Review Article

Functions and the related signaling pathways of the neurotrophic factor neuritin

Jin-jing YAO, Qian-ru ZHAO[#], Jun-mei LU[#], Yan-ai MEI^{*}

Institutes of Brain Science, State Key Laboratory of Medical Neurobiology and School of Life Sciences, Fudan University, Shanghai 200433, China

Abstract

Neuritin is a member of the neurotrophic factor family, which is activated by neural activity and neurotrophins, and promotes neurite growth and branching. It has shown to play an important role in neuronal plasticity and regeneration. It is also involved in other biological processes such as angiogenesis, tumorigenesis and immunomodulation. Thus far, however, the primary mechanisms of neuritin, including whether or not it acts through a receptor or which downstream signals might be activated following binding, are not fully understood. Recent evidence suggests that neuritin may be a potential therapeutic target in several neurodegenerative diseases. This review focuses on the recent advances in studies regarding the newly identified functions of neuritin and the signaling pathways related to these functions. We also discuss current hot topics and difficulties in neuritin research.

Keywords: neuritin; neurotrophic factor; insulin receptor; MEK-ERK pathway; PI3K-Akt-mTOR pathway; Ca²⁺-CaN-NFATc4 pathway

Acta Pharmacologica Sinica (2018) 39: 1414–1420; doi: 10.1038/aps.2017.197; published online 29 Mar 2018

Introduction

Neuritin, discovered by Nedivi and colleagues in 1993^[1], is an activity-induced neurotrophin factor, named for its function of promoting neurite outgrowth. Neuritin mRNA is predominantly expressed in the nervous system, although minor expression can also be detected in other organs^[2]. Although most studies regarding neuritin have focused primarily on its regulation and functions in neuroprotection and regeneration, other functions including roles in angiogenesis, tumorigenesis, and immunomodulation have also been reported. For example, new evidence has shown that neuritin expression can be down-regulated or up-regulated among different cancer types^[3–5]. Another study found that recombinant neuritin protein increased human umbilical vein endothelial cell migration and overexpression of neuritin significantly promoted tumor angiogenesis^[6]. In addition, Barbi *et al* found that neuritin could maintain and promote regulatory T cell (Treg) function in autoimmune diseases and cancer. This study further characterized neuritin as underappreciated immunoregulatory molecule and a potential target for therapies aimed at the fine-tuning of Treg function in cancer and inflammatory/autoim-

mune diseases^[7].

Although several reviews^[8, 9] have discussed the physiological and pathological functions of neuritin in nerve and non-nervous tissue, the primary mechanisms of neuritin, including whether or not it acts through a receptor or which downstream signals might be activated following binding, are not fully understood. In this brief review, in addition to the biological characteristics of neuritin and its neuronal functions, we also discuss work published by this lab and others regarding advances in uncovering the mechanisms involved in neuritin-mediated signaling^[10–12].

The biological characteristics of neuritin

Using Northern-blot analysis, scientists discovered that neuritin is expressed in many organs of the human body during puberty and adulthood. Among these organs, the highest expression levels are found in the brain, followed by liver, lung, heart, and skeletal muscles, respectively. Since its discovery nearly two decades ago, new techniques have aided in identifying the genes which encode for neuritin^[2, 13].

The structure of the neuritin gene and its subsequent activation

The human neuritin gene (candidate plasticity gene 15, CPG15) is located on chromosome 6 (6p25.1); is 2072 bp long; and is highly conserved across species, with a homology of 97% between human and mouse (*cpg15*). The open reading

[#]These authors contributed equally to this work.

^{*}To whom correspondence should be addressed.

E-mail yamei@fudan.edu.cn

Received 2017-09-20 Accepted 2017-12-08

frame is 426 bp long and contains two introns and three exons which encode the amino acid sequences 1–19, 20–67, 68–142, separately. The entire coding DNA sequence (CDS) of *cpg15* encodes 142 amino acid residues; the first 27 of which act as a signal peptide; while residues 116–142 residues act as the glycosylphosphatidylinositol (GPI) anchor. The remaining 88 amino acid residues are considered as the core of neuritin^[2].

The immediate-early gene, *cpg15*, can be induced by Ca^{2+} influx through *N*-methyl-D-aspartate (NMDA) receptor and L-type voltage-gated calcium channels (VGCC). Influx of Ca^{2+} can further activate the Ca^{2+} /calmodulin-dependent protein kinase pathway and also the mitogen-activated protein kinase (MAPK) pathway, thus initiating transcription of the *cpg15* gene^[2]. Although the activity-dependent expression of neuritin does not require the activation of protein kinase A (PKA), Fujino and colleagues found that *cpg15* could be induced by cyclic adenosine monophosphate (cAMP) in active neurons^[14]. Furthermore, they proved that CREB can bind to the promoter region of *cpg15* *in vivo* and partially regulate the activity-dependent expression of neuritin.

