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Actions of GDNF on Midbrain Dopaminergic Neurons: The Signaling Pathway

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1. Introduction

Parkinson's disease (PD) is a degenerative disease of the central nervous system (CNS) predominantly occurring in older adults, and is particularly common after the age of 60. The primary pathologic characteristics of PD are neuronal degeneration and cell death of dopaminergic (DA-ergic) neurons, leading to a decrease in the dopamine (DA) level in the striatum. Originally extracted and purified in 1993, glial cell line-derived neurotrophic factor (GDNF) is a novel neurotrophic factor which exerts neurotrophic effects upon DA-ergic neurons in the midbrain, sympathetic neurons in the superior cervical ganglion and motor neurons in the spinal cord. Specially, GDNF protects midbrain DA-ergic neurons, and promotes their differentiation and survival (Lin *et al.*, 1993). These studies provide impetus to delve more deeply into the underlying mechanisms of GDNF, and suggest potential future applications of GDNF. The present review focuses on the correlates of neurotrophic factors and PD treatments, and the mechanisms of action of GDNF.

2. Parkinson's disease and neurotrophic factors

2.1 Parkinson's disease

Parkinson's disease (PD) is also known as paralysis agitans. As a degenerative disease of the central nervous system (CNS), PD is characterized by static tremors, bradykinesia, myotonia and unstable posture. It is a disease normally occurring in older adults, especially after the age of 60. Thus far, the etiology and mechanisms underlying this disease have not been fully elucidated (Daly *et al.*, 1999), though most scholars support the dopamine hypothesis and the oxidative stress hypothesis of PD. According to the dopamine hypothesis, dopamine synthesis decreases following the pathologic degeneration of DA-ergic neurons in the substantia nigra pars compacta (SNpc), leading to a decrease in inhibition of acetylcholine in the corpus striatum and a relative increasing excitability of acetylcholine. The disequilibrium between dopamine and acetylcholine results in the hyper-functioning of the extrapyramidal system, thus leading to paralysis agitans. The oxidative stress hypothesis explains the causes of degeneration of DA-ergic neurons in the SNpc. In PD, hydrogen peroxide (H_2O_2) and superoxide anions, produced during the process of oxidative metabolism under oxidative stress, are catalyzed by Fe^{2+} in the SNpc, and then formed into hydroxy radicals with more significant noxious properties. Meanwhile, the activity of complex I of the mitochondrial respiratory chain in DA-ergic neurons decreases, causing

antioxidants (esp. glutathione) to disappear. At this point, the free radicals can no longer be removed. Thus, by oxidizing the lipoids of neurilemma, destroying the functions of the membranes and directly demolishing DNA in DA-ergic neurons, the free radicals cause the degeneration and death of the neurons.

2.2 Neurotrophic factors

Neurotrophic factors (NTFs), discovered at the end of the 20th century, are dissoluble polypeptides derived from tissues (e.g. muscles) which are innervated by nerves, or from astrocytes. They can promote and maintain the growth, survival and differentiation of specific neurons, as well as influence synaptic plasticity. More than twenty NTFs are known to exist, including brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF) and glial cell line-derived neurotrophic factor (GDNF). Sharing as much as 50 - 60% homology in amino acid sequence, NTFs are quite congenerous. In general, the NTFs enter the nerve terminal through receptor-mediated endocytosis, then transport to the cell soma by retrograde axoplasmic transport. There, they promote the synthesis of numerous proteins supporting the growth, development and functional integrity of neurons. Recently, some NTFs were found to be produced by neurons and transported to nerve endings by anterograde axoplasmic transport, where they play a role in supporting the integrity of shape and function in postsynaptic neurons.

Neurotrophic factors, especially GDNF, exert protective effects on DA-ergic neurons, correlating with the etiology of PD. Some studies have demonstrated that the amount of NTFs is decreased in PD patients, compared with control. It is highly likely that this decrease could lead to the degeneration and death of DA-ergic neurons and induce the clinical symptoms of PD. In addition, some scholars propose that the apoptosis of numerous neurons during the process of development may be related to a decrease in NTFs. Studies based on animal models have demonstrated that the application of NTFs-medium can increase the survival rate of fetal DA-ergic neurons and reduce apoptosis *in vitro* (Siegel & Chauhan, 2000). Currently, no evidence exists addressing whether defects of NTFs or decreased expression of receptors for NTFs occur during the pathogenic progress of PD.

3. The structure, expression and basic physiological properties of GDNF

3.1 The structure of GDNF

The GDNF family ligands (GFLs) belong to the transforming growth factor-β (TGF-β) super-family, and include four neurotrophic factors: glial cell line-derived neurotrophic factor (GDNF), neurturin (NRTN), artemin (ARTN) and persephin (PSPN). These factors possess structures similar to other factors in the TGF-β super-family, and function as homodimers (Ibáñez, 1998). GFLs transduce signals through a receptor system, which is a protein complex consisting of RET tyrosine protein kinase and the GFRα family of proteins, playing an important role in the biological processes of cell survival, differentiation and migration.

The GFLs are secretory proteins, derived from the precursor preproGFL. ProGFLs can be activated by proteolytic cleavage. The diffusion and local concentration of proGFLs are influenced by the combination of GFLs and the lateral chain of heparan sulfate in the extracellular matrix (Hamilton *et al.*, 1998). Despite the fact that the binding sites for the GFLs have been extensively studied, the gene regulation, mechanisms of secretion and the precursors leading to the activation of GFLs have not been fully elucidated (Golden *et al.*,

1999). Similarly, the protease responsible for the breakdown and activation of the GFL precursor has yet to be revealed. Recent evidence has uncovered the biological activities for the precursor of secretory neurotrophic factors (Lee *et al.*, 2001). The secreted NGF and BDNF are sheared outside the cell by serine protein kinases and selective matrix metalloproteinases. ProNGF has a high affinity for p75NTR, a receptor responsible for the apoptosis of neurons in culture media, and maintains mild activation of the pathway, accompanied by differentiation and survival of cells mediated by TrkA. Whether the proteolytic cleavage mediates the biological activities of the GFLs, and whether the functional GFLs are different from other ligands, are major areas of interest to current scholars.

Of the 4 members of the GFLs, GDNF and NRTN were the first to be extracted. Subsequently, ARTN and PSPN were identified through basic data analysis and the use of homogeneous clones (Baloh *et al.*, 2000). The GDNF gene for both humans and mice has been cloned and expressed in prokaryotic and eukaryotic vectors. The human GDNF gene is located on chromosome 5p13. 1-13. 3, and is composed of two exons and one intron. In humans and mice, two types of GDNF mRNA with different lengths exist: a large fragment of 633bp and small fragment of 555bp, encoding for polypeptides of GDNF precursors with 211 amino acids and 185 amino acids, respectively. After the removal of 26 amino acids in the N terminal, the large fragment becomes smaller and the two fragments (large and small) are eventually transformed into polypeptides consisting of 134 amino acids (Trupp *et al.*, 1995). The mature protein of GDNF consists of 7 cysteine residues, and as such, 3 intrachain disulfide bonds are formed between the sites of amino acids 41 and 102, 68 and 131 and 72 and 133. The cysteine residues in the side chain of amino acid 101 form interchain disulfide bonds, thus forming the structure of the homodimer of GDNF (Haniu *et al.*, 1996). The structural features are quite similar to that of the TGF-β family members. Two glycosylation sites lie in the polypeptide chain, which has a molecular weight of 20 kD. The molecular weight of the natural GDNF dimer, which contains a heparan biding site, is 40 - 45 kD. X-ray methods have demonstrated that the structure of rat GDNF contains two finger-like structures lying in the rostral and caudal parts. Additionally, the amino acids in the middle form a helical structure; two monomers are connected by a pair of disulfide bonds, and the four finger-like structures form a plane (Eigenbrot *et al.*, 1997) (Figure 1). GDNF has conservation in evolution, as evidenced by the significant similarities between humans and mice in amino acid sequences in the mature proteins of GDNF (as high as 93%). Human GDNF, produced by genetic engineering, exerts activities on murine DA-ergic neurons. These lines of evidence indicate that GDNF has cross-species activity.

