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REVIEW ARTICLE

The journey towards physiology and pathology: Tracing the path of neuregulin 4

Min Chen, Jieying Zhu, Hongyang Luo, Wangjing Mu, Liang Guo*

School of Exercise and Health and Shanghai Frontiers Science Research Base of Exercise and Metabolic Health, Shanghai University of Sport, Shanghai 200438, China

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Abstract Neuregulin 4 (Nrg4), an epidermal growth factor (EGF) family member, can bind to and activate the ErbB4 receptor tyrosine kinase. Nrg4 has five different isoforms by alternative splicing and performs a wide variety of functions. Nrg4 is involved in a spectrum of physiological processes including neurobiogenesis, lipid metabolism, glucose metabolism, thermogenesis, and angiogenesis. In pathological processes, Nrg4 inhibits inflammatory factor levels and suppresses apoptosis in inflammatory diseases. In addition, Nrg4 could ameliorate obesity, insulin resistance, and cardiovascular diseases. Furthermore, Nrg4 improves non-alcoholic fatty liver disease (NAFLD) by promoting autophagy, improving lipid metabolism, and inhibiting cell death of hepatocytes. Besides, Nrg4 is closely related to the development of cancer, hyperthyroidism, and some other diseases. Therefore, elucidation of the functional role and mechanisms of Nrg4 will provide a clearer view of the therapeutic potential and possible risks of Nrg4.

Introduction

Nrg4, a member of the epidermal growth factor (EGF) family, is found to be expressed in 27 tissues,[1] with its most abundant expression in brown fat.[2] Nrg4 is known to have five different isoforms (designated A1, A2, B1, B2, and B3) and these isoforms can be located in different subcellular sites. Thereby, the physiological functions mediated by different Nrg4 isoforms may be variable.[3] The Nrg4 A1/2 isoforms are localized to the plasma membranes, and their extracellular juxtamembrane domains are capable of being hydrolyzed to release the EGF-like domain.[4] Nrg4 is involved in a variety of physiological and pathological processes mainly through binding with ErbB4.[5,6] Compared to other members of the ErbB family, ErbB4 contains 4 different isoforms; these 4 naturally occurring receptor

* Corresponding author.
E-mail address: guoliang@sus.edu.cn (L. Guo).
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isoforms provide a new level of diversity to the control of growth factor-stimulated cellular responses. Thus, upon binding with the ligands, the ErbB4 isoforms may have distinct and specific roles in signal transduction and biological processes.[6] Under physiological conditions, Nrg4 has been widely studied in neurobiogenesis,[7] glucose metabolism, lipid metabolism,[8,9] thermogenesis,[10] and angiogenesis.[11] Furthermore, studies have indicated that dysregulation of Nrg4 is involved in the occurrence and development of a variety of diseases, including cancers,[12] chronic inflammation,[13] metabolic syndromes,[9,14] and some other diseases.[15,16] Although an increasing number of studies have shown the important role of Nrg4 in many physiological and pathological processes, the underlying mechanisms are still not fully understood. In this paper, the properties of Nrg4 and its role and mechanism in physiological and pathological processes will be reviewed and discussed, which is helpful for better understanding the biological characteristics of Nrg4 and its potential roles in the prevention and treatment of diseases.

The molecular feature of Nrg4 and its receptors

The molecular feature of Nrg4

Nrg4 was initially detected in the human pancreas and muscles.[5] Then, by using multiple DNA sequencing technologies, Nrg4 was found to be expressed in 27 tissues,[1] with its most abundant expression in brown adipose tissue.[2] The human *Nrg4* gene is located on chromosome 15q24.2, with a coding sequence (CDS) length of 348 nt. By comparing the genomic sequence of *Nrg4* with the mRNA sequence, Nandini et al found that the *Nrg4* gene encodes at least five different isoforms (designated A1, A2, B1, B2, and B3) by alternative splicing (Fig. 1A).[3] Each isoform has the same exon 1, which contains the start codon, and exon 2. The different *Nrg4* isoforms are generated by variable splicing between exons 3 and 9. The mRNA sequence of *Nrg4* A1 and A2 encodes a complete EGF region (exon 2 and a part of exon 6) and transmembrane structural domain (a part of exon 6). The difference is that *Nrg4* A1 contains a long cytoplasmic tail (encoded by exon 8 and exon 9), while *Nrg4* A2 contains a new exon (exon 7) that encodes a short cytoplasmic tail.[3,4] However, *Nrg4* B1, B2, and B3 do not contain exon 6, so they all share a common truncated EGF-like motif that only includes the first four cysteine residues in the EGF-like motif, and they also lack the transmembrane region.[3] Prior to the stop codon, *Nrg4* B1 is spliced from exon 2 into exon 5 and *Nrg4* B2 is spliced from exon 2 into exon 4. *Nrg4* B3 is spliced from exon 2 into exon 3, which contains a predicted nonreceptor tyrosine kinase (NRTK) phosphorylation site.[3]

The different isoforms of *Nrg4* have different protein structures, which may determine their subcellular location (Fig. 1A). From the N-terminal to the C-terminal, the structure of Nrg4 protein mainly includes the extracellular EGF-like motif, extracellular sequence proximal to the membrane, transmembrane domain, and intracellular domain. The isoform A1-encoding Nrg4 precursor (pro-Nrg4) has 115 amino acids, of which amino acids 1—62 are outside the cell membrane, amino acids 63—83 are the transmembrane region, and amino acids 84—115 are the intracellular region.[4] The N-terminal of Nrg4 has an EGF-

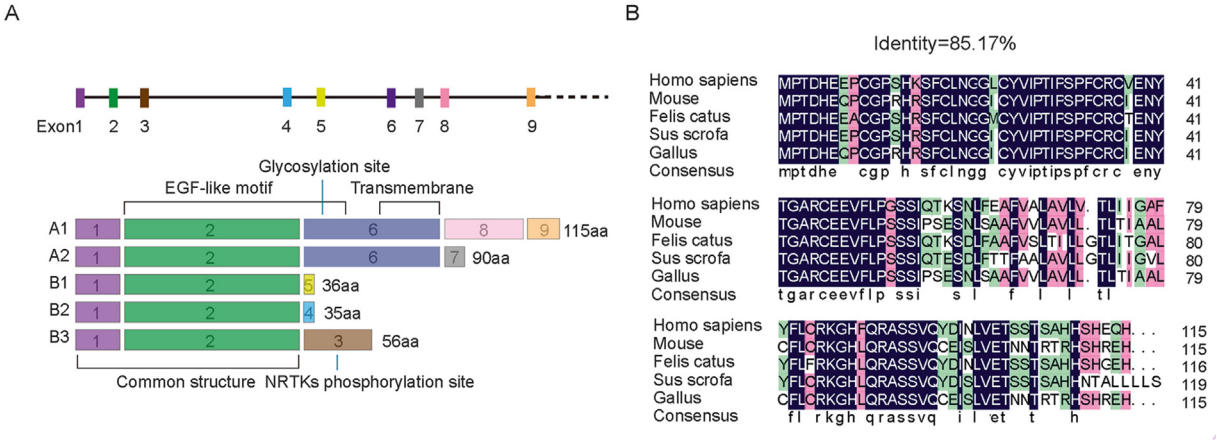

Figure 1 The variants of *Nrg4* gene and the sequence alignment of Nrg4 proteins among species. **(A)** The human *Nrg4* gene encodes five different isoforms, namely A1, A2, B1, B2, and B3. These isoforms are the result of alternative splicing of exons 1—9 of *Nrg4* gene, and all isoforms contain exons 1 and 2. Both *Nrg4* A1 and A2 encode a complete epidermal growth factor (EGF) like region (containing codon encoding a glycosylation site) and transmembrane domain (exon 2 and exon 6). The difference is that the cytoplasmic tails of *Nrg4* A1 and A2 are encoded by different exons (exons 8 and 9 for A1, and exon 7 for A2). Furthermore, *Nrg4* B1 is spliced from exon 2 into exon 5 and *Nrg4* B2 is spliced from exon 2 into exon 4, prior to the stop codon. *Nrg4* B3 is spliced from exon 2 into exon 3, and exon 3 contains a codon encoding a predicted nonreceptor tyrosine kinase (NRTK) phosphorylation site. **(B)** Comparison of Nrg4 A1 protein sequences of human, mouse, cat, pig, and chicken. Among the 5 sequences, 3, 4, and 5 identical amino acids are represented in green, pink, and dark blue, respectively. A homology of 85.17% is detected among the Nrg4 proteins of different species.

like motif between 5 and 46 amino acids,[5] and this motif contains an N-linked glycosylation site (NYT) at residue 39[4].