In addition to *cpg15* and its encoded protein, neuritin, Fujino *et al* also identified the only paralogue of *cpg15* in the mouse and human genome, *cpg15-2*^[15]. Specifically, they found that both *cpg15* and *cpg15-2* were regulated by neuronal activity; the proteins for which they encoded shared basic biochemical properties; and both proteins had similar functions in neurite growth and neuronal survival. However, *cpg15* and *cpg15-2* were found to be differentially expressed, with *cpg15* mRNA most abundant in the brain and liver, while *cpg15-2* mRNA was most abundant in the eye and brain. Within the central nervous system (CNS), *cpg15* mRNA was most abundant in the cerebral cortex, followed by the hippocampus and thalamus, respectively; whereas *cpg15-2* was expressed most abundantly in the retina, followed by the olfactory bulb and striatum, respectively. As a result of the discovery of *cpg15-2*, several researchers termed the protein encoded by this gene as neuritin-2. However, to date, additional studies regarding neuritin-2 have been lacking.

Neuritin expression and regulation

Neuritin is concentrated in the neuronal soma and has been shown to be unevenly dispersed along neurite projections in the rat brain^[2]. Sato *et al* showed that neuritin is expressed in puncta throughout cell bodies, dendrites, and axons; and its expression in axon terminals is particularly strong^[16]. A recent study by Merianda *et al* showed that neuritin mRNA shifted from neuronal cell bodies to axons, predominantly after nerve crush injury. Interestingly, neuritin mRNA also showed different localization elements for CNS and PNS axons: the 3' untranslated region (UTR) drove localization in hippocampal neurons and the 5' UTR drove localization in sensory axons^[17].

As *cpg15* is an activity-regulated gene, late neuritin expression is contemporaneous with critical periods for activity-dependent plasticity and requires action potential activity. However, neuritin is also expressed in an activity-independent manner during early brain development, prior to circuit for-

mation and mutations^[18].

Added to the complicated regulation of neuritin expression, scientists found two forms of neuritin expressed in the CNS; one being membrane-bound and the other soluble. Membrane-bound neuritin is attached to the cell plasma membrane through a GPI linkage and has been shown to function non-cell autonomously to regulate growth of opposing dendritic and axonal arbors; and also promotes synaptic maturation^[19]. Putz *et al* found that soluble neuritin expressed in the embryonic brain could regulate survival of cortical progenitors by preventing caspase-mediated apoptosis^[20]. These observations suggest that: neuritin has different expression patterns at different development stages; and the two forms of neuritin (membrane-bound and soluble) have diverse functions.

The neuronal functions of neuritin

As a neurotrophic factor, neuritin has multiple effects in the nervous system and many studies related to its neuronal functions have been investigated primarily through studies involving the overexpression, gene silencing, and application of neuritin. To date, scientists have showed that neuritin is important in neuritogenesis, neurite arborization, and neurite extension^[9].

Neuritin promotes neuritogenesis and neuronal regeneration

Naeve *et al* found that treatment of primary cultured rat hippocampal and cortical neurons with recombinant neuritin over 4 days produced extensive neuritogenesis compared with control cultures^[2]. Other groups have found similar neuritogenesis effects in PC12 cells and in motor neurons^[21–23]. Moreover, Picard *et al* indicated that the absence of neuritin by global knock-out of *cpg15* induced aberrant development and plasticity of excitatory visual cortical networks^[24]. In addition, the effect of neuritin in promoting neuritogenesis also has been found in hippocampal neurons isolated from depression models; whereby viral-mediated expression of neuritin in the hippocampus prevented atrophy of dendrites and dendritic spines caused by chronic stress^[25]. Taken together, these studies indicate that neuritin plays an important role in neurodevelopment and neural plasticity.

Neuritin is also a potential target in the study of neuronal regeneration after injury. Di Giovanni *et al* found that in neurons and axons in an experimental rat spinal cord injury (SCI) model, neuritin mRNA and protein levels were down-regulated 24 h post injury; followed by strong re-induction during the following 2 weeks, which paralleled functional recovery^[26]. This finding was recently confirmed by Gao *et al*, where they suggested that neuritin created an environment that promoted nerve cell survival and neurite regeneration after SCI, thus contributing to nerve regeneration and recovery of motor function^[27]. Besides spinal cord injury, results obtained by Zhao *et al* suggest that soluble neuritin secreted by astrocytes promoted the recovery of injured hippocampal neurons via adherence to the surface of neurons in ischemic brains^[28].

Similar neuroprotective and regenerative effects were

observed in retina, optic nerve and sciatic nerve fiber after injury. Using an optic nerve crush mouse model, Xu *et al* found that neuritin mRNA and protein were transiently increased in retina and optic nerve after injury. They also found infection of rat retina with neuritin-expressing lentivirus significantly increased the number of retinal ganglion cells (RGCs) and optic nerve axons compared with controls; these effects were subsequently confirmed by functional tests^[29]. Using a similar optic nerve crush model, Sharma *et al* evaluated the axogenic properties of neuritin on axotomized RGCs *in vitro* and on the optic nerve *in vivo*, and found that recombinant neuritin significantly increased RGCs survival and neurite outgrowth. Furthermore, significant increases in Gap43, a classical marker of axonal regeneration, were observed within the optic nerves of the adeno-associated virus (AAV)2-neuritin group, suggesting that neuritin preserved RGC function on axotomized RGCs *in vitro* and after axonal injury *in vivo*^[30]. Using a sciatic nerve injury model in rats, Wang *et al* reported that recombinant human neuritin stimulated nerve regeneration and restored motor function, as assessed by the walking-track test and nerve conduction velocity; and thus promoted the repair of injured sciatic nerves^[31]. Although, neuritin can be both down-regulated and up-regulated at different stages in different injury models, the overall levels of neuritin increase after nerve injury; indicating that axonal neuritin protein synthesis may provide a spatially restricted mechanism for promoting axonal growth. Taken together, these data suggest that neuritin plays a critical role in nerve regeneration and is a potential new target for optic and sciatic nerve injury therapy.

