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Balancing neurotrophin pathway and sortilin function: its role in human disease.

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Abstract

Neurotensin receptor-3 or sortilin is a vacuolar protein sorting 10 protein domain (Vps10p) has been firstly discovered in the human brain, it acts as receptor or co-receptor of the cell and traffics different proteins within the cell. Sortilin deregulation contributes to the development of several diseases, including neurological diseases and cancer. On the other hand, neurotrophins which are a family of proteins essential for the nervous system development, function and plasticity. The first discovered member is the nerve growth factor; other members are brain-derived growth factor, neurotrophin-3 and neurotrophin-4. Nerve growth factor and brain-derived growth factor are the common neurotrophins that have a role in cancer. Neurotrophins initiate their signals through interaction with tyrosine receptor kinases TrkA, TrkB, and TrkC; each member has an affinity for a specific receptor to stimulate cell survival, while the interaction with $p75^{NTR}$ initiates cell apoptosis pathway by forming a complex with sortilin and neurotrophin precursors. A number of therapeutic approaches are emerging to target the neurotrophins pathway as well as sortilin.

Abbreviations:

AKT, RAC-alpha serine/threonine-protein kinase ;BDNF, brain-derived growth factor; ER, endoplasmic reticulum; ERK, extracellular signal-regulated kinase; GLUT4, glucose transporter type 4; GWAS, Genome-Wide Association Study; MAPK, mitogen activated protein kinase; NGF, Nerve growth factor; NSCLC, non-small cell lung cancer; NT, neurotensin; NT-3, neurotrophin-3; NT-4, neurotrophin-4; PI3K, phosphatidylinositol 3-kinase; PLC-γ1, phospholipase C-γ1; SCLC- small cell lung cancer; TGN, trans Golgi network; Trk, tyrosine receptor kinase; Vps10p, vacuolar sorting protein 10 protein domain.

Introduction

Sortilin, also known as neurotensin receptor 3, is a member of vacuolar protein sorting 10 protein (Vps10p) domain family, which was first discovered in the human brain, but it is also expressed in many tissues [1]. Sortilin is encoded by SORT1 gene, located on chromosome 1p13.3. Sortilin acts as a receptor or co-receptor responsible for cell survival, signal transduction, and sorting or trafficking proteins such as neurotrophins to the plasma membrane, lysosomes or endocytic pathway [2, 3]. Neurotrophins compose a protein family that are important for the nervous system development, function and plasticity. They exert their functions either by interacting with the tyrosine kinase receptors (Trks) to induce different pathways such as Rat Sarcoma (Ras), phosphatidylinositol 3-kinase (PI3K), and phospholipase C-γ1 (PLC-γ1) responsible for cell survival [4] or with p75 ^{NTR} neurotrophin receptor to induce activation of NF-κB and Jun N-terminal kinase (JNK) and modulates RhoA activity that ends up with cell apoptosis or survival [5]. Sortilin forms a heteromeric complex with p75^{NTR} at the cell surface that transduces the pro-neurotrophin cell death signals, while with Trks, sortilin enhances neurotrophins signals by facilitating the anterograde transport along axons. Therefore, the defect of sortilin expression in many human cell lines might affect neurotrophins trafficking and release [6]. Hence, the imbalance of neurotrophin signalling has been involved in various diseases, such as Alzheimer's disease (AD), Parkinson's disease (PD), cardiovascular disease (CVD), type II diabetes mellitus (T2 DM), as well as cancer. In AD, sortilin mediates the trafficking of β-secretase, thus increasing the beta-amyloid precursor protein cleavage [7], while in diabetes formation of GLUT-4 storage vesicles are affected by sortilin expression [8]. In CVDs, sortilin correlates with the levels of circulatory low-density lipoprotein, in addition to SNPs located on chromosome 1p13.3 that associated with sortilin and cardiovascular phenotypes [9]. Sortilin expression is elevated in many types of carcinomas, along with alteration in Trks expression [8]. Herein, we discuss the effect of sortilin dysregulation in neurotrophins trafficking, their role in developing of various diseases, and the ongoing therapeutic applications that target sortilin and neurotrophin receptors.

Sortilin family, structure and function

In 1997, Petersen and colleagues discovered sortilin by receptor-associated protein affinity chromatography through isolation, identification and purification of membrane glycoprotein (95kDa), this protein was initially called gp95 and later named as sortilin (also known as neurotensin receptor 3). After cDNA cloning and screening, the receptor structure and the sequence homologies with vacuolar sorting protein 10 protein domain (Vps10p) was identified [1, 10]. Sortilin is a 100kDa type-1 membrane glycoprotein which belongs to the Vps10p receptor family. The Vps10 protein family include heterogenous type-1 transmembrane receptors, which consists of sortilin (100 kDa), SorLA, SorCS1, SorCS2, and SorCS3 [11]. The pre-fix "sor" is used as an abbreviation for the names of Vps10 family members "sorting receptor-related" [12]. It consists of two domains; the large segment is named the extracellular domain, while the short tail is the cytoplasmic or intracellular domain [1]. The extracellular domain contains Vps10p domain, which in turn consists of structural domains. The first structure is known as a ten bladed β-propeller which situated in the N-terminal domain, followed by two small domains, 10CC-a and 10CC-b which represents the ten conserved cysteines (10CC) in the C-terminal segment. Both 10CC-a and 10CC-b interact extensively with the β-propeller [13].

Several studies demonstrated that the sortilin family are synthesised as a precursor at the ER and then converted to the mature form at the trans-Golgi network (TGN) through the removal of the N-terminal preprotein that contains convertase consensus cleavage sequence (RXXR). This cleavage opens the structure of sortilin by exposing a ligand-binding region [2, 14]; additional motif found in the N-terminal domain is Fibronectin-type III which shows different function between Vps10p members [11]. However, the β-propeller consists of ten blades in a wheel-shape with a conical tunnel that contains a ligand-binding site, with a diameter of 25 by 37 Å in the cross-section. Each blade has a four-stranded β-sheet stabilised by an Asp-box repeat, which is a short repeat motif between strands 3 and 4 to support the structural integrity in β-propellers [13].

While the cytoplasmic domain contains motifs established from several signal sequences to be involved in binding to adaptor proteins including (AP-1, AP-2), Golgi-localised, γ ear-containing, ADP ribosylation factors (ARF) -binding proteins (GGA1, GGA2 and GGA3), [15, 16], Ras-related protein (Rab7b) and phosphofurin acidic cluster-sorting protein 1 (PACS-1). The well-defined sorting sites in sortilin are C-terminal acidic cluster combined with dileucine

which targeted by AP-1, -2, GGA proteins and PACS-1 and involved in Golgi-endosome trafficking; and the second site is tyrosine-based motif that located at the upper third of the cytoplasmic part, contributes to endocytosis through AP-2 binding and also could participate in the trafficking pathway between Golgi and endosome by AP-1, in addition to its binding to p21 activated kinases (PAK 1-3) that have an effect on neuronal and non-neuronal cells growth, shape and motility [17]. Also, sortilin shares similarity to the mannose-6 phosphate receptor; thereby, sortilin could guide lysosomal enzymes from TGN to endosomes and lysosomes [18]. Sortilin plays a role in the delivery of sphingolipid activator proteins (SAPs) and sphingomyelinase acid to lysosomes [19].

Under the effect of various stimuli such as a protein kinase (PKC)-dependent activator of metalloproteases, neurotensin (NT) receptor-3 (NTSR3) could be released from the plasma membrane by shedding producing the soluble form (sNTSR3) [20]. The sNTSR3 which is able by itself and upon binding to cells to stimulate Akt, Src and FAK phosphorylation thus can regulate intracellular pathways related to cell survival but not cell growth through its ability to bind and to be internalised in colon cancer cells [21]. Moreover, sNTSR3 plays a role in regulating the morphology of the cell, properties of cell-cell and the cell-matrix via reduction of integrins expression and modifying desmosomes structure resulting in initiation of cell separation and spreading leading to cancer metastasis [22]. While its role in CVD, DM and dyslipidaemia, a study found that levels of circulating sortilin associated with both CVD and DM when measuring the plasma sortilin levels from CVD and DM patients with higher levels of sortilin compared to the controls. Thus, sortilin levels could be used as a biomarker for CVD and DM, while validating these results further studies are required [23]. Another study demonstrated that activated platelet could release soluble sortilin upon collagen stimulation which act as a platelet stimulator, and this release can be suppressed by treatment with aspirin. They found that higher levels of sortilin in plasma were observed in patients having cardiovascular risk factors such as hypertension, dyslipidaemia and/or diabetes, hence high plasma soluble sortilin levels could be related to platelet activation and a risk factor for atherothrombosis *in vivo* [24]. In other studies, investigating circulating sortilin levels in type II DM patients with lower limb peripheral artery disease (PAD). Sortilin levels were found to be significantly high in this group and was independently associated with lower limb PAD and could be considered as a promising biomarker for atherosclerosis of the lower limb [25]. Gustafsen and colleagues found that PCSK9 secretion which is implicated in lipid metabolism is regulated by sortilin and there is a positive link between circulating PCSK9 and sortilin

whereby sortilin expression affects the circulating levels of PCSK9 in humans [9]. Another study investigated the relationship between serum sortilin levels and metabolic profiles of subjects newly diagnosed with type II DM and found the circulating levels of sortilin is lower in patients with newly diagnosed type II DM than in controls, also has a negative correlation with insulin resistance and undesirable lipid profiles, while a positive correlation with high-density lipoprotein cholesterol in newly diagnosed type II DM subjects [26]. High serum sortilin levels found in patients with depression as explained by Buttenschøn *et al.,* study and also found there is a link between the serum level of sortilin and BDNF in addition to vascular endothelial growth factor A (VEGF) which is a neurotrophic factor that has a role in depression pathophysiology [27].

