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Tropomyosin-Receptor-Kinases Signaling in the Nervous System

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ABSTRACT

The development and function of the nervous system is dependent on many growth factors and their signaling. Tropomyosin-receptor-kinase receptor family controls synaptic strength and plasticity in the mammalian nervous system. Dysregulation of Tropomyosin-receptor-kinase receptors signaling can lead to neural developmental disorders and has been reported in certain diseases of the nervous system. Apart from their role in the nervous system, these tyrosine kinase receptors are also involved in cancer biology. Tropomyosin-receptor-kinases and their ligands, neurotrophins, are also involved in neural precursor stem cells differentiation. This review focuses on Tropomyosin-receptor-kinases, the most abundant receptors in mammalian nervous system.

INTRODUCTION

Tropomyosin-receptor-kinase (Trk) receptors belong to a family of growth factor receptors with tyrosine kinase activity that controls synaptic strength and plasticity of the mammalian nervous system. Growth factor receptor tyrosine kinases (GFR-TK) are transmembrane proteins that are involved in cell growth, survival, differentiation and apoptosis.

The first tyrosine kinase receptor, Bcr-Abl, which is a result of the t(9;22)(q34;q11) translocation, was discovered more than three decades ago, by Janet Rowley (1). Since that moment on, many members of this family of cell-surface receptors have become known as key regulators of critical cellular processes, such as proliferation and differentiation, cell survival and metabolism, cell migration, and cell-cycle control (2-4). Over the past 30 years, a number of 58 GFR-TKs has been identified in human, which have been divided into 20 subfamilies (4).

There is high similarity in molecular structure of GFR-TKs, all of them consisting of li-

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gand-binding domains in the extracellular section of the protein, one transmembrane helix, a cytoplasmic section that has the protein tyrosine kinase activity and a regulatory domain, adjacent to the cell membrane (4). The whole topology of GFR-TKs, their mechanism and key components of the intracellular signaling pathways are highly preserved in evolution from the nematode *Caenorhabditis elegans* to humans. Moreover, many maladies are triggered by genetic alterations or abnormalities that modify the action, frequency, cellular spreading, or regulation of GFR-TKs. Diabetes and cancer are the most frequent diseases, related to abnormal function of GFR-TKs and their signaling transduction pathways.

GFR-TKs are activated by ligand binding through receptor dimerization (3). It is important to mention that a subset of GFR-TKs forms oligomers even in the absence of ligand, however, receptor activation required ligand binding.

For instance, the insulin receptor (IR) and insulin like growth factor 1 receptor (IGF-1R) are expressed on the extracellular side as disulfide-linked $(\alpha\beta)_2$ dimers (5). Structural changes within these dimeric receptors are induced by binding of insulin or IGF-1. These act on tyrosine kinase activity and cell signaling.

Epidermal growth factor (EGF) binding to pre-existing oligomers of its receptor, has been proposed by several reports (6,7). But, the exact nature and size of these oligomers have not yet been identified. Furthermore, activation of certain GFR-TKs, such as Tie2 and Eph receptors, may evidently require the formation of larger oligomers (8,9). Activation of the receptor still asks for the bound ligand to stabilize a specific relationship between individual receptor molecules in an "active" dimer or oligomer, even if the "inactive" state is monomeric or oligomeric. A more accurate view of how ligand binding can induce receptor dimerization has been provided by structural studies of the GFR-TK proteins. Although its exact role is not known yet, the single membrane-spanning α helix may participate to receptor dimerization in several cases. It has also been suggested that in the ligand- receptor complex association of the cell surface domain controls the intracellular regions to form a dimeric structure which in turn stimulates the catalytic tyrosine kinase domains.

A theoretically simple mechanism for ligand-induced dimerization was suggested to be the concomitant bivalent interactions between ligand and two receptors that can facilitate the cross-linking of the receptors into a single dimeric complex. This type of receptor dimerization is proposed to be held by crystal arrangements of several pieces of the ligand-binding domains from GFR-TKs bound to their appropriate ligands. This theory is supported by molecular structure of several receptors, such as: stem cell factor receptor KIT, (10), the Flt1 vascular endothelial growth factor (VEGF) receptor (11,12), the nerve growth factor (NGF)/neurotrophin receptor TrkA (13), Axl (14), Tie2 (8), and Eph receptors (9).

Tropomyosin-receptor-kinases and their ligands neurotrophins

Trks are part of the GFR-TK family of receptors, regulating synaptic strength and plasticity in the vertebrate nervous system (15). Like all GFR-TKs members, structure of Trks receptors consists of an extracellular ligand-binding domain, a transmembrane domain and a cytoplasmic section that have tyrosine kinase activity (Figure 1).

In general, neuronal existence and differentiation are dependent on all Trk receptors, through numerous signal cascades (16). The oncogene *trk* was first identified in colon cancer, and has been reported to be activated in approximately 25% of thyroid papillary cancer (17). Its identification led to the discovery of the first member of TRKs family, the TrkA (15).

Trk receptor family is composed by three transmembrane receptors TrkA, TrkB, and TrkC (15). Trk receptors are activated by neurotrophic factors known as protein nerve growth factors or neurotrophins that are present in a family of growth factors, critically involved in the function of the nervous system (18). In addition, the p75 neurotrophin receptor (p75NTR), which belong to the tumor necrosis factor (TNF) receptor family, is also activated by neurotrophins (19).

Neurotrophins are involved in neuronal development, growth, and differentiation in nervous system and play an important role in neurons apoptosis (20).

The neurotrophins family is composed of four classes of neurotrophic factors: Nerve Growth Factor (NGF), Brain Derived Neurotrophic Factor (BDNF), Neurotropin 3 (NT-3), and Neurotrophin 4/5 (NT-4/5) (18). There is a different binding affinity for each type of neu-

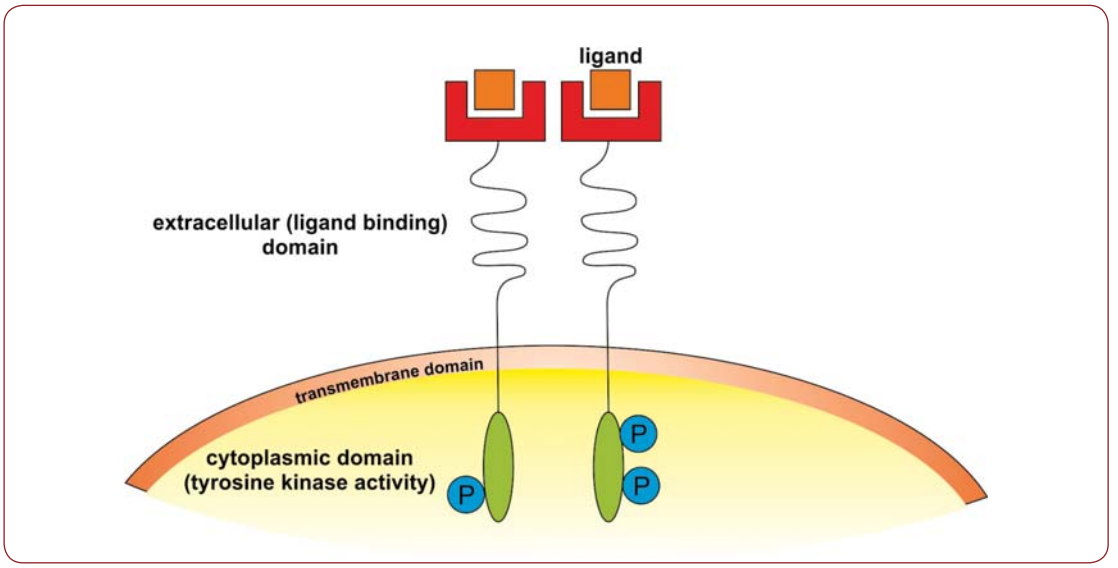

FIGURE 1. Structure of Trks Receptor.

rotrophin considering its corresponding Trk receptor. Binding of neurotrophin ligand to Trk leads to receptors activation, starting a signal cascades that result in promoting cell survival. The neurotrophins are produced in an immature undeveloped forms, proneurotrophins, and then changed in the final processed form, by intracellular protease cleavage. Undeveloped neurotrophins have high specificity to p75NTR. In a processed final form, neurotrophins, bind with low affinity to p75NTR and high affinity to their corresponding Trk receptors (18). Neurotrophins exercise their effect by binding to specific Trk receptors. The binding affinity of neurotrophins is specific to each Trk receptor, and generates different intracellular signaling. The differences in signaling are very important because they can generate a large variety of biological events.

There is a high affinity between TrkA and the binding nerve growth factor (NGF) (21,22). The NGF/TrkA complex undergoes internalization and endocytosis and then trafficked retrogradely from the periphery into the cell where it activates an NGF-dependent transcriptional program.

Several studies have reported that NGF binding to TrkA and p75, and both NGF/TrkA and NGF/p75 complexes, control neuronal survival, differentiation, and the level of innervation, during the development of the central and peripheral nervous system (21,22). The neurotrophin/TrkA complex promotes cell growth and survival *via* two major pathways: the Ras/MAPK and the PI3K/Akt signaling pathways (22,23).

Referring to TrkB, it has the highest affinity to the binding of BDNF and NT-4 (24). BDNF is a growth factor that plays important roles in the survival and function of neurons in the central nervous system. BDNF is also reported to improve the survival of neurons in the hippocampus and cortex, cholinergic neurons of the basal forebrain and affects the survival and function of neurons in the central nervous system, particularly in brain regions susceptible to degeneration in Alzheimer's Disease (25). The binding of the BDNF to TrkB, activates many intercellular proteins, which control motor neurons development, survival, plasticity, and apoptosis (26). Even if both BDNF and NT-4 have high specificity to TrkB, the BDNF/ TrkB and NT-4/ TrkB complexes exercise different function in the cell (27). For example, when BDNF expression was replaced by NT-4 in a mouse model study, the mouse exhibited reduced body weight and decreased fertility (27). Data from several research studies showed that BDNF enhances the differentiation of EGF-generated neuronal precursors (28).

In the recent studies, it has been demonstrated that TrkB receptor is associated with Alzheimer's disease (26).

TrkC was reported to be activated by binding to NT-3 and to have little affinity for other ligands. NT-3 was shown to be involved in both *in vivo* and *in vitro* viability and differentiation of neurons.

It was shown that proprioceptive sensory neurons express the majority of TrkCs. In an animal model, a substantial loss of proprioceptiveness was found in NT-4 knockout mice (18). The binding affinity and specificity of Trk receptors to their neurotrophins ligands is affected by p75NTR (29). Even if the dissociation constants of p75NTR and TrkA are almost the same, their kinetics is quite different. In a study by D Esposito et al, it has been reported that the formation of high-affinity binding sites on TrkA is prevented by mutations in genes that encode the cytoplasmic and transmembrane domains of either TrkA or p75NTR (30). The ligands binding to p75NTR is not demanded to support high-affinity binding. The conformation of TrkA, especially the form with high-affinity binding site for NGF, is influenced by the presence of p75NTR (18).

Apart from their role in the nervous system, the Trks are also involved in cancer biology. TrkA and p75 were reported to be expressed in human colon cancer and melanoma, respectively (31,32). NGF expression was found in sarcoma and its alteration was also found in other several cancer types: neuroblastoma, medulloblastoma, glioblastoma, prostate cancer, pancreatic carcinoma etc. (33-35).

Tropomyosin-receptor-kinase activation

The activation of Trks is induced by neurotrophins ligand binding or by transactivation, in response to G-protein coupled receptor signaling (36). In a study by Lee and Chao, it was reported that the ligands of the G-protein-coupled receptor can activate TrkA and TrkB, in the absence of NGF or BDNF (37).

Recently, Geng-Xian Shi et al. demonstrated that pituitary adenylate cyclase-activating polypeptide (PACAP), a neuropeptide with neurotrophic and neurodevelopmental properties, was involved in Src family kinase-dependent TrkA receptor transactivation by Rit GTP-ase and contributes to PACAP-dependent neuronal differentiation (38).

Trks activation can also occur in presence of adenosine and adenosine agonists, by a mechanism involving the adenosine A2A receptor. In addition, Trks transactivation by adenosine was suggested to increase neuronal cell survival (23, 39).

The mechanism behind Trks transactivation effects is not well understood, but the ability of several receptors to activate each other, was suggested to cross-activate several intracellular signaling proteins, such as the extracellular signal-regulated kinase (ERK) and mitogen-activated protein kinase (MAPK) (40).

Trk signal transduction includes several proteins: SH2 containing proteins, Grb2, APS, FRS2, and phospholipase Cg (PLC-g). Trk activation by neurotrophins was also shown to induce overphosphorylation of Src, which in turn activates the PI3K/Akt and the Ras-MAPK pathways (23,25,41).

Trks activation by neurotrophin has also been reported to induce phosphorylation of phospholipase C (PLC). The breakdown of lipids to diacylglycerol and inositol (1,4,5) is catalyzed by an enzyme, induced by this phosphorylation of PLC.

PI3 kinase or other protein kinase C (PKC) isoforms may be indirectly activated by diacyglycerol, whereas the release of calcium from intracellular stores is promoted by inositol (1,4, 5) (18).

The activation of Ras family of intracellular proteins (H-Ras and K-Ras) and RAP is determined by phosphorylation of tyrosine residues in the Trk receptors (18).

Two alternative MAP kinase pathways have been proved to be induced as a result of Trks activation, both of them being mediated by PI3K. In addition, PI3K activation by trk/neurotrophin complexes can also induce the activation of PDK-1 and Akt kinases pathways which, in turn, activate FRK, BAD, and GSK-3 signaling proteins (18).

The signaling pathway K-Ras, Raf1, and MEK 1,2 activates Erk 1,2 protein, while the B-Raf, MEK5, and Erk 5 pathway stimulates ERK5. It has also been reported that TrkA/NGF complex, preferential activates the Ras/MAPK pathway, while TrkC/NT3 complex activates the PI3 pathway coupling (18). The Trks activity is controlled by ubiquitination and recent studies which indicate that these receptors are differentially regulated by ubiquitination to modulate the survival of neurons (42).

After activation, Trk receptors are subjected to internalization process, undergoing axonal transport, endocytosis and finally they will be either recycled or degraded (43,44). Abnormal phosphorylation of Trks, induced by binding of neurotrophins, has been shown in several types of cancer, including CNS tumors. *In vitro* and *in vivo* evidence of Trk inhibitors efficacy in the treatment of cancer has been reported by many research groups (45-49). ❑

CONCLUSIONS

Tropomyosin-receptor-kinases are a family of transmembrane receptors with a key role in function and survival of the neurons in the central nervous system. Trks function is complex, their activation inducing many intracellular changes that regulate neuronal plasticity, neuronal regeneration and apoptosis, both *in vitro* and *in vivo*. Disregulation of Trks function has frequently been observed in neurodegenerative diseases, such as Alzheimer's and Parkinson's diseases. Trks are also involved in cancer biology. Trks alterations were found in several human neoplasms: neuroblastoma, medulloblastoma, glioblastoma, prostate cancer, pancreatic carcinoma. For this reason, many pharmacological strategies have been developed to inactivate TRKs or to suppress their expression in several diseases and disorders. ❑

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