Neuritin promotes synaptic maturation, stability and neurotransmitter release

The formation of CNS circuits is characterized by the development of neuronal structure and synaptic function. The effect of neuritin in promoting synaptic maturation was first reported by Cantallops and colleagues^[29]. Using whole-cell recording from *Xenopus* optic tectal neurons, they found that neuritin expression promoted retinotectal synapse maturation by recruiting functional AMPA receptors to synapses. In addition, they found that when a truncated form of neuritin (lack of GPI anchor) was expressed, it did not promote axon arbor growth and actually blocked synaptic maturation^[19, 32]. These results not only suggest that neuritin can coordinately increase the growth of pre- and postsynaptic structures and also strengthen their synaptic contacts; but that different forms of neuritin coordinate different processes. To investigate the requirement for neuritin in maturation of synapses, Fujino *et al* generated a *cpg15* knockout (*cpg15* KO) mouse and found that in these mice, there was a developmental delay in axonal and dendritic arborization. Electrophysiological studies also showed that synaptic maturation was delayed, and electron microscopy confirmed that many dendritic spines initially lacked functional synaptic contacts^[33]. These findings established a role for *cpg15* in the maturation of synapses and efficient circuit formation.

The frequency of miniature excitatory post-synaptic currents (mEPSCs) in neuron recordings has been used for the evaluation of synaptic efficiency; including number of synapses and the probability of transmitter release. Picard *et al* showed that the density of spine protrusions was lower in neurons of *cpg15* KO mice, which was consistent with the reduced mEPSC frequency measured at P28. These findings indicated that loss of neuritin likely resulted in the abnormal postnatal development of the excitatory network in the visual cortex and associated disruption in development of visual receptive field properties, suggesting loss of normal excitatory synapse maturation in neuritin deficient mice^[24]. In accordance with the Picard *et al* study in the visual cortex, data from this lab demonstrated that the overexpression of hippocampal neuritin by an AAV vector significantly increased neuritin levels and also dendritic spine density of hippocampal neurons^[34]. However, it is worth noting that a recent study from this lab involving the medial prefrontal cortex revealed that, although short-term incubation of cortical neurons with recombinant neuritin resulted in an increase in mEPSC frequency, this increase was not induced by increased dendritic spines density; but rather by the release of glutamate. Moreover, up-regulation of the T-type VGCC α -subunit ($\text{Ca}_v3.3$) at the membrane surface was also involved. This work provides direct evidence for neuritin-mediated modification of synaptic plasticity and synaptic transmission in cortical neurons^[12].

The role of neuritin in Alzheimer's disease (AD) and psychiatric disorder-related cognitive function impairment

As synaptic plasticity has been associated with depressive disorders, schizophrenia, and cognitive performance, studies regarding the potential therapeutic use of neuritin in combating loss of cognitive function have been reported. Additionally, a possible role for neuritin gene variations in psychiatric disorder etiology have also been reported recently. Son *et al* found that in a chronic unpredictable stress-induced rat depression model, neuritin expression in the hippocampus was decreased. Furthermore, viral-mediated expression of neuritin in the hippocampus produced antidepressant effects and prevented the atrophy of dendrites and spines; as well as depressive and anxiety behaviors caused by chronic unpredictable stress^[25]. These results demonstrate a unique action of neuritin in depressive disorders. A recent study from this lab also indicated that exposure of mice to extremely low-frequency (50 Hz) electromagnetic fields (ELF MFs) significantly decreased hippocampal dendritic spine density; and resulted in deficits in recognition memory, as detected by a novel object recognition test^[34]. AAV-mediated overexpression of neuritin in the hippocampus was found to reverse both ELF MFs exposure-induced reduction in dendritic spine density and deficits in recognition and novel object associative recognition memory^[34].

Another potential effect of neuritin is against AD-related neural and cognitive defects^[35]. Using the AD model Tg2576 mouse, An *et al* observed that exogenous application of recombinant neuritin fully restored dendritic complexity as

well as spine density in hippocampal neurons prepared from Tg2576 mice; without affecting neurite branching of neurons from wild-type littermates. Chronically infused neuritin into Tg2576 mouse brains also aided in the restoration long-term potentiation in acute hippocampal slices^[35]. Similarly, Choi *et al* indicated that viral-mediated overexpression of neuritin in the dentate gyrus of 13-month-old Tg2576 mice attenuated a deficit in learning and memory, as assessed by the Morris water maze test. This neuritin-mediated rescue effect on AD-induced cognitive impairments resulted in an approximate 3.6-fold increase in synaptophysin protein levels (a presynaptic marker). In addition, Choi *et al* also demonstrated that levels of neuritin mRNA were significantly decreased in the hippocampus and cerebral cortex of AD patients compared to age-matched control subjects^[36]. Consistent with this study, Fei *et al* found that microRNA-574 (miR-574) was up-regulated in the hippocampi of 5-month-old APP/PS1 mice; while overexpression of miR-574 lowered neuritin and synaptic protein expression in A β ₂₅₋₃₅-treated primary hippocampal neurons^[37]. These studies indicated that neuritin could be used as a potential therapeutic approach for patients with AD.