There are several trafficking pathways for sortilin. Firstly, fusion of sortilin with the cell surface through the secretory vesicles, which is known as *constitutive secretory pathway*; at the cell surface, sortilin extracellular domain can be cleaved and released extracellularly; this represents about 5-10% of sortilin molecules, while the remaining intact sortilin functions as a ligand-binding site to transduce a signal or mediate endocytosis by AP2. Following the latter, the ligand may undergo lysosomal degradation while sortilin is transported back to the TGN with the help of retromer and AP1. Secondly, the anterograde sortilin transport which involves the movement of sortilin from TGN to early endosome by GGAs and AP1. Lysosomes usually degrade ligands and a portion of sortilin, while mainly sortilin is palmitoylated and transported reversely to TGN [28]. Thirdly, sortilin assists with ligand incorporation into secretory granules followed by extracellular signal stimulation in order to regulate the secretory pathway in cells [29].

Many studies have been performed to support the hypothesis that sortilin could be a protein sorting receptor, transporting ligands to the membrane surface, or between the Golgi and late endosomes through to endocytosis [2, 3, 30]. Thus, the physiological role of sortilin involves many different functions, including cell survival, induction of post-traumatic neuronal apoptosis, in addition to signal transduction, ligand-binding, and engaging in intracellular sorting. Also, sortilin mediates lipoprotein lipase, neurotensin and the pro-form of nerve growth factor (pro-NGF) endocytosis, transporting proteins from Golgi to the late endosomes, and act as a co-receptor with $p75^{NTR}$ on the cell membrane to induce neural death [19, 31, 32]. Also, sortilin has anti-apoptotic effects when it interacts with other neurotensin receptors; a study found that NTSR3/sortilin forms a functional complex with NTSR2 in pancreatic β-TC3 cells which is important to stimulate the protective role of neurotensin against apoptosis by Akt

activation [33]. Another study showed that human corneal keratocytes cultured with JMV449 (pseudopeptide NT agonist) expressed NTSR1 and NTSR3, and JMV449 increased the cell proliferation and reduced apoptosis through NTRS1 along with NTRS3 which partially mediates the JMV449 effect on cell proliferation [34]. A study by da Silva *et al.,* investigated the inflammatory response stimulated by lipopolysaccharide (LPS) in skin dendritic cells through NT effect, and they found that foetal-skin dendritic cells (FSDCs) expressed NTSR1 and NTSR3 and treatment with LPS induces the expression of neurotensin which leads to downregulation of inflammatory signalling pathways NF-κB and JNK, and cytokines IL-6, TNF-α, IL-10 and vascular endothelial growth factor (VEGF) expression, while upregulating the ERK and epidermal growth factor (EGF) survival pathways [35]. Also, high sortilin expression found on the surface of B cell chronic lymphocytic leukaemia (B-CCL) than in healthy subjects and a correlation between sortilin and CD23 expression was also observed in leukemic cells suggesting that it is responsible for leukemogenesis of CCL cells, as a result sortilin could be used as a diagnostic and therapeutic biomarker for CCL patients [36].

Sortilin ligand binding sites

Neurotensin (NT) is a tridecapeptide, firstly isolated from the hypothalamus of bovine in 1973 and then from the small intestine [37], has both central and peripheral actions depending on its interaction with specific receptors on the plasma membrane of the targeted cells [2]. NT binds to three different receptors, neurotensin receptor 1 (NTSR1), NTSR2 and NTSR3 (sortilin). NTSR1 and 2 are seven-transmembrane G-protein coupled receptors, whereas NTSR3 or sortilin is a single transmembrane domain sorting receptor [38]. A study by Quistgaard and colleagues illustrated the 3D structure of sortilin and ligand binding sites. They found that the ectodomain structure of sortilin forms a complex with neurotensin through its C-terminal part in the tunnel of a ten-bladed β-propeller domain [39]. However, most ligands of Vps10p domain compete for binding which likely depends on steric hindrance rather than on identical binding sites, such as neurotensin act as a competitive inhibitor for most sortilin ligands. Furthermore, ten-bladed β-propeller of Vps10p domain has a fourfold increase in the volume of the tunnel and provide binding sites for an extended set of large protein ligands and only one ligand at a time as it confines the ligand binding to the tunnel rather than on an outer surface [40]. Moreover, the interaction with cell membrane or with transmembrane receptors, including p75NTR or TRKs family receptors has been proposed through two protruding hydrophobic

loops [39]. Another study used hydrogen/deuterium exchange mass spectrometry to examine sortilin conformational response when it binds to biological ligands, such as neurotensin, pro-peptide of sortilin, progranulin, and pro-NGF and found that inside the sortilin β-propeller tunnel, ligands have two binding sites and also progranulin and pro-NGF stabilise the residues in the 10CC-b domain [41].

Neurotrophin pathway

Neurotrophins are a family of proteins required for vertebrate nervous system development, function and plasticity. Nerve growth factor (NGF) is the first characterised factor discovered during a search for survival factor; there are four neurotrophins, NGF, brain-derived growth factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin-4 (NT-4). They are similar in their sequence and structure [42]. Initially, neurotrophins are synthesized as a precursor, which require cleavage to produce the mature protein. Their cleavage occurs inside the cell at the endoplasmic reticulum (ER) and Golgi by furin or pro-convertase at dibasic amino-acid cleavage site producing C-terminal mature neurotrophins [43]. While extracellular cleavage by the enzyme matrix metalloproteinase 7 (MMP-7) [44] or serine protease plasmin [45]. The amino terminal part of the precursor neurotrophins is important for neurotrophin folding [46] and sorting neurotrophins to either constitutive or regulated secretory pathways [45]. While the mature domains are the secreted ligands responsible for initiating the biological effects of neurotrophins [43].

Neurotrophins bind to specific receptors according to their affinities, nerve growth factor (NGF) binds to tropomyosin receptor A (TrkA); BDNF and neurotrophin 4 bind to tropomyosin receptor kinase B (TrkB); while neurotrophin 3 (NT3) to tropomyosin receptor kinase C (TrkC). However, the low affinity of binding NGF to TrkA, and of BDNF to TrkB, can be modified by dimerisation of the receptor, structural modification, and association with $p75^{NTR}$ receptor, which can also increase the ligand selectivity. The $p75^{NTR}$ receptor acts as a co-receptor for Trk receptors that can bind to each neurotrophin [47-49]. A study evaluated the selectivity and ability of proneurotrophins and mature neurotrophins binding to p75NTR and Trk receptors; they found that pro-NGF bound to p75NTR with an affinity of five times higher than mature NGF, this means that pro-NGF is the ligand of p75NTR not TrkA and p75NTR has more affinity to bind to pro-NGF than mature NGF to induce apoptosis [45]. Thus, the effect of neurotrophins on neuronal survival/death depends on if mature neurotrophins or pro-

neurotrophins are secreted and bind to either Trk receptors or the complex p75NTR/sortilin to induce downstream signalling pathways [43]. $P75^{NTR}$ is a member of the tumour necrosis superfamily, consists of four cysteine-rich motifs in the extracellular domain, a transmembrane domain and a death domain in the cytoplasmic domain [50]. The extracellular domain contains four tandemly arranged domains for ligands binding followed by juxtamembrane stalk region of around 60 amino acids; the transmembrane domain has a sequence of 60 amino acids specific chopper module of $p75^{NTR}$ that is connected to the death domain in the intracellular or cytoplasmic domain (Figure 1) [51]. Compared to the structure of Trk receptors, which contain the extracellular domain in which there is cysteine-rich cluster followed by three leucine-rich repeats, another cysteine-rich cluster and two immunoglobulin-like domains, transmembrane span ended with tyrosine kinase domain in the cytoplasm that have several tyrosines responsible for phosphorylation and act as docking sites for cytoplasmic adaptors and enzymes (Figure 1) [52].

