

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

YA-TING GONG^{1*}, MU-YAO WU^{1*}, JIA-FENG TANG¹, JIN-CHAO SHEN²,
JIE LI³, RONG GAO⁴, BAO-QI DANG¹ and GANG CHEN⁵

Departments of ¹Rehabilitation, ²Anesthesiology, ³Intensive Care Unit and ⁴Neurosurgery, Zhangjiagang TCM Hospital Affiliated to Nanjing University of Chinese Medicine, Suzhou, Jiangsu 215600; ⁵Department of Neurosurgery and Brain and Nerve Research Laboratory, The First Affiliated Hospital of Soochow University, Suzhou, Jiangsu 215006, P.R. China

Received November 17, 2020; Accepted February 22, 2021

DOI: 10.3892/mmr.2021.12032

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

1. Introduction
2. Role of WNK3 in neurological diseases
3. Conclusion

Correspondence to: Dr Rong Gao, Department of Neurosurgery, Zhangjiagang TCM Hospital Affiliated to Nanjing University of Chinese Medicine, 77 Changan Southern Road, Suzhou, Jiangsu 215600, P.R. China
E-mail: 714866001@qq.com

Dr Bao-Qi Dang, Department of Rehabilitation, Zhangjiagang TCM Hospital Affiliated to Nanjing University of Chinese Medicine, 77 Changan Southern Road, Suzhou, Jiangsu 215600, P.R. China
E-mail: zhenjiangdbq@163.com

*Contributed equally

Key words: with-no-lysine kinase 3, epilepsy, ischemic brain injury, intracerebral hemorrhage, autism, glioma, schizophrenia, neurological disease

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 hyperkalemia (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 granula 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

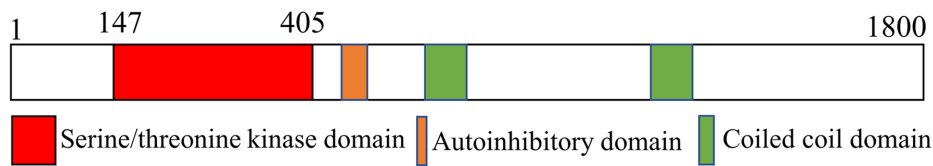


Figure 1. Schematic of the structure of WNK3. WNK3 is composed of 1,800 amino acid residues (numbers in the figure represent amino acids). The WNK3 gene is made up of a highly conserved serine/threonine kinase domain, an autoinhibitory domain and two-coiled coil domains. WNK3, with-no-lysine kinase 3.

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, $\text{TNF-}\alpha$ and $\text{IL-1}\beta$ (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 <i>et al</i> , 2012	Autism	<i>In vitro</i> model in 293 cells	WNK3/FOX-1	<i>In vitro</i> 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 <i>et al</i> , 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 <i>et al</i> , 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 <i>et al</i> , 2017	Ischemia brain injury	<i>In vivo</i> 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 <i>et al</i> , 2015	Ischemia brain injury	<i>In vitro</i> 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 <i>et al</i> , 2020	Intracerebral hemorrhage	<i>In vivo</i> 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)
Haas <i>et al</i> , 2011	Glioma	Intracerebral hemorrhage <i>In vitro</i> model in human glioma cells	<i>In vitro</i> model in neurons WNK3/NKCC1	Previous studies with human glioma cells revealed that WNK3 stimulated glioma invasion by regulating the cellular volume through NKCC1.	(31)
Garzon <i>et al</i> , 2012	Glioma	<i>In vitro</i> 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.