Recent studies have demonstrated that there is an association between neuritin and psychiatric diseases, including schizophrenia, schizophrenia-spectrum disorders, and bipolar disorders. Chandler *et al* and Fatjo-Vilas *et al* analyzed common variants along the neuritin sequence and their corresponding association with intelligence and schizophrenia-spectrum disorders by testing neuritin gene sequence variations on susceptibility to schizophrenia and on general cognitive ability in patients and non-psychiatric control subjects. Results suggested that certain allelic variants in the neuritin gene modulated the risk for developing schizophrenia-spectrum disorders and also reduced general cognitive performance. Thus, the association between variations in neuritin and schizophrenia suggests a role for neuritin as a modifier of cognitive function^[38, 39]. A subsequent study by Prats *et al* found that multiple genes were involved in this modification process and gene-gene interactions between dysbindin-1 and neuritin increased the risk of schizophrenia-spectrum disorders. Interestingly, both of these genes are known to be involved in synaptic maturation, connectivity, and glutamate signaling^[40]. These results suggest that the neuritin gene could be used as a potential marker for schizophrenia etiology.

Potential receptors of neuritin and related signaling pathways

Although previous studies implied that neuritin may function as a ligand^[15, 41], few have investigated the receptor/receptors responsible for neuritin signal transduction. For several years, this lab has worked on neuritin related pathways and the results may aid in the understanding of the role neuritin plays in the CNS.

Neuritin may be a potential ligand for the insulin receptor (IR)

We previously showed that incubation of cerebellar granule neurons (CGNs) with neuritin increased the density of

transient outward potassium currents (I_A) in a time- and concentration-dependent manner. Further studies revealed that these increases were due to the enhanced transcription and translation of Kv4.2, the main α -subunit of I_A ^[10]. Interestingly, as administration of insulin induces I_A density increases as well as increases in Kv4.2 expression, we hypothesized that the enhanced expression of Kv4.2 caused by neuritin may be through the activation of the IR. Indeed, biochemical analyses showed neuritin could activate IR and that pharmacological blockade of IR completely offset the effect caused by neuritin. To date though, neuritin is still considered as an orphan ligand and its receptor remains unknown. Although there is a lack of evidence indicating that neuritin may directly bind to the IR, our data suggest neuritin can be a potential ligand of IR.

However, in CGNs, insulin-like growth factor type 1 receptor (IGF-1R) is also present^[42]. Although IR and IGF-1R differ in ligand binding kinetics and functions, they share a high degree of structural homology^[43]. Therefore, it cannot be discounted that neuritin may also bind to IGF-1R. However, results indicated that pharmacological inhibition of IGF-1R affected neither neuritin induced upregulation of I_A density nor neuritin induced increases in Kv4.2 expression^[10]. Interestingly, Yan *et al* recently have shown that exogenous IGF-1 can rescue Schwann cells from hyperglycemia-induced apoptosis. In the same study, they found that neuritin mediated the rescue of IGF-1 and they considered neuritin as a downstream element of the IGF-1-PI3K signaling pathway^[44]. Taken together, these studies indicate that neuritin may not directly activate the IGF-1R.

Neuritin activates the MEK-extracellular signal-regulated kinase (ERK) and PI3K-Akt-mTOR pathways

ERK is an important regulator of neuronal functions, including synaptic plasticity, and long term memory formation^[45, 46]. Previous studies indicated that the ERK pathway is a target for growth factors such as NGF and also IR signal transduction^[47, 48]. Studies from this lab have shown that: incubation of CGNs with neuritin for 30 min dramatically increased the phosphorylation of ERK1/2; and elevated phosphorylation levels of ERK1/2 returned to baseline levels after 60 min of incubation. Notably, co-incubation of neuritin and U0126, an inhibitor of the MEK-ERK pathway, significantly blocked both neuritin induced Kv4.2 expression and increases in I_A density.

In addition to the MEK-ERK pathway, we also tested if neuritin activated the PI3K-Akt-mTOR pathway. This signaling pathway is essential for several forms of synaptic plasticity for learning and memory^[49, 50]. Similar to ERK, following incubation of CGNs with neuritin, the phosphorylation of both Akt and mTOR increased. However, unlike the quick response of ERK, the onset of Akt phosphorylation occurred 1 h after incubation and lasted up to 6 h. Interestingly, increased phosphorylation of mTOR was not found until 18 h after neuritin incubation, and lasted up to 24 h. Similarly, co-incubation of neuritin with either the PI3K inhibitor, LY294002, or the mTOR blocker, rapamycin, reversed neuritin-mediated increases in Kv4.2 expression and elevated I_A density.

Neuritin-mediated expression of *Kv4.2* requires the activation of Ca^{2+} -CaN-NFATc4 pathway

Most studies regarding neuritin have focused on determining how and when the neuritin-encoding gene, *cpg15*, is activated^[14, 18, 20]. As neuritin is also a neurotrophic factor, it may modulate the expression of other genes; for example, *Kv4.2*. Recently, we found that the Ca^{2+} -calcineurin (CaN)-nuclear factor of activated T-cells (NFAT) c4 axis is required for this modulation effect.