Neurotrophins are involved in many different signalling pathways through interaction with two types of receptors on the cell surface (Trks and $p75^{NTR}$) promoting survival, differentiation and many various activities by interactions with other receptors and ion channels [46].

Ion Channels

The interaction of tyrosine kinase receptors and ion channels has been discovered in many studies [53]. A study found that activation of TrkB receptor by BDNF in the rat olfactory bulb neurons uses Kv1.3 potassium channel as a substrate for the phosphorylation of tyrosine residue in the receptor and the function of this channel is altered depending on duration of trophic factor stimulation and previous odour sensory experience, this result applied for TrkB and have no effect on TrkA and TrkC as the majority of these receptors exist as truncated forms [54]. Another study found that binding of BDNF to TrkB can depolarise the membrane via rapid activation of tetrodotoxin (TTX)-insensitive sodium channel Na_v 1.9 in HEK-293 cells [55]. There are many questions still unclear, such as the cellular mechanism and signalling cascades that are responsible for the modulation of ion channels by BDNF, understanding of the action of BDNF on ion channels, and the mode of ion channel activation; all these questions will add new insights to understanding the role of neurotrophins function in the brain.

Neurotrophin signalling pathway

Ligand binding to extracellular part of tyrosine receptor kinases induces oligomerisation and autophosphorylation which represent the general mechanism for the growth factor and cell surface receptor activation [56]. Binding of neurotrophins to $p75^{NTR}$ and Trk family members is by the formation of dimers; dimerization of Trk receptor results in autophosphorylation and activation of intracellular signalling pathways [57]. The phosphorylated-tyrosines and the surrounding amino acid residues within the activated Trk molecule create binding sites for proteins having phosphotyrosine-binding (PTB) or Src homology 2 (SH2) domains. The Trk receptors activated pathways are Proto-Oncogene Proteins p21 (Ras), phosphatidylinositol 3-kinase (PI3K), phospholipase C-γ1 (PLC-γ1), and their downstream effectors (Figure 2) [4].

Proto-Oncogene Proteins p21 (Ras)

Ras activation is essential for the differentiation of neural cells and promotes many neural subpopulation survival. Neurotrophin (NGF) binds Trk receptor resulting in dimerization and autophosphorylation of the tyrosine residues [58]. For transient activation of Ras, the adaptor protein Src homology 2 domain-containing transforming protein 1 (Shc) links phosphor-Y490 on Trk to another adaptor Growth factor receptor-bound protein 2 (Grb2) which in turn forms a complex with son of sevenless (SOS), a nucleotide exchange factor that functions to activate Ras by replacing GDP with GTP. Active Ras induces downstream signalling of PI3K and MAPK pathways [5]. For MAPK activation, active Ras interacts with the RAF proto-oncogene serine/threonine-protein kinase, which phosphorylates and activates Mitogen-activated ERK kinase (MEK) and MAP kinase isoforms. Finally, MAPK translocates to the nucleus, causing phosphorylation of transcription factors required for neural cell differentiation [58]. Termination of the signalling pathway is via phosphorylation of intermediates and activation of phosphatase, such as phosphorylation of SOS results in the SOS-Grb2 complex dissociation (Figure 2) [5].

PI3K pathway

PI3K pathway can be activated via Ras-dependent and independent pathways [5]. Ras-dependant pathway depends on Shc phosphorylation by Trk that causes an increase in Ras activity and extracellular signal-regulated kinase (ERK) or known as MAP kinase, which in

turn induces transcriptional events, like cyclic adenosine monophosphate response element-binding (CREB) transcription factor, that affects cell cycle, neurite outgrowth and synaptic plasticity [57]. While the Ras-independent pathway depends on recruiting GRB2-associated-binding protein 1 (GAB1) via phosphorylated Grb2 initiating the subsequent activation of the PI3K signalling pathway [5]. The 3-phosphoinositides produced by PI3K activates two protein kinases, the serine-threonine kinase AKT and RAC-PK (Related to A and C protein kinase) [59]. AKT responsible for controlling the protein activities involved in promoting cell survival, such as substrates that control the caspase cascade, for example, Bcl-2-associated death promoter (BAD) (Figure 2) [60].

PLC-γ pathway

Also, PLC-γ binds to activated Trk receptors and induces an intracellular signalling cascade, leading to the production of inositol triphosphate (IP3) and diacylglycerol (DAG) by phosphatidylinositol 4,5-bisphosphate hydrolysis. IP3 stimulates mobilisation of cytoplasmic Ca^{2+} stores, while DAG regulates protein kinase C isoforms, both signalling molecules resulting in the activation of intracellular enzymes, such as protein kinase C isoforms and Ca^{2+}-calmodulin-dependent protein kinases (Figure 2) [61].

EGFR pathway

Epidermal growth factor (EGF) receptor belonging to the type I receptor tyrosine kinases of ErbB receptors and consists of extracellular domain (ECD), transmembrane segment, and intracellular domain (ICD) which has a tyrosine kinase domain [62]. Ligand binding to the ECD stimulates receptor dimerization and autophosphorylation at tyrosine residues of ICD to mediate various signalling pathways, such as RAS/RAF/MEK/ERK, PI3K/AKT/mTOR and JAK/STAT pathways that are involved in cell proliferation, survival, migration, invasion, anti-apoptosis, and pro-angiogenesis. Termination of EGFR signalling is related to EGFR internalisation and degradation after binding to ligand, thus EGFR trafficking dysregulation participates in uncontrolled cell proliferation and survival such as in cancer cells [62, 63]. Following the study by Wilson's group that illustrated sortilin plays a role in transporting and loading EGFR into extracellular vesicles by endocytosis [64], another study showed that

sortilin regulates EGFR internalisation and limiting the proliferative signalling, thus loss of sortilin promotes continuous EGFR signalling and accelerates tumor growth *in vivo* [65].

Cell survival versus cell death

Sortilin binds TrkA, -B, and -C to facilitate the anterograde axonal transport of these receptors and thus enhance the signalling of neurotrophins; and sortilin is a positive modulator of neuronal survival induced by neurotrophins [66]. In TGN, sortilin interacts with Trk receptors to shuttle them to the nerve terminals along the axonal path where they can form a heterodimeric complex with $p75^{NTR}$ and transduce mature NTs signals [67]. The $p75^{NTR}$ receptor signalling pathway involves the activation of NF-κB and Jun N-terminal kinase (JNK) and modulates RhoA activity. These are initiated by binding of adaptor proteins to the cytoplasmic domain of $p75^{NTR}$, such as neurotrophin-receptor interacting factor (NRIF), neurotrophin-associated cell death executor (NADE), neurotrophin-receptor-interacting MAGE homologue (NRAGE), Schwann cell 1 (SC1), and receptor-interacting protein 2 (RIP2), which have effects on apoptosis and non-apoptotic or survival response [68, 69]. Apoptosis pathway involves the formation of the sortilin complex with $p75^{NTR}$, which in turn binds with high affinity to pro-neurotrophins [70]. The interaction takes place in the extracellular domain of sortilin and $p75^{NTR}$ to form a heterodimer that is strengthened by the pro-neurotrophin ligands, while the intracellular domain has no role in this interaction; the intracellular domain of sortilin plays a role in regulating the cleavage rate of $p75^{NTR}$ through its regulatory function in p75-regulated intramembrane proteolysis and apoptosis [51], whereas binding of neurotrophins to $p75^{NTR}$ activates Jun kinase-signalling cascade resulting in the activation of p53 and apoptosis and also promotes Fas ligand expression resulting in apoptosis through Fas receptor. Activation of NF-κB has a role in neural survival; neurotrophins initiates the signalling through the association of TNF receptor-associated factor 6 (TRAF6) to $p75^{NTR}$ cytoplasmic domain, after recruitment of several kinases the transcription factor NF-κB released. While Rho activity controls growth cone motility (Figure 2) [5]. Pro-neurotrophins are ligands that bind selectively to the $p75^{NTR}$ compared to the mature form and are more effective at inducing apoptosis by their interaction to $p75^{NTR}$ [45], thereby the regulation of biological function of neurotrophins is by their proteolytic cleavage, with the mature forms activating the Trk receptor and inducing cell survival and the pro-forms activate $p75^{NTR}$ to promote apoptosis.

Sortilin and human diseases

The discovery of sortilin in 1997 illustrated the physiological function of the protein. The modification in its expression can lead to the development of several human diseases in both the central and peripheral organs [8]. Sortilin has a role in neurological disorders, such as Alzheimer's and Parkinson's diseases; it also affects lipid and glucose homeostasis regulation. In cancer, sortilin is dysregulated in different cancer types. As sortilin controls the trafficking of neurotrophins and acts as a receptor or co-receptor. Thus, any defect in this regulation and imbalance in cellular homeostasis can lead to CVD, DM, and cancer as well as its role in genetic disorders.