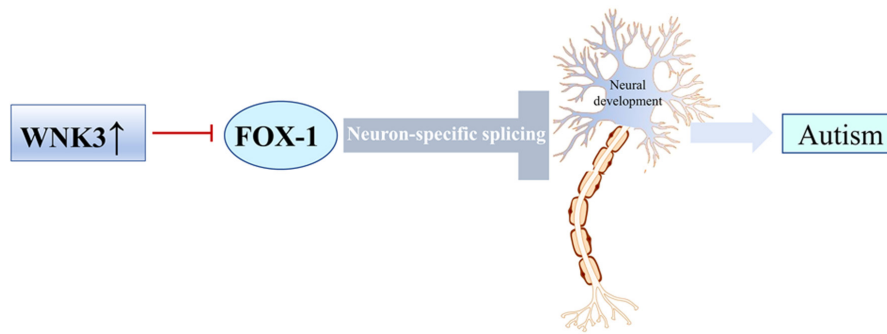


Figure 2. Underlying mechanism of the WNK3 signaling pathway in autism. When the expression levels of WNK3 are upregulated in the brain, FOX-1 neuron-specific splicing is inhibited, thus affecting the development and function of neurons and promoting the occurrence of autism. WNK3, with-no-lysine kinase 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 (OXSRI) 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 OXSRI/STK39 binding motif (97). These findings suggested that WNK3 may regulate NKCC1 expression through OXSRI, 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 OXSRI 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 OXSRI 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 OXSRI and WNK3 expression levels were markedly upregulated in patients with schizophrenia compared with healthy subjects (1,97). In schizophrenia, upregulated WNK3 and OXSRI expression levels led to increased phosphorylation and

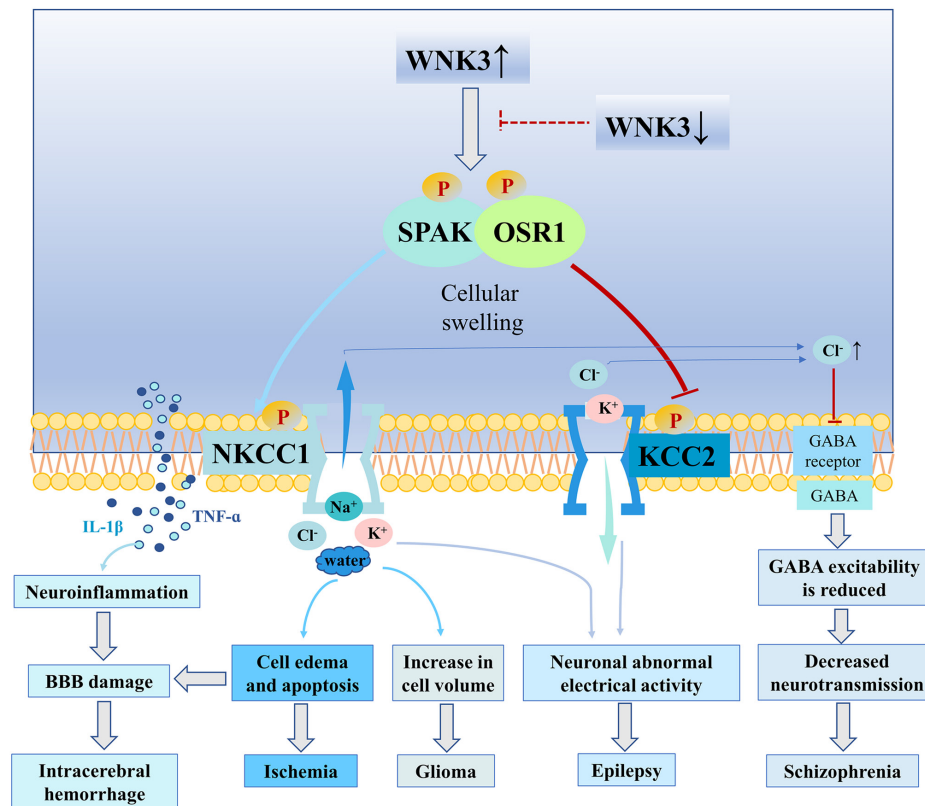


Figure 3. Mechanisms through which WNK3 signaling regulates ion channels and neural excitability in neurological diseases. WNK3 can phosphorylate downstream NKCC1 and inhibit the activity of KCC2 to regulate neuronal excitability by phosphorylating SPAK/OSR1, which contributes to the induction of neuronal 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-1 β , 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.

Acknowledgements

Not applicable.

Funding

The present study was supported by the Zhangjiagang Science and Technology Project (grant no. ZKS1914) and Zhangjiagang Health Youth Science and Technology Project (grant no. ZJGQNKJ202030).

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.