Using Ca^{2+} -sensitive fluorescent dye, Fura-2, *in vitro* calcium imaging data showed that neuritin-incubation for 15 min increased intracellular Ca^{2+} ($[\text{Ca}^{2+}]_i$) levels in mouse CGNs at both basal and high K^+ conditions. Potentiated $[\text{Ca}^{2+}]_i$ at high K^+ conditions could be a consequence of modulated T-type or L-type VGCCs^[51], while elevated $[\text{Ca}^{2+}]_i$ at basal levels may depend on increased release of calcium from endoplasmic reticuli (ER) through inositol 1,4,5-triphosphate receptors and/or ryanodine receptors^[52-54]. Additionally, increased $[\text{Ca}^{2+}]_i$ could also be the result of inhibited up-take of Ca^{2+} into ER by IR pathway activation^[55]. A recent study showed that neuritin up-regulated the surface expression of the $\text{CaV}3.3$ α -subunit^[9]. Although the precise mechanisms of increased $[\text{Ca}^{2+}]_i$ by neuritin need further exploration, there is little doubt that elevated $[\text{Ca}^{2+}]_i$ activate related pathways. Indeed, when neuritin was co-incubated with calmidazolium chloride, a calmodulin (CaM) inhibitor, the effects of neuritin on *Kv4.2* expression and I_A density were blocked.

It is well established that activated CaM modulates CaN, a Ca^{2+} and CaM dependent serine/threonine protein phosphatase^[56]. CaN may activate NFATc4, a transcription factor, via dephosphorylation. Activated NFATc4 is then translocated into the nucleus, where it regulates gene expression^[57]. Using the CaN inhibitor, cyclosporine A, or ascomycin (FK520), we found that translocation of NFATc4 was blocked, resulting in the enhanced transcription of *Kv4.2* and also increased I_A density; both mediated by neuritin. More importantly, in *nfatc4*^{-/-} mice, neuritin no longer affected *Kv4.2* expression or I_A density. Taken together, these data suggest that neuritin elevates $[\text{Ca}^{2+}]_i$, which subsequently activates the CaN-NFATc4 pathway resulting in up-regulation of *Kv4.2* and increased I_A density.

Other possible signaling pathways

Although few studies have focused on the mechanism or signaling pathways involved in neuritin-related functions, several groups have done interesting work related to potential signaling pathways^[58, 59]. Using sensory neurons in an experimental diabetic neuropathy, Karamoysoyli *et al* found in rat L4/5 small-diameter dorsal root ganglia neurons, neuritin was colocalized with the tyrosine kinase A (TrkA) receptor^[58]; however, there was no direct evidence indicating that neuritin activated the TrkA receptor. Recently, Shimada *et al* reported that neuritin and fibroblast growth factor (FGF) cooperated in inducing mossy fiber sprouting through FGF signaling pathways^[59]. In addition, they found that neuritin activated the MAPK, ERK, PI3K-Akt, and phospholipase C- Ca^{2+} pathways;

Figure 1. Schematic illustration depicting signaling pathways involved in neuritin-induced *Kv4.2* expression and the subsequent increase in I_A channel density.

which is consistent with our results. Interestingly, they also found that activation of these pathways could be blocked by inhibiting the FGF receptor. This latter finding suggests that interaction partners of neuritin may vary by cellular location. Recently, Zhang *et al* reported a mechanism connecting neuritin with Notch signaling. Using yeast two-hybrid and co-immunoprecipitation assays, they discovered an interaction between neuritin and Neuralized E3 Ubiquitin Protein Ligase 1 (NEURL1), an important regulator of Notch. Notably, recombinant neuritin restored the retraction of neurites caused by activation of Notch, and neurite growth stimulated by neuritin was partially blocked by NEURL1; suggesting that neuritin is an upstream and negative regulator of NEURL1 that inhibits Notch signaling to promote neurite growth^[23]. This mechanism provides a valuable foundation for further investigation into the role of neuritin in neurodevelopment and neural plasticity.

It is well established that neuritin is a GPI-linked protein^[2], and that GPI-linked proteins can act as heterophilic or homophilic adhesion molecules, which act as co-receptors for slit FGF and laminin^[60]. Although neuritin lacks the common immunoglobulin G domain of GPI-linked adhesion molecules, it does have some structural similarity to ephrins; a family of GPI-linked ligands that can activate their receptors by cell-cell contact^[61]. This indicates that GPI-linked neuritin could possibly be activated by the interaction of neuritin from adjacent neurons or between neuron and the extracellular matrix.

Conclusion and perspectives

Neuritin was discovered more than two decades ago and a large body of research exists regarding its functions, yet little is known in regards to possible receptors of this protein or what signaling pathways may be activated. As mentioned in this review, neuritin-mediated activation of IR triggered the MEK-

ERK, PI3K-Akt-mTOR, and Ca^{2+} -CaM-CaN-NFATc4 pathways resulting in the expression of Kv4.2 α -subunit in mouse CGNs: A proposed model is shown Figure 1. We also confirmed neuritin enhanced synaptic transmission in mouse mPFC neurons, a function that requires the activation of both IR and the MEK-ERK pathways. It remains to be elucidated whether there are other receptors and related signaling pathways for neuritin in different tissues and cells.