Neurological disorders

- **Alzheimer's diseases (AD)**

Alzheimer's disease (AD) is the most common neurodegenerative disease. According to the WHO report in 2016, the number of people affected with AD is about 46.8 million in the world, and the number is expected to be double to 74.7 million by 2030 [71]. It is caused by tangles and senile plaques accumulation, that leads to neuronal loss in the hippocampus region of the brain. Senile plaques consist of amyloid-β peptides, which come from the two proteolytic enzymes action on β-amyloid precursor protein (APP), known as β- and γ- secretase. APP is firstly cleaved by β-secretase-amyloid precursor protein-cleaving enzyme 1 (BACE1), accompanied by γ-secretase to produce amyloid peptides and an intracellular fragment [7, 72]. Some studies found that the over-expression of sortilin (not other VPS10p receptors, like sorLA) tend to be high in the post-mortem brains of patients [73, 74], while another study did not discover that the sortilin expression is significantly changed in AD [75]. Saadipour and colleagues illustrated that Aβ42 presence in culture media is enough to increase the expression levels of both mRNA and protein of sortilin mediated through $p75^{NTR}$ in SH-SY5Y cells in a time and dose-dependent manner [74]. However, the sortilin cytoplasmic domain can regulate BACE1 subcellular distribution, and thus affect the proteolytic process of APP mediated by BACE1, which facilitates retrograde transport of the enzyme. In addition, sortilin over-expression correlates with increased cleavage of APP by BACE1 through the interaction of BACE1 with sortilin at the TGN [73].

Moreover, sortilin ectodomain proteolysis is mediated by transmembrane metalloprotease A disintegrin and metalloproteinase domain-containing protein 10 (ADAM10) separating the ligand-binding domain from the trafficking motifs encoded by intracellular domain, and sortilin shedding can occur at the cell surface and intracellularly. The significant biological role of this cleavage is unclear, a study demonstrated that the cytoplasmic tail of sortilin interacts with pro-domain of brain-derived neurotrophic factor (BDNF) to facilitate the trafficking of BDNF through the secretory pathway [76]. The cleavage of sortilin ectodomain by A disintegrin and metalloproteinase domain-containing protein 10 (ADAM10) represents the regulatory switch for BDNF delivery to either the secretory pathway or to the lysosome [77]. A study from Fleitas and colleagues, implicates pro-BDNF neurotoxic signalling in AD pathology primarily by three mechanisms: one is increased expression of $p75^{NTR}$ and sortilin [78] while the others are increased p75 basal levels processing found in AD, and increased stability of proBDNF by post-translational modification induced by reactive oxygen species (ROS). They studied BDNF, pro-BDNF and their receptors' relative levels and found there is a significant increase of sortilin and pro-BDNF in the hippocampus. In AD pro-BDNF-p75/sortilin signalling contribute to the pathogenesis and increase in cell death with neural differentiation impairment. The reactive oxygen species (ROS) induced glycation end products to cause pro-BDNF modification, which prevents pro-BDNF from maturing and increases the pathogenicity [78]. A recent report showed that the levels of full-length sortilin were increased in the neocortical lysates from aged and AD cases that is relative to middle-aged cases [79]. In this study, they characterised the full length sortilin normal expression pattern in adult human cerebrum by using two antibodies binding the extracellular and intracellular domains, and they found that there were extracellular deposits of sortilin C-terminal antibody in the human brains with cerebral amyloid pathology, these deposits present as mini-plaques, densely packed and mature looking plaques with a ring-like shape, these deposits are fragments with 15kDa and are elevated in the cortical lysates from the human brains of AD accompanied with amyloid pathology compared to aged/mid-age controls [79].

Another study found clusterin (CLU) is one of the genes contributing to the risk of a late-onset type of AD (LOAD); also it is heat shock family member that is highly conserved and participates in physiological processes, such as cell cycle, apoptosis, and adhesion, inflammation, lipid transport and membrane recycling. Clusterin binds to Aβ, prevent the aggregation of Aβ and promote their lysosomal degradation. Also, they found that the expression of sortilin increased with a reduction in clusterin level due to Aβ 1-42 treatment in

primary hippocampal neurons in the rat and M17 neuroblastoma cells of human. Sortilin binds clusterin and targeting it to lysosomal degradation, thereby, knockdown of sortilin prevents clusterin degradation by Aβ induction [80].

AD also, characterised by propagated prions (misfolded tau proteins). Johnson *et al.* (2017) found that formation and histopathologic deposition of tau prion is highly confined to the hindbrain [81]. They used prion propagation assay and found that tau prion replication inhibited by inhibitors derived from forebrain such as sortilin. This inhibition is mediated through sortilin interaction with prion protein and direct it to the lysosomes for degradation and knockdown of sortilin accelerates prion disease. Sortilin plays a role in neuroprotection and in the presence of sortilin prevents the replication of tau prion and can be considered as a therapeutic target in tau-related disease [81].

In addition, other Vps10p members are being challenged in drug discovery, such as SorL1 for AD therapy [82]. A component of ginger called 6-shogaol has neuronal cell anti-inflammatory and antioxidant effects. This has been investigated to see whether 6-shogaol increases the levels of sortilin-related receptor 1 (SORL1) which is a neural sorting protein responsible for reducing the trafficking of amyloid precursor protein (APP) and thus prevent the generation of Aβ. They found that inhibiting SORL1 by siRNA results in an increase in BACE, secreted APP-β (sAPPβ) and Aβ. While, SORL1 activated via 6-shogaol and significantly downregulate BACE, sAPPβ and Aβ both in vitro and in vivo [82]. Further studies are required to elucidate the role of sortilin in AD pathogenesis and as well as the development of new therapies for the treatment of this devastating disease.

Parkinson's Disease (PD)

PD is a common neurodegenerative disease that occurs commonly in the elderly population and rare before 50 years old, with an incidence ranges from 5 to over 35 new cases per 100,000 cases every year worldwide [83]. PD is caused by progressive impairment and death of dopaminergic neurons in the substantia nigra. Both Lewy bodies and Lewy neuritis are the hallmarks of PD that consist of aggregated alpha-synuclein (α-syn) found in assemblies of fibrillar protein in neural cells [84, 85]. Szego *et al.* (2013) showed that gene expression of NGF and TrkA was upregulated in wild type mice treated with 1-methyl 4-phenyl-1,2,3,6-

tetrahydropyridine hydrochloride (MPTP), in contrast, sortilin and $p75^{NTR}$ genes were upregulated in the A30P α-synuclein-expressed mice [86].

Diabetes mellitus (DM)

Diabetes mellitus (DM) is a metabolic disorder, classified into type I and type II. DM is characterised by chronic hyperglycaemia, with an incidence of about 415 million people worldwide, 90% of cases had type II DM mainly in low- and middle- income countries [87], and causes death of around 1.6 million people in 2016 [88]. Type I DM is juvenile-onset diabetes, caused by T-cell mediated attack on the beta cells of the pancreas that produce insulin, with a worldwide estimate of 542,000 affected children in 2015 [89]. Type II DM is the most common and is caused by insulin resistance with partial insulin deficiency, associated with ageing, obesity and physical inactivity [90].

In adipocytes, sortilin colocalised with glucose transporter type 4 (GLUT4) and is one of the major proteins in the GLUT4 storage vesicles. Sorting protein sortilin is responsible for the formation of insulin-responsive GLUT4 storage vesicles in adipocytes and myocytes to stimulate the insulin-regulated glucose uptake. Thus, the decrease in sortilin levels may prevent the trafficking and secretion of GLUT4 which could be linked to type II DM. However, further studies are required to establish the role of sortilin in type II DM [91, 92]. The defect in glucose transport is likely to be correlated with insulin resistance and modulation of sortilin expression levels in obesity and diabetes [93]. In addition, they found that the TNF-α controls the sortilin expression in adipose tissues and low-grade inflammatory state in obesity that could contribute to insulin resistance [93]. A study has found that regulating NT and sortilin have reduced the chance of developing obesity, metabolic disorders and hepatic steatosis, which could be an exciting avenue to target in therapy [94]. Sortilin facilitates the trafficking of acid sphingomyelinase (aSMase) to the cell surface, resulting in metabolic changes in diet-induced obesity (DIO) and sortilin knockdown promotes insulin signalling in adipose tissue with a reduction in acid sphingomyelinase (aSMase) activity end with attenuation of hepatic steatosis and inflammation [94].