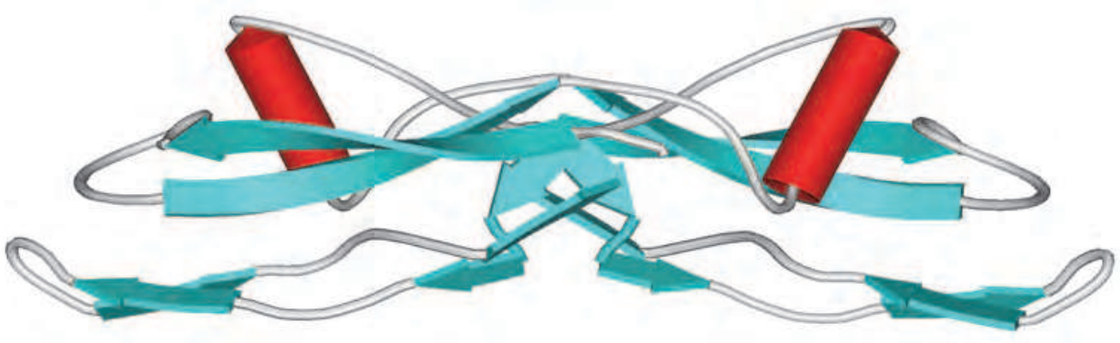


Fig. 1. The molecular structure for the crystal monomer of GDNF.

The monomer structure of GDNF includes a central α-spiral and β-fold (A and B). The amino acids of A include two reverse and parallel β-folds. The C-terminal of B contains four β-folds. The dimer, with a reverse and parallel structure, presents a symmetrical receptor binding site. A and B are crucial binding sites for GFRα1.

3.2 GDNF distribution and expression

In mammalian brains, GDNF is mainly expressed in the target cells of DA-ergic neurons in the midbrain substantia nigra, such as granular cells in the corpora striatum, nucleus accumbens, thalamic nuclei, tuber olfactorium, hippocampus, cerebellum, callosal gyrus and olfactory bulb, where it acts as the original neurotrophic factor (Sonntag *et al.*, 2005). In the kidneys and gastrointestinal tract, GDNF is expressed in media cells and smooth muscle cells, respectively. Additionally, GDNF induces the differentiation of neural crest cells and Wolffian ducts in intestinal neurons and renal ducts, respectively (Bohn *et al.*, 2000). The peripheral expression of GDNF can also been seen in tissues innervated by sensory neurons (trigeminal ganglion, nodosal ganglion and dorsal root ganglion) and autonomic neurons. The distribution of GDNF in the human brain, which is identical to that in the rat brain, includes high expression in the caudate nucleus (innervated by the substantia nigra), low expression in the dorsal caudate putamen and no expression in the substantia nigra (Schaar *et al.*, 1994). GDNF has also been reported to be expressed in the hippocampus, cortex and spinal cord (Springer *et al.*, 1994). In general, the GDNF expression in peripheral organs is higher than that of neuronal tissues.

3.3 The functions of GDNF

In the CNS, GFLs maintain the survival and activity of DA-ergic neurons in the midbrain and motor neurons of the cornu anterius medullae spinalis. In addition, GDNF, NRTN and ARTN maintain the differentiation and survival of some peripheral neurons, such as sympathetic neurons, parasympathetic neurons, sensory neurons and neurons in the intestinal wall. Outside of the nervous system, GDNF plays vital roles in the processes of kidney development and spermatic production, along with many other processes in the body (Airaksinen & Saarma, 2002; Manié *et al.*, 2001; Airaksinen *et al.*, 1999).

In the midbrain of adult animals, GDNF promotes the survival of DA-ergic neurons (Springer *et al.*, 1994). Some scholars have proposed that GDNF plays an essential role in supporting the survival and maintenance of mature DA-ergic neurons (Pascual *et al.*, 2008). Microinjection of 6-OHDA into the corpora striatum leads to the degeneration of DA-ergic neurons in the substantia nigra. This degeneration can be attenuated by pretreatment with GDNF in the substantia nigra, thus abrogating the injury induced by 6-OHDA and hindering the death of DA-ergic neurons. Other evidence has confirmed that GDNF promotes the regeneration of DA-ergic neurons after brain injury (Beck *et al.*, 1995; Tomac *et al.*, 1995; Hou *et al.*, 1996), which correlates with the fact that GDNF promotes the differentiation of DA-ergic neurons after brain injury. For example, GDNF promotes increase of the cell body, prolongation of axons and an increase in dopamine synthesis. Additionally, studies have demonstrated that GDNF can selectively protect DA-ergic neurons against the neurotoxic effects exerted by methylamphetamine (Cass, 1996). Thus far, GDNF, as a potential therapeutic agent for PD, has been studied extensively (Azzouz *et al.*, 2004; Eslamboli *et al.*, 2005). Further research has demonstrated that GDNF can modulate neuronal excitability and transmitter release (Wang *et al.*, 2003; Kobori *et al.*, 2004). For

example, *in vitro*, GDNF can rapidly activate the MAPK signaling pathway after acting on midbrain DA-ergic neurons, and increase neuronal excitability (Yang *et al.*, 2001). In addition, the GDNF signaling system can mediate the migration and chemotaxis of neurons (Tang *et al.*, 1998). Some researchers have also demonstrated that GDNF can potentiate analgesia in neuropathic pain (Sakai *et al.*, 2008). Further, GDNF plays critical roles in the neuronal development of sympathetic and motor neurons, and synapse formation in the hippocampus (Moore *et al.*, 1996; Ledda *et al.*, 2007).

4. The signal pathway for GDNF activation

4.1 GDNF receptors and structures

4.1.1 GFRα

The biological actions exerted by GDNF on cells must be mediated via trans-membrane signal transmission, i.e. membrane receptors, as GDNF is a secreted protein (Josso & di Clemente, 1997). The receptor system for GFLs is divided into two parts. One part, a binding receptor, anchors to the cell surface via GPI and binds to GDNF directly. The identified GFLs include GFRα1, GFRα2, GFRα3 and GFRα4, each with its own binding receptor. For example, GDNF binds to GFRα1, NRTN binds to GFRα2, ARTN binds to GFRα3 and PSPN binds to GFRα4. The other part of the GFLs receptor system consists of the trans-membrane signal transduction receptor, RET, as well as a tyrosine kinase receptor. In the process of signal transduction, the members of the GDNF family share the same RET, but a distinct GFRα (Airaksinen *et al.*, 1999). The homology between GFRα1 and GFRα2 is as high as 48%, and both are expressed in the neurons and tissues of the superior cervical ganglion and dorsal root ganglion (Baloh *et al.*, 1997). The expression of GFRα3 is strictly limited, and is not seen in the CNS (Widenfalk *et al.*, 1998). In contrast, GFRα1 and GFRα2 are widely expressed in the CNS and peripheral organs (Nomoto *et al.* 1998). The expression of GFRα4 has only been observed in avian cells (Saarma & Sariola, 1999). In addition, GFRα contains three corresponding cysteine-rich domains, with different lengths for three structural domains. The second domain binds to GFL, while the functions of the first and third domains are not clear. The first domain is not involved in the binding of RET and GFRα1, and is not expressed in GFRα4.

4.1.2 RET

RET is a trans-membrane tyrosine kinase receptor encoded by the oncogene *Ret*. *Ret* was first discovered in mice NIH/3T3 cells. Identical to other tyrosine kinase receptors, the RET protein is composed of an extracellular ligand-binding domain, a trans-membrane domain and an intracellular functional tyrosine kinase domain. The RET protein contains 21 exons and many spliceosomes, with four cadherin-like domains and a cysteine-rich domain.

RET can be expressed as many sub-types based on the selective splicing of the primary *Ret* transcript (Tahira *et al.*, 1990; Lorenzo *et al.*, 1995). Two subtypes, RET9 and RET51, have been further studied, and the functional difference between the two is ascribed mainly to structural differences in the tails. The C-terminal end of RET9 contains nine amino acids, and the entire protein consists of 1072 amino acids. RET51 is longer than RET9, with a total of 1114 amino acids, including 51 amino acids in the tail. Both RET9 and RET51 have Tyr1062 in their C-terminal. Tyr1062 is phosphorylated during RET activation and anchoring of the receptor protein. Some researchers have demonstrated the regulation of lateral sequences in the C-terminal of Tyr 1062, which leads to different activities of the two

subtypes (Wong *et al.*, 2005). Moreover, RET51 contains two tyrosine residues, Tyr 1090 and Tyr1096, which interact with SHC and GRB2, respectively (Lorenzo *et al.*, 1997; Alberti *et al.*, 1998; Borrello *et al.*, 2002).

RET, a common signal transduction receptor for GFLs, mediates many signal pathways for GDNF and induces apoptosis (Bordeaux *et al.*, 2000), and also inhibits tumor growth (Cañibano *et al.*, 2007) when GDNF is absent. The mutation of *Ret* may lead to diseases with functional depletion. Activating mutations correlate with thyroid cancer and type-II familial polyendocrine adenomatosis. A deactivating mutation of *Ret* is the cause of Hirchsprung disease. In adult animals, the depletion of RET leads to the depletion of DA-ergic cells in the nigrostriatal system. This indicates that RET is a key factor in maintaining the nigrostriatal system. The degradation of RET occurs through Cb-1 ubiquitin ligase mediated receptor recruitment and ubiquitination in the proteasome pathway.