Although neuritin is anchored to the cell membrane, it has been shown that it can be cleaved by phospholipase C, producing a soluble form of neuritin^[19]. Most studies associated with neuritin have used expression plasmids which produce GPI-linked neuritin, while several labs including our own, used plasmids which produced the soluble form of neuritin. Although studies have proven that both GPI-linked and soluble neuritin are functional^[19, 59], it is not known whether these two different forms use the same receptor or similar activate signaling pathways. Future research is needed to clarify these questions.

Fujino *et al* discovered *cpg15-2* in both humans and mice^[15]. Interestingly, besides having a similar gene sequences, *cpg15-2* and *cpg15* also have similar temporal and spatial expression patterns. Furthermore, the two isoforms (netrin 1 and 2) encoded by these genes can form heterodimers. Future studies are warranted to investigate whether the function and regulation of neuritin-2 is different from neuritin-1. In addition, more work is needed in order to fully understand neuritin and its possible therapeutic role in neurodegenerative and psychiatric diseases.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (NSFC 31370827 and 31428003) and Shanghai Leading Academic Discipline Project (No B111).

References

- Nedivi E, Hevroni D, Naot D, Israeli D, Citri Y. Numerous candidate plasticity-related genes revealed by differential cDNA cloning. *Nature* 1993; 363: 718–22.
- Naeve GS, Ramakrishnan M, Kramer R, Hevroni D, Citri Y, Theill LE. Neuritin: a gene induced by neural activity and neurotrophins that promotes neuritogenesis. *Proc Natl Acad Sci U S A* 1997; 94: 2648–53.
- Le Jan S, Le Meur N, Cazes A, Philippe J, Le Cunff M, Leger J, *et al*. Characterization of the expression of the hypoxia-induced genes neuritin, TXNIP and IGFBP3 in cancer. *FEBS Lett* 2006; 580: 3395–400.
- Bosserhoff AK, Schneider N, Ellmann L, Heinzerling L, Kuphal S. The neurotrophin Neuritin1 (cpg15) is involved in melanoma migration, attachment independent growth, and vascular mimicry. *Oncotarget* 2017; 8: 1117–31.
- Yuan M, Li Y, Zhong C, Li Y, Niu J, Gong J. Overexpression of neuritin in gastric cancer. *Oncol Lett* 2015; 10: 3832–6.
- Han D, Qin B, Liu G, Liu T, Ji G, Wu Y, *et al*. Characterization of neuritin as a novel angiogenic factor. *Biochem Biophys Res Commun* 2011; 415: 608–12.
- Barbi JJ, Vignali PDA, Yu H, Pan F, Pardoll D. The neurotrophic factor neuritin maintains and promotes the function of regulatory T cells in autoimmunity and cancer. *J Immunol* 2016; 196: 58.12.
- Zhou S, Zhou J. Neuritin, a neurotrophic factor in nervous system physiology. *Curr Med Chem* 2014; 21: 1212–9.
- Shimada T, Sugiyama H, Yamagata K. Neuritin: A therapeutic candidate for promoting axonal regeneration. *World J Neurol* 2013; 3: 6.
- Yao JJ, Gao XF, Chow CW, Zhan XQ, Hu CL, Mei YA. Neuritin activates insulin receptor pathway to up-regulate Kv4.2-mediated transient outward K^+ current in rat cerebellar granule neurons. *J Biol Chem* 2012; 287: 41534–45.
- Yao JJ, Zhao QR, Liu DD, Chow CW, Mei YA. Neuritin up-regulates Kv4.2 alpha-subunit of potassium channel expression and affects neuronal excitability by regulating the calcium-calcineurin-NFATc4 signaling pathway. *J Biol Chem* 2016; 291: 17369–81.
- Lu JM, Liu DD, Li ZY, Ling C, Mei YA. Neuritin enhances synaptic transmission in medial prefrontal cortex in mice by increasing Cav3.3 surface expression. *Cereb Cortex* 2017; 1–14.
- Nedivi E, Javaherian A, Cantalalops I, Cline HT. Developmental regulation of CPG15 expression in *Xenopus*. *J Comp Neurol* 2001; 435: 464–73.
- Fujino T, Lee WC, Nedivi E. Regulation of cpg15 by signaling pathways that mediate synaptic plasticity. *Mol Cell Neurosci* 2003; 24: 538–54.
- Fujino T, Wu Z, Lin WC, Phillips MA, Nedivi E. cpg15 and cpg15-2 constitute a family of activity-regulated ligands expressed differentially in the nervous system to promote neurite growth and neuronal survival. *J Comp Neurol* 2008; 507: 1831–45.
- Sato H, Fukutani Y, Yamamoto Y, Tatara E, Takemoto M, Shimamura K, *et al*. Thalamus-derived molecules promote survival and dendritic growth of developing cortical neurons. *J Neurosci* 2012; 32: 15388–402.
- Merianda TT, Gomes C, Yoo S, Vuppalaanchi D, Twiss JL. Axonal localization of neuritin/CPG15 mRNA in neuronal populations through distinct 5' and 3' UTR elements. *J Neurosci* 2013; 33: 13735–42.
- Harwell C, Burbach B, Svoboda K, Nedivi E. Regulation of cpg15 expression during single whisker experience in the barrel cortex of adult mice. *J Neurobiol* 2005; 65: 85–96.
- Cantalalops I, Cline HT. Rapid activity-dependent delivery of the neurotrophic protein CPG15 to the axon surface of neurons in intact *Xenopus* tadpoles. *Dev Neurobiol* 2008; 68: 744–59.
- Putz U, Harwell C, Nedivi E. Soluble CPG15 expressed during early development rescues cortical progenitors from apoptosis. *Nat Neurosci* 2005; 8: 322–31.
- Cappelletti G, Galbiati M, Ronchi C, Maggioni MG, Onesto E, Poletti A. Neuritin (cpg15) enhances the differentiating effect of NGF on neuronal PC12 cells. *J Neurosci Res* 2007; 85: 2702–13.
- Marron TU, Guerini V, Rusmini P, Sau D, Brevini TA, Martini L, *et al*. Androgen-induced neurite outgrowth is mediated by neuritin in motor neurones. *J Neurochem* 2005; 92: 10–20.
- Zhang P, Luo X, Guo Z, Xiong A, Dong H, Zhang Q, *et al*. Neuritin inhibits notch signaling through interacted with neuralized to promote the neurite growth. *Front Mol Neurosci* 2017; 10: 179.
- Picard N, Leslie JH, Trowbridge SK, Subramanian J, Nedivi E, Fagioli M. Aberrant development and plasticity of excitatory visual cortical networks in the absence of cpg15. *J Neurosci* 2014; 34: 3517–22.
- Son H, Banasr M, Choi M, Chae SY, Licznernski P, Lee B, *et al*. Neuritin produces antidepressant actions and blocks the neuronal and behavioral deficits caused by chronic stress. *Proc Natl Acad Sci U S A* 2012; 109: 11378–83.
- Di Giovanni S, Faden AI, Yakovlev A, Duke-Cohan JS, Finn T, Thouin M, *et al*. Neuronal plasticity after spinal cord injury: identification of a gene cluster driving neurite outgrowth. *FASEB J* 2005; 19: 153–4.