On the other hand, a large precursor that is responsible for the synthesis of neurotensin (NT) and contains a six amino acid NT-like peptide known as neuromedin N (NN). The C-terminal region of the precursor (pro-NT/NN) contains NT and NN separated by three Lys-Arg sequences. However, depending on the tissue that express the precursor the three Lys-Arg sites

are differentially processed to give rise to NT and NN [95]. A study showed the relation of plasma pro-neurotensin levels and the risk of developing diabetes among populations from Middle East and Caucasus [96]. They found that plasma pro-neurotensin levels are higher in Middle Eastern than Caucasian populations and effects the glucose control, thus this suggests that pro-neurotensin could be a type II diabetes strong determinant in populations from Middle East compared to Caucasian populations [96]. Another study showed there is a significant relation between fasting pro-neurotensin and developing diabetes, cardiovascular disease, and breast cancer in women. This study concluded that there is an interaction between sex and pro-neurotensin while confirmation of this phenomena still need further experiments to study the same outcome on men [97].

Cardiovascular Disease

Cardiovascular disease (CVD) is the leading cause to morbidity and mortality in Western countries, with an incidence of about 17.9 million people die every year, which represents 31% of all global death [98]. CVD involves the heart and/or blood vessels. Age, genetics, sex, smoking habits, diabetes, hypertension and hypercholesterolemia are the common risk factors that can contribute to developing CVD. Advances in technologies gave rise to next generation sequencing (NGS) which paved the way for Genome-Wide Association Studies (GWAS) of population-based cohorts [99-102]. Functional roles of sortilin in CVD, studied using NGS data, suggest that high expression of sortilin leads to increased LDL internalisation by reducing plasma level [103, 104] and transcriptional regulation of sortilin expression and LDL concentration in the circulation through CCAAT/enhancer-binding protein (C/EBPα) [105]. Recently, a study demonstrated that sortilin colocalised with proprotein convertase subtilisin/Kexin type 9 (PCSK9), a variant implicated in lipid metabolism in the TGN and causes its secretion from hepatocytes. As a result, sortilin expression mirrors PCSK9 plasma level and overexpression of sortilin confer high plasma PCSK9 [9].

Moreover, GWAS has helped to reveal the association between CVD and sortilin by finding out novel candidate genes or loci responsible for the cardiac phenotypes. A gene cluster, CELSR2-PSRC1-MYBPHL-SORT1 at loci 1p13.3 has many single nucleotide polymorphisms which may, in turn, affect the normal function of proteins and are associated with CVD. The intergenic region between PSRC1 and CELSR2 and downstream of SORT1 and MYBPHL has all the single-nucleotide polymorphisms. Where SORT1 encodes sortilin, PSRC1 encodes

proline/serine-rich coiled-coil protein 1, MYBPHL encodes myosin-binding protein H like and CELSR2 encodes cadherin EGF LAG (epidermal growth factor laminin A G-type) seven-pass G-type receptor 2. These studies help to use sortilin as a cardiovascular risk marker and as a potential drug target [106, 107]. Other studies based on ethnicity; demonstrated by whole genome sequence and lipoprotein (a) Lp(a) which is a risk factor for CVD with different concentrations in African and European ancestry. They found that genetic determinants have many unique determinants between Europeans and Africans, and the lipoprotein(a) Lp(a) rs12740374 a common variant independent of LDL cholesterol is the expression quantitative trait loci (eQTL) for SORT1. Both African and European people have up to 90% Lp(a) heritability by twin studies, and its concentration is linked to atherosclerotic CVD. Thus, Lp(a) can be used as a promising target in the treatment of CVD [108].

In addition, sortilin has a role in glucose and lipid metabolism regulation. Thus, any defect can lead to atherosclerosis and obesity. To study this role, a study showed that female mouse model with a deficiency in both low-density lipoprotein receptor ($Ldlr^{-/-}$) and sortilin ($Sort1^{-/-}$) causes suppression of Niemann-Pick type C1-Like 1 (NPC1L1) mRNA levels; NPC1L1 a protein responsible for the absorption of intestinal cholesterol [109]. This suppression causes a decrease in body weight and white adipose tissue, which store lipid and improves the function of brown adipose tissue that takes part in energy expenditure and lipid metabolism via partial downregulation of the transcription of Kruppel-like factor 4 and liver X receptor [109].

Other Diseases

α-1 antitrypsin deficiency (α1ATD)

α1ATD is a genetic disorder that leads to the accumulation of abnormal α1AT protein in liver cells due to a decrease in the activity of α-1 antitrypsin in the blood and lungs. The incidence of the disease in Europe is about 1 in 600 and 1 in 2000 individuals, while between 1 in 3000 and 1 in 6000 individuals in the USA [110, 111]. It is caused by a mutation that gives rise to the Z variant of AT (α1ATZ) and creates a defect in folding and secretion. As α1ATZ builds up in the liver, it aggregates and accumulates in the liver, and at the same time leads to a decrease in α1AT in the lungs [112]. However, the severity of ATZ-associated liver disease could be altered by modification of sortilin levels; a study demonstrated that ATZ after being

routed from TGN is degraded in the vacuole, and sortilin acts as a receptor that can deliver the aberrant proteins to the vacuole [113].

Lysosomal storage disease (LSD)

LSD is an inborn error of metabolism, affecting the normal function of the lysosome. Mutations in the lysosomal protein-encoding genes cause LSD by affecting the integrity of lysosomal glycosidases, proteases, transporters, integral membrane proteins, and modifiers or activator enzymes [114]. Globally LSD has an incidence of 1 in 7000 to 1 in 8000 live births [115]. Mutations produce malfunctioned proteins, which in turn cause the accumulation of substrates in the lysosome and result in cellular dysfunction and death [114]. The standard LSD classification includes storage molecules like sphingolipidoses, mucopolysaccharidoses and glycoproteinosis and recently updated with lysosomal membrane proteins, activator proteins, transport proteins and non-lysosomal proteins [116].

Lysosomes are vesicles containing digestive enzymes, like glucosidases, proteases, and sulfatases. The ER is responsible for the synthesis of lysosomal enzymes that are transported to the Golgi apparatus then directed to lysosomes by the addition of mannose-6-phosphate label [115]. Sortilin plays a role in the mannose-6-phosphate independent pathway and binds to various ligands, such as neurotensin and receptor-associated protein (RAP). Sortilin mediates trafficking of proteins, like sphingolipid activator proteins including prosaposin and GM2 activator proteins, acid sphingomyelinase, cathepsins D and H, to lysosomes [19, 117-119]. However, the most recent therapeutic approaches for LSD are enzyme replacement therapy, gene therapy or hematopoietic stem cell transplantation, pharmacologic chaperone for defective enzyme stabilisation or substrate reduction therapy [116].

Cancer

Sortilin, one of Vps10p sorting receptor family members, is involved in several roles, including intracellular protein transport and as a co-receptor**.** Sortilin interacts with the neurotrophin Trk receptors (Trk-A, -B, and -C) and enhances their signalling pathways. Therefore, a defect in sortilin expression effects neurotrophin signalling pathways and could contribute to the development of different types of cancer. However, deregulation of sortilin expression has been studied in several carcinomas such as breast, colorectal, thyroid, lung, and glioblastoma.

Breast Cancer

Breast cancer is the most common cause of death among women, with a chance of about 1 in 4 cancer cases among women and about 2.1 million cases were diagnosed in females in 2018 [120]. Most breast cancer cells growth and maintenance dependant on oestrogen hormones. Breast cancer is divided into three major subtypes due to the presence or absence of molecular markers for the oestrogen or progesterone receptor and human epidermal growth factor 2 (ERBB2; previously known as HER2). Approximately, 70% of patients are hormone receptor (HER2) negative, 15-20% are (HER2) positive, and 15% are triple-negative, all markers are absent [121]. Tamoxifen is the selective oestrogen receptor modulator, which until now used as a standard therapy; also, oestrogen receptor pathway correlates with human epidermal growth factor receptor (HER2) pathway with about 22% of HER2 is overexpressed in breast cancer [122].

Several studies have shown that sortilin participates in breast cancer cells invasion [123, 124]. Pro-NGF dependent autocrine signalling is mediated by overexpressed TrkA and sortilin, activating Akt and Src, in breast cancer cell invasion. [123]. In addition, NGF promotes breast cancer cell proliferation with less expressed TrkA and overexpressed $P185^{HER2}$ [125]. Moreover, progression of breast carcinoma from a primary tumour to pleural effusion is mediated by overexpressed phospho-Trk-A receptor (activated receptor) [126].