4.1.3 NCAM

In the CNS, the regions of expression of GFRα1 and RET are not identical, e.g. GFRα1, but not RET, is expressed in the cortex and hippocampus (Trupp *et al.*, 1997; Glazner *et al.*, 1998; Golden *et al.*, 1998; Burazin & Gundlach, 1999; Golden *et al.*, 1999). The absence of RET expression in these regions indicates the existence of RET-independent GDNF signal pathways. In 2003, Paratcha *et al.* demonstrated that GDNF has the ability to transmit signals through directly binding to NCAM (Paratcha *et al.*, 2003). NCAM belongs to the cell adhesion molecule super family, and is encoded by a singular gene consisting of 26 exons (Lin *et al.*, 1993). The selective splicing of the gene can produce almost 120 NCAM subtypes, three of which are named NCAM-120, NCAM-140 and NCAM-180. These three subtypes possess an identical extracellular N-terminus, which contains five immunoglobulin-like domains and two domains located on the cell surface that are homologous with type-III fibronectin. Both NCAM-140 and NCAM-180 are trans-membrane proteins possessing the same trans-membrane domains but different intracellular domains. NCAM-120 has no trans-membrane or intracellular domains, and anchors to the extracellular surface via GPI. NCAM-180 and NCAM-120 are expressed predominantly in fetal neurons and neuroglia, respectively, while NCAM-140 is expressed in both (Noble *et al.*, 1985). Further research has demonstrated that the NCAM-140 subtype mediates the biological activities of GDNF. NCAM is not only involved in the adherence of cells, but also induces the intracellular signal transduction, and thus promotes axon growth and neuronal survival (Ditlevsen *et al.*, 2008) and inhibits the proliferation of astrocytes (Krushel *et al.*, 1998). The action of GDNF on DA-ergic neurons in the midbrain can be attenuated by blocking the function of NACM using anti-NACM antibodies, indicating that GDNF is activated via an NACM-transmitted signaling pathway, even in brain regions that express RET (Chao *et al.*, 2003).

4.1.4 Integrins and N-cadherin

Integrins are a family of cell surface receptors of cell adhesion molecules (CAMs). As a transmembrane glycoprotein, integrin is distributed on many types of cells, and can interact with extracellular ligands, the intracellular cystoskeleton and signal molecules to integrate the intra-with the extracellular environmental signals. Integrin is a heterodimer composed of an α and a β subunit, coupled by non-covalent bonds. Studies have indicated that integrin assembles some protein complexes via its β subunit, which is responsible for the specificity of those proteins and the subsequent signals. FAK, a non-receptor tyrosine

kinase, plays an important role in this process. First, FAK binds to corresponding proteins in the C terminus and phosphorylates itself. The phosphorylated tyrosine then binds to an SH2 domain in Src. Next, many amino acids sites in FAK are phosphorylated by Src which initiates a series of signals. The extracellular segment of the α subunit can bind to other proteins on the membrane, which would, in turn, bind to other SHCs. The phosphorylated tyrosines in SHC then bring the Grb2-SOS complex to the cell membrane and activate Ras. Integrins can bind to many extracellular matrix proteins, including laminin and fibronectin, which play vital roles in cell movement, adherence, synaptogenesis, proliferation, apoptosis, neural development and the inflammatory response (Chao *et al.*, 2003; Anton *et al.*, 1999). Some studies have indicated that GDNF is effective at increasing the expression of integrin αV, and the trans-membrane signal may include integrin β1. Hence, integrin αV and integrin β1 are thought to be candidate receptors for GDNF (Cao *et al.*, 2008).

N-cadherin, a member of the cadherin (cell adhesion molecule) super-family, is widely distributed in the CNS, and has a molecular weight of 130 kD. Cadherin super-family molecules are cell surface glucoproteins whose main functions include the regulation of calcium-mediated cell adhesion, cell polarity and morphogenesis, and involvement in the mechanisms underlying intercellular recognition and signal transduction. The recognition of N-cadherin is derived from its actions on cellular differentiation and growth in the CNS. N-cadherin is a canonical trans-membrane protein, with five extracellular segments (cadherin domains) composed of 110 amino acid residues. Research has demonstrated that the entire extracellular segment of N-cadherin is very similar to that of RET (Anders *et al.*, 2001) (Figure 2). The extracellular segments of N-cadherin can interact with catenin and other proteins, so as to regulate intracellular adhesion and recombination, and promote the survival and migration of cells.

Accumulating evidence has demonstrated that both fibroblast growth factor (FGF) and epidermal growth factor (EGF) can exert their actions by binding to N-cadherin via their receptors. Recently, our studies (using plasmid construction and cell transfection) have demonstrated that inhibition of the biological functions of N-cadherin may influence the protective effects exerted by GDNF upon DA-ergic neurons. At the same time, we discovered that GDNF can bind to N-cadherin or RET directly (data not shown). Further studies should be undertaken to determine whether GDNF activation of downstream signal pathways is dependent on the interaction of its receptor and N-cadherin (a process that is identical to FGF and EGF).

The distance from the neuronal adherent proteins' domain to the cell membrane is similar to that of RET. The encoding of the RET residues is consistent with the naïve RET molecule. The black solid line indicates the inserted sequence for the RET extracellular segment. The black solid circle indicates coherent calcium binding sites. CD = binding site for cadherin, TK = tyrosine kinase, Cys = cysteine-rich domain, Cyt = cytoplasmic domain (adapted from Anders *et al.*,,2001)

4.1.5 Heparan sulfate glycosaminoglycans

Aside from RET and GFRα1, heparan sulfate proteoglycans, including syndecan and glypican, are essential in mediating the signal pathway of GDNF (Barnett *et al.*, 2002). Bespalov *et al.* demonstrated that syndecan, exerts its function via binding to heparan sulfate with high affinity (Bespalov *et al.*, 2011).

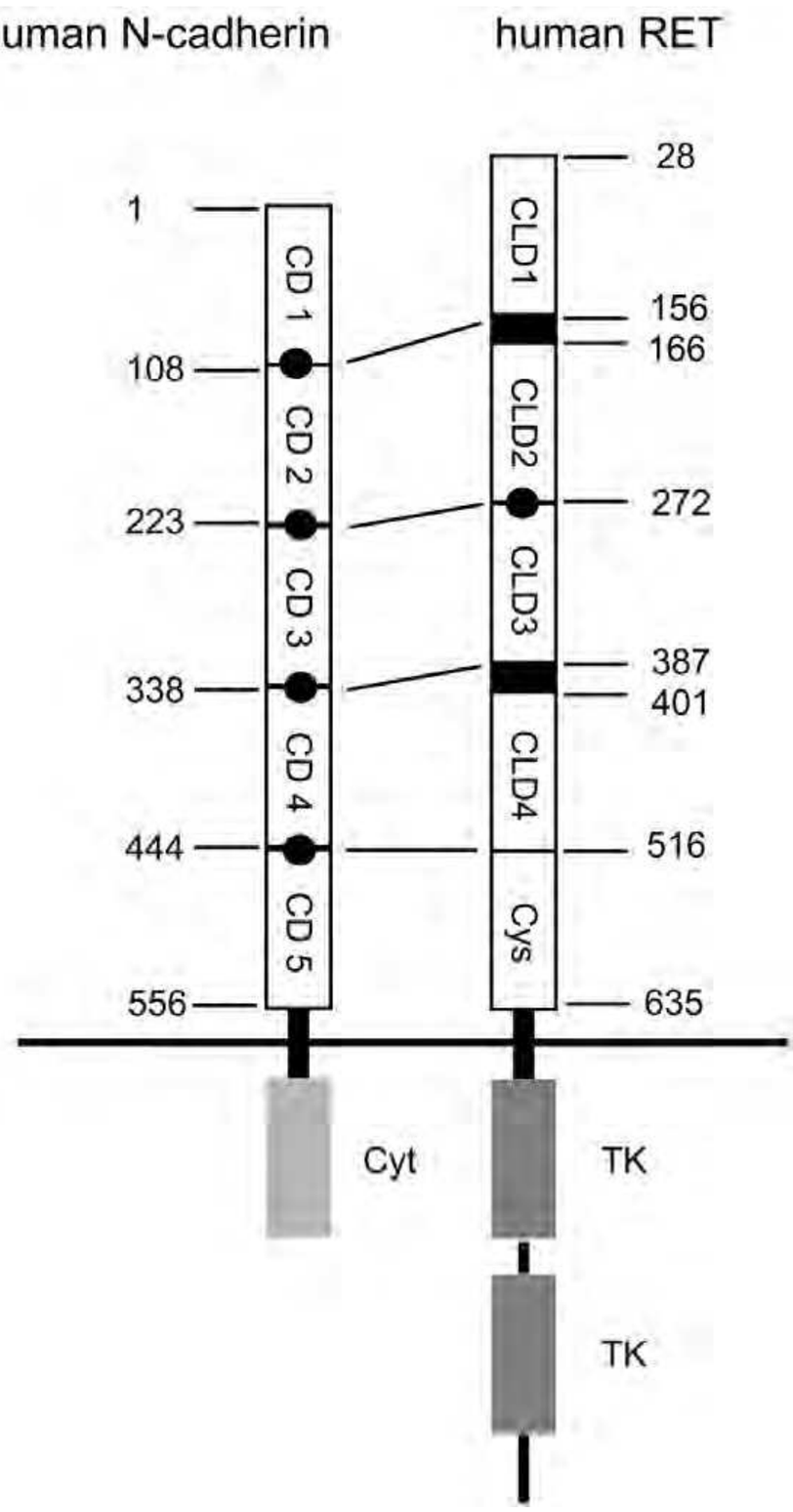


Fig. 2. The comparison of human neuronal adherent proteins and the RET extracellular segment.

