: of these the tyrosine kinase type A is the specific, also called high-affinity receptor and it triggers the cell survival signaling events [11]. The genes of tropomyosin related to the tyrosine kinase A is localizated on chromosome 17 [12]. Two forms of TrkA can be demonstrated in the cell extracts: the 110 kDa N-glycosylated form ($gp110^{TrkA}$) and the 140 kDa fully maturated form ($gp140^{TrkA}$) [11,13]. The NGF binding leads to the dimerization and activation of TrkA, inducing the downstream phophorylation of the tyrosines and the adaptor proteins (Figure 2) [14,15]. From the synthesis to the degradation, the TrkA receptor undergoes internalization via the endocytic pathway that directs to various localizations within the cells; the TrkA receptor returns to the cell surface via the recycling pathway or degrades in the lysosomes [16-18]. The extracellular ligand-binding domain of the TrkA receptor contains multiple repeatitions of leucine-rich motifs, two cysteine clusters, and two immunoglobulin-like domains [1,19]. The binding specifity of the receptor is mostly determined by the second immunoglobulin-like domain. The catalytic and extracellular domains of the cytoplasmic tyrosine kinase show a high degree of identity with the neurotrophin receptors, approximately 80% for the intra- and 30 % for the extracellular domains, respectively [1].

The 140 kDa form of TrkA translocates rapidly to the cell surface, its half-life is 138 ± 4 minutes, which is shortened to 86 ± 8 minutes after NGF treatment [11,16]. NGF induces the clearing of TrkA receptors from the cell surface, by increasing their lysosomal degradation through the internalization. The expressions of both NGF receptors (TrkA and $p75^{NTR}$) exhibit dependency on the cell cycle phases (M: mitotic cell cycle, G1: cell cycle during interphase, S: synthesis phase, G2: cell cycle arrest) [20]. In PC12 cells (derived from pheochromocytoma cells of the rat adrenal medulla), TrkA is

expressed in the M and the early G1 phases but not in the late G1, S and G2 phases when $p75^{NTR}$ has expressed on the cell surface. The cellular pools of TrkA are found within the different cellular compartments.

The binding of NGF to TrkA initiates the autophosphorylation of the receptor-specific tyrosine residues; this contributes to the activation of phosphatidylinositol 3-kinase (PI-3K), mitogen-activated protein kinase (MAPK) and phospholipase Cγ (PLCγ) [21].

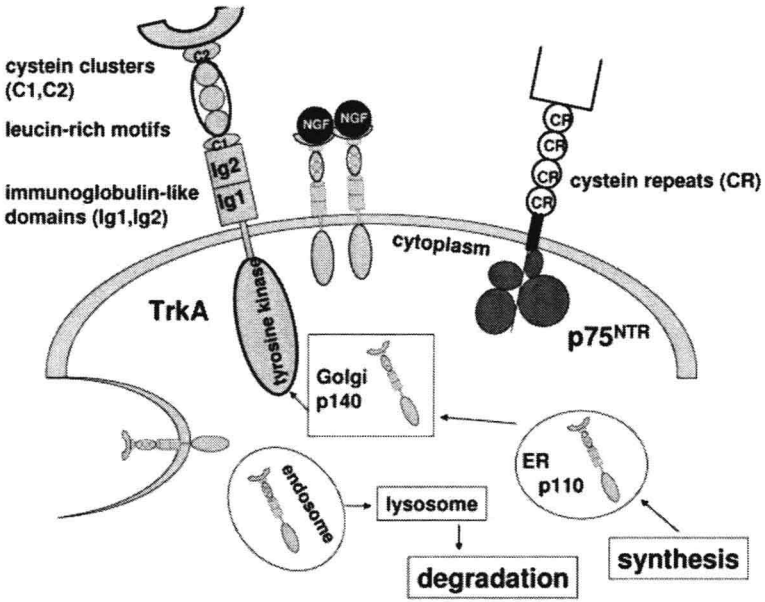

Figure 2. Nerve growth factor receptor trafficking. Tyrosine kinase receptor A (TrkA) undergoes internalization from synthesis to degradation. $p75^{NTR}$: low-affinity tyrosine kinase receptor; ER: endoplasmic reticulum.

2.2. $p75^{NTR}$ Receptor

The $p75^{NTR}$ receptor displays a structural and sequential similarity to the receptors of the tumor necrosis factor (TNF) family, which includes Fas (CD95, apoptosis protein), CD40 (co-stimulatory protein of the antigen-presenting cells), CD30 (cell membrane protein of the tumor necrosis factor receptor family) and CD27 (tumor necrosis factor receptor) [22-24]. The $p75^{NTR}$ receptor is mainly considered a death receptor, but its exact role has not been highligthed. The activation of the $p75^{NTR}$ receptor can modify the

activity of the TrkA receptor due to their co-expression on the cell surface [11,21]. The ligand-binding extracellular domain of the $p75^{NTR}$ receptor contains repeats of cysteine [1]. None of these receptors exhibit any intrinsic catalytic activity. Their cytoplasmic domain is identical to the death domain.