- 27 Gao R, Li X, Xi S, Wang H, Zhang H, Zhu J, et al. Exogenous neuritin promotes nerve regeneration after acute spinal cord injury in rats. *Hum Gene Ther* 2016; 27: 544–54.
- 28 Zhao JJ, Hu JX, Lu DX, Ji CX, Qi Y, Liu XY, et al. Soluble cpg15 from astrocytes ameliorates neurite outgrowth recovery of hippocampal neurons after mouse cerebral ischemia. *J Neurosci* 2017; 37: 1628–47.
- 29 Xu J, Cao W, Liu W, Ma H, Qi A, Huang T, et al. Neuritin, a new potential target for optic nerve injury therapy. *FASEB J* 2016; 30: 564.3–3.
- 30 Sharma TP, Liu Y, Wordinger RJ, Pang IH, Clark AF. Neuritin 1 promotes retinal ganglion cell survival and axonal regeneration following optic nerve crush. *Cell Death Dis* 2015; 6: e1661.
- 31 Wang H, Li X, Shan L, Zhu J, Chen R, Li Y, et al. Recombinant hNeuritin promotes structural and functional recovery of sciatic nerve injury in rats. *Front Neurosci* 2016; 10: 589.
- 32 Cantallops I, Haas K, Cline HT. Postsynaptic CPG15 promotes synaptic maturation and presynaptic axon arbor elaboration *in vivo*. *Nat Neurosci* 2000; 3: 1004–11.
- 33 Fujino T, Leslie JH, Eavri R, Chen JL, Lin WC, Flanders GH, et al. CPG15 regulates synapse stability in the developing and adult brain. *Genes Dev* 2011; 25: 2674–85.
- 34 Zhao QR, Lu JM, Yao JJ, Zhang ZY, Ling C, Mei YA. Neuritin reverses deficits in murine novel object associative recognition memory caused by exposure to extremely low-frequency (50 Hz) electromagnetic fields. *Sci Rep* 2015; 5: 11768.
- 35 An K, Jung JH, Jeong AY, Kim HG, Jung SY, Lee K, et al. Neuritin can normalize neural deficits of Alzheimer's disease. *Cell Death Dis* 2014; 5: e1523.
- 36 Choi Y, Lee K, Ryu J, Kim HG, Jeong AY, Woo RS, et al. Neuritin attenuates cognitive function impairments in tg2576 mouse model of Alzheimer's disease. *PLoS One* 2014; 9: e104121.
- 37 Li F, Wei G, Bai Y, Li Y, Huang F, Lin J, et al. MicroRNA-574 is involved in cognitive impairment in 5-month-old APP/PS1 mice through regulation of neuritin. *Brain Res* 2015; 1627: 177–88.
- 38 Chandler D, Dragovic M, Cooper M, Badcock JC, Mullin BH, Faulkner D, et al. Impact of Neuritin 1 (NRN1) polymorphisms on fluid intelligence in schizophrenia. *Am J Med Genet B Neuropsychiatr Genet* 2010; 153B: 428–37.
- 39 Fatjo-Vilas M, Prats C, Pomarol-Clotet E, Lazaro L, Moreno C, Gonzalez-Ortega I, et al. Involvement of NRN1 gene in schizophrenia-spectrum and bipolar disorders and its impact on age at onset and cognitive functioning. *World J Biol Psychiatry* 2016; 17: 129–39.
- 40 Prats C, Arias B, Moya-Higueras J, Pomarol-Clotet E, Parellada M, Gonzalez-Pinto A, et al. Evidence of an epistatic effect between Dysbindin-1 and Neuritin-1 genes on the risk for schizophrenia spectrum disorders. *Eur Psychiatry* 2017; 40: 60–4.
- 41 Nedivi E, Wu GY, Cline HT. Promotion of dendritic growth by CPG15, an activity-induced signaling molecule. *Science* 1998; 281: 1863–6.
- 42 Tu H, Xu C, Zhang W, Liu Q, Rondard P, Pin JP, et al. GABAB receptor activation protects neurons from apoptosis via IGF-1 receptor transactivation. *J Neurosci* 2010; 30: 749–59.
- 43 Denley A, Cosgrove LJ, Booker GW, Wallace JC, Forbes BE. Molecular interactions of the IGF system. *Cytokine Growth Factor Rev* 2005; 16: 421–39.