GDNF mediates axonal growth and promotes neuronal migration via binding to syndecan 3. Of course, syndecan 3 may mediate the GFL signaling pathway, or may submit GFLs to the RET receptor pathway. With regard to other receptors, activation of the MET tyrosine kinase receptor by heparan sulfate proteoglycans is essential for the NCAM mediated GDNF pathway (Sariola & Saarma, 2003). The effects of heparan sulfate proteoglycans on the integrin αV/β1 and N-cadherin mediated GDNF signal pathway is under investigation.

The paucity of heparan sulfate proteoglycans may inhibit GDNF-dependent RET phosphorylation, GDNF mediated axon growth and differentiation of endothelial cells (Barnett *et al.*, 2002). Substantial evidence in mice indicates that a lack of syndecan or GDNF may lead to a decrease in GABA-releasing neurons, which implies that the two molecules are involved in cortex development.

4.1.6 Heat shock protein 27

Heat shock protein 27 (HSP27) is a protein with high conservation that is selectively synthesized after being stimulated. It resides ubiquitously in the cell membrane, cytoplasm and nucleus of all prokaryotes and eukaryotes. HSP27 is involved in maintaining microfilaments, signal transduction of cytokines, maintaining the integrity of the cell membrane under stimuli and protection of cells against some stress injuries (Nakamoto & Vígh L, 2007). In 2009, Hong Z *et al.* discovered that 92 proteins were altered under the action of GDNF; among the altered proteins, the phosphorylation of HSP27 significantly increased, accompanied by nuclear translocation. The GDNF induced axonal growth of PC 12 cells is significantly inhibited by interference of HSP27 mRNA, which implies that many proteins are involved in GDNF-mediated neuronal differentiation of DA-ergic neurons. HSP27 is a novel signaling molecule involved in GDNF-mediated axon growth of DA-ergic neurons (Hong *et al.*, 2009).

4.2 RET-dependent signaling pathway

GFRα1 and RET are expressed in the DA-ergic neurons of the substantia nigra and ventral tegmental area, as well as in the α-motor neurons of the ventral spine and motor nuclei in the brain stem, such as the hypoglossal nucleus, facial nucleus, nuclei of the trigeminal nerve and the nucleus nervi abducentis (Glazner *et al.*, 1998). RET is mainly located in non-lipid regions where GDNF action is absent (Paratcha *et al.*, 2001).

GDNF activates RET via two pathways, in cis and in trans. When expressed in the same cell, the combination of GFRα1-RET is mediated by a cis signaling system (Yu *et al.*, 1998; Paratcha *et al.*, 2001), delineated as: (1) activation of the cis system, (2) GDNF binds to GPI-anchored GFRα1 on the membrane, (3) c-RET is recruited and activated, which is independent of tyrosine kinase activity. On the other hand, when GFRα1 is dissoluble, its binding to GFRα1-RET via a trans signaling system is delineated as: (1) GDNF and dissoluble GFRα1 (sGFRα1) are released from consecutive cells, (2) activation of extracellular c-RET, (3) the activated receptors couple and phosphorylate SHC, (4) c-RET is recruited to the membrane via activation of its tyrosine kinase and phosphorylation of Tyr-1062. Compared with the cis system, the recruitment of RET to the membrane mediated by dissoluble GFRα1 is delayed and persistent, and is dependent on the activation of domains of the RET self-kinase (Yu *et al.*, 1998; Paratcha *et al.*, 2001).

RET is recruited to the membrane and binds to GDNF-GFRα1 via the two pathways mentioned above. After recruitment to the membrane, a dimer is formed, which is followed by self-phosphorylation of RET intracellular segments (rendered as tyrosine kinase activity), recruitment of downstream signal molecules and activation of several intracellular signal pathways via a series of enzymatic reactions (Airaksinen *et al.*, 1999). In this way, cell survival, differentiation, proliferation, migration and chemotaxis are regulated. The binding site of SHC receptor proteins, tyrosine 1062, plays vital roles in the activated intracellular signal cascade reactions. After binding to the intracellular tyrosine of RET, SHC forms a complex with the GAB1/2 receptor protein and GRB2/SOS, and induces activation of the PI3K/Akt and RAS/ERK signaling pathways, respectively. After combination of tyrosine 1096 and GRB2, GAB1/2 binds to the p85 subunit of PI3K, causing the activation of PI3K (Airaksinen & Saarma, 2002). GDNF induces the formation of neuronal lamellar parapodia, which is related to the formation of the neural axis (Van Weering & Bos, 1997) and DA-ergic differentiation *in vitro* (Pong *et al.*, 1998). Jun N-terminal Kinase (JNK) is activated by Rhc/Ras-related small molecules (Chiariello *et al.*, 1998), such as GTPase and CDC42. As key regulatory factors for survival of neurotrophin-dependent neurons, JNK and PI3K/Akt may be involved in the trophic action of GDNF (Kaplan & Miller, 1997). Tyrosine 981 binds to Src, and activated p60Src is a key factor for GFL-induced signal cascade reactions. The GDNF signaling pathway regulates axonal epitaxy, distribution of ureters and neural survival (Airaksinen & Saarma, 2002). After binding to tyrosine 1015, PLC is activated and hydrolyzes the second messenger produced by IP_3, which increases intracellular calcium and activates multi-signal transmissions such as gene expression. The Ras-MAPK and PI3K/Akt signal pathways are involved in DA-ergic neurons, and GDNF activates cAMP/PKA/CREB in brain neurons in the fetus. The activation of Src kinase and PLC is involved in sensory neurons (Sariola & Saarma, 2003) (Figure 3). RET possesses other tyrosine residues, including Tyr687, Tyr826 and Tyr 1029, which correlate with GFL binding. The mechanisms involved in signal transmission, however, are still unclear.

RET, but not GFR, is expressed in the cerebellum, olfactory tubercles and nuclei in the hypothalamus. This could be explained by the hypothesis that the actions of GFR are independent on RET, and GFR is only active in trans-formation (Saarma & Sariola, 1999).

The extracellular domain of RET contains four cadherin-like structures and a cysteine-rich region. Phosphorylated Y1062 is the binding site for several proteins, which subsequently activates the RAS/ERK, JNK and PI3K/Akt signal pathways via binding to SHC, FRS2, IRS1/2, Dok1-5 and Enigma. Phosphorylated Y905, Y981, Y1015 and 1096 bind to GRB7/10, Src, PLCγ and GRB2, respectively, and mediate a series of signal pathways. (Adapted from Wells & Santoro, 2009).

4.3 RET-independent signaling pathway

In spite of the vital roles played by RET-mediated signaling pathways, the GFRα1-RET signal system is not an essential pathway for neuronal development (demonstrated in knockout mice). Recently, a series of studies has demonstrated that GDNF may exert its biological actions via RET-independent signaling pathways.