The Trk-independent effects of $p75^{NTR}$ have been demonstrated including the retrograde transport of the NGF-TrkA complex [25]. The gene expression of proteins involved in the cell migration, the cellular differentiation and the apoptosis events, can be regulated alone by $p75^{NTR}$ [21,26]. The importance of $p75^{NTR}$ was exhibited during the neurogenesis in the regulation of the programmed cell death [27,28]. The role of cell death could be confirmed in the developmental processes of the retina, optic nerve, spinal cord in mice with the generation of the retinal ganglion cells and the initial phase of the axonal elongation [29].

3. Nerve Growth Factor Receptor Signaling Mechanisms

3.1. Tyrosine Kinase Receptor A Signaling

Three main signaling cascades are activated by TrkA receptors and their substrates [30]. The first activation pathway is the RAS/RAF/MEK/MAPK (RAS, RAF: oncogens; MEK: MAPK or ERK kinase; MAPK: mitogen-activated protein kinase; ERK: extracellular signal-regulated kinase) for the development and differentiation of the neurons. The second is the activation of PI-3K (phosphatidylinositol-3 kinase) for the neuronal survival via PKB/AKT kinase activation (PKB: protein kinase B; Akt: serine/threonine kinase), membrane trafficking, proliferation, and differentiation. The third pathway is the activation of phospholipase Cγ for the neurotrophin-mediated neurotrophin release and the synaptic plasticity [1]. The activation of the same Trk receptors by the different ligands leads to distinct signaling events. The activation of the TrkA receptor alone is sufficient to induce morphogenic and survival signals [15]. Competitive signaling processes, which are responsible for the cell survival, could be demonstrated between the TrkA and $p75^{NTR}$ receptors [31]. The selective interplay between the tyrosine kinases and the cytokine receptors highlights a new, alternate mechanism during the cellular events [32].

A/ The binding of NGF to the TrkA receptors triggers the dimerization and recruitment of various adapter molecules, such as the Shc/Grb2/SOS complex (Shc: adapter protein, Grb2: growth factor receptor-binding protein

2; SOS: the son of sevenless protein) for the RAS/RAF/MEK/MAPK cascade (Figure 3) [21]. The adapter molecules and the PI-3K bind to the tyrosine residues located in the juxtamembrane region of the TrkA receptor. The phosphorylated tyrosine recruits phospholipase Cγ in the C terminal part of the TrkA.

NGF promotes the association of TrkA with the Shc adapter protein, eliciting the formation of the Shc/Grb2/SOS complex. The RAS oncogene activation is not receptor bound, and it contributes to the activation of ERKs. Other growth factors, such as the epidermal growth factor (EGF) and insulin, also activate the RAS oncogene and ERKs [33,34]. The MAPK cascade associates with the activation of the c-fos and c-jun effector transcription factors through the RAF, MEK and ERK cascades [35,36]. MAPK plays a central role in the control of cell growth. The activation of the RAS/ERK pathway via NGF leads to the induction of the immediate-early genes [37]. The MAPK pathway could be amplified and integrated by signals from extracellular stimuli (growth factors, hormones, inflammatory, environmental and oxidative stress, and cytokines) [32,38]. The phosphorylated tyrosines of Trk A bind to the Shc adapter protein and one domain of Grb2, which has no catalytic activity at the inner surface of the membrane [21]. Another domain of Grb2 binds to SOS protein. The RAS oncogene, the guanine nucleotide exchanging factor stimulates the replacement of GDP (guanosine diphosphate) with GTP (guanosine triphosphate). The RAS-GTP complex recruits the RAF oncogene to the membrane, promoting it to become an active phosphokinase. The active RAF oncogene initiates the MAPK signaling cascade (Figure 4). NGF leads to the activation of two distinct MAPK pathways: RAS/RAF/-MEK/ERK and p38. The p38 MAPK pathway contributes to the phosphorylation of the cAMP (cyclic adenosine monophosphate) response element binding protein (CREB). CREB and the RAS/ERK complex potentiate the synthesis of proteins.

B/ PI-3K in the NGF responses plays an additional signaling route. The insulin receptor substrates - IRS-1 and IRS-2 - binding to the Gab-1 adapter molecule activate PI-3K [39]. PI-3K does not act directly on the TrkA receptors. PI-3K is implicated in the neuronal survival signaling pathway via