Nrg4 A1/A2 proteins are located on the plasma membrane. Fluorescence staining showed that Nrg4 A1 was localized in membrane folds, while Nrg4 A2 was more distributed on the membrane.[4] Nrg4 A1/A2 has a complete EGF-like motif.[4] Hayes et al found that the N-terminal of Nrg4 A1 and A2 are in the extracellular space, indicating that the EGF-like motif containing glycosylation sites is outside the cell membrane.[4] Because Nrg4 B isoform lack exon 6, they do not contain transmembrane structural domains and complete EGF-like motifs. Therefore, Nrg4 B isoforms are predicted to be soluble and located in the intracellular area.[17] From the biological and structural point of view, the structure and location of the protein may determine its biological function and activity. Because Nrg4 A1/A2 exists on the plasma membrane, they may remain at this subcellular location as anchored molecules or may be cleaved and released. The cleavage of Nrg4 at the extracellular region allows Nrg4 to travel to cells or tissues located a distance from their site of production. Because Nrg4 B1−3 do not have the transmembrane structure and are mainly expressed intracellularly, they may have certain biological roles inside the cells, which needs further exploration. Furthermore, the Nrg4 A1 protein is conserved in humans, mice, cats, pigs, and chickens (Fig. 1B). The high homology of Nrg4 proteins among species suggests its critical role in biological processes.

Receptors for Nrg4

Cell-to-cell signaling is an essential feature of multicellular organisms and plays an important role in regulating the physiological actions and homeostasis of the organisms. A large number of growth factor receptors with tyrosine kinase activity play a key role in this process. ErbB receptor tyrosine kinases (ErbBs) are classical members of the human epidermal growth factor receptor (HER) family, which are activated and dimerized by ligands. This is followed by autophosphorylation catalyzed by tyrosine kinases, leading to signal transduction and downstream signal cascades. Nrg4 is a transmembrane protein that contains a unique EGF-like motif, and this EGF-like motif is required for the activation of ErbBs.[5] Nrg4 contains proteolytic cleavage sites.[4] Therefore, after being cleaved on the plasma membrane, the extracellular EGF-like motif of Nrg4 will be released to recognize and activate ErbB4, which in turn triggers signal transduction.[5] As a result, Nrg4 can act on different target cells, and then combine with ErbB4 to trigger signal transduction.

ErbB4 is a glycoprotein composed of a glycosylated extracellular domain (ECD), a single transmembrane domain, and an intracellular domain (ICD) containing a functional tyrosine kinase and a C-terminal tail.[6] Four structurally different ErbB4 isoforms have been identified from human and mouse tissues (Fig. 2A), all of which can recognize Nrg4.[6] ErbB4 isoforms are different in their extracellular domain near the juxtamembrane (JM) or in the cytoplasmic region near the C-terminal. The JM isoforms differ by having a 23-amino acid (JM-a) or 13-amino acid (JM-b) insert in the extracellular JM domain, and only the JM-a isoforms with a stretch of 23 unique amino acids in the JM domain possess a cleavage site for a metalloproteinase, tumor necrosis factor-α converting enzyme (TACE).[18−20] In addition, the cytoplasmic (CYT) variants differ by having (CYT-1) or not having (CYT-2) a 16-amino acid insert in the cytoplasmic tail.[21] CYT-1 contains 16 amino acids (with a binding site for the p85 subunit of PI3K), while CYT-2 lacks the 16 amino acids (without a binding site for the p85 subunit of PI3K).[18,21] Therefore, Nrg4-mediated activation of ErbB4 CYT-1 stimulates PI3K activity, which promotes PI3K/AKT signaling pathway (Fig. 2A, B).[22] The generic shell script compiler (Shc), a scaffold protein in receptor tyrosine kinase signaling, can activate the mitogen-activated protein kinase (MAPK) signaling pathway.[23] All ErbB4 variants contain Shc binding sites in the cytoplasmic structure, which can couple with Shc, and Shc in turn activates MAPK (Erk-1/2) signaling pathway (Fig. 2A, B).[22] In addition, in the JM structure, JM-a contains the TACE site. Activated ERBB4 is first cleaved within the JM region by TACE.[20] Subsequent cleavage of TACE-processed ErbB4 by γ-secretase releases ICD from cellular membranes (Fig. 2A).[24] After ErbB4 ICD is cut and released, part of ErbB4 ICD accumulates in the nucleus. The ErbB4 accumulated in the nucleus directly regulates the function of signal transducer and activator of transcription 5a (STAT5a) in the transcriptional regulation of target genes expression (Fig. 2A, B).[25] Both CYT isoforms of ErbB4 can interact with and phosphorylate STAT5a.[26] However, the physiological significance of ectodomain shedding is still unclear. It remains to be elucidated whether the cleavage of ErbB4 JM-a simply contributes to ICD shedding or whether the cleaved ErbB4 ECD fragment can act as a signal in the extracellular environment. Therefore, ErbB4 signaling is further complicated by the existence of four distinct isoforms that would lead to differential activation of downstream pathways and mediate distinct biological processes.

The formation of dimeric complexes is essential for the function of ErbBs. ErbB4 is a fully functional receptor tyrosine kinase that can act as both a homodimer and a heterodimer (Fig. 2B). When Nrg4 is combined with ErbB4, ErbB4 can recruit ErbB1 or ErbB2 to form heterodimers.[5] ErbB2 has no known ligand but is the preferred heterodimerization partner for the other ErbB receptors. ErbB3 lacks intrinsic kinase activity and is only active after forming a heterodimer.[27] Upon the binding of Nrg4 to ErbB3/ErbB4 heterodimer, ErbB3 phosphorylation increased significantly.[2] Under some physiological conditions, ErbB4 alone may be insufficient to elicit a biological response to Nrg4, and it requires a co-receptor such as ErbB3 to transduce its signal.[2]

Physiological roles of Nrg4

Neurobiogenesis

Neurons act as the center of information transmission in the body. A typical neuron can receive thousands of messages at a time via synapses. Most tissues of the organism are rich in neural structures, so neurogenesis is important for the rate of information transmission via the nerves and the executive