Progranulin is an 88-kDa secreted glycoprotein cleaved by neutrophil elastase and matrix metallopeptidase 12 (MMP12) to produce biologically active 6-kDa granulin peptide domain [127]. Progranulin acts on differentiated cells that express sortilin and initiate tumour progression partially by triggering dedifferentiation and increasing the cancer stem cell pool proliferation. Thus, inhibition of sortilin blocks the propagation induction by progranulin in the breast cancer stem cells and prevent metastasis and infiltration [127]. On the other hand, EphA2 was identified, which is a membrane receptor tyrosine kinase through a study on proteins interacting with TrkA upon pro-NGF stimulation [128]. In breast cancer cells, signaling through sortilin and TrkA by pro-NGF binding recruits EphA2, which in turn causes Src activation in a TrkA phosphorylation-independent manner. As an assumption, proNGF/TrkA/EphA2 axis can be used both as a marker for diagnosis and target for therapy. [128]. Zhang and colleagues studied TrkA signalling pathway because of its role in the proliferation of breast cancer cells that are resistant to chemotherapy [129]. They found that

knockdown of TrkA by small interference RNA (siRNA) results in the inhibition of cancer cell proliferation and cell cycle arrest at G0/G1 phase through NF-κB p65 inactivation and induces apoptosis through activation of caspase-3. Thereby, to enhance breast cancer's chemotherapy effectiveness, TrkA pathway inhibition could be a potential pharmacologic target [129].

Colorectal Cancer (CRC)

Colorectal Cancer (CRC) is the third cancer-related deaths worldwide [130]. CRC incidence is steadily increasing in the world, particularly in developing countries with an incidence of about 2 million new cases and 1 million deaths expected in 2018 [131]. Many risk factors are associated with the development of CRC, including age and hereditary factors, while environmental and lifestyle are modifiable factors heavily contributing to the high incidence of the disease [132]. Many tyrosine kinase receptors, such as epidermal growth factor receptor (EGFR), vascular EGFR and tropomyosin-related kinase B (TrkB), are involved in the initiation, progression and metastasis of CRC. TrkB is overexpressed in tumorous compared to non-tumorous tissues and correlate with the density and metastasis to the lymphatic vessels [133]. The BDNF and its receptor TrkB, are also overexpressed in colorectal cancer cell lines and BDNF expression and release are increased by blockage of a gastrin-releasing peptide by EGFR-dependant mechanism [134].

In contrast, it has been suggested that TrkB function is to protect CRC cells from anoikis in the circulatory and lymphatic systems [135]. A study explained the pathway involved in the development of CRCs through the phosphorylation of Akt via the upstream phosphorylation of the complex focal adhesion kinase and steroid receptor coactivator (FAK-Src) by small concentrations of soluble sortilin (10nM). This pathway regulates several downstream intracellular pathways responsible for cell migration and proliferation [136]. Stress culture conditions enhance autocrine secretion of BDNF and relocate TrkB to the cell surface of the CRC cells, which is a major mechanism in cancer cell survival [137]. As a result, BDNF and TrkB are important for the growth of CRC cells *in vitro* and in tumours. In another study the dual inhibition of TrkB/BDNF and autophagy pathways reduces the tumour volume followed by long term treatment and thus can be considered as a new therapeutic approach for CRC [138].

Thyroid Cancer (TC)

Thyroid cancer (TC) causes around 567,000 cases, with an incidence rate of 10.2 per 100,000 women around the world, which means it is three times higher in women than men, and ranking in ninth place [120]. The follicular or papillary variants (differentiated thyroid cancer) comprises over 90% of thyroid cancers [139]. It is suggested that hormonal factors play a role in the aetiology of thyroid cancer [140, 141]. Furthermore, studies showed that thyroid-stimulating hormone (TSH) may be involved in the development of thyroid carcinomas, with epidemiological evidence that smoking, and alcohol consumption may be inversely associated with the production of TSH [142, 143].

In thyroid cancer, the pro-NGF is overexpressed and suggests the role in pathogenesis [144]. However, there is no clear status of NGF/pro-NGF receptors in thyroid tissue. A study explained that pro-NGF binds to the complex of sortilin-$p75^{NTR}$ receptor and initiates several signalling pathways, such as NF-κB, RhoA, and JNK. Also, neurotrophin tyrosine receptor kinase 1 (NTRK1 or TrkA) activated by pro-NGF [145]. Targeting overexpressed TrkA, sortilin and $p75^{NTR}$ in thyroid cancers resulted in growth and invasion reduction, which could be used as a therapeutic target [146].

Lung Cancer

Lung cancer is the leading cause of cancer-related mortality worldwide, with about 2.1 million cases and 1.8 million deaths reported in 2018, which represents around 18.4% cancer deaths [120]. There are two histological types of lung cancer, small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). NSCLC represents about 85% of all lung cancer cases, with common subtypes named adenocarcinoma and squamous cell carcinoma [147]. Cigarette smoking is the common cause of lung cancer [148, 149] and is responsible for over 40% of deaths from lung diseases such as cancer according to WHO. Also, cumulated exposure to air pollution can cause lung cancer as an adverse effect to this exposure. Also, exposure to carcinogens leads to increased risk of lung and other cancers [150, 151]. Genetic factors may contribute to developing lung cancer by gene mutation, and those with one copy of the mutated gene have 30% risk of developing cancer, compared to around 70% in those with two copies [152-154].

Tyrosine kinase receptors, such as EGFR, transmit information into the cell from the microenvironment thereby activating the relevant signalling pathways and inducing cell proliferation. Sortilin has an important role in the communication between the NSCLC and endothelial cells through exosomes. This is achieved through the interaction of sortilin with two tyrosine kinase receptors (TrkB and EGFR) called the TES complex, which is found inside lung cancer exosomes. The TES-containing lung cancer exosomes were shown to control cell migration and activate angiogenesis [64]. Sortilin was also shown to regulate EGFR internalisation and loss of sortilin in tumour cells induced the proliferation by maintaining EGFR signalling at the cell surface [65]. A study on the expression of NGF, pro-NGF, TrkA, sortilin and $p75^{NTR}$ in a series of lung cancers compared to normal lung tissues demonstrated that the expression of NGF, pro-NGF increased in squamous cell carcinoma and adenocarcinoma and TrkA in squamous cell carcinoma, while $p75^{NTR}$ was increased in all lung cancer types, compared to sortilin which was shown to be highly expressed in adenocarcinoma and small cell carcinoma. This implies that targeting NGF-TrkA axis in squamous cell carcinomas can be used for therapeutic effects [155]. On the other hand, TrkB can be used as a therapeutic target in metastatic lung adenocarcinoma as explained [156]. In this study, they found that TrkB expression is directly upregulated by hypoxia-inducible factor-1α [156]. The data suggest that TrkB drives migration via partial activation of Akt signalling and its knockdown in human lung cancer cells decreases migration and metastasis significantly [156]. NTRK2 gene encodes two isoforms, TrkB-FL which is a full-length receptor responsible for the regulation of several pathways related to cancer, and TrkB-T1 which is a truncated form having a different cytoplasmic domain encoded in a unique 3' terminal exon of the mRNA. A study demonstrated that the most abundant mRNA is TrkB-T1 in oral, laryngeal, oesophageal and lung squamous cell carcinoma examined compared to normal cell types. TrkB-FL binds BDNF and activates different signalling pathways such as the PI3K/AKT pathway, ERK/MAPK pathway, and phospholipase C pathway. While the TrKB-T1 isoform can form heterodimers with the active forms of kinase and act as dominant-negative of TrkB-FL, also can compete for BDNF, and alter the proliferation and survival phenotypes of neural cells [157].

The oncogenic driver neurotrophin receptor kinase (NTRK) gene fusions (NTRK+) are rare but present in solid tumours such as lung cancer. NTRK family consists of three members (NTRK1, NTRK2, NTRK3). A large number of Chinese lung cancer patients were screened over three years and they were found positive for NTRK1 fusions and other genetic alterations

that coexisted with NTRK1 fusions, such as mutations in tumour suppressor genes Tp53, neurofibromin (NF1), and retinoblastoma (RB1) [158]. It was concluded that NTRK1+ lung cancer accounts about 0.1% of NSCLC cases, and one half of NTRK+ NSCLC cases has a resistance mechanism to EGFR tyrosine kinase inhibitors (TKIs) regardless of which generation of EGFT TKIs used. This study did not characterise NTRK2/3 fusions, thus study of NTRK2/3 will be important to assess in future studies [158].

Glioblastoma multiforme (GBM)

The most common and deadly type; affecting adult males with 40% cases progressing from a lower-grade brain tumour to the malignant type, while the remaining 60% are *de novo* [159, 160]. Also, it represents stage IV of gliomas and considered as the most aggressive type with the median survival time being 15 months, even after treatment with chemotherapy, radiotherapy and surgical procedures [161]. GBM is the most aggressive primary malignant brain tumour with an incidence of less than 10 per 100,000 people globally and accounts for 60% of brain tumours in adults [162]. Also, it is the third cancer-causing death with 2.5% deaths between the ages of 15 to 35 years [163].