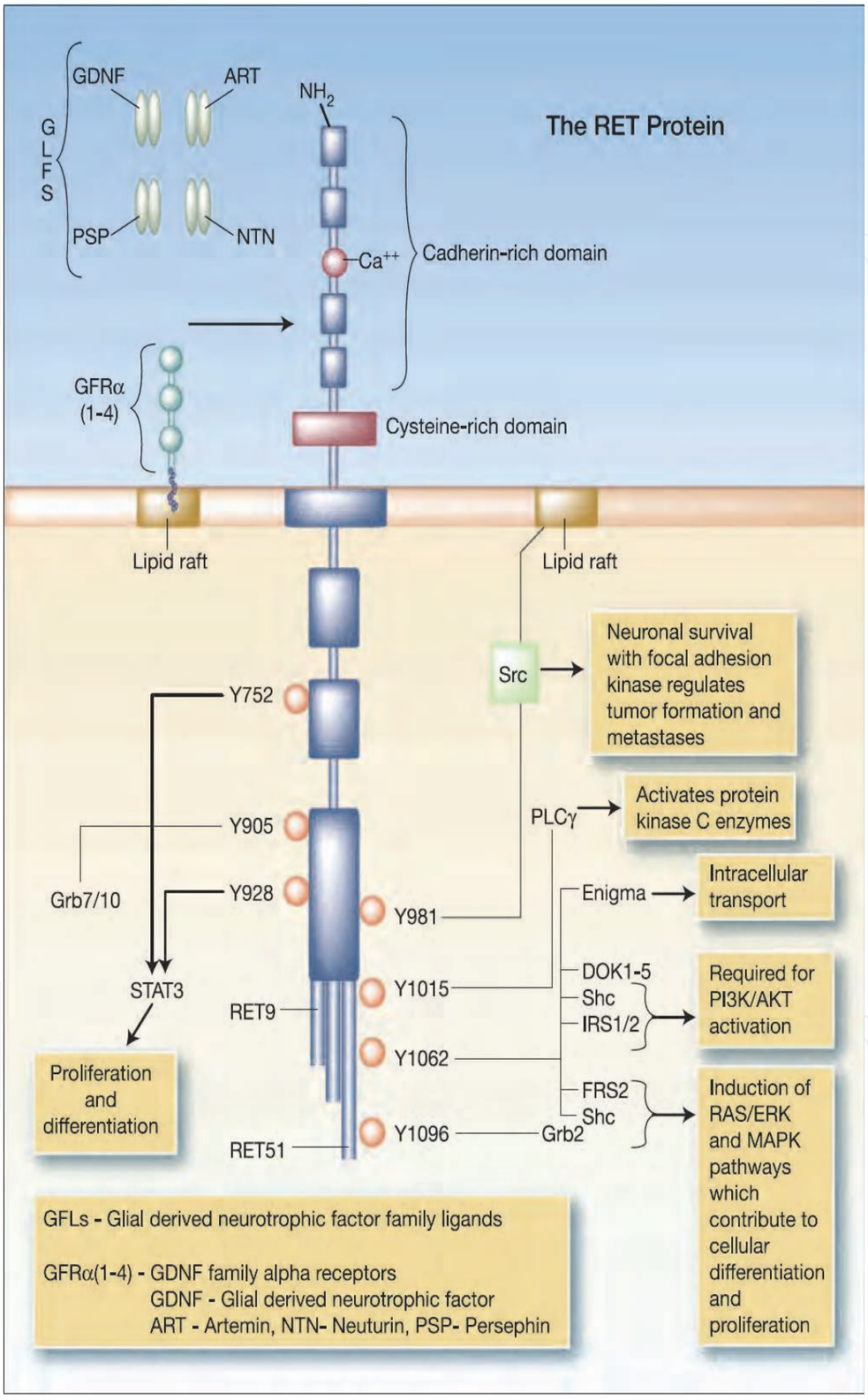


Fig. 3. The structure of RET and its signal pathway mediation.

4.3.1 GDNF and Src

As mentioned previously, the activation of Src family kinases mediated by GDNF is independent of RET. Src family kinases are non-receptor type tyrosine kinases. The Src family is divided into three sub-families: the SrcA sub-family, consisting of Src, Yes, Fyn, and Fgr; the SrcB sub-family, consisting mainly of Lck, Hck, Blk, and Lyn; and another sub-family composed of Frk. SrcA and SrcB sub-families are specific to vertebrates. The activation of Src family kinases is closely related to a series of biological functions, such as cell division, migration, apoptosis and differentiation. Poteryaev *et al.* demonstrated that GDNF activates Src-family tyrosine kinases, after binding to the GFRα1-GPI complex, in mice DRG neurons (RET tyrosine kinase paucity); phosphorylation of MAPK kinase, PLC2γ and CREB ensue, and induce the third messenger IP_3 to increase calcium concentration, eventually promoting cell survival and Fos expression (Paratcha & Ibáñez, 2002). Popsueva *et al.* also demonstrated that GDNF can promote the differentiation of MDCK cells (GFRα1 positive and RET negative) via activation of the Src family kinases (Popsueva *et al.*, 2003). Trupp also demonstrated, using an immortalized neuronal precursor cell line, RN33B (high expression of GFRα1 and no expression of RET), that GDNF does not activate the Ras/ERK signaling pathway, in these cells; instead GDNF induces the activation of Src family kinases, rapid phosphorylation of CREB and up-regulation of c-fos mRNA (Trupp *et al.*, 1999). This study provides additional evidence supporting the notion that the functions of GDNF are independent of RET.

4.3.2 GDNF and MET

The differential expression models for GFR and RET indicate that: (1) GFR functions via signal transduction pathways that do not involve RET, and (2) other GDNF ligands (binding to GFR) are involved. MET is a hepatocyte growth factor receptor (HGFR) that is normally expressed in epithelium-derived cells. The expression of hepatocyte growth factor is limited in desmohemoblast-derived cells. The primary precursor proteins of MET are cleaved to produce α and β subunits. The mature receptor is formed via a disulfide bond, and plays preliminary roles in embryonic development and wound healing. After stimulating hepatocyte growth factor, MET induces several biological reactions. In many RET-absent but GFRα1-rich cells, GDNF induces the phosphorylation of MET, and then activates the Src family kinases. This provides impetus for research on a RET-independent GDNF signaling system. The RET-independent activation of Src and MET, induced by GDNF, may be regulated by heparan sulfate and NCAM. However, *in vivo* immunoprecipitation studies have demonstrated that MET does not bind to GDNF, implying an uncertain role for GDNF-induced MET activation.

4.3.3 GDNF and NCAM

The GFRα receptor is expressed more widely than RET in many regions of the nervous system, especially the procerebrum, cortex and internal ear (Trupp *et al.*, 1997; Kokaia *et al.*, 1999), indicating that the signal transduction in neurons and glials, and GLF-protein binding may not always be dependent on RET. Other studies demonstrated that GFRα1, but not RET, is expressed in the RN33B cell line, and the signal pathway induced by GDNF overlaps with that induced by NCAM. In 2003, Paratcha *et al.* demonstrated that NCAM may mediate actions via RET-independent trans-membrane signaling. Further studies demonstrated that NCAM is involved in the promotion effects, engendered by GDNF, affecting the survival of DA-ergic neurons (Paratcha *et al.*, 2003). When GFRα is absent, the binding between GFLs

and NCAM is low. In contrast, GDNF binds to NCAM140 closely when GFRα1 binds to NCAM; this leads to the phosphorylation of Fyn, a molecule conjugated to the intracellular segments of NCAM, followed by phosphorylation of FAK, and eventually activation of the MAPK signal pathway via normal signal transduction. Interestingly, the combination of GFRα1 and NCAM may downregulate NCAM-mediated cellular adherence when GDNF is absent (Cao *et al.*, 2008). This regulation is indicative of the independent roles played by GFRα1-NCAM and the GDNF-GFRα1-NCAM signaling pathway. In the RET-independent model, GDNF stimulates the migration of neurilemma cells and the growth of synapses. This finding indicates that the GFRα protein, GFLs, or their combination with NCAM, activates distinct pathways to regulate differential signal pathways.

4.3.4 GDNF and integrins

GDNF is a crucial neurotrophic factor for DA-ergic neurons. Transmembrane signal transduction is mediated by a special receptor system, which includes GFRα, RET, and NCAM140. Thus far, we have identified that another transmembrane cell adhesion molecule, integrin (a heterodimer consisting of α and β subunits), can regulate the signal transduction of GDNF. Some studies have demonstrated that chronic injection of GDNF into the substantia nigra increases the expression of integrin αV and NCAM (Chao *et al.*, 2003). This implies that GDNF exerts special effects on the increase of integrin αV expression, and that integrin αV may be a selective receptor for functional GDNF.

Under the influence of GDNF, the combination of phosphorylated SHC and intracellular integrin β1 increases. Data from co-immunoprecipitation demonstrate that integrin β1 and GFRα1 form a complex. Additionally, phosphorylation of SHC in the cytoplasmic domain of integrin β1 was shown to increase after stimulation with GDNF. Other data from molecular models demonstrated the presence of four hydrogen bonds between integrin β1 and GFRα1. These data indicate that integrin β1 may be involved in the transmembrane signaling of GDNF, and that integrin β1 may even be a selective signal receptor for GDNF (Cao *et al.*, 2008).