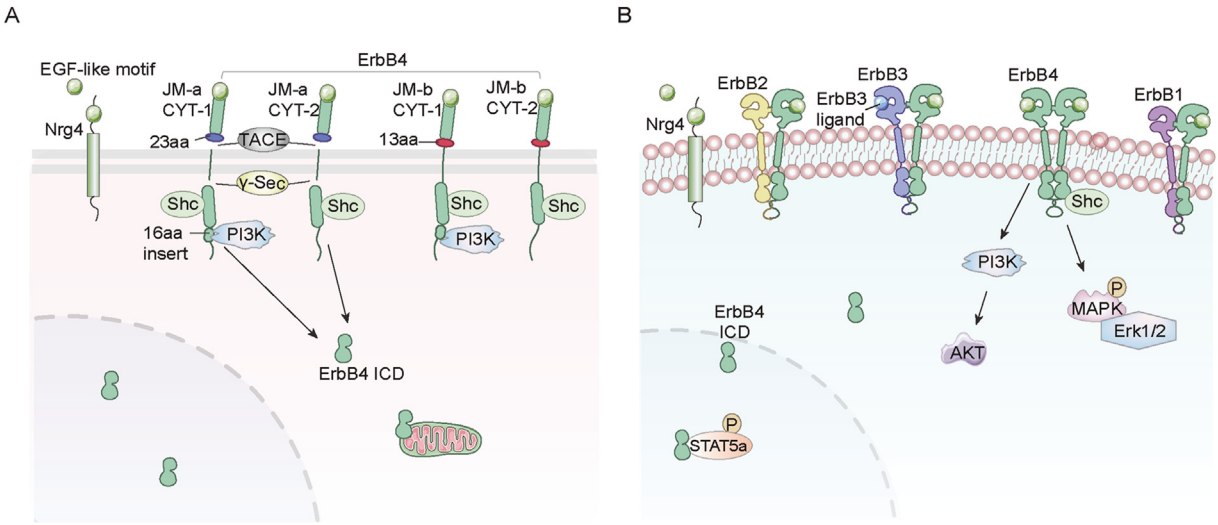

Figure 2 The Nrg4 receptor ERBB4 has different variants and mediates different signal transduction. **(A)** ErbB4 has four different isoforms, all of which can recognize Nrg4. ErbB4 isoforms are different in their extracellular domain near the juxtamembrane (JM) or in the cytoplasmic region near the C-terminal. The JM isoforms differ by having a 23-amino acid (JM-a) or 13-amino acid (JM-b) inserted. JM-a contains the tumor necrosis factor-α-converting enzyme (TACE) site. Activated ERBB4 is first cleaved within the JM region through the activity of TACE. The TACE-processed ErbB4 is then cleaved by γ-secretase, leading to the release of the intracellular domain (ICD) from cellular membranes. ErbB4 ICD is able to play a functional role in the cytoplasm and nucleus. The cytoplasmic (CYT) variants differ by having (CYT-1) or not having (CYT-2) a 16-amino acid insert in the cytoplasmic tail. CYT-1 contains the 16 amino acids, which form a binding site for PI3K, while CYT-2 lacks the 16 amino acids. All ErbB4 variants contain shell script compiler (Shc) binding sites in the cytoplasmic region. **(B)** The EGF-like motif of Nrg4 can be cleaved and released, and then recognize and activate ErbB4, which in turn triggers signal transduction. Nrg4-mediated activation of ErbB4 can stimulate PI3K/AKT signaling pathway. Activated ErbB4 can couple with shell script compiler (Shc), and Shc in turn activates the mitogen-activated protein kinase (MAPK) (Erk-1 and Erk-2) signaling pathway. Moreover, the ICD of ErbB4 accumulated in the nucleus directly regulates the function of signal transducer and activator of transcription 5 a (STAT5a) in the transcriptional regulation of target genes. In addition, ErbB4 can combine with itself to form a homodimer, or it can recruit ErbB1, ErbB2, or ErbB3 to form heterodimers.

functions of the organism.[28,29] Neuregulins including Nrg4, have been shown to be extensively involved in many aspects of neural development and function (Fig. 3).[7,30–33] In central neurodevelopment, the apical and basal dendrites of neocortical pyramidal neurons, including the frontal/motor cortex and the most rostral region of the somatosensory cortex, are significantly less developed in *Nrg4*$^{-/-}$ neonates compared to *Nrg4*$^{+/+}$ littermates, with significantly shorter dendrites and fewer dendritic spines.[7] The reduction in axonal and dendritic length and branching in pyramidal neurons of *Nrg4*$^{-/-}$ mice were fully restored to wild-type levels by administration of the mice with recombinant Nrg4.[7] By further investigating the effect of Nrg4 deletion on vertebral neuron morphology, it was found that *Nrg4* deletion was able to reduce not only the size and complexity of neuronal dendritic spines but also the cytosolic area of neurons.[32] Moreover, *Nrg4* deficiency led to a reduction in the motor cortex pyramidal neuron soma size.[32] Most embryonic cortical neurons co-express Nrg4 and ErbB4, suggesting that Nrg4 may promote the development of pyramidal cortical neurons through the ErbB4-mediated signaling.[7,32] Therefore, in cortical pyramidal neurons, Nrg4 may affect the morphology of pyramidal neurons through the autocrine/paracrine mechanisms. Additionally, imaging of early developing cutaneous trigeminal, brachial plexus, and thoracic nerve fibers in mice showed that nerve fiber length and branching density were significantly reduced in *Nrg4*$^{-/-}$ embryos compared to *Nrg4*$^{+/+}$ embryos.[31] On the other hand, after cold exposure, norepinephrine (NE) promotes the upregulation and secretion of Nrg4 in adipocytes, which in turn signals to neurons and promotes axonal growth.[34] Thus, Nrg4 may have a potential role in enhancing the innervation of sympathetic nerves in adipose tissue. However, the above studies showed that the effect of Nrg4 on neurons was limited to the early stages of growth and development in mice. Overall, Nrg4 may influence the morphology and growth of mouse neurons through autocrine/paracrine manners.

Lipid metabolism

Lipid metabolism is an important and complex biochemical reaction in organisms, which is important for energy storage and energy supply in the body.[35–37] Nrg4 is widely expressed in adipose tissue, especially within brown fat.[38] Circulating Nrg4 levels in obese patients were negatively correlated with adipsin levels.[39] Adipsin has been documented to promote adipocyte differentiation and to increase lipid accumulation.[40–42] Similarly, *Nrg4* transcript levels were significantly lower in epididymal white fat and liver of obese mice compared to lean mice.[8] Upon being fed with a high-fat diet (HFD), *Nrg4 null* mice gained slightly more weight, accompanied by a reduction in the percentage of lean mass and an elevation in the percentage of fat mass, and higher levels of plasma and liver triglyceride (TG) content.[2] However, *Nrg4* gene transfer in mice blocked

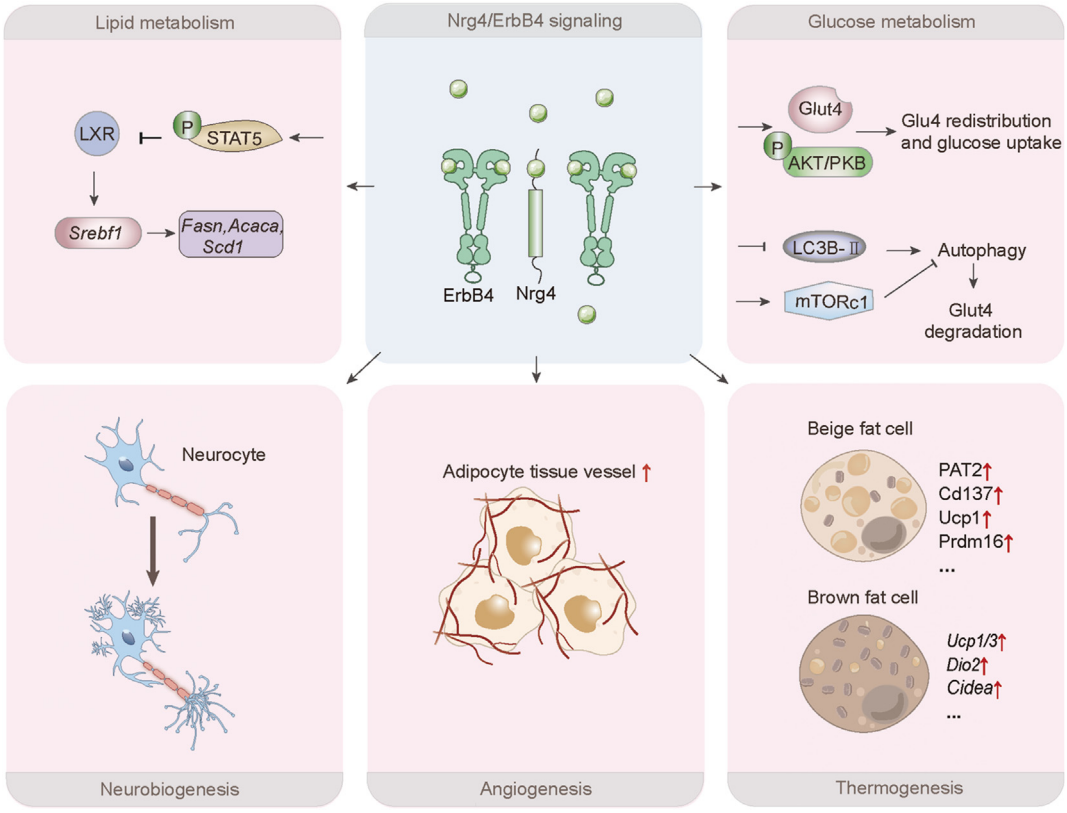

Figure 3 Nrg4 is involved in a spectrum of physiological processes including lipid metabolism, glucose metabolism, neurobiogenesis, angiogenesis, and thermogenesis. In lipid metabolism, Nrg4 can promote the phosphorylation of STAT5 significantly to repress the transcriptional activity of the liver-X receptor (LXR), leading to cell-autonomous repressed expression of the sterol regulatory element binding transcription factor 1 (*Srebf1*), acetyl coenzyme A carboxylase (*Acaca*), stearoyl-CoA desaturase 1 (*Scd1*), and fatty acid synthase (*Fasn*) in hepatocytes. In glucose metabolism, Nrg4/ErbB4 signaling may promote glucose uptake by increasing glucose transporter 4 (Glut4) redistribution to the plasma membrane. Moreover, Nrg4 can promote AKT/PKB phosphorylation to increase the uptake of glucose in 3T3-L1 adipocytes. In addition, Nrg4 increases the activity of the mammalian target of rapamycin complex 1 (mTORc1) and decreases the expression of autophagy marker microtubule-associated protein light chain 3B form II (LC3B-II), which can block Glut4 storage vesicle (GSV) degradation by autophagy in adipocytes. In neurobiogenesis, Nrg4 can promote the growth of axons and dendrites of neurons, and increase the length and complexity of dendrites. In angiogenesis, Nrg4 overexpression resulted in increased vascular density in adipose tissue. In thermogenesis, Nrg4 is able to stimulate the expression of thermogenic genes in BAT, including *Ucp1*, *Ucp3*, *Cidea*, and *Dio2*, and Nrg4 can promote the expression of beige fat markers PAT2 and CD137.

hypertrophy in epididymal white fat tissue (eWAT) and inguinal white fat tissue (iWAT) by more than 50% and increased the expression of adipose triglyceride lipase (ATGL) and adiponectin, which can prevent HFD-induced weight gain.[8] Cellular studies showed that Nrg4 treatment significantly reduced lipid content in 3T3-L1 adipocytes, but did not affect differentiation.[43] Notably, in the livers of *Nrg4*-deficient mice, the expression of genes involved in *de novo* lipogenesis was significantly higher than those in wild-type control mice, including encoding glucose kinase (*Gck*), malic enzyme 1 (*Me1*), sterol regulatory element binding transcription factor 1 (*Srebf1*), fatty acid synthase (*Fasn*), stearoyl-CoA desaturase 1 (*Scd1*), and ELOVL fatty acid elongase 5 (*Elovl5*).[2] Similarly, Nrg4 overexpression significantly suppressed the expression of lipogenic genes in mice livers.[8] Mechanically, Nrg4 signaling in hepatocytes can significantly activate STAT5a to repress the transcriptional activity of nuclear hormone receptor liver-X receptor (LXR), leading to cell-autonomous repression of the sterol regulatory element-binding protein 1c (Srebp-1c)-induced *de novo* lipogenesis pathway (Fig. 3).[2] Similarly, Zhu et al confirmed by further studies that intraperitoneal injection of Nrg4 significantly blunted the increase of Fasn and Srebp-1c expression in the liver of HFD-fed mice.[44]

In *Nrg4* transgenic mice, fatty acid profiling of the mice's livers demonstrated that the levels of the major fatty acid species, including palmitic acid (C16:0), oleic acid (C18:1), and linolenic acid (C18:2), were lower than those in the control wild type mice. *Nrg4* transgenic mice exhibited significantly higher plasma β-hydroxybutyric acid concentration after overnight fasting.[45] The concentrations of CoA-enzyme (CoA), acetyl-CoA, and malonyl-CoA, but not succinyl-CoA, were increased significantly in the liver of *Nrg4* transgenic mice, compared to the control mice.[45] Therefore, in the case of starvation, Nrg4 may promote fatty acid β-oxidation and ketogenesis, and elevated malonyl-CoA may result from substrate accumulation due to reduced flux through the *de novo* lipogenesis and increased β-oxidation. Peroxisome proliferator-activated receptor α (*Pparα*) and its target gene carnitine

palmityl transferase 1a (*Cpt1a*), which facilitates fatty acid oxidation, were found to be down-regulated in aged HFD mice, but Nrg4 treatment was able to restore it.[44] Therefore, Nrg4 plays an important role in regulating the homeostasis of lipid metabolism. However, there are few studies investigating the role of Nrg4 in lipid transport, another important process for lipid metabolism. Whether Nrg4 could promote the secretion of apolipoproteins and whether it could promote the transport of lipids by apolipoproteins need to be further explored.

Glucose metabolism

Glucose metabolism provides energy support for the body's activities, and Nrg4 can promote the uptake and transport of glucose (Fig. 3).[9,43] Nrg4 significantly increases glucose uptake in eWAT of wild-type mice.[43] However, in *ErbB4*-deficient mice, Nrg4 could not increase glucose uptake in adipocytes.[43] This suggests that Nrg4/ErbB4 signaling may promote glucose uptake. Glucose transporter 4 (Glut4) is the major glucose transporter for glucose uptake in adipose tissue. In 3T3-L1 cells, Glut4 redistribution to the plasma membrane was enhanced to significantly increase glucose uptake after Nrg4 treatment.[43] On the contrary, in 3T3-L1 cells with Nrg4 knockdown (KD), the uptake of glucose analogs 2-D-deoxyglucose induced by insulin was completely blocked and the level of AKT/PKB phosphorylation upon insulin stimulation was also reduced.[9] Furthermore, in Nrg4 KD adipocytes, autophagic flux was increased significantly, which could lead to the degradation of Glut4 storage vesicle (GSV). However, blocking autophagy can restore the reduction of Glut4 and GSV content in adipocytes.[9] Insulin activates the mammalian target of rapamycin complex 1 (mTORc1), which regulates cell growth and metabolism and represses autophagy.[46] Compared to the control group, there is a significantly higher level of autophagy in Nrg4 KD adipocytes, and mTORc1 activity is significantly lower, even in the presence of insulin stimulation. Similarly, the autophagy marker microtubule-associated protein light chain 3B form II (LC3B-II)[47] is significantly increased in Nrg4 KD adipocytes.[9] Therefore, Nrg4 KD leads to the inhibition of mTORc1 activity, which may lead to the degradation of Glut4 through autophagy and insulin resistance in adipocytes. Further studies are needed to investigate the role of the above Nrg4-mediated pathways in glucose metabolism *in vivo*.

Thermogenesis

Mammals have three types of fat, white fat (WAT), brown fat (BAT), and beige fat. White adipocytes are important for energy homeostasis, as it is able to metabolize glucose as well as to store and release energy-rich fatty acids.[48,49] Beige adipocytes and brown adipocytes are enriched in mitochondria that allow energy dissipation as heat.[50,51] Nrg4 is expressed mainly in adipose tissue, especially in BAT,[2] and several studies have shown that Nrg4 expression is increased significantly in WAT and to a lesser extent in BAT after cold exposure.[2] Beige fat mass in *Nrg4* KO mice was less than that in wild-type mice even after being exposed to a 6 °C temperature for 6 days.[10] Meanwhile, analysis of *Nrg4* transgenic mice revealed that Nrg4 was able to significantly up-regulate genes involved in mitochondrial function and energy metabolism in eWAT, accompanied by increased basal metabolic rate and energy expenditure.[45] Brown and beige adipose tissue can dissipate chemical energy into heat through thermogenic respiration, a process that requires thermogenic genes.[50] Population-based cohort studies have shown that *Nrg4* gene expression is positively correlated with the expression of thermogenic genes (*Ucp1*, *Ucp3*, and *Tmem26*)[52] in subcutaneous adipose tissue. Meanwhile, the expression of Ucp1 was reduced in BAT of the mice with brown adipocyte-specific knockout *Nrg4* (*Nrg4*-bKO), compared with that of control mice.[11] *Nrg4* gene transfer was able to stimulate the expression of thermogenic genes in BAT, including *Ucp1*, *Ucp3*, *Cidea*, and *Dio2*.[8] Similarly, after 3T3-L1 cells were treated with exogenous Nrg4 for 48 h, the beige fat markers (PAT2 and Cd137) and the brown fat markers (Ucp1 and Prdm16) increased significantly (Fig. 3).[43] However, the exact effect of Nrg4 on thermogenesis in humans needs to be further explored.

Angiogenesis

The features of angiogenesis involve the migration, growth, and differentiation of endothelial cells, which line the interior wall of blood vessels.[53] Vascular endothelial growth factor (VEGF) has an important role in angiogenesis.[54] Adipose tissue contains well-developed vascular networks. Nrg4 has a potential protective effect on vascular tissue (Fig. 3).[55] Related studies have shown that Nrg4 expression level in iWAT is positively correlated with VEGF levels and it can promote subcutaneous adipose angiogenesis.[56] Meanwhile, recombinant Nrg4 significantly promoted proliferation and reduced apoptosis of endothelial cells.[11] On the contrary, reduced vascularity was observed in WAT and BAT of *Nrg4* KO mice.[11] Conditional *Nrg4* KO in BAT was also shown to reduce BAT vascularity and exacerbate the extent of obesity.[11] Through further studies, Nugroho et al found that Nrg4/ErbB4 signaling played a major role in Nrg4-mediated angiogenesis in adipose tissues.[57] In mice with adipose tissue-specific overexpression of Nrg4 (aP2-*Nrg4*-Tg), it was found that the vascular density in WAT was significantly increased and the hypoxia of WAT was significantly improved, while the improvement of WAT angiogenesis was eliminated by using ErbB4 inhibitors to inhibit Nrg4/ErbB4 signal transduction.[57] Angiogenesis plays an important role in various biological processes. Nrg4/ErbB4 signal may be a potential therapeutic target for improving angiogenesis in adipose tissue. The detailed mechanism of Nrg4-mediated angiogenesis in adipose tissue and whether it has the same benefits in other tissues need further studies.

Pathological role of Nrg4

Inflammation diseases

Inflammatory bowel disease (IBD) is a chronic illness with relapsing inflammatory injury in the gastrointestinal tract. Nrg4 expression was decreased in human IBD samples and

mouse models of colitis.[58] McElroy et al found that exogenous treatment of Nrg4 could reduce the severity of necrotizing enterocolitis (NEC) in rats, and the intraperitoneal injection of *Nrg4* could completely block the drug-induced NEC (Fig. 4A).[58] The mechanism is that Nrg4/ErbB4 blocked Paneth cell loss in the ileum of NEC model. In addition, Nrg4 can promote epithelial cell survival. IEC-6 ileal epithelial cells were infected by NEC-related bacteria, and Nrg4/ErbB4 signaling could block the apoptosis of IEC-6 cells induced by the bacteria.[58] Another study showed

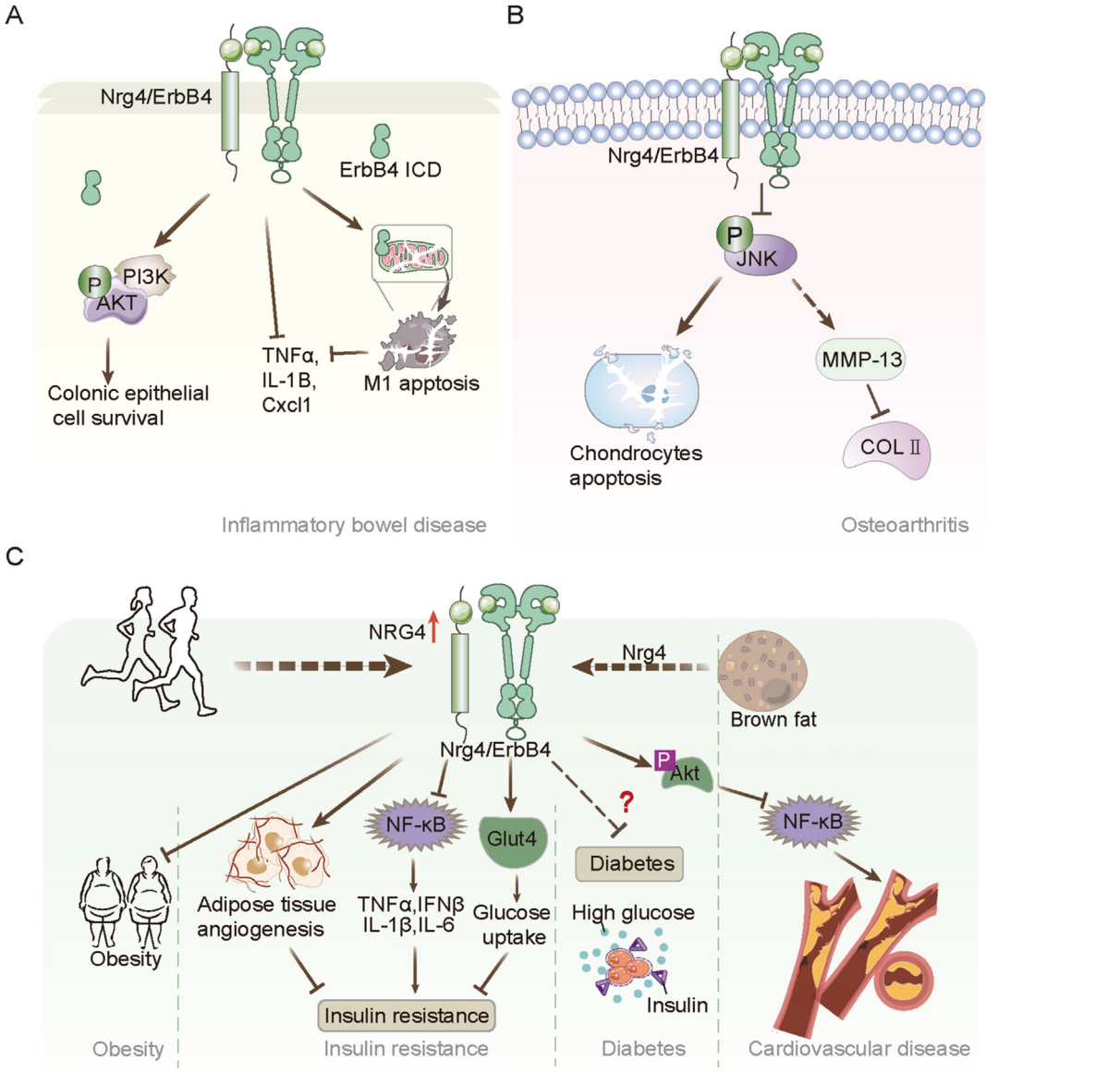

Figure 4 The effects of Nrg4 on inflammatory diseases, obesity, insulin resistance, diabetes, and cardiovascular diseases. **(A)** In inflammatory bowel disease, Nrg4 can activate PI3K/AKT signaling pathway, which in turn promotes colonic epithelial cell survival. In addition, Nrg4 can promote M1 macrophage apoptosis through ErbB4 ICD binding to mitochondria, which reduces the production of TNFα, Cxcl1, and IL-1β from macrophages. **(B)** In osteoarthritis, Nrg4 inhibits the activation of JNK phosphorylation and reduces chondrocyte apoptosis. Moreover, Nrg4-mediated inhibition of JNK signaling also results in the reduction of the matrix metalloproteinase 13 (MMP-13), an enzyme for the degradation of type II collagen (COL II), thereby inhibiting the degradation of COL II in chondrocytes. **(C)** Nrg4 may have an anti-obesity effect. In addition, Nrg4 can increase Glut4 levels on the plasma membranes to promote glucose transport and uptake in 3T3-L1 adipocytes. Nrg4 suppresses the activation of the transcription factor NF-κB and the expression of its downstream inflammatory factors (including TNFα, IL-1β, IL-6, and IFNβ) in 3T3-L1 adipocytes. Besides, Nrg4/ErbB4 signaling leads to increased adipose tissue angiogenesis to improve adipose tissue function and preserve metabolic health. These effects above help to ameliorate insulin resistance. The beneficial role of Nrg4 in diabetes needs further exploration. In cardiovascular disease, BAT-derived Nrg4 activates Akt signaling through ErbB4 receptor, which inhibits the NF-κB activity to reduce endothelial inflammation and injury, thereby ameliorating atherosclerosis. In addition, the levels of circulating Nrg4 were significantly elevated after exercise, suggesting the potential roles of Nrg4 in exercise-mediated metabolic health.

that Nrg4 treatment, either in cultured cells or in mice, blocked colonic epithelial apoptosis induced by TNFα and IFN-γ, and Nrg4 treatment could block inflammation and injury in dextran sulfate sodium (DSS) induced murine colitis model.[13] Nrg4 treatment of colonic epithelial cells promoted the phosphorylation of Akt, an anti-apoptotic mediator.[13] Consistently, pharmacological inhibition of PI3K/Akt signaling reversed the anti-apoptotic effects of Nrg4,[13] which illustrates the mechanism of Nrg4-mediated promotion of colonic epithelial cell survival. It should be noted that the proliferation and migration of colonic epithelial cells were not affected by Nrg4.

Inflammatory injury is a prominent feature of IBD and macrophages are central in the process. Nrg4 expression is inhibited during the pro-inflammatory activation of macrophages in IBD, while Nrg4/ErbB4 activation induces apoptosis of pro-inflammatory macrophages in IBD, which in turn ameliorates the symptoms of IBD.[19] Nrg4 was able to reduce TNFα, Cxcl1, and IL-1β expression in macrophages (Fig. 4A), thereby alleviating colorectal inflammation.[59] Another study demonstrated that Nrg4 could selectively inhibit the growth and survival of M1 macrophages through binding to ErbB4 on these cells.[19] By further study, it was found that the mitochondrial transmembrane potential (ΔΨ m) was reduced in Nrg4-treated M1 macrophages, supporting the fact that Nrg4 could induce the mitochondrial apoptosis pathway in M1 macrophages.[19] The above pathway requires the involvement of the hydrolyzed ICD of ErbB4 receptor, which binds to mitochondria and thus stimulates apoptosis in M1 macrophages (Fig. 4A).[19] ICD is a soluble intracellular fragment generated by the cleavage of ErbB4 receptor, and it can be localized in the cytoplasm, nucleus, and mitochondria.[19] In inflammatory response, macrophages can perform pro-inflammatory (classical M1 activation) or anti-inflammatory (alternative M2 activation) functions. Nrg4 can alleviate IBD by inhibiting the growth and survival of M1 macrophages. However, in IBD, the effect of Nrg4 on M2 macrophages is not clear, including whether Nrg4 could promote the activation of M2 macrophages and the transformation of M1 macrophages to M2 macrophages, which merits further investigation.

Osteoarthritis (OA) is characterized by chondrocyte apoptosis and increased degradation of type II collagen (COL II).[60] Nrg4 deficiency aggravated the severity of OA, while Nrg4 restoration alleviated the damage of articular cartilage in mice.[61] The underlying mechanism is that Nrg4 can reduce mouse chondrocyte apoptosis. *Nrg4* KO increased the levels of IL-1 β, IL-6, and TNFα in the synovial fluid of the knee joint of mice. On the contrary, murine recombinant Nrg4 suppressed inflammation and apoptosis of chondrocytes *in vitro*.[61] JNK signaling pathway is important for inflammation and apoptosis in chondrocytes. Nrg4 can inhibit JNK phosphorylation and reduce chondrocyte apoptosis (Fig. 4B).[60,62] Additionally, the degradation of type II collagen is a characteristic feature of OA. Nrg4-mediated inhibition of JNK signaling also resulted in the reduction of the matrix metalloproteinase 13 (MMP-13, an enzyme for the degradation of COL II), thus helping to elevate the level of COL II (Fig. 4B).[61] Therefore, Nrg4 could alleviate chondrocyte inflammation and apoptosis, thereby mitigating the progression of OA.

Obesity, insulin resistance, and diabetes

Nrg4 is an adipokine that is sensitive to metabolic conditions and has a potential role in the combat against overweight and obesity (Fig. 4C). Nrg4 expression was significantly reduced in adipose tissue and liver of obese mice compared to control lean mice, and Nrg4 overexpression prevented HFD-induced weight gain in mice.[8] Furthermore, by measuring the circulating Nrg4 levels of 1212 obese individuals, it was found that the circulating Nrg4 levels of obese individuals with metabolic syndrome were lower than those with simple obesity.[39] Similarly, in 44 obese men after 12 weeks of resistance training, it was found that plasma Nrg4 and adiponectin levels increased, while plasma leptin levels decreased.[63] The data above suggest that Nrg4 could improve obesity, and may be involved in exercise-mediated amelioration of obesity and its related metabolic disorders.

Hydrodynamic injection of pLIVE-Nrg4 plasmids in mice promoted the expression of adiponectin, ATGL, and thermogenic genes (*Ucp1*, *Ucp3*, *Dio2*, and *Cidea*)[8] in BAT. Meanwhile, *Nrg4* gene transfer reduced insulin resistance and chronic inflammation in obese mice and significantly reduced the expression of macrophage marker genes such as *F4/80*, *Cd68*, *Cd11*, and the inflammatory factor monocyte chemotactic protein 1 (*Mcp1*) in eWAT.[8] In the meantime, *Nrg4* gene transfer enhanced the expression of the M2 macrophage marker gene *Cd163*.[8] Moreover, Nrg4 facilitates glucose uptake and transport in adipocytes by promoting the expression of insulin receptors and Glut4 protein.[9] Similarly, in 3T3-L1 cells, Nrg4 treatment significantly reduced lipid content and promoted glucose uptake.[43] Insulin resistance is known to be associated with chronic low-grade inflammation in WAT, which requires the activation of the transcription factor NF-κB.[64] The expression of NF-κB and its downstream inflammatory factors (including TNFα, IL-1β, IL-6, and IFN-β) was significantly elevated in Nrg4 KD (shNrg4) 3T3-L1 adipocytes, and treatment with recombinant Nrg4 can suppress the inflammation in adipocytes (Fig. 4C).[9] Obesity causes vascular thinning in BAT and WAT, which can increase the hypoxia of WAT and BAT. Increasing the vascular density in WAT and BAT can significantly improve adipose hypoxia, which can alleviate metabolic stress. Related studies have shown that Nrg4/ErbB4 signaling deficiency led to reduced adipose tissue angiogenesis,[11] resulting in increased levels of adipose tissue inflammation and exacerbated insulin resistance and that *Nrg4* transgenic mice promoted adipose tissue angiogenesis and improved insulin resistance.[57] Taken together, by using cellular and animal models, Nrg4 was shown to ameliorate insulin resistance (Fig. 4C).

Cross-sectional and case—control studies have shown that circulating Nrg4 levels are significantly lower in patients with diabetes compared to healthy populations.[65,66] Lower circulating Nrg4 could be a risk factor for gestational diabetes mellitus (GDM),[67] and Nrg4 serum levels are negatively associated with HOMA-IR.[68] Moreover, circulating levels of Nrg4 are negatively associated with the severity of type 2 diabetic peripheral neuropathy.[69,70] However, a human study exhibits an opposite conclusion. In this study, it was found that serum NRG4 level was negatively correlated with insulin sensitivity and positively associated with the level of high-sensitivity C relative protein (hsCRP).[71] At present, there are relatively

few works reporting whether and how Nrg4 can affect diabetes, and further studies are needed to dissect the specific role and mechanism of Nrg4 in diabetes.

One study showed that Nrg4 levels in the liver of diabetic mice increased and Nrg4 could promote liver gluconeogenesis.[14] Nrg4 expression was significantly induced by cAMP activator in mouse primary hepatocytes, along with the up-regulation of phosphoenolpyruvate carboxy kinase (PEPCK) and glucose-6-phosphatase (G6Pase) expression that are key enzymes for hepatic gluconeogenesis.[14] Peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) is a crucial transcriptional coactivator that can stimulate PEPCK and G6Pase expression under the fasting state.[72] Hepatic Nrg4 KD in db/db mice improved pyruvate tolerance, with down-regulation of PEPCK, G6Pase, and PGC-1α.[14] This study indicates that Nrg4 is an important positive regulator of hepatic gluconeogenesis, which contradicts previous murine experiments in which Nrg4 was found to improve insulin sensitivity. This inconsistency may be due to the different genetic backgrounds of mice that were used in the different studies, which needs to be further verified.

Cardiovascular diseases

Neuregulins have been shown to be beneficial in the prevention of cardiovascular disease (CVD), and Nrg4 also appears to have a similar function (Fig. 4C). Circulating Nrg4 concentrations are inversely associated with subclinical atherosclerosis in obese adults, indicating that low level of circulating Nrg4 could play a role in identifying patients at high risk for CVD.[73] Additionally, a clinical study has shown that serum Nrg4 levels were significantly lower in 144 patients with acute coronary syndrome (ACS) and were negatively correlated with serum HDL-c[74]. Moreover, there was a significant association between higher Nrg4 level and lower risk of ACS. Therefore, reduced serum Nrg4 levels are believed to be a possible independent risk factor for ACS.[74] A recent study shows that BAT-derived Nrg4 serves as a potential cross-talk factor between BAT and arteries. It attenuates endothelial inflammation and cellular adhesion responses, inhibits leukocyte homing, and reduces endothelial injury and atherosclerosis in a manner involving Akt—NF—κB signaling.[75] In more detail, BAT-derived Nrg4 activates Akt signaling through ErbB4 receptor, which inhibits the NF-κB activity to reduce endothelial inflammation and injury.[75] This study provides us with new ideas for the treatment of atherosclerosis.

Furthermore, appropriate exercise has been shown to be beneficial in improving metabolism and CVD.[76] Three different types of exercise, including moderate-intensity continuous training (MICT), high-intensity interval training (HIIT), and circuit resistance training (CRT), were found to increase circulating Nrg4 levels and were accompanied by decreased levels of HOMA-IR, serum LDL, and TC.[76] The above study reported that HIIT and CRT led to a greater reduction in serum LDL, insulin, and HOMA-IR than the MICT.[76] Circulating Nrg4 was increased more significantly by HIIT and CRT than by MICT. Compared to MICT, HIIT and CRT have higher intensity, and produce greater physiological and metabolic stress during exercise, resulting in more physiological adaptations.[76] Consequently, the circulation levels of Nrg4 may be positively related to exercise intensity, and more studies are needed to elucidate the mechanisms by which exercise training regulates the expression of Nrg4.

Non-alcoholic fatty liver disease

Non-alcoholic fatty liver disease (NAFLD) is a continuum of liver abnormalities ranging from nonalcoholic fatty liver (NAFL) to nonalcoholic steatohepatitis (NASH), which can further lead to cirrhosis and liver cancer.[77-79] The liver is the direct target tissue of Nrg4. Nrg4 activates ErbB4 receptor tyrosine kinases, which in turn attenuates hepatic lipogenesis.[2] A case—control study showed that decreased serum Nrg4 level is prevalent in NAFLD subjects compared to non-NAFLD controls, and is an independent risk factor for NAFLD.[80,81] Autophagy is important for maintaining metabolic homeostasis. Promoting autophagy can reduce liver steatosis and liver injury in NAFLD mice, while dysfunctional autophagy can lead to lipid accumulation in hepatocytes.[82] Nrg4 can promote hepatocyte autophagy and reduce HFD-induced hepatic steatosis in aged mice.[44] *In vivo* studies showed that HFD feeding decreased the level of autophagy marker protein LC3B-II and increased P62 levels, while intraperitoneal injection of recombinant Nrg4 resulted in increased LC3B-II levels and decreased P62 levels.[44] In LO2 hepatocytes, it was further verified that Nrg4 could decrease P62 and increase LC3B-II levels in a dose-dependent manner and that Nrg4 administration increased autophagic flux in LO2 cells.[44] The mTOR kinase is an important regulator of autophagy. Activated mTOR can inhibit autophagy, while AMPK promotes autophagy by negative regulation of mTOR. Through further verification, this study showed that p-AMPK levels were elevated and p-mTOR levels decreased in the liver of mice treated with Nrg4, indicating that Nrg4 could activate AMPK signaling and inhibit mTOR signaling *in vivo*.[44] In addition, in LO2 cells, treatment of Nrg4 significantly decreased cellular lipid accumulation. However, after inhibition of AMPK or autophagy, the inhibitory effect of Nrg4 on lipid accumulation in hepatocytes was weakened.[44] These data suggest that Nrg4 may induce autophagy through the AMPK and mTOR signaling pathway, which in turn attenuates hepatic steatosis in aged mice (Fig. 5A). Further studies are needed to investigate the functional role of the above pathway in young mice or humans.

In vivo, Nrg4 was able to suppress the expression of lipogenic genes (*Srebf1*, *Acaca*, *Fasn*, and *Scd1*) in the liver (Fig. 5A).[8] *In vitro*, Nrg4 activated ErbB4 to improve oleic acid (OA)-induced lipid accumulation in HepG2 cells and reduced the mRNA levels of *de novo* lipogenesis genes such as *Srebf1*, *Acaca*, *Scd1*, and *Fasn*, but had no significant effect on the mRNA levels of fatty acid β-oxidation genes.[83] Similarly, adenovirus-mediated Nrg4 overexpression in primary mouse hepatocytes inhibited the expression of genes related to *de novo* lipogenesis but did not affect fatty acid β-oxidation

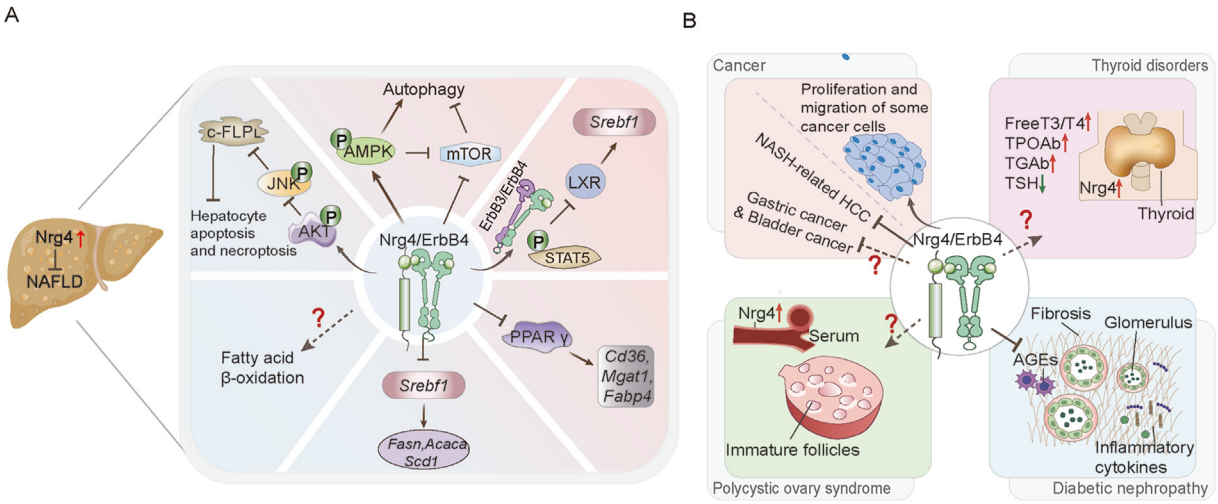

Figure 5 The potential roles of Nrg4 in NAFLD, cancer, and thyroid disorders. **(A)** Nrg4 has a protective role against non-alcoholic fatty liver disease (NAFLD). In hepatocytes, Nrg4 may induce autophagy through the activation of AMPK signaling and the inhibition of mTOR signaling. This in turn attenuates hepatic steatosis in aged mice. Nrg4/ErbB4 signaling can increase phosphorylation of endogenous ErbB3 and STAT5 proteins, and activated STAT5 inhibits LXR transcriptional activity, which inhibits the expression of *Srebf1*, the gene encoding Srebp-1c that promotes lipogenesis. Moreover, Nrg4 inhibits the expression of PPARγ and its target genes (*Cd36*, *Mgat1*, and *Fabp4*) in the liver, thus preventing hepatic steatosis. Nrg4 is able to suppress the expression of lipogenic genes (*Srebf1*, *Acaca*, *Fasn*, and *Scd1*) in the liver. Whether Nrg4 could ameliorate NAFLD by promoting fatty acid β-oxidation needs further investigation. Furthermore, Nrg4 can inhibit the JNK1/2 phosphorylation by activating AKT and then reduces the ubiquitination and proteosomal degradation of FADD-like apoptosis regulator (c-FLIP$_L$) to exert cytoprotective effects, which hinders the development of hepatic steatosis to NASH. **(B)** Nrg4 is shown to play potential roles in cancer, thyroid disorders, diabetic nephropathy, and polycystic ovary syndrome. In cancer, Nrg4 is highly expressed in melanoma, prostate cancer, and malignant lymphoma in the gastrointestinal tract, and it can promote the proliferation of the above cancer cells. However, Nrg4 is down-regulated in a mouse model of NASH-related hepatocellular carcinoma (HCC). Nrg4 can inhibit NASH-related HCC by impairing tumor-prone liver immune microenvironment. In addition, Nrg4 and its receptor ErbB4 were significantly reduced in cancerous tissues from patients with bladder cancer and gastric cancer. The exact roles of Nrg4 in different cancers need further exploration. In thyroid disorders, Nrg4 levels were positively correlated with serum-free T3, free T4, thyroid peroxidase antibody (TPOAb), and thyroglobulin antibody (TGAb) levels, but negatively correlated with thyroid stimulating hormones (TSH). In diabetic nephropathy, Nrg4 can attenuate renal function injury, tubulointerstitial fibrosis, inflammation and suppress the expression levels of advanced glycosylation end products (AGEs). In polycystic ovary syndrome, serum Nrg4 levels are significantly elevated in patients with polycystic ovary syndrome, and the role and mechanism of Nrg4 in polycystic ovary syndrome need to be further explored.

genes expression.[2] Furthermore, in hepatocytes, Nrg4, through binding to ErbB4, was able to increase phosphorylation of endogenous ErbB3 and STAT5 proteins, and activated STAT5 led to inhibition of LXR transcriptional activity, which suppressed the expression of lipogenic genes, including *Srebf1* and its downstream target genes.[2] PPARγ can promote lipogenesis. A study has shown that transgenic expression of Nrg4 inhibits the expression of PPAR γ and its target genes (*Cd36*, *Mgat1*, and *Fabp4*) in the liver (Fig. 5A), thus preventing hepatic steatosis.[8] In general, Nrg4 can alleviate hepatic lipid accumulation by inhibiting the *de novo* lipogenesis in the liver. Because Nrg4 may promote fatty acid β-oxidation in mice under starvation,[45] which could be independent of the regulation of fatty acid β-oxidation gene expression, further studies are needed to investigate whether enhanced fatty acid β-oxidation is involved in Nrg4-mediated alleviation of NAFLD.

NASH is characterized by persistent liver injury, chronic inflammation, and liver fibrosis; hepatocyte death is an important driver for the development of NASH. A previous study showed that Nrg4 protected hepatocytes from apoptosis and necroptosis and prevented the transition from hepatic steatosis to NASH in mice.[84] *Nrg4* null mice fed with the NASH-inducing diet accelerated hepatocyte death, inflammation, and hepatocyte fibrosis, while *Nrg4* transgenic expression in adipose tissue improved the severity of diet-induced NASH in mice.[84] Hepatocyte apoptosis and necroptosis in NASH are related to an increase in JNK1/2 phosphorylation and a decrease of FADD-like apoptosis regulator (c-FLIP$_L$) protein stability, in which c-FLIP$_L$ is a negative regulator of apoptosis and necroptosis. Moreover, Nrg4 can inhibit JNK1/2 phosphorylation by activating AKT, which reduces the ubiquitination and proteasomal degradation of c-FLIP$_L$ and exerts cytoprotective effects. The

above effect hinders the progression of hepatic steatosis to NASH.[84] Therefore, Nrg4 can inhibit apoptosis and necroptosis of hepatocytes to protect against NASH. Liver fibrosis is an important feature of NASH. Whether Nrg4 can directly affect the activation of hepatic stellate cells to control liver fibrosis during the initiation and progression of NASH needs to be further explored.

Cancer

Nrg4 is involved in the occurrence and development of many cancers (Fig. 5B). Nrg4 was shown to be highly expressed in many cancer cells, including the ones of melanoma,[85] prostate cancer,[3] breast cancer,[86] and malignant lymphoma in the gastrointestinal tract.[12] A high level of Nrg4 expression is detected in prostate cancer, and the expression level is positively correlated with the grades of prostate tumor development. In prostate cancer, all five splice variants of Nrg4 can be detected and they are expressed mainly in the epithelial cells.[3] However, because of the structural differences, different variants of Nrg4 have different subcellular localizations, with Nrg4 A variants being expressed mainly on the cell membrane and Nrg4 B mainly in the intracellular compartments.[3] In addition, in prostate cancer cell migration assay, Nrg4 A1/A2 containing the EGF domain promoted the formation of lamellipodia and filopodia pseudopods as well as cell migration.[87] More research is required to further confirm whether Nrg4 A1 and Nrg4 A2 can facilitate the proliferation and metastasis of prostate tumor cells. Similarly, Nrg4 was highly expressed in lymphoma cell lines.[12] Immunohistochemical analyses of malignant lymphoma clinical samples revealed that Nrg4 and ErbB4 were mainly expressed in mucosa-associated lymphoid tissue (MALT) and follicular lymphoma.[12] Additionally, recombinant Nrg4 induced the tyrosine phosphorylation of ErbB4 and activated the proliferation of malignant lymphoma cells in the gastrointestinal tract.[12] These studies indicate that Nrg4 is highly expressed in some cancer tissues, which in turn may play a role in promoting the proliferation and migration of these cancer cells. However, at present, the mechanism of the Nrg4/ErbB4 signaling pathway in facilitating the proliferation and migration of cancer cells is still not clear. Understanding the above mechanism is important for Nrg4-based cancer therapy.

In hepatocellular carcinoma (HCC), the expression of Nrg4 was found to be down-regulated.[88] In the diet-induced NASH-related HCC, Nrg4 deficiency exacerbated the induction of tumor-prone liver immune microenvironment, including tumor-associated macrophage (TAM)-like macrophages and exhaustion of cytotoxic $CD8^+$ T cells in the liver.[88] On the contrary, transgenic Nrg4 overexpression elicited a protective role against NASH-related HCC in mice.[88] In addition, some studies have shown that the expression of Nrg4 and its receptor ErbB4 was significantly reduced in cancerous tissues from patients with bladder cancer[89,90] and gastric cancer.[91] It is not clear how the decrease of Nrg4 expression affects the occurrence and development of bladder cancer and gastric cancer. The exact role and mechanism of Nrg4 in various cancers have not been fully elucidated and need to be further explored.

Other diseases

Thyroid hormone (TH) is required for normal development and metabolism. Compared to the healthy population, serum Nrg4 concentrations were significantly higher in hyperthyroid patients.[15] Serum Nrg4 levels were positively correlated with serum-free T3, free T4, thyroid peroxidase antibody (TPOAb), and thyroglobulin antibody (TGAb) levels, but negatively correlated with thyroid stimulating hormones (TSH).[15] However, after thionamide treatment (a hyperthyroidism treatment drug), the patients' TH levels returned to normal and serum Nrg4 concentrations were also significantly reduced.[15] TH plays a significant role in regulating body metabolism. Similarly, Nrg4 plays an important role in maintaining metabolic homeostasis. Therefore, further studies are needed to investigate the possible association and interplay between TH and Nrg4 in the regulation of body metabolism and whether TH can directly regulate the expression of Nrg4.

Diabetic nephropathy (DN) is identified as a cause of end-stage renal disease (ESRD), and tubulointerstitial fibrosis (TIF) is a key event in DN development. In DN patients, serum Nrg4 levels were significantly reduced.[16] Animal studies have shown reduced levels of Nrg4 in the serum and renal tissue of DN rats.[92] However, exogenous Nrg4 treatment was able to attenuate renal function injury, tubulointerstitial fibrosis, inflammation and suppress the expression levels of advanced glycosylation end products (AGEs) in DN rat kidney, and the levels of renal function marker serum creatinine (Scr), blood urea nitrogen (BUN), and urine creatinine were also significantly reduced by Nrg4 treatment.[92] Further studies revealed that Nrg4 may ameliorate DN-induced fibrosis and attenuate AGEs and inflammation levels via inhibition of TNF receptor 1 (TNF-R1) expression in human kidney-2 cells.[92] Therefore, Nrg4 has a potential role in mitigating TIF during the development of DN.

In addition, several studies have shown that serum Nrg4 levels are significantly elevated in patients with polycystic ovary syndrome.[93,94] However, the current study is limited to correlation analysis, and the role and mechanism of Nrg4 in polycystic ovary syndrome need to be further explored.

Concluding remarks

Studies in cellular and animal models have shown that Nrg4 is involved in a variety of physiological processes. Nrg4 is able to influence neuronal morphology and growth during early development in mice, and further studies are needed to determine whether this affects behavioral expression in adulthood. Moreover, Nrg4 alleviates lipid accumulation by inhibiting lipid synthesis in the liver. In addition, Nrg4 plays a role in alleviating insulin resistance by promoting glucose uptake in adipocytes. Nrg4 can promote the expression of thermogenic genes in adipose tissue, but its mechanism

needs to be further clarified. Nrg4 has five variants and Nrg4 A variant is able to act on the target tissue in the form of a secreted factor. The EGF-like structure of Nrg4 can interact with its receptor ErbB4 to mediate different signal transduction and then participates in a wide range of physiological processes. Therefore, the exploration of the target tissues affected by Nrg4 and the clarification of the roles and mechanisms of its variants may provide more insights into the physiological processes regulated by Nrg4.

In pathological conditions, Nrg4 alleviates inflammatory diseases by promoting apoptosis of M1 macrophages and inhibiting the release of inflammatory factors. Obesity is associated with abnormal lipid metabolism, insulin resistance, elevated levels of inflammation, and impaired adipose tissue angiogenesis, while Nrg4 overexpression has been shown to alleviate the above disorders. However, the exact role of Nrg4 in diabetes needs to be clarified. In addition, circulating Nrg4 levels were significantly lower in individuals with cardiovascular disease. Exercise can promote the level of circulating Nrg4, which may contribute to the improvement of obesity-related disorders and cardiovascular disease. Whether Nrg4 plays an important role in exercise-mediated metabolic improvement remains to be studied in depth. In addition, Nrg4 alleviates hepatic steatosis by promoting autophagy and inhibiting lipogenesis in the liver. It can also prevent the progression from simple fatty liver to steatohepatitis by inhibiting apoptosis and necroptosis of hepatocytes. Nrg4 is closely associated with the development of cancers, and its effect on the proliferation and metastasis of cancer cells has not been fully elucidated and needs to be further explored. Besides, Nrg4 is also closely associated with a variety of other diseases, including hyperthyroidism, diabetic nephropathy, and polycystic ovary syndrome. Moreover, the regulation and pathophysiological role of Nrg4 splice variants are not well defined, which merits further investigation. Therefore, further elucidation of the functional role and mechanism of Nrg4 and its splice variants in different diseases would provide a clearer understanding of the therapeutic potential and possible risks of Nrg4.

Author contributions

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Conflict of interests

The authors declare no conflict of interests.

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