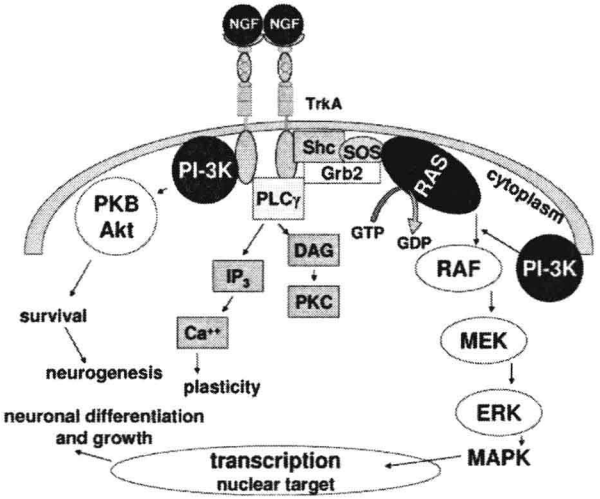

Figure 3. Nerve growth factor signaling cascades through tyrosine kinase A receptors. The binding of nerve growth factor (NGF) to its high-affinity tyrosine kinase receptor (Trk A) triggers the dimerization of the receptors, initiating three signaling pathways: RAS/RAF/ MEK/MAPK, PKB/Akt and PKC. PI-3K: phosphatidylinositol 3-kinase; IP_3: inositol trisphosphate; PLCγ: phospholipase Cγ; PKC: protein kinase C; DAG: diacylglycerol; Shc: adapter protein; SOS: son of sevenless protein; Grb2: growth factor receptor-binding protein; RAF, RAS: oncogenes; MEK: MAPK or ERK kinase; MAPK: mitogen-activated protein kinase; ERK: extracellular signal-regulated kinase; PKB: protein kinase B; Akt: serine/threonine kinase; GTP: guanosine triphosphate; GDP: guanosine diphosphate.

the activation of protein kinase B/Akt kinase, but it also plays a pivotal role in a wide range of cellular processes mediating a variety of extracellular stimuli, e.g. growth factors and hormones [40-42]. Akt is characterized as a serine/threonine kinase and a proto-oncogene product playing a role as a second messenger for the PI-3K [43]. Akt takes place in the various effector signaling routes, which control the proliferation, the migration, the invasion and the survival of cells. The PI-3K/Akt signaling pathway seems to be critical for the survival of endothelial cells, the migration and the cord formation which are relevant in angiogenesis [44]. PI-3K is composed of regulatory (p85) and catalytic (p110) subunits. The p85 regulatory subunit displays a common substrate for many upstream regulators. PI-3K phosphorylates the phosphatidylinositol 4,5-bisphosphate (PIP_2), producing phosphatidylinositol 3,4,5-trisphosphate (PIP_3), which acts as a second lipidmessenger in the stimulation of Akt.

C/ The association of phospholipase Cγ with TrkA leads to the regulation of the intracellular Ca2+ levels. It also leads to the enzyme activity of protein kinase C (PKC) via cleaving the substrate phosphatidylinositol 4,5-bisphosphate into diacylglycerol (DAG) and inositol trisphosphate (IP_3). The phospholipase Cγ pathway not only regulates the release of neurotrophin and the synaptic plasticity; it also regulates the synthesis of neuron-specific intermediate filament protein, the peripherin [45,46]. Diacylglycerol induces the activation of protein kinase C, while phosphatidylinositol 3,4,5-trisphosphate leads to a release of calcium from the intracellular stores.

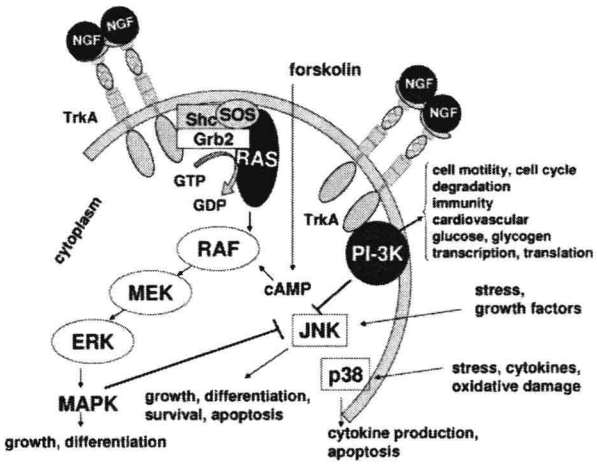

Figure 4. Mitogen-activated protein kinase cascade is modulated by various factors. The nerve growth factor (NGF) initiated mitogen-activated protein kinase (MAPK) signaling pathway is modulated by various factors influencing the cAMP and JNK activities, which may turn the signaling events into apoptosis. PI-3K: phosphatidylinositol 3-kinase; TrkA: tyrosine kinase receptor A; cAMP: cyclic adenosine monophosphate; Shc: adapter protein; SOS: son of sevenless protein; Grb2: growth factor receptor-binding protein; RAF, RAS: oncogenes; MEK: MAPK or ERK kinase; ERK: extracellular signal-regulated kinase; GTP: guanosine triphosphate; GDP: guanosine diphosphate; JNK: c-jun NH_2-terminal kinase; p38: mitogen-activated protein kinase; forskolin: cAMP generation stimulating substance; → : action direction; ⊣ : action inhibition.

3.2. Cell Signaling via $p75^{NTR}$ Receptor

$p75^{NTR}$ can bind to each neurotrophin and modify the affinity of the Trk receptors [1,14]. The precursor form of NGF displays high-affinity binding to $p75^{NTR}$, but the receptor is considered having low-affinity in comparison with