4.3.5 GDNF and N-cadherin

N-cadherin is a transmembrane adhesion protein, whose cytoplasmic region can interact with various intracellular proteins (Drees *et al.*, 2005; Reynolds, 2007). When its extracellular domain binds a ligand, the intracellular domain of N-cadherin can activate the PI3K/Akt and Ras/Raf/MAPK signaling pathways (Hulit *et al.*, 2007). It is well-known that the structural and functional characteristics of N-cadherin are somewhat similar to that of NCAM and integrin β1, and that it mediates not only adherence, but also signal transduction. In addition, the binding site for the extracellular domains of RET and the GDNF/GFRα1 complex, is a cadherin-like domain. Our previous studies have demonstrated that N-cadherin can also bind to GDNF, and the phosphorylation of the N-cadherin intracellular domain (Tyr860) is mediated by GDNF. Further studies, using gene silencing and immunoblotting, have demonstrated that GDNF activates the intracellular PI3K/Akt signaling pathway via N-cadherin, thus protecting DA-ergic neurons. Results from studies, using flow cytometry and Hoechst 33258 staining, indicate that GDNF interferes with the expression of N-cadherin, and that the apoptosis of injured DA-ergic neurons increases. Additional results from immunoblotting indicate, under the same conditions, that phosphorylated Akt, but not total Akt, decreases in the cytoplasm. Results from immunohistochemistry indicate a decrease in total N-cadherin, phosphorylated N-cadherin (Tyr860) and phosphorylated Akt, however,

total Akt does not change. Finally, through immunoblotting it was discovered that the levels of phosphorylated N-cadherin (Tyr860) and phosphorylated Akt are dose-dependent on GDNF, and that the peak levels of both occur at 50 ng/ml (*in vitro*) and 13 ng/μl (*in vivo*) of GDNF. The levels of phosphorylated N-cadherin (Tyr860) and phosphorylated Akt are also time-dependent, and the peak levels of both occur at 15 min (*in vitro*) and 30 min (*in vivo*) after GDNF actions. Statistical analyses show that the two phosphorylations are positively related. Thus, it may be concluded that GDNF activates the PI3K/Akt pathway via N-cadherin to protect DA-ergic neurons.

5. Effects of membrane lipid rafts on GDNF actions

Tiny regions in cell membrane exist that differ in composition from other parts of the membrane. In these regions, the extracellular lipids contain rich sphingolipids and cholesterol molecules, which gather and form a mobile platform of fluid double-lipids, i.e. lipid raft. The main components of lipid rafts include GPI-coupled proteins (extramembrane), trans-membrane tyrosine kinases (intermembrane) and two Src family tyrosine kinases (intramembrane) (Simons & Ikonen, 1997). The lipid raft is an essential structure in the membrane for signal transduction, and plays a vital role in cell adherence, axon guidance and synaptic transmission (Paratcha & Ibáñez, 2002).

Substantial studies have focused on GDNF and its receptors, but the studies on the function of lipid rafts and the relationship between GDNF and its receptors are few and far between. To determine whether receptor-mediated GDNF is dependent on the functional lipid rafts, we investigated four transmembrane proteins in the lipid rafts (RET, NCAM140, integrin β1 and N-cadherin) and their involvement in mediating GDNF signal pathways. To this end, the binding of these proteins and GDNF was analyzed, using co-immunoprecipitation, based on the integrity of the membrane lipid rafts. The results demonstrated that all proteins bound to GDNF when the lipid raft was complete, and only RET and N-cadherin bound to GDNF when the lipid raft was not complete. In addition, extraction tests for the membrane lipid rafts have demonstrated that RET and NCAM, but not N-cadherin and integrin β1, may be shifted to the lipid rafts by GDNF. This series of tests indicates the importance of lipid rafts in mediating the interaction of GDNF with its receptors (Figure 4).

RET can mediate the protective actions exerted by GDNF upon DA-ergic neurons (Trupp *et al.*, 1996; Durbec *et al.*, 1996). After GDNF stimulation, RET induces multiple signals to mediate the signal pathways of GDNF via two models (intra-lipid raft and extra-lipid raft) (Tansey *et al.*, 2000). Membrane lipid rafts are the basis for the shift and functional activation of RET (Poteryaev *et al.*, 1999). Activated RET influences different downstream targets in lipid rafts. The RET activated extra-raft preferentially binds to SHC, and the RET activated intra-raft binds preferentially to FRS2, thus mediating a series of signal pathways. The membrane lipid rafts provide abundant micro-environments for the binding of RET and kinases/receptor proteins in the raft, and is involved in the GFL signaling pathway. For example, Src family kinases, as proteins in the raft, mediate a series of functions of GDNF signal transduction, after binding to activated RET. In the lipid rafts rich in GFRα1 and Src, GDNF activates Src via a GFRα1 mediated pathway (Tansey *et al.*, 2000). Furthermore, the location of RET on the lipid raft enhances its binding to Src, whose activation is essential for GDNF mediated promotion of survival (Encinas *et al.*, 2001). In a lipid raft, RET cannot be degraded by proteases, and this protective effect may be related to its downstream actions of regulating receptor functions (Pierchala *et al.*, 2006).

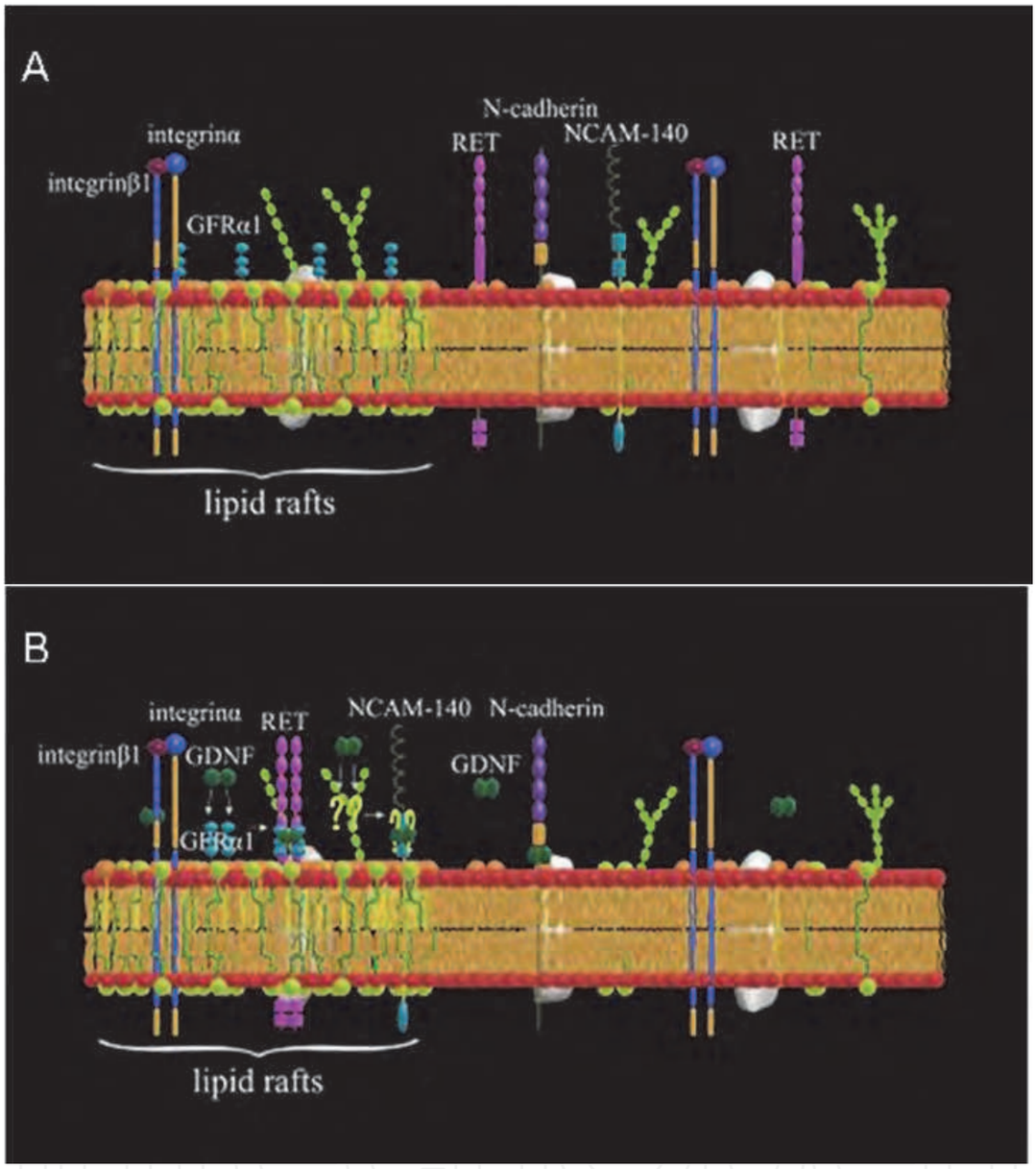


Fig. 4. A membrane lipid raft is an ultrastructure rich in cholesterol and phosphosphingolipids.