The aetiology of brain cancers still not fully understood, and they are highly incurable. The expected carcinogenetic cause is the exposure to high ionizing radiation dose. At the same time, there is no conclusive effect of environmental factors like, smoking, cell phones, electromagnetic field, severe head injury, and diets with GBM initiation [164]. Several studies explained the role of neurotrophins and their receptors for glioma invasion and proliferation [165, 166]. Neurotrophins have been predicted to promote tumour growth. Singer and colleagues have demonstrated NGF treatment stimulates cell proliferation of glioblastoma cell lines, as well as enhancing the secretory pathway of NGF [167]. Another study demonstrated the relationship between the expression of neurotrophin receptors and YKL-40 expression (YKL-40 abbreviation derived from the first three N-terminal amino acids code, tyrosine (Y), lysine (K), and leucine (L) with the molecular weight) in the cells, which plays a role in the progression and aggressiveness of glioblastoma. YKL-40, is one of the mammalian chitinase-like glycoprotein family members, encoded by CHI3L1 gene, and has a role in GBM cell migration, survival, invasion and angiogenesis. Silencing of YKL-40 in U87-MG cells results in the reduction of TrkB, sortilin and $P75^{NTR}$ expressions, and thus a decrease in GBM

aggressiveness [168]. Another study proved that sortilin level correlates with GBM aggressiveness and poor prognosis. Also, promotes glioblastoma invasion and mesenchymal transition through Glycogen Synthase Kinase 3 beta (GSK-3β)/ β-catenin/ Twist pathway [169]. Tong *et al.,* investigated $p75^{NTR}$ expression and regulation in hypoxic glioma cells *in vivo* and *in vitro* [170]. They found that $p75^{NTR}$ regulates the glioma aggressiveness in hypoxia, such as migration, invasion and stemness [170]. The expression and protein levels of $p75^{NTR}$ are upregulated in primary hypoxic human glioma cells. The $p75^{NTR}$ stabilizes hypoxia inducing factors (HIF-α, HIF-β). Knockdown of $p75^{NTR}$ results in a decrease of expression of both HIF-α and HIF-β at hypoxia. While knocking down of one of HIFs did not affect the $p75^{NTR}$ level, this explains that $p75^{NTR}$ is the regulator of hypoxic response of glioma cells and thereby can be represented as a target to treat brain tumors [170].

Targeting neurotrophins for the treatment of disease

The imbalance in neurotrophin signalling characterising neurodegenerative diseases suggests that activation of Trk receptors using agonists or blocking the effect of $p75^{NTR}$ through antagonists represent a promising therapeutic approach. However, clinical trials utilising exogenous neurotrophin agonists have failed due to poor pharmacokinetic and pharmacodynamic properties as well as unintended targeting of $p75^{NTR}$ [171]. In Alzheimer's disease, a β-turn peptidomimetic (D3) which acts as TrkA agonist and stimulates dimerization and phosphorylation of the receptor [172]; also was showed an improvement in learning and short term-memory when tested on Alzheimer's mouse module [173]. A natural product Gambogic amide activates TrkA by kainic acid and decreases neural death [174]. Whereas, Paecilomycine A [175] and Deoxygedunin [176] has TrkB agonist's effect that induces neurogenesis and neural survival, respectively. Also, Deoxygedunin has a neuroprotective effect when studied in Parkinson's disease animal models [177]. Moreover, competitive ligands with pro-NGF and NGF on their binding to $p75^{NTR}$ could lead to neuroprotection; such ligands are LM11A-24, and LM11A-31 have been tested on Alzheimer's disease mouse model [178].

Variations in the expression and activity of Trk receptors such as NTRK gene alterations and fusions are considered as cancer drivers; therefore clinical trials are ongoing to test pan-TRK inhibitors that target TrkA, -B, and -C on several cancer types [179, 180]. Efforts in developing a more specific sortilin inhibitor (A38469) which could be utilised in the study

of sortilin function and its role in disease [181]. A recent study in Western diet (WD)-fed mice showed that using SORT1 inhibitor (AF38469) by oral route reduces the plasma cholesterol level and provides evidence that it could be used as a pharmacological target to treat dyslipidaemia [182]. On the other hand, ongoing clinical trial studying the effect of AL001, which is a recombinant human anti-human SORT1 monoclonal IgG1 antibody has an FDA orphan drug designation to treat frontotemporal dementia. It is designed to target GRN mutations, thus could increase the progranulin level in the plasma and cerebrospinal fluid (CSF) in a dose-dependent manner [183, 184]. Another study based on conjugating a cytotoxic drug to peptide ligands that can selectively bind an exclusively expressed receptor on cancerous cells. They used conjugated Doxorubicin to a sortilin binding peptide (KA-peptide) in ES-2 and SKOV-3 ovarian cancer cell line models [185]. This study paves the way to use personalised therapy in the treatment of cancers through targeting sortilin [183].

Conclusion and future perspective:

Herein, we have explored the connections between the neurotrophin pathway and sortilin function in a number of life-threatening human diseases. Sortilin is a Vps10p domain receptor present inside the cell or on the cell surface encompassing different roles in the cell as a receptor, co-receptor or protein transporter in the secretory pathway. The interaction of sortilin with neurotrophins through tyrosine receptor kinases and p75 initiate signals for cell survival and apoptosis respectively. A common denominator is that the neurotrophins pathway are linked to signalling pathways including the EGFR pathway by cross-talk between three major MAPK pathways and PI3K/AKT survival pathway. Interestingly, sortilin interacts with EGFR/and or TrkB and traffics these co-receptors to the nucleus or are secreted from the cell as exosomes for cellular communication. The function and expression of sortilin is finely tuned to facilitate normal physiological processes and thus an imbalance may promote the development of many human diseases such as AD, CVD, type II DM and cancer. The expression of sortilin at the transcript and protein level is closely regulated in the different human diseases with strong evidence of a genetic risk and direct association. To note, the monitoring of sortilin levels in the circulation could be a useful biomarker for the presence of diseases such as CVD and type II DM. Targeting the neurotrophins pathway could provide a promising route to combat a number of human diseases such as neurodegeneration and cancer. With this endeavour, a number of potential therapeutic strategies are emerging into the clinic.

There is hope that these drug discovery strategies will challenge the role of neurotrophins in human disease. Still, the ideal therapeutics would correct the imbalance or dysregulation of the neurotrophin pathway. Further studies are required to understand the cellular mechanism of sortilin and neurotrophins in normal versus disease progression and to determine specific active molecules that can be targeted in the site of action avoiding other tissues.

Figure legends

Figure 1 Neurotrophins and their tyrosine receptor kinases (Trks) and p75NTR. The precursor form of neurotrophins (pro-NGF, pro-BDNF, proNT-3, and proNT-4) interact with high affinity to $p75^{NTR}$). After cleavage, the mature form neurotrophin NGF interacts with a high affinity to TrkA, BDNF and NT-4 to TrkB, and NT-3 to TrkC. Sortilin forms a complex with p75NTR and induces cell death upon proneurotrophins binding, while the complex that involve Trk receptors is included in cell survival pathways. CR1-CR4 cysteine-rich motif; C1/C2 cysteine-rich clusters; LRR1-3 leucine-rich repeats; Ig1/Ig2 immunoglobulin-like domains.

Figure 2 Neurotrophins signalling. This illustrates neurotrophin interaction with Trk, sortilin and p75 receptors, and the main intracellular signalling pathways activated through these receptors. The p75 receptor regulates three different pathways. Neurotrophins interaction with p75NTR activates NF-κB pathway that induces cell survival by TRAF6 association with $p75^{NTR}$ cytoplasmic domain and activation of IκB kinase (IKK) that phosphorylates and degrades IκB proteins to liberate NF-κB to enter the nucleus. While Jun kinase pathway promotes cell apoptosis through the activation of Jun kinase cascade followed by downstream activation JUN kinase (JNK) which in turn activates P53 and Bax that induces apoptosis, also phosphorylates c-JUN that translocates to nucleus while Rho activation controls growth cone motility. As mentioned in the text, interaction of p75 with sortilin and pro-neurotrophins stimulate pro-apoptotic action through JNK apoptotic pathway. The Trk receptor also stimulates three signalling pathways by forming complex with p75NTR and interact with neurotrophins. MAP Kinase signalling pathway induces cell differentiation by recruiting Shc that promotes Grb2-SOS complex formation which in turn recruits Ras and stimulates MAPK pathway, PI3K promotes cell survival and growth through GAB1 recruitment by phosphorylated Grb2 to activate protein kinase Akt control other proteins important in cell survival. PLC-γ activation results in calcium activation by IP3 and PKC by DAG, which both control synaptic plasticity.