- 44 Yan L, Xie M, Lu H, Zhang H, Shi M, Zhang Y, et al. Anti-apoptotic effect of IGF1 on Schwann exposed to hyperglycemia is mediated by neuritin, a novel neurotrophic factor. *Mol Neurobiol* 2018; 55: 495–505.
- 45 Sweatt JD. Mitogen-activated protein kinases in synaptic plasticity and memory. *Curr Opin Neurobiol* 2004; 14: 311–7.
- 46 Davis S, Vanhoutte P, Pages C, Caboche J, Laroche S. The MAPK/ERK cascade targets both Elk-1 and cAMP response element-binding protein to control long-term potentiation-dependent gene expression in the dentate gyrus *in vivo*. *J Neurosci* 2000; 20: 4563–72.
- 47 Boulton TG, Nye SH, Robbins DJ, Ip NY, Radziejewska E, Morgenbesser SD, et al. ERKs: a family of protein-serine/threonine kinases that are activated and tyrosine phosphorylated in response to insulin and NGF. *Cell* 1991; 65: 663–75.
- 48 Xie Y, Tisi MA, Yeo TT, Longo FM. Nerve growth factor (NGF) loop 4 dimeric mimetics activate ERK and AKT and promote NGF-like neurotrophic effects. *J Biol Chem* 2000; 275: 29868–74.
- 49 Kumar V, Zhang MX, Swank MW, Kunz J, Wu GY. Regulation of dendritic morphogenesis by Ras-PI3K-Akt-mTOR and Ras-MAPK signaling pathways. *J Neurosci* 2005; 25: 11288–99.
- 50 Hoeffler CA, Klann E. mTOR signaling: at the crossroads of plasticity, memory and disease. *Trends Neurosci* 2010; 33: 67–75.
- 51 Sato M, Suzuki K, Yamazaki H, Nakanishi S. A pivotal role of calcineurin signaling in development and maturation of postnatal cerebellar granule cells. *Proc Natl Acad Sci U S A* 2005; 102: 5874–9.
- 52 Zhu H, Bhattacharyya BJ, Lin H, Gomez CM. Skeletal muscle IP3R1 receptors amplify physiological and pathological synaptic calcium signals. *J Neurosci* 2011; 31: 15269–83.
- 53 Steinbeck JA, Henke N, Opatz J, Gruszczynska-Biegala J, Schneider L, Theiss S, et al. Store-operated calcium entry modulates neuronal network activity in a model of chronic epilepsy. *Exp Neurol* 2011; 232: 185–94.
- 54 Mattson MP, LaFerla FM, Chan SL, Leissring MA, Shepel PN, Geiger JD. Calcium signaling in the ER: its role in neuronal plasticity and neurodegenerative disorders. *Trends Neurosci* 2000; 23: 222–9.
- 55 Xu GG, Gao ZY, Borge PD Jr, Wolf BA. Insulin receptor substrate 1-induced inhibition of endoplasmic reticulum Ca²⁺ uptake in beta-cells. Autocrine regulation of intracellular Ca²⁺ homeostasis and insulin secretion. *J Biol Chem* 1999; 274: 18067–74.
- 56 Klee CB, Crouch TH, Krinks MH. Calcineurin: a calcium- and calmodulin-binding protein of the nervous system. *Proc Natl Acad Sci U S A* 1979; 76: 6270–3.
- 57 Hogan PG, Chen L, Nardone J, Rao A. Transcriptional regulation by calcium, calcineurin, and NFAT. *Genes Dev* 2003; 17: 2205–32.
- 58 Karamoysoyli E, Burnand RC, Tomlinson DR, Gardiner NJ. Neuritin mediates nerve growth factor-induced axonal regeneration and is deficient in experimental diabetic neuropathy. *Diabetes* 2008; 57: 181–9.
- 59 Shimada T, Yoshida T, Yamagata K. Neuritin mediates activity-dependent axonal branch formation in part via FGF signaling. *J Neurosci* 2016; 36: 4534–48.
- 60 Bloechlinger S, Karchewski LA, Woolf CJ. Dynamic changes in glypican-1 expression in dorsal root ganglion neurons after peripheral and central axonal injury. *Eur J Neurosci* 2004; 19: 1119–32.
- 61 Knoll B, Drescher U. Ephrin-As as receptors in topographic projections. *Trends Neurosci* 2002; 25: 145–9.