NCAM can function as a receptor of GDNF signal transduction (Sjöstrand *et al.*, 2007; Sjöstrand & Ibáñez, 2008). Under the influence of GDNF, NCAM140 binds to Fyn and is recruited to lipid rafts, followed by stimulation of the migration of Schwann cells and promotion of neuronal axonal growth (Paratcha *et al.*, 2003). In membrane lipid rafts, the binding of NCAM and Fyn leads to recruitment of FAK and the activation of the Ras-Raf-ERK1/2 pathway (Beggs *et al.*, 1997; Niethammer *et al.*, 2002).

Integrin β1 is a transmembrane cell adhesion molecule, which is expressed in DA-ergic neurons in the substantia nigra of adult mice (Cao *et al.*, 2008). Inhibitory antibodies can counteract the effects of GDNF on the promotion of survival and growth of DA-ergic

neurons (Chao *et al.*, 2003). Some reports have demonstrated that the membrane lipid rafts provide a perfect environment for the activation of integrins, mediated by growth factors, in the system of survival for oligodendrocytes. The shift of activated integrin to membrane lipid rafts is an important cue, which provides rationale for the actions of functional T cells and the basis for the study of integrin in other cells (Leitinger & Hogg, 2002).

N-cadherin, a calcium dependent cell adhesion molecule similar to NCAM140 and integrin β1, is a trans-membrane protein that mediates the axonal growth of astrocytes and Schwann cells. As mentioned above, the intracellular domain of N-cadherin is similar to the extra-domain of RET. Additionally, N-cadherin can activate the PI3K/Akt and Ras/Raf/MAPK signal pathways (*et al.*,) [73], which provides the rationale for further delving into GDNF and its function.

It is closely related to signal transduction in the membrane and provides a platform for anchoring proteins. A: GFRα1 and integrin β1 integrate into the membrane lipid raft of DA-ergic neurons, but RET, N-cadherin, and NCAM-140 are outside of the raft. B: A GDNF dimer combines with GFRα1 and forms a GDNF-GFRα1 complex, which binds to RET and gathers in the membrane lipid raft. The combination of the GDNF dimer and NCAM dimer requires another substance. N-cadherin and integrin do not gather in the membrane lipid raft, but N-cadherin can bind to GDNF outside of the raft.

6. GDNF treatment in Parkinson's disease

The degeneration of DA-ergic neurons in the substantia nigra of the midbrain is the main pathological characteristic of PD. Based on this characteristic, the most predominant therapeutic strategies are to administer DOPA agents to supply dopamine to the brain, or to employ dopamine agonists to activate the DA-receptor directly. However, the efficacies of these drugs tend to wane because the residual DA-ergic neurons decrease during the progression of PD. In 1993, GDNF, as a growth factor that promotes the survival and growth of DA-ergic neurons in the midbrain, was extracted and qualified (Lin *et al.*, 1993). Early studies demonstrated that GDNF elevates the affinity of neurons to DA, increases the uptake of DA and the expression of tyrosine hydroxylase and promotes the growth of neurapophysis in cultured midbrain DA-ergic neurons. In addition, GDNF was demonstrated to act as neurotrophic factor in spinal motor neurons (Henderson *et al.*, 1994) and noradrenergic neurons (Arenas *et al.*, 1995) in the CNS. Further, GDNF was found to maintain a low level in PD patients and in elder rodents (Yurek & Fletcher-Turner, 2000; Jenner and Olanow, 1998). Thus, this neurotrophic factor is viewed as a potential treatment for neurodegenerative disease.

In cultured embryonic DA-ergic neurons, the paucity of GDNF initiates a novel non-classical death pathway in which the mitochondria does not release cytochrome to the cytoplasm, Bax is not activated and over-expressed Bcl-xL does not inhibit death. However, death is inhibited by the caspase inhibitor BAF, and mutants of caspase-9, caspase-3 and caspase-7, indicating that caspases are essential for the process. Another study demonstrated that in the MLK-c-Jun pathway, caspase-2 and caspase-7 are essential for GDNF-paucity-induced death. In neuroblastoma cells, the GDNF response is similar.

Based on this principle, researchers have adopted different models to supply GDNF in PD animal models. The results demonstrated that GDNF inhibits the variation of Bcl-2 and Bax, up-regulates Bcl-XL, inhibits the combination of CYT-C and Apaf-1 as well as the activity of caspase-3 (Ghribi *et al.*, 2001) reverses the death of DA-ergic neurons induced by

neurotoxins and stimulates functional recovery. In the presence of Aβ peptide, GDNF can inhibit Aβ neurotoxin via blocking the activation of gad153, JNK and ERK induced by ER stress, and promote cell survival. In granular cells of the cerebellum, GDNF inhibits the activation of p38-MAPK via PI3K, and prevents the injury induced by TGF-β in the protoplasmic membrane (Subramaniam *et al.*, 2008).

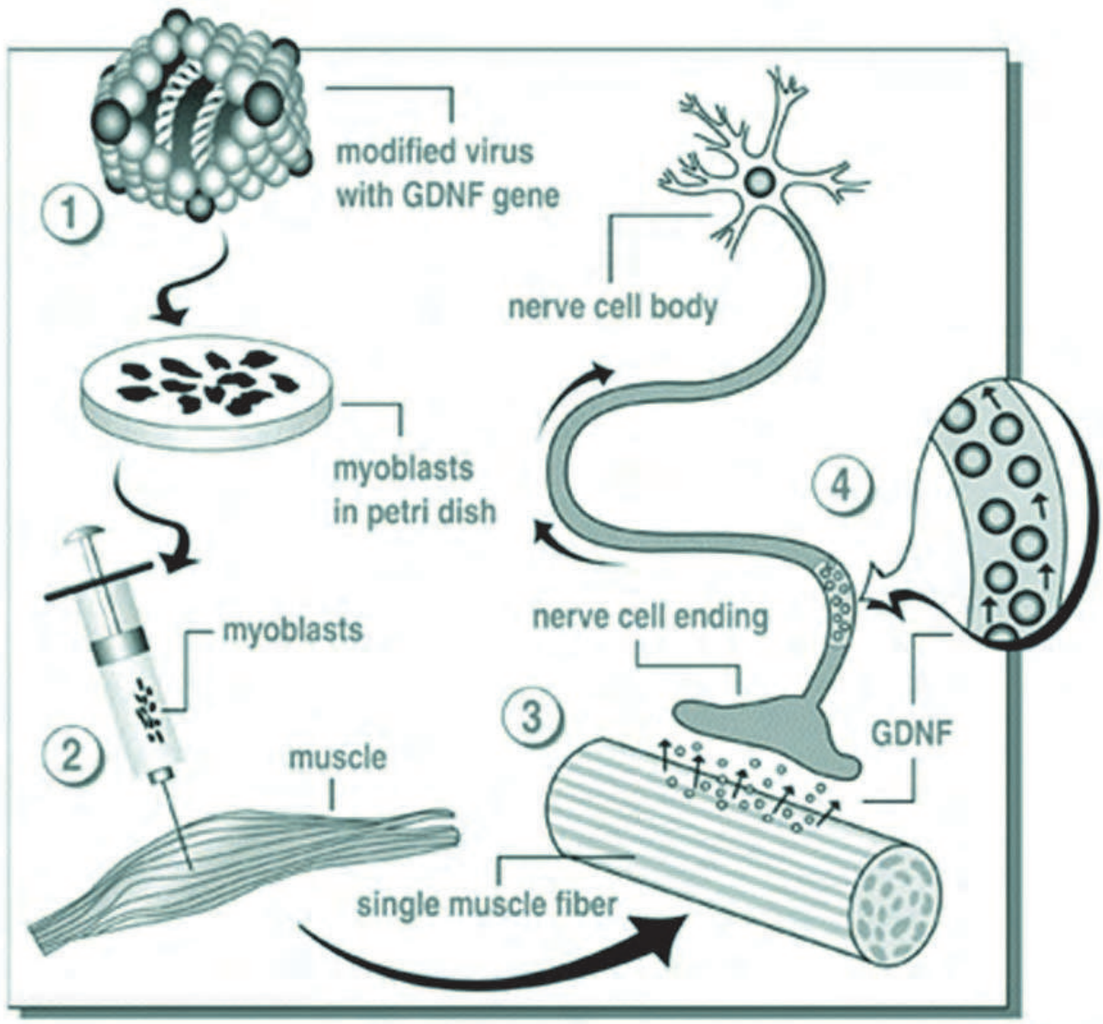
The summary of research progress on different GDNF supplementation methods is as follows:

Direct injection of GDNF: Some studies have demonstrated that injection of GDNF into the substantia nigra only decreases the apoptosis of DA-ergic neurons, but injection into the striatum corpora repairs the neuronal process of TH-positive neurons and improves the PD symptoms (Bohn *et al.*, 2000). Injection of GDNF into the striatum corpora can decrease the death rate of DA-ergic neurons up to 60%, as well as increase the ispilateral TH-positive immunoreactivity and synaptic growth in the nigrostriatal system. Additionally, injection of GDNF into the striatum corpora can increase the amount, volume and synaptic length of DA-ergic neurons as well as the uptake of dopamine.

