Advances in the study of the role and molecular mechanism of with-no-lysine kinase 3 in nervous system diseases (Review)

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Abstract. With-no-lysine kinase 3 (WNK3) is a serine/threonine kinase that functions by regulating downstream signaling molecules. WNK3 mainly regulates intracellular and extracellular Na^+, Cl^- and K^+ levels by regulating downstream ion transporters, the disruption of which has been associated with cerebral ischemia, epilepsy, glioma and other diseases. In addition, WNK3 was demonstrated to regulate neuronal splicing factor RNA binding fox-1 homolog-1 to influence autism. Over the past 20 years, accumulating evidence has reported that dysfunctional WNK3 signaling was involved in the pathologies of various neurological disorders; therefore, WNK3 has become a promising therapeutic target for ameliorating the corresponding symptoms of such disorders. The present review aimed to provide a general overview of the expression patterns and physiological functions of WNK3 signaling and its pathophysiological roles in neurological diseases, such as epilepsy, ischemic brain injury, intracerebral hemorrhage, autism, glioma and schizophrenia.

Contents

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1. Introduction

With-no-lysine kinases (WNKs) are a class of protein kinases that were initially discovered in multicellular organisms (1). Four WNK genes, namely WNK1, WNK2, WNK3 and WNK4, have been identified in the human genome, which are encoded by genes on chromosome 12, 9, X and 17, respectively (2). A defining characteristic of WNK family members is the absence of a catalytic lysine residue specifically located in the N-terminal kinase domain (3). WNKs have been reported to play an important role in cell and body physiology (4). For example, WNK1 was found to be expressed in a variety of tissues, including the kidney, heart and brain (5), and two major transcripts have been identified: One is mainly produced in the heart, muscles and brain and is called L-WNK1, while the other shorter transcript is primarily located in the kidney, thus is also known as kidney-specific WNK1, as it is only expressed in distal convoluted tubules and connecting tubules (6,7). WNK1 is widely expressed and has been reported to be involved in the regulation of numerous cellular processes (8). For example, WNK1 in regulation of the podocyte actin cytoskeleton, biophysical properties of glomerular capillaries and slit diaphragm structure, all of which are essential (9). Notably, due to the observed association between WNK1 mutations and familial hypertension and autonomic neuropathy, the function of WNK1 in the kidney and nervous system has been extensively studied (10). WNK1 is self-phosphorylated on serine residues, and mutations in the gene encoding WNK1 were found to cause high blood pressure in humans (7). Similar to WNK1, WNK4 is also expressed in the kidney and has been closely associated with hypertension (11). The loss of introns in the WNK1 and WNK4 genes has been discovered to lead to pseudoaldosterone deficiency type II, a disease associated with salt-sensitive hypertension and hyperkalaemia (12,13). This may be due to the influence of WNK1 and WNK4 on the ion reabsorption signaling pathway (14). WNK1 and WNK4 can also activate the Na^+-Cl^- cotransporter (NCC) of distal concentric tubules through the serine/threonine-protein kinase STE20/serine/threonine kinase 39 (SPAK)/odd-skipped related transcription factor 1 (OSR1) signaling pathway, forming the WNK/SPAK/OSR1/NCC phosphorylation cascade, which was reported to be involved in the regulation of renal pressure homeostasis by regulating intracellular ions and water (15,16).

Unlike other WNKs, WNK2 is not expressed in the kidney (17). WNK2 is a neuron-rich kinase mainly expressed in neocortical pyramidal cells, thalamic relay cells, cerebellar granulosa cells and Purkinje cells in the brain (18). Through large-scale genomic and epigenomic analyses of human invasive gliomas, WNK2 was identified to be a tumor suppressor gene (19-21). In addition, epigenetic silencing of WNK2 was found to occur in all grades of meningiomas (19).