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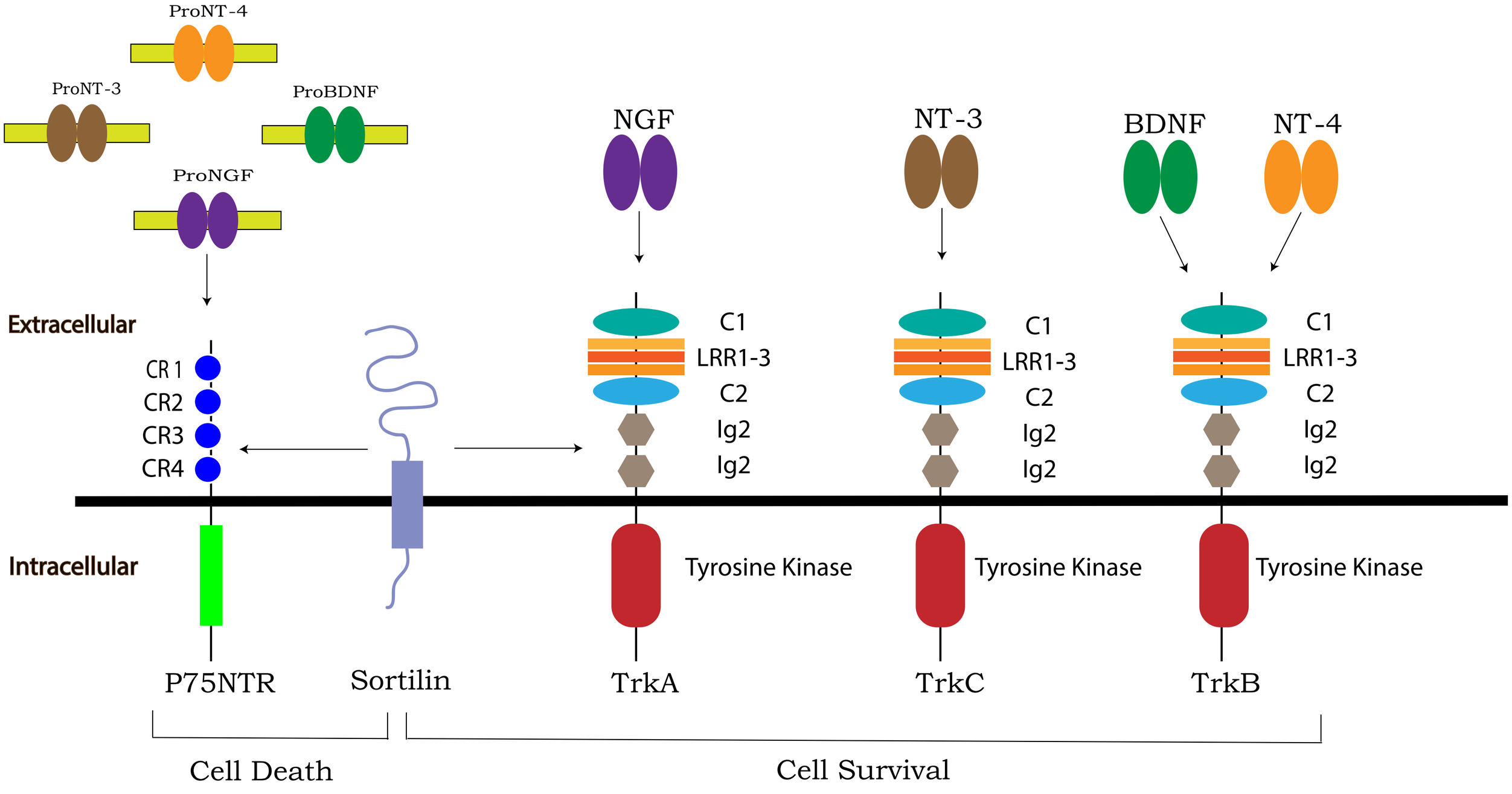
Figure 1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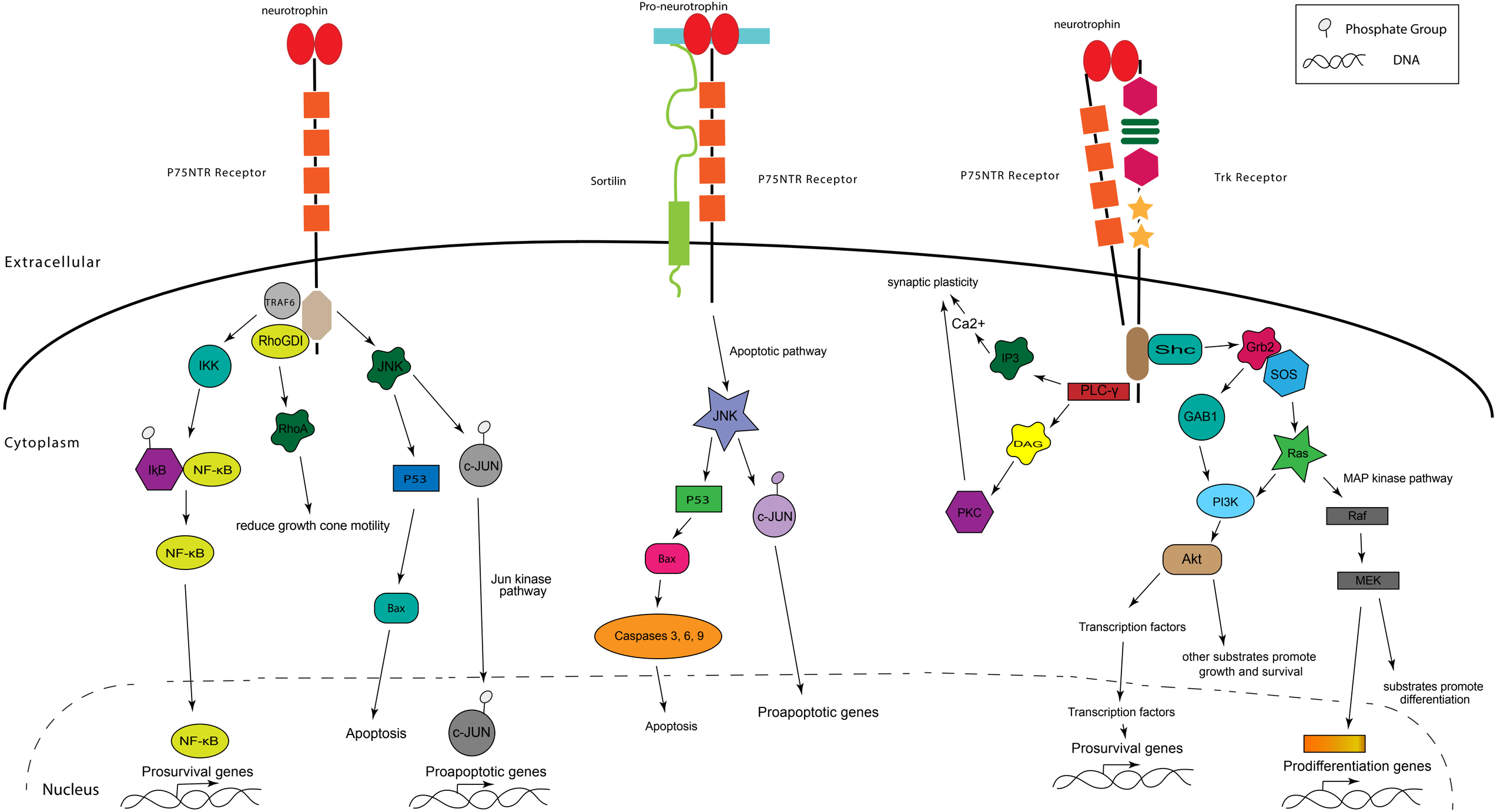
ProNT-3
ProBDNF
ProNGF
NGF
NT-3
BDNF
NT-4
Extracellular
CR 1
CR2
CR3
CR4
C1
LRR1-3
C2
Ig2
Ig2
Intracellular
Tyrosine Kinase
Tyrosine Kinase
Tyrosine Kinase
P75NTR
Sortilin
TrkA
TrkC
TrkB
Cell Death
Cell Survival

Figure 2


neurotrophin
P75NTR Receptor
Pro-neurotrophin
Sortilin
P75NTR Receptor
neurotrophin
P75NTR Receptor
Trk Receptor
Phosphate Group
DNA
Extracellular
Cytoplasm
TRAF6
RhoGDI
IKK
IkB
NF-κB
NF-κB
RhoA
reduce growth cone motility
JNK
P53
Bax
c-JUN
Jun kinase pathway
Apoptotic pathway
JNK
P53
Bax
Caspases 3, 6, 9
c-JUN
Proapoptotic genes
synaptic plasticity
Ca2+
IP3
PLC-γ
DAG
PKC
Shc
Grb2
SOS
GAB1
Ras
PI3K
Akt
MAP kinase pathway
Raf
MEK
Transcription factors
other substrates promote growth and survival
Transcription factors
Prosurvival genes
substrates promote differentiation
Prodifferentiation genes
Nucleus
NF-κB
Prosurvival genes
Apoptosis
c-JUN
Proapoptotic genes
Apoptosis