References

1. Siew K and O'Shaughnessy KM: Extrarenal roles of the with-no-lysine[K] kinases (WNKs). *Clin Exp Pharmacol Physiol* 40: 885-894, 2013.
2. Xu B, English JM, Wilsbacher JL, Stippec S, Goldsmith EJ and Cobb MH: WNK1, a novel mammalian serine/threonine protein kinase lacking the catalytic lysine in subdomain II. *J Biol Chem* 275: 16795-16801, 2000.
3. Akella R, Drozd MA, Humphreys JM, Jiou J, Durbacz MZ, Mohammed ZJ, He H, Liwocha J, Sekulski K and Goldsmith EJ: A phosphorylated intermediate in the activation of WNK kinases. *Biochemistry* 59: 1747-1755, 2020.
4. Thomson MN, Cuevas CA, Bewarder TM, Dittmayer C, Miller LN, Si J, Cornelius RJ, Su XT, Yang CL, McCormick JA, *et al*: WNK bodies cluster WNK4 and SPAK/OSR1 to promote NCC activation in hypokalemia. *Am J Physiol Renal Physiol* 318: F216-F228, 2020.
5. Gao JL, Peng K, Shen MW, Hou YH, Qian XB, Meng XW, Ji FH, Wang LN and Yang JP: Suppression of WNK1-SPAK/OSR1 attenuates bone cancer pain by regulating NKCC1 and KCC2. *J Pain* 20: 1416-1428, 2019.
6. Bergaya S, Vidal-Petiot E, Jeunemaitre X and Hadchouel J: Pathogenesis of pseudohypoaldosteronism type 2 by WNK1 mutations. *Curr Opin Nephrol Hypertens* 21: 39-45, 2012.
7. Naray-Fejes-Toth A, Snyder PM and Fejes-Toth G: The kidney-specific WNK1 isoform is induced by aldosterone and stimulates epithelial sodium channel-mediated Na^+ transport. *Proc Natl Acad Sci USA* 101: 17434-17439, 2004.
8. Huang CL, Jian X and Yuh CH: Wnk1-Ors1/spak kinase cascade is important for angiogenesis. *Trans Am Clin Climatol Assoc* 131: 140-146, 2020.
9. Liu Z, Yoon J, Wichaidit C, Jaykumar AB, Dbouk HA, Embry AE, Liu L, Henderson JM, Chang AN, Cobb MH and Miller RT: Control of podocyte and glomerular capillary wall structure and elasticity by WNK1 kinase. *Front Cell Dev Biol* 8: 618898, 2020.
10. Chi RA, Wang T, Huang CL, Wu SP, Young SL, Lydon JP and DeMayo FJ: WNK1 regulates uterine homeostasis and its ability to support pregnancy. *JCI Insight* 5: e141832, 2020.
11. Zhao X, Lai G, Tu J, Liu S and Zhao Y: Crosstalk between phosphorylation and ubiquitination is involved in high salt-induced WNK4 expression. *Exp Ther Med* 21: 133, 2021.
12. Sie ZL, Li RY, Sampurna BP, Hsu PJ, Liu SC, Wang HD, Huang CL and Yuh CH: WNK1 kinase stimulates angiogenesis to promote tumor growth and metastasis. *Cancers (Basel)* 12: 575, 2020.
13. Rafael C, Chavez-Canales M and Hadchouel J: New perspective on the role of WNK1 and WNK4 in the regulation of NaCl reabsorption and K^+ secretion by the distal nephron. *Med Sci (Paris)* 32: 274-280, 2016 (In French).
14. Delaloy C, Lu J, Houot AM, Disse-Nicodeme S, Gasc JM, Corvol P and Jeunemaitre X: Multiple promoters in the WNK1 gene: One controls expression of a kidney-specific kinase-defective isoform. *Mol Cell Biol* 23: 9208-9221, 2003.
15. Furusho T, Uchida S and Sohara E: The WNK signaling pathway and salt-sensitive hypertension. *Hypertens Res* 43: 733-743, 2020.
16. Anderregg MA, Albano G, Hanke D, Deisl C, Uehlinger DE, Brandt S, Bhardwaj R, Hediger MA and Fuster DG: The sodium/proton exchanger NHA2 regulates blood pressure through a WNK4-NCC dependent pathway in the kidney. *Kidney Int* 99: 350-363, 2020.
17. Klebe D, Iniaighe L, Burchell S, Reis C, Akyol O, Tang J and Zhang JH: Intracerebral hemorrhage in mice. *Methods Mol Biol* 1717: 83-91, 2018.
18. Rinehart J, Vazquez N, Kahle KT, Hodson CA, Ring AM, Gulcicek EE, Louvi A, Bobadilla NA, Gamba G and Lifton RP: WNK2 kinase is a novel regulator of essential neuronal cation-chloride cotransporters. *J Biol Chem* 286: 30171-30180, 2011.
19. Costa AM, Pinto F, Martinho O, Oliveira MJ, Jordan P and Reis RM: Silencing of the tumor suppressor gene WNK2 is associated with upregulation of MMP2 and JNK in gliomas. *Oncotarget* 6: 1422-1434, 2015.
20. Alves ALV, Costa AM, Martinho O, da Silva VD, Jordan P, Silva VAO and Reis RM: WNK2 inhibits autophagic flux in human glioblastoma cell line. *Cells* 9: 485, 2020.

21. Moniz S, Martinho O, Pinto F, Sousa B, Loureiro C, Oliveira MJ, Moita LF, Honavar M, Pinheiro C, Pires M, *et al.*: Loss of WNK2 expression by promoter gene methylation occurs in adult gliomas and triggers Rac1-mediated tumour cell invasiveness. *Hum Mol Genet* 22: 84-95, 2013.
22. Holden S, Cox J and Raymond FL: Cloning, genomic organization, alternative splicing and expression analysis of the human gene WNK3 (PRKWKN3). *Gene* 335: 109-119, 2004.
23. Moniz S and Jordan P: Emerging roles for WNK kinases in cancer. *Cell Mol Life Sci* 67: 1265-1276, 2010.
24. Kahle KT, Ring AM and Lifton RP: Molecular physiology of the WNK kinases. *Annu Rev Physiol* 70: 329-355, 2008.
25. Verissimo F, Silva E, Morris JD, Pepperkok R and Jordan P: Protein kinase WNK3 increases cell survival in a caspase-3-dependent pathway. *Oncogene* 25: 4172-4182, 2006.
26. de Los Heros P, Pacheco-Alvarez D and Gamba G: Role of WNK kinases in the modulation of cell volume. *Curr Top Membr* 81: 207-235, 2018.
27. Wu D, Lai N, Deng R, Liang T, Pan P, Yuan G, Li X, Li H, Shen H, Wang Z and Chen G: Activated WNK3 induced by intracerebral hemorrhage deteriorates brain injury maybe via WNK3/SPAK/NKCC1 pathway. *Exp Neurol* 332: 113386, 2020.
28. Pacheco-Alvarez D and Gamba G: WNK3 is a putative chloride-sensing kinase. *Cell Physiol Biochem* 28: 1123-1134, 2011.
29. Begum G, Yuan H, Kahle KT, Li L, Wang S, Shi Y, Shmukler BE, Yang SS, Lin SH, Alper SL and Sun D: Inhibition of WNK3 kinase signaling reduces brain damage and accelerates neurological recovery after stroke. *Stroke* 46: 1956-1965, 2015.
30. Tang BL: (WNK)ing at death: With-no-lysine (Wnk) kinases in neuropathies and neuronal survival. *Brain Res Bull* 125: 92-98, 2016.
31. Haas BR, Cuddapah VA, Watkins S, Rohn KJ, Dy TE and Sontheimer H: With-no-lysine kinase 3 (WNK3) stimulates glioma invasion by regulating cell volume. *Am J Physiol Cell Physiol* 301: C1150-C1160, 2011.
32. Shekarabi M, Zhang J, Khanna AR, Ellison DH, Delpire E and Kahle KT: WNK kinase signaling in ion homeostasis and human disease. *Cell Metab* 25: 285-299, 2017.
33. Schreck KA and Richdale AL: Sleep problems, behavior, and psychopathology in autism: inter-relationships across the lifespan. *Curr Opin Psychol* 34: 105-111, 2020.
34. Fakhoury M: Autistic spectrum disorders: A review of clinical features, theories and diagnosis. *Int J Dev Neurosci* 43: 70-77, 2015.
35. Richdale AL and Schreck KA: Sleep problems in autism spectrum disorders: Prevalence, nature, and possible biopsychosocial aetiologies. *Sleep Med Rev* 13: 403-411, 2009.
36. Horvath GA, Stowe RM, Ferreira CR and Blau N: Clinical and biochemical footprints of inherited metabolic diseases. III. Psychiatric presentations. *Mol Genet Metab* 130: 1-6, 2020.
37. Fogel BL, Wexler E, Wahnich A, Friedrich T, Vijayendran C, Gao F, Parikshak N, Konopka G and Geschwind DH: RBFOX1 regulates both splicing and transcriptional networks in human neuronal development. *Hum Mol Genet* 21: 4171-4186, 2012.
38. Wen M, Yan Y, Yan N, Chen XS, Liu SY and Feng ZH: Upregulation of RBFOX1 in the malformed cortex of patients with intractable epilepsy and in cultured rat neurons. *Int J Mol Med* 35: 597-606, 2015.
39. Voineagu I, Wang X, Johnston P, Lowe JK, Tian Y, Horvath S, Mill J, Cantor RM, Blencowe BJ and Geschwind DH: Transcriptomic analysis of autistic brain reveals convergent molecular pathology. *Nature* 474: 380-384, 2011.
40. Sebat J, Lakshmi B, Malhotra D, Troge J, Lese-Martin C, Walsh T, Yamrom B, Yoon S, Krasnitz A, Kendall J, *et al.*: Strong association of de novo copy number mutations with autism. *Science* 316: 445-449, 2007.
41. Qiao Y, Liu X, Harvard C, Hildebrand MJ, Rajcan-Separovic E, Holden JJ and Lewis ME: Autism-associated familial microdeletion of Xp11.22. *Clin Genet* 74: 134-144, 2008.
42. Edens AC, Lyons MJ, Duron RM, Dupont BR and Holden KR: Autism in two females with duplications involving Xp11.22-p11.23. *Dev Med Child Neurol* 53: 463-466, 2011.
43. Lee AY, Chen W, Stippec S, Self J, Yang F, Ding X, Chen S, Juang YC and Cobb MH: Protein kinase WNK3 regulates the neuronal splicing factor Fox-1. *Proc Natl Acad Sci USA* 109: 16841-16846, 2012.
44. Chung BH, Drmic I, Marshall CR, Grafodatskaya D, Carter M, Fernandez BA, Weksberg R, Roberts W and Scherer SW: Phenotypic spectrum associated with duplication of Xp11.22-p11.23 includes autism spectrum disorder. *Eur J Med Genet* 54: e516-e520, 2011.
45. Gehman LT, Stoilov P, Maguire J, Damianov A, Lin CH, Shiue L, Ares M Jr, Mody I and Black DL: The splicing regulator Rbfox1 (A2BP1) controls neuronal excitation in the mammalian brain. *Nat Genet* 43: 706-711, 2011.
46. Piton A, Gauthier J, Hamdan FF, Lafrenière RG, Yang Y, Henrion E, Laurent S, Noreau A, Thibodeau P, Karemura L, *et al.*: Systematic resequencing of X-chromosome synaptic genes in autism spectrum disorder and schizophrenia. *Mol Psychiatry* 16: 867-880, 2011.
47. Navidhamidi M, Ghasemi M and Mehranfarid N: Epilepsy-associated alterations in hippocampal excitability. *Rev Neurosci* 28: 307-334, 2017.
48. Zhou Y, Liu M and Liang WN: Progress on the epidemiological study of epilepsy. *Zhonghua Liu Xing Bing Xue Za Zhi* 28: 92-94, 2007 (In Chinese).
49. Thurman DJ, Hesdorffer DC and French JA: Sudden unexpected death in epilepsy: Assessing the public health burden. *Epilepsia* 55: 1479-1485, 2014.
50. Devinsky O, Vezzani A, O'Brien TJ, Jette N, Scheffer IE, de Curtis M and Perucca P: Epilepsy. *Nat Rev Dis Primers* 4: 18024, 2018.
51. Chen Z, Brodie MJ, Liew D and Kwan P: Treatment outcomes in patients with newly diagnosed epilepsy treated with established and new antiepileptic drugs: A 30-year longitudinal cohort study. *JAMA Neurol* 75: 279-286, 2018.
52. Shima A, Nitta N, Suzuki F, Laharie AM, Nozaki K and Depaulis A: Activation of mTOR signaling pathway is secondary to neuronal excitability in a mouse model of mesio-temporal lobe epilepsy. *Eur J Neurosci* 41: 976-988, 2015.
53. Schmeiser B, Zentner J, Prinz M, Brandt A and Freiman TM: Extent of mossy fiber sprouting in patients with mesiotemporal lobe epilepsy correlates with neuronal cell loss and granule cell dispersion. *Epilepsy Res* 129: 51-58, 2017.
54. Jeong KH, Kim SH, Choi YH, Cho I and Kim WJ: Increased expression of WNK3 in dispersed granule cells in hippocampal sclerosis of mesial temporal lobe epilepsy patients. *Epilepsy Res* 147: 58-61, 2018.
55. Kahle KT, Rinehart J, de Los Heros P, Louvi A, Meade P, Vazquez N, Hebert SC, Gamba G, Gimenez I and Lifton RP: WNK3 modulates transport of Cl⁻ in and out of cells: Implications for control of cell volume and neuronal excitability. *Proc Natl Acad Sci USA* 102: 16783-16788, 2005.
56. Huberfeld G, Blauwblomme T and Miles R: Hippocampus and epilepsy: Findings from human tissues. *Rev Neurol (Paris)* 171: 236-251, 2015.
57. Eftekhari S, Mehvari Habibabadi J, Najafi Ziarani M, Hashemi Fesharaki SS, Gharakhani M, Mostafavi H, Joghataei MT, Beladimoghdam N, Rahimian E and Hadjighassem MR: Bumetanide reduces seizure frequency in patients with temporal lobe epilepsy. *Epilepsia* 54: e9-e12, 2013.
58. Loscher W, Puskarjov M and Kaila K: Cation-chloride cotransporters NKCC1 and KCC2 as potential targets for novel antiepileptic and antiepileptogenic treatments. *Neuropharmacology* 69: 62-74, 2013.
59. Silayeva L, Deeb TZ, Hines RM, Kelley MR, Munoz MB, Lee HH, Brandon NJ, Dunlop J, Maguire J, Davies PA and Moss SJ: KCC2 activity is critical in limiting the onset and severity of status epilepticus. *Proc Natl Acad Sci USA* 112: 3523-3528, 2015.
60. Chen Y, Zhou H, Jin T, Ye T and Xie W: Clinical observation of the phased acupuncture for ischemic stroke hemiplegia. *Zhongguo Zhen Jiu* 38: 1027-1034, 2018 (In Chinese).
61. Hu YY, Li L, Xian XH, Zhang M, Sun XC, Li SQ, Cui X, Qi J and Li WB: GLT-1 upregulation as a potential therapeutic target for ischemic brain injury. *Curr Pharm Des* 23: 5045-5055, 2017.
62. Tuttolomondo A, Puleo MG, Velardo MC, Corpora F, Daidone M and Pinto A: Molecular biology of atherosclerotic ischemic strokes. *Int J Mol Sci* 21: 9372, 2020.
63. Zhao H, Nepomuceno R, Gao X, Foley LM, Wang S, Begum G, Zhu W, Pigott VM, Falgout LM, Kahle KT, *et al.*: Deletion of the WNK3-SPAK kinase complex in mice improves radiographic and clinical outcomes in malignant cerebral edema after ischemic stroke. *J Cereb Blood Flow Metab* 37: 550-563, 2017.
64. Demian WL, Persaud A, Jiang C, Coyaud E, Liu S, Kapus A, Kafri R, Raught B and Rotin D: The ion transporter NKCC1 links cell volume to cell mass regulation by suppressing mTORC1. *Cell Rep* 27: 1886-1896.e6, 2019.
65. Yan Y, Dempsey RJ, Flemmer A, Forbush B and Sun D: Inhibition of Na⁽⁺⁾-K⁽⁺⁾-Cl⁽⁻⁾ cotransporter during focal cerebral ischemia decreases edema and neuronal damage. *Brain Res* 961: 22-31, 2003.

66. Chen H, Luo J, Kintner DB, Shull GE and Sun D: Na(+)-dependent chloride transporter (NKCC1)-null mice exhibit less gray and white matter damage after focal cerebral ischemia. *J Cereb Blood Flow Metab* 25: 54-66, 2005.
67. Krueger M, Hartig W, Reichenbach A, Bechmann I and Michalski D: Blood-brain barrier breakdown after embolic stroke in rats occurs without ultrastructural evidence for disrupting tight junctions. *PLoS One* 8: e56419, 2013.
68. Chen H, Kintner DB, Jones M, Matsuda T, Baba A, Kiedrowski L and Sun D: AMPA-mediated excitotoxicity in oligodendrocytes: Role for Na(+)-K(+)-Cl(-) co-transport and reversal of Na(+)/Ca(2+) exchanger. *J Neurochem* 102: 1783-1795, 2007.
69. Hossain Khan MZ, Sohara E, Ohta A, Chiga M, Inoue Y, Isobe K, Wakabayashi M, Oi K, Rai T, Sasaki S and Uchida S: Phosphorylation of Na-Cl cotransporter by OSR1 and SPAK kinases regulates its ubiquitination. *Biochem Biophys Res Commun* 425: 456-461, 2012.
70. Zhang J, Gao G, Begum G, Wang J, Khanna AR, Shmukler BE, Daubner GM, de Los Heros P, Davies P, Varghese J, *et al*: Functional kinomics establishes a critical node of volume-sensitive cation-Cl-cotransporter regulation in the mammalian brain. *Sci Rep* 6: 35986, 2016.
71. Zhang P, Wang T, Zhang D, Zhang Z, Yuan S, Zhang J, Cao J, Li H, Li X, Shen H and Chen G: Exploration of MST1-mediated secondary brain injury induced by intracerebral hemorrhage in rats via hippo signaling pathway. *Transl Stroke Res* 10: 729-743, 2019.
72. Kamel H and Hemphill JC III: Characteristics and sequelae of intracranial hypertension after intracerebral hemorrhage. *Neurocrit Care* 17: 172-176, 2012.
73. Honner SK, Singh A, Cheung PT, Alter HJ, Dutaret CG, Patel AK and Acharya A: Emergency department control of blood pressure in intracerebral hemorrhage. *J Emerg Med* 41: 355-361, 2011.
74. Zheng H, Chen C, Zhang J and Hu Z: Mechanism and therapy of brain edema after intracerebral hemorrhage. *Cerebrovasc Dis* 42: 155-169, 2016.
75. Dang G, Yang Y, Wu G, Hua Y, Keep RF and Xi G: Early erytholysis in the hematoma after experimental intracerebral hemorrhage. *Transl Stroke Res* 8: 174-182, 2017.
76. Hemphill JC III, Greenberg SM, Anderson CS, Becker K, Bendok BR, Cushman M, Fung GL, Goldstein JN, Macdonald RL, Mitchell PH, *et al*: Guidelines for the management of spontaneous intracerebral hemorrhage: A guideline for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke* 46: 2032-2060, 2015.
77. Zhang X, Gu Y, Li P, Jiang A, Sheng X, Jin X, Shi Y and Li G: Matrix metalloproteases-mediated cleavage on β -dystroglycan may play a key role in the blood-brain barrier after intracerebral hemorrhage in rats. *Med Sci Monit* 25: 794-800, 2019.
78. Mracsko E and Veltkamp R: Neuroinflammation after intracerebral hemorrhage. *Front Cell Neurosci* 8: 388, 2014.
79. Wu X, Fu S, Liu Y, Luo H, Li F, Wang Y, Gao M, Cheng Y and Xie Z: NDP-MSH binding melanocortin-1 receptor ameliorates neuroinflammation and BBB disruption through CREB/Nr4a1/NF- κ B pathway after intracerebral hemorrhage in mice. *J Neuroinflammation* 16: 192, 2019.
80. Tian Y, Guo SX, Li JR, Du HG, Wang CH, Zhang JM and Wu Q: Topiramate attenuates early brain injury following subarachnoid haemorrhage in rats via duplex protection against inflammation and neuronal cell death. *Brain Res* 1622: 174-185, 2015.
81. Digregorio M, Lombard A, Lumapat PN, Scholtes F, Rogister B and Coppieters N: Relevance of translation initiation in diffuse glioma biology and its therapeutic potential. *Cells* 8: 1542, 2019.
82. Giese A and Westphal M: Glioma invasion in the central nervous system. *Neurosurgery* 39: 232-250, 1996.
83. de Paula LB, Primo FL and Tedesco AC: Nanomedicine associated