As a member of the WNK family, WNK3 is composed of 1,800 amino-acid residues (22). The WNK3 gene is made up of an amino-terminal domain, a highly conserved serine/threonine kinase domain, an autoinhibitory domain, and at least two coiled-coil domains (Fig. 1) (23). The human WNK3 gene is located on chromosome Xp11.22 (24). The molecular weight of WNK3 is 192 kDa and the protein kinase catalytic domain of WNK3 is located between residues 147 and 405 (25). WNK3 is found in almost all tissues of the human body, but was found to be highly expressed in the brain (26). Among all members of the WNK family, the WNK3 expression was reported to be highest in the brain (27). In the brain, WNK3 transcriptional products were found to be located in the cortex, thalamus, hypothalamus (including the suprachiasmatic nucleus and supraoptic nucleus), raphe nuclei, cerebellar Purkinje cell layer, locus coeruleus and reticular structures (28). These expression patterns suggested that WNK3 may play an important role in brain diseases.

The present review searched relevant literature from PubMed (https://pubmed.ncbi.nlm.nih.gov/), Ovid (https://ovidsp.ovid.com/), Cochrane Library (https://www.cochranelibrary.com/library), Embase (https://www.embase.com/) and CNKI (https://www.cnki.net/) databases. The retrieval time was set to be from database establishment to August 2020. To avoid omissions, the full text of the literatures was scanned and the reference lists from the included studies were also screened manually and classified into different neurological diseases. Then, the role and molecular mechanisms of WNK3 in neurological diseases were analyzed, and the applications of WNK3 and its downstream signaling pathways in neurological diseases were systematically described. Neurological diseases are complex and difficult to treat. Currently, there is a lack of knowledge of the role and mechanism of action of WNK3 in the nervous system (27). Therefore, the present review summarized the current knowledge of the potential role of WNK3 in the nervous system. The present review aimed to enhance the present knowledge by reviewing and summarizing the underlying neurological mechanism of WNK3, which may provide an intervention target for neurological diseases, and thus provide novel possibilities and directions for clinical treatment.

2. Role of WNK3 in neurological diseases

At present, to the best of our knowledge, there are few published studies investigating WNK3 signaling in the nervous system; however, its dysfunction in the brain has been associated with the occurrence of several neurological diseases, including epilepsy (29), ischemic brain injury, intracerebral hemorrhage (27), autism (30), glioma (31), schizophrenia and autonomic nerve pain (Table I) (32). It has been suggested that WNK3 signaling may play distinct roles in different brain diseases, which are further discussed in more detail in the following sections.

Role of WNK3 signaling in autism. Autism comprises a heterogeneous range of neurodevelopmental conditions characterized by symptoms such as communication/language deficits, repetitive/restricted patterns of behavior and inadequate social interactions (33,34). Individuals with autism have difficulty with social communication and interactions, increased rates of restricted/repetitive patterns of behavior and increased sensory sensitivities (35). Autism is also accompanied by several other complications, such as insomnia, intellectual disabilities, epilepsy, self-injurious behavior, aggression, anxiety, attention-deficit hyperactivity disorder and depression (33,36). WNK3 is known to regulate the activity of the neuronal splicing factor, RNA binding Fox-1 homolog 1 (FOX-1). FOX-1 is a neuron-specific splicing factor which has been predicted to regulate neuronal splicing networks that are clinically implicated in neurodevelopmental diseases, including autism spectrum disorder (37,38). Comparative profiling of splicing in brains from patients with autism spectrum disorder and normal brains revealed that FOX-1 expression was strongly associated with autism (39). FOX-1 regulated the excitability of neurons by specific splicing (40). Previous studies have shown that WNK3 regulated FOX-1-mediated alternative splicing and subsequently affected autism (39,41,42). FOX-1 and WNK3 has several intersections, which are strongly related to the control of neuronal excitability (43). FOX-1 has been shown to regulate alternative splicing of neuronal transcripts by binding the sequence (U) GCAUG in introns flanking alternative exons and is responsible for generating proper alternative splicing variants required for normal neuronal excitability and synaptic transmission (40). WNK3 was discovered to affect the splicing activity of FOX-1 by affecting the subcellular localization of FOX-1 and neuronal transcripts cannot be spliced normally, leading to reduced neuronal excitability, which has an important influence on the pathogenesis of autism (43). In addition, FOX-1-mediated exon inclusion bodies were significantly downregulated after co-expression with wild-type WNK3, while inactive WNK3 only exerted a marginal effect (39,44,45). Due to the role of WNK3 and FOX-1 in disorders of neuronal development, WNK3 may represent a target for treatment of FOX-1-induced autism (Fig. 2) (43,46).

Role of WNK3 signaling in epilepsy. Epilepsy consists of a group of recurrent episodes of abnormal neuronal firing caused by temporary central nervous system dysfunction (47). The typical clinical manifestations of epilepsy comprise sudden loss of consciousness, muscle spasms, rigidity and convulsions; it can also be accompanied by urinary incontinence, asphyxia or other symptoms (48,49). Epilepsy is second only to stroke in the number of years of potential life lost due to a neurological disease. The prevalence of epilepsy is 6.4 per 1,000 individuals and the annual incidence is 67.8 cases per 100,000 person-years (50), which poses a major public health burden (51-53). A previous study observed that WNK3 immunoreactivity was increased in dispersed granule neurons in patients with epilepsy, suggesting that WNK3 may play a role in neuronal hyperexcitability (54). Furthermore, WNK3 phosphorylated the Na^+-K^+-Cl^- cotransporter (NKCC) and inhibited the K^+-Cl^- cotransporter (KCC) to regulate neuronal

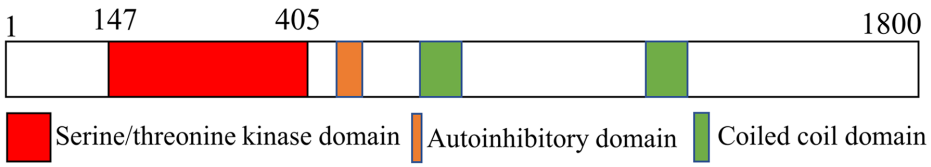

Figure 1. Schematic of the structure of WNK3. WNK3 is composed of 1,800 amino acid residues (numbers in the figure represent amino acids). The WNK3 gene is made up of a highly conserved serine/threonine kinase domain, an autoinhibitory domain and two-coiled coil domains. WNK3, with-no-lysine kinase 3.

excitability (55). NKCC1 expression levels were upregulated in dispersed granule cells within postmortem tissues from patients with epilepsy (56). In addition, previous studies have demonstrated that restoration of KCC2 function and controlling the regulation of NKCC1 have potential as antiepileptic therapies (57-59). These findings suggested that the sustained activation of WNK3, which is upstream of NKCC1 and KCC2, may contribute to the induction of neuronal hyperexcitability and result in the development of abnormal electrical activity within the hippocampus. Thus, WNK3 expression may be a novel therapeutic target for mitigating epileptogenesis (Fig. 3) (54).

Role of WNK3 signaling in ischemic brain injury. Ischemic brain damage, also known as stroke, is a sudden cessation in cerebral blood flow. Clinical manifestations include sudden fainting, unconsciousness, sudden mouth/eye skewness, hemiplegia and intellectual disability (60). Moreover, ischemic brain injury is one of the most common causes of mortality and disability worldwide, affecting 30 million people (61,62). A previous study in mouse models revealed that inhibition of WNK3 modulation of SPAK/OSR1 and NKCC1 signaling in the brain ameliorated both gray- and white-matter damage and promoted neurological recovery following ischemic stroke (54). In addition, after cerebral ischemic injury, the WNK3/SPAK/OSR1/NKCC1 signaling cascade was found to be activated, WNK3 expression levels were upregulated, and SPAK and OSR1 were phosphorylated, which resulted in the increased phosphorylation of downstream NKCC1 (63). NKCC1 was also discovered to play an important role in the pathophysiology of ischemia, where it regulated cellular volume by regulating the entry of Na^+, K^+ and Cl^- into cells (64). Under ischemic conditions, NKCC1 induced excessive amounts of Na^+, K^+ and Cl^- to enter into cells, leading to intracellular ionic overload that damaged the endoplasmic reticulum and mitochondria, and led to necrosis and apoptosis (65,66). Hence, it was suggested that WNK3 may upregulate NKCC1 to promote ischemic brain damage (67-69). A previous study using stroke model mice demonstrated that WNK3-knockout mice had a reduced infarct volume, cerebral edema and axonal demyelination following a stroke episode compared with wild-type mice (29). These findings suggested that cerebral ischemia may aggravate ischemic brain injury by increasing the phosphorylation of NKCC1 through the WNK3/SPAK/OSR1 signaling pathway. Furthermore, the knockdown of WNK3 significantly inhibited the activity of NKCC1, suggesting that the knockdown of WNK3 expression may protect nerve cells by regulating ion and water transport (26). WNK3/SPAK inhibition also prevented acute cellular swelling in response to osmotic stress and ameliorated brain swelling by simultaneously increasing the stimulatory phosphorylation of NKCC1 and inhibiting KCC phosphorylation (70). Notably, in another previous study, following WNK3/SPAK inhibition, the damage caused by cerebral ischemia was alleviated; however, the mechanisms by which cerebral ischemia activated the WNK3/SPAK/OSR1/NKCC1 signaling pathway remain unclear (Fig. 3) (63).

Role of WNK3 signaling in intracerebral hemorrhage. Intracerebral hemorrhage is a common type of stroke, which is accompanied by a mortality rate of ~50%, and ~2/3 of patients have a poor prognosis and are unable to live independently (17,71). An intracerebral hemorrhage forms a hematoma, which squeezes the brain tissue to cause intracranial hypertension and pathophysiological changes in the brain, which can lead to secondary brain injury (72). At present, reducing intracranial pressure and blood pressure is one of the emergency treatment methods for intracerebral hemorrhage (73,74). Therefore, determining endogenous intervention measures is one of the treatment approaches to improve secondary brain injury following intracerebral hemorrhage (75,76). Brain edema caused by blood brain barrier (BBB) damage is a common secondary brain injury following intracerebral hemorrhage (77). After intracerebral hemorrhage, WNK3 expression levels were found to be upregulated in brain tissue, which activated NKCC1 in microglia cells by phosphorylating SPAK (27). The activation of the microglia and release of the inflammatory factors, TNF-α and IL-1β (78), leads to brain inflammation, which further aggravates brain damage (79,80). The overexpression of WNK3 could also increase the phosphorylation of NKCC1, and phosphorylated NKCC1 stimulated the microglia to secrete inflammatory factors, which simultaneously expanded the cell volume, destroyed the tight connection of cells, accelerated the diffusion of inflammatory factors, destroyed the BBB, led to neuronal apoptosis and aggravated brain edema (27). Conversely, knocking out WNK3 expression had the opposite effects. These findings indicated that inhibiting the WNK3 signaling pathway may play a role in brain protection, and WNK3 may improve secondary brain injury caused by intracerebral hemorrhage (Fig. 3) (27).

Role of WNK3 signaling in glioma. Brain tumors originate from glial cells, and gliomas are among the most problematic primary cancers to treat (81). Furthermore, gliomas are the most common type of tumor of the central nervous system (82). Gliomas invade by diffusing into the surrounding brain parenchyma, thereby making surgical resection difficult (83). Glioma cells change in morphology

Table I. Published studies on the expression patterns and effects of WNK3 signaling in brain diseases.

Authors, year	Disease	Study model	Molecule/s	Conclusion	(Refs.)
Lee *et al*, 2012	Autism	*In vitro* model in 293 cells	WNK3/FOX-1	*In vitro* data revealed that the KO of WNK3 expression in 293 cells suppressed FOX-1-dependent splicing. Thus, WNK3 may represent a target for treatment of FOX-1-induced diseases.	(43)
Qiao *et al*, 2008	Autism	Clinical patients	WNK3	Studies in patients with autism suggested that the deletion of family sequence similarity 120C and WNK3 genes from Xp11.22 may be involved in the pathogenesis of autism.	(41)
Jeong *et al*, 2018	Epilepsy	Clinical patients	WNK3/NKCC1/KCC2	WNK3 resulted in the development of abnormal electrical processes within the hippocampi in patients with epilepsy. Thus, WNK3 may represent a novel therapeutic target in epilepsy.	(54)
Zhao *et al*, 2017	Ischemia brain injury	*In vivo* model in mice	WNK3/SPAK-NKCC1	WNK3-KO and SPAK-KO mice exhibited a reduction in cerebral edema, significantly less demyelination and improved neurological outcomes. Thus, inhibition of WNK3 or SPAK exhibited therapeutic effects in middle cerebral artery stroke.	(63)
Begum *et al*, 2015	Ischemia brain injury	*In vitro* model in primary cortical neurons	WNK3/SPAK/OSR1/NKCC1	KO of WNK3 in primary cortical neurons revealed that the WNK3/SPAK/OSR1 signaling pathway may represent a therapeutic target for neuroprotection following ischemic stroke.	(29)
Wu *et al*, 2020	Intracerebral hemorrhage	*In vivo* model in rats	WNK3/SPAK/NKCC1	WNK3/SPAK/NKCC1 signaling pathway induces neuronal apoptosis. After intracerebral hemorrhage, inhibition of WNK3 inhibited the induced neuronal apoptosis and reduced brain edema.	(27)
		Intracerebral hemorrhage	*In vitro* model in neurons		
Haas *et al*, 2011	Glioma	*In vitro* model in human glioma cells	WNK3/NKCC1	Previous studies with human glioma cells revealed that WNK3 stimulated glioma invasion by regulating the cellular volume through NKCC1.	(31)
Garzon *et al*, 2012	Glioma	*In vitro* model in human brain tumor cells	WNK3/NKCC1	WNK3-mediated NKCC1 phosphorylation following the activation of the PI3K/AKT signaling pathway may promote glioma cell migration.	(86)
Arion and Lewis, 2011	Schizophrenia	Clinical patients	WNK3/OXSR1/NKCC1/KCC2	By comparing the brain tissue from normal individuals with brain tissue from individuals with schizophrenia, the results revealed that the expression levels of OXSR1 and WNK3 were upregulated in patients with schizophrenia.	(97)

WNK3, with-no-lysine kinase 3; SPAK, serine/threonine kinase 39; NKCC, Na^+-K^+-Cl^- cotransporter; KCC, K^+-Cl^- cotransporter; OSR1, odd-skipped related transcription factor 1; OXSR1, oxidative stress responsive kinase 1; KO, knockout; FOX-1, RNA binding fox-1 homolog 1.

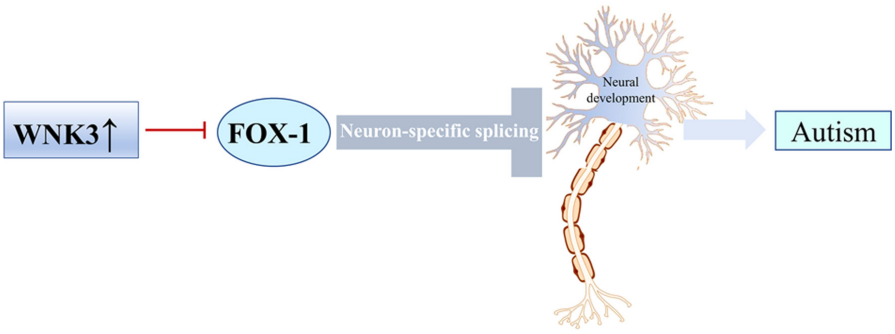

Figure 2. Underlying mechanism of the WNK3 signaling pathway in autism. When the expression levels of WNK3 are upregulated in the brain, FOX-1 neuron-specific splicing is inhibited, thus affecting the development and function of neurons and promoting the occurrence of autism. WNK3, with-no-lysine kinase 3; FOX-1, RNA binding Fox-1 homolog 1.

and volume to migrate to adjacent brain parenchyma, and the transport of Na^+, Cl^- and water was discovered to play a crucial role in this process (84,85). Compared with those in the normal brain, the expression levels of WNK and SPAK/OSR1 were found to be upregulated in the brain tissues of patients with glioma (31). In addition, WNK3 was discovered to be involved in the regulation of cellular volume, which is an important parameter for the migration of glioma (31). Previous studies have shown that WNK3 facilitates intracellular entry of Cl^- and water, resulting in changed cell volume; cell volume serves a significant role in tumor migration (86). Changes in cellular volume have also been increasingly recognized as an important requirement for cancer cell invasion and metastasis (87). During the migration of tumor cells, ions must accumulate in cells to allow them to flow along an electrochemical gradient (88). NKCC1 was demonstrated to regulate the entry of ions into cells and played an indispensable role in regulating intracellular pressure and tumor cell migration (89). In addition, a previous study revealed that NKCC1 co-transported water and ions, making it ideally suited to transporting salt and water across the plasma membrane during cytoplasmic cell-volume regulation (90,91). A previous study by Haas *et al* (31) showed that the knockdown of WNK3 with small interfering RNA promoted loss of NKCC1 function, which alleviated cell-volume changes associated with cellular invasion. These findings suggested that WNK3 may influence glioma migration through its regulation of NKCC1. Thus, it has been hypothesized that SPAK/OSR1 may participate in the regulation of WNK3 over NKCC1 to inhibit the signaling pathway, which may help reduce intracellular ionic influx and prevent cellular volumes from being too large to inhibit the metastasis and invasion of gliomas (31,86). Therefore, understanding the role of WNK3 in facilitating glioma migration and invasion may provide evidence to suggest the potential of WNK3 as a therapeutic target for glioma (Fig. 3) (28,30).

Role of WNK3 signaling in schizophrenia. At the beginning of the 20th century, psychiatrists considered blunted affect and emotional withdrawal as key symptoms of schizophrenia (92,93). The main clinical manifestations of patients with schizophrenia comprise anhedonia, loneliness, avolition, emotional immaturity and asociality (94). These emotional and cognitive deficits are caused by neural-network dysfunctions, which may, at least partly, be due to abnormal neurotransmission of γ-aminobutyric acid (GABA) in the dorsolateral prefrontal cortex (95,96). According to previous studies, the upregulated expression levels of WNK3 and oxidative stress responsive kinase 1 (OXSR1) modulated the activity of Cl^- transporters, which led to a change in the concentration of Cl^- ions inside and outside of neurons that affected GABAergic neurotransmission in patients with schizophrenia (55,97). WNK3 was also found to effectively activate NKCC1, which is co-expressed in neurons, and was found to have a OXSR1/STK39 binding motif (97). These findings suggested that WNK3 may regulate NKCC1 expression through OXSR1, thereby regulating the flow of Cl^- ions and increasing intracellular Cl^- concentrations to inhibit GABA receptors, which ultimately reduces the excitability and hyperpolarization of GABA (98). Upregulated expression levels of WNK3 were found to be accompanied by upregulated OXSR1 expression levels and enhanced NKCC1 activity, which promoted the flow of Cl^- ions and led to abnormal GABAergic transmission (99,100). Previous studies have also reported that WNK signaling not only affected downstream NKCC1, but it also influenced the flow of Cl^- ions inside and outside the cell by regulating the expression of members of the KCC family (101,102). It is well established that WNK3 is highly expressed in the brain. Several previous studies investigating the WNK kinase family have reported that WNK3 could simultaneously activate NKCC1 and inhibit KCC2 (55, 97,103). The regulatory effect of WNK3 on NKCC1 and KCC2 altered the flow of Cl^- ions, facilitating Cl^- accumulation in cells to produce higher concentrations of intracellular Cl^-, thus affecting the excitability of GABA receptors (97). In addition, the expression levels and kinase activities of WNK3 and OXSR1 were upregulated in patients with schizophrenia, which further increased the activity of NKCC1 and reduced the activity of KCC2, resulting in a high concentration of intracellular Cl^- and increased depolarization of the postsynaptic membrane; these effects ultimately altered the function of the GABA receptors (97,104). A small clinical trial involving 42 patients with schizophrenia and 42 matched healthy subjects revealed that OXSR1 and WNK3 expression levels were markedly upregulated in patients with schizophrenia compared with healthy subjects (1,97). In schizophrenia, upregulated WNK3 and OXSR1 expression levels led to increased phosphorylation and

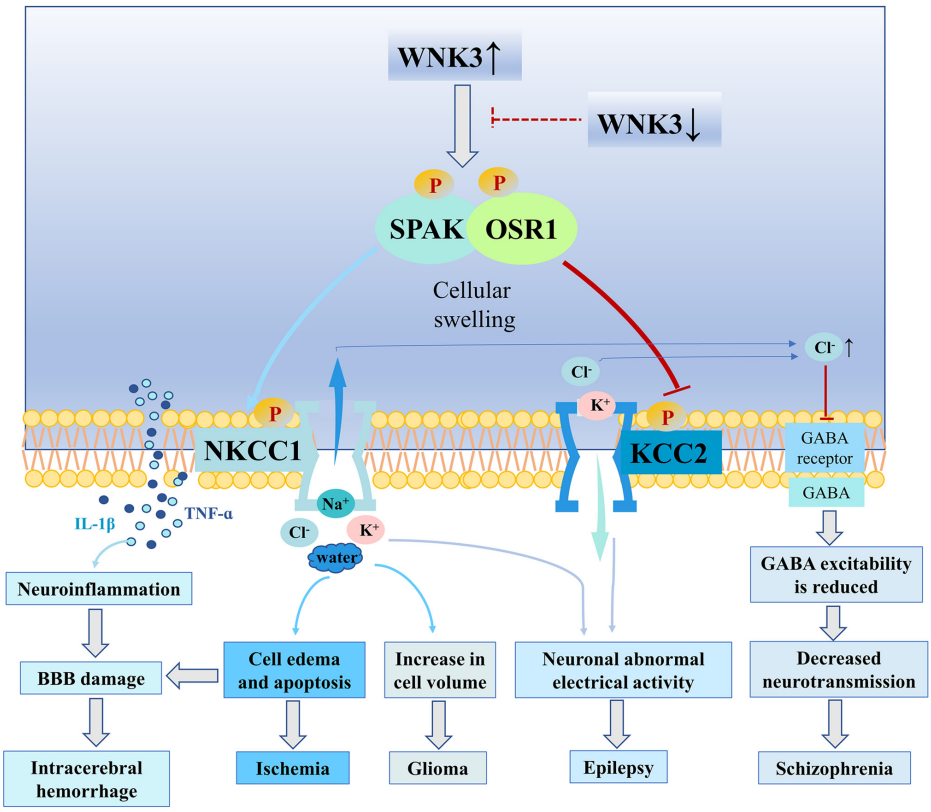

Figure 3. Mechanisms through which WNK3 signaling regulates ion channels and neural excitability in neurological diseases. WNK3 can phosphorylate downstream NKCC1 and inhibit the activity of KCC2 to regulate neuronal excitability by phosphorylating SPAK/OSR1, which contributes to the induction of neuronal hyperexcitability and results in the development of abnormal electrical activity within the hippocampus, which can lead to epilepsy. Upregulated WNK3 expression levels can also regulate ion channels such that increased concentrations of Na^+, K^+ and Cl^- enter the cell, while KCC2 regulates K^+ and Cl^- outflow, thereby regulating cell size and facilitating the translocation of glioma cells. In addition, WNK3 can facilitate NKCC1 regulates the entry of ions and water into cells, resulting in excessive cell edema and apoptosis, which further contributes to brain edema that aggravates ischemia. WNK3/SPAK/NKCC1 signaling can also promote the release of the inflammatory cytokines, TNF-α and IL-1β, leading to neuroinflammation and affecting intracerebral hemorrhage. WNK3 in its kinase-active state can increase Cl^- inflow, decrease Cl- outflow and increase intracellular Cl^- concentration, so GABA receptors are inhibited and GABA excitability is reduced, which affects normal nerve transmission and leads to schizophrenia. On the contrary, downregulation of WNK3 inhibited these signaling pathways. WNK3, with-no-lysine kinase 3; SPAK, serine/threonine kinase 39; OSR1, odd-skipped related transcription factor 1; P, phosphorylated; GABA, γ-aminobutyric acid; NKCC1, Na^+-K^+-Cl^- cotransporter 1; BBB, blood brain barrier; KCC2, K^+-Cl^- cotransporter 2.

consequently increased NKCC1 activity and decreased KCC2 activity, thereby increasing the intracellular Cl^- concentration (97). Thus, when GABA receptors are activated, Cl^- influx is reduced and the GABAergic neurotransmission is altered. At present, although there are relatively few studies reporting the association between WNK3 and schizophrenia, WNK3 is expected to represent a promising target for alleviating and/or treating schizophrenia (Fig. 3).

3. Conclusion

WNK3 expression levels have been reported to be upregulated in the brain, and among the members of the WNK family, the expression levels of WNK3 are the highest in the brain (105). This distribution pattern suggests that WNK3 may play an important role in brain diseases. The present review summarized the current roles and applications of WNK3 in the nervous system, and discovered that the WNK3 signaling pathway may be involved in the regulation of a number of neurological diseases. Thus, WNK3 is suggested to play an important role in nervous system diseases both physiological and pathological processes. Briefly, the aforementioned studies indicated that WNK3 may increase the activity of proapoptotic pathways in central nervous system diseases, partially accelerate the progression of numerous diseases, and worsen the poor prognosis of nervous system diseases and secondary brain damage (27,31).

The mechanism through which the WNK3 signaling pathway may regulate the pathology of brain diseases remains complex and at present, the understanding of the role of WNK3 in nervous system diseases is not complete. WNK3 has been shown to play different roles in the pathological processes of nervous system diseases by regulating multiple different signaling pathways. The most common mechanism identified to date is that WNK3 may regulate downstream ionic transport by phosphorylating SPAK/OSR1 (27). Increases in intracellular ionic concentrations via NKCC1 inhibit KCC activity, which regulates ion influx/efflux across the plasma membrane, increases the number of ions and water molecules inside the cell, alters the cellular structure/volume, and destroys cytoskeletal structures within glia and endothelial cells (97). These processes were discovered to contribute to brain edema and trigger apoptosis, which compromises the normal physiological functioning of the brain. The regulation of this signaling pathway was demonstrated to serve an important role in glioma, intracerebral hemorrhage and ischemic brain injury (29,31,63). In addition, WNK3 is involved in regulating

the flow of Cl^- ions and promoting the accumulation of Cl^- in cells, thereby affecting the excitability of GABA receptors, which may be related to schizophrenia (97). WNK3 was also reported to lead to the increase in intracellular Cl^- concentrations by regulating NKCC1 and KCC expression, which promoted abnormal electrical activity in the hippocampus and thereby induced epilepsy (55). In addition, WNK3 was found to be closely associated with autism by inhibiting the shearing activity of FOX-1, which ultimately affected neural development (43). Previous studies have also shown that WNK3 was associated with neuropathic pain (104), spasticity (1) and other related diseases of the nervous system. However, to the best of our knowledge, currently, the underlying mechanisms of how nervous system diseases may induce WNK3 activation remain unclear.

Although the potential of WNK3 as a novel therapeutic target for brain diseases has been discussed in the present study, whether therapeutic strategies that target WNK3 signaling will be successful in the clinic remains unknown. However, since compounds that inhibit WNK3 have been discovered or developed, such as WNK463 (106), WNK3 inhibitors may represent promising novel targets for the treatment of nervous system diseases.

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Availability of data and materials

The literatures analyzed during the current study are available in the PubMed (https://pubmed.ncbi.nlm.nih.gov/), Embase (https://www.embase.com/), Ovid (https://ovidsp.ovid.com/), CNKI (https://www.cnki.net/) and Cochrane Library (https://www.cochranelibrary.com/library) repository.

Authors' contributions

GC and BQD conceived and designed the study. YTG and MYW wrote the manuscript. JCS created the figures. JFT and JL assisted in the literature search and analyzing the literature. RG reviewed and revised the manuscript. BQD and GC confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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