Injection of GDNF via virus vehicle: This method can transfer the neurotrophic factors into the CNS (Figure 5). In 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) animal models, a lentivirus encoding GDNF was injected into the caudate nucleus, putamen and substantia nigra; a lentivirus encoding for the β-galactosidase enzyme was injected in the control group. Three months later, the PD system scores in the test groups were improved compared to that of the control group (Kordower *et al.*, 2000). Bowenkamp *et al.* used 6-OHDA animal models and obtained similar results (Bowenkamp *et al.*, 1996). These findings indicate that GDNF plays a positive role in neuronal reconditioning and synaptic growth in the substantia nigra. However, over-expressed GDNF may also play a negative role in PD treatment. Eslamboli *et al.* reported that high levels of GDNF expression altered bilateral DA transport and synthesis, and produced toxic or adverse effects (Eslamboli *et al.*, 2003; Eslamboli *et al.*, 2005). Thus, low and sustained expression seems to be most prudent. In Huntington's disease, the repeated gene amplification of the huntingtin protein leads to neurological disorders, which can be attenuated by adenovirus-vehicle mediated GDNF expression.

Injection of GDNF via cell mediation: Researchers are searching for other efficacious ways to import GDNF. For example, the BHK-21 cell, transformed by genetic engineering, can persistently express GDNF in neural stem cells (Behrstock & Svendsen, 2004). Here, we introduce a method of delivering GDNF termed encapsulated cell delivery (ECD). This method requires a catheter-like device with a diameter of no more than 1 mm, which is used to inject agents into the striatum corpora. A mobile device, made up of a hollow fiber membrane (< 1.5 cm), is used to control the diffusion of nutrients and injected GDNF. Cell lines of high quality are selected via this fiber membrane. In the process, the membrane is a barrier preventing immunological rejection of allogenic cells.

GDNF permeable to the blood-brain barrier: Recently, a novel GDNF was developed. It is a fusion protein formed by the combination of GDNF and cell perforin mediated by the trans-activator of the HIV virus. Diez *et al.* demonstrated, in the mouse PD model (i.p. injection), that Tat-GDNF could cross the blood-brain barrier and enter into the nigrostriatal system (Dietz *et al.*, 2006). Unfortunately, Tat -GDNF could not protect injured DA-ergic neurons or improve PD symptoms in MPTP models.



1. Immature myogenous cells are infected by improved GDNF-carrier genes.
2. The infected myogenous cells are injected into muscle in amyotrophic lateral sclerosis (ALS) mice
3. The infected cells mix with myogenous cells, and produce and secrete GDNF, which is absorbed by nerve fibers.
4. GDNF is transferred into the body of neurons and maintains the survival of the cells.

(Figure source: www.epfl.ch)

Fig. 5. Injection of GDNF into the CNS to transfer neurotrophic factors via virus-vehicle.

Results from animal models provide invaluable information for clinical research. In 2002, Steven Gill and colleagues injected GDNF directly into the brain of PD patients, which significantly attenuated the PD symptoms (Gill *et al.*, 2003). The purpose of this Phase I clinic trial was to evaluate the safety of GDNF injections. Previous data, based on animal models, showed that GDNF can prolong the survival time of DA-ergic neurons and enhance their functions. In this study, researchers pumped GDNF into the striatum corpora of five PD patients at dose of 40 mg/d, for 18 months. These researchers believed that the cells closest to tip of the catheter absorbed the GDNF first. After permeating the brain tissues, GDNF reached the DA-ergic cells. The results demonstrated that the symptoms were attenuated,

the motor functions were elevated by 39%, the quality of life was improved and daily activities were increased by 61%; no adverse effects were reported up to one year later. Results from PET scans demonstrated that GDNF enhanced dopamine in brain. This therapy also attenuated L-dopa induced tremors. Unfortunately, in 2004, Amgen conducted a larger-scale trial, and GDNF did not exert effects as expected. Further, the company expressed concern over the safety of GDNF. After that, the direct application of GDNF was all but abandoned. However, exploration into mechanisms of action of GDNF is an alternative avenue that may aid in the development of new therapies for PD treatment.

7. Conclusion

After the identification of GDNF, three other family members (NTN, PSP and ART) were identified, and as further studies are undertaken, more family members may be uncovered. The receptors of these factors are composed of two parts: the GDNF family receptor and the transmembrane tyrosine kinase RET. The GPI-coupled GFR determines their specific signal transduction.

Initially, researchers assumed that only one signal pathway for GDNF existed: via GFRα1/2, anchored by receptor tyrosine kinase RET and glycosyl-phosphatidyl inositol. In 2003, *in vitro* studies demonstrated that the adhesion molecule NCAM was a receptor for GFLs that was independent of RET signaling. Recently, studies have indicated that integrin αV/β1 and N-cadherin may also be selective receptors for GDNF. As more evidence comes to light, it is evident that the signal pathway for GDNF protection of DA-ergic neurons is a complex one.

In DA-ergic neurons, GDNF activated Ras-MAPK and PI3K/Akt signaling pathways play vital roles in neuronal survival and axon growth. Present studies indicate that NCAM, a new GFI receptor, is also involved in the signaling pathways of GDNF-GFRα1-MET and NGF-TrkA-RET, which also makes the biological characteristics of GDNF complex.

Some progress has been made in therapeutic trials for neuronal degenerative diseases based on the neurotrophic actions of GDNF. Repeated, direct injection of GDNF is especially valuable for treating myeleterosis such as amyotrophic lateral sclerosis (ALS). Because of the features of the blood-brain barrier and brain tissues, direct injections of GDNF to treat PD and other neurodegenerative diseases have limited use. Thus, direct GDNF gene injection mediated by vehicle or transgenic cellular transplant is a promising alternative. Currently, the challenge lies in the development of a safe, novel vehicle for the delivery of genes that can be specially expressed in tissues, can induce gene expression and can replace the virus vehicle.

In the management of PD, the application of drugs with low molecular weights is another choice for recombining neurotrophic factors. Other GFLs may be of potential value for treatment. Present studies demonstrate that, in murine PD models, PSPN, which is transferred into brains via neuronal stem cells, exerts effects as well as GDNF, on the survival of DA-ergic neurons in the midbrain (Akerud *et al.*, 2002). As we know, the expression of GFRα4, which is the receptor of PSPN, is more limited than that of GFRα1, the receptor of GDNF. Thus, we can propose that, even if high concentrations of PSPN are used, the adverse effects may be fewer than using GDNF (Lindahl *et al.*, 2000). The next challenge is to identify novel drugs, with low molecular weights and natural neurotrophic properties, to influence intracellular signal transduction. These explorations should be based on the comprehensive recognition of GFLs and the 3D structure of its receptor, as well as molecules, cells and disease.

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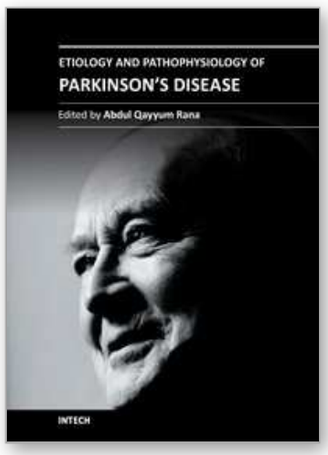
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This book about Parkinsonâ€™s disease provides a detailed account of etiology and pathophysiology of Parkinsonâ€™s disease, a complicated neurological condition. Environmental and genetic factors involved in the causation of Parkinsonâ€™s disease have been discussed in detail. This book can be used by basic scientists as well as researchers. Neuroscience fellows and life science readers can also obtain sufficient information. Beside genetic factors, other pathophysiological aspects of Parkinsonâ€™s disease have been discussed in detail. Up to date information about the changes in various neurotransmitters, inflammatory responses, oxidative pathways and biomarkers has been described at length. Each section has been written by one or more faculty members of well known academic institutions. Thus, this book brings forth both clinical and basic science aspects of Parkinsonâ€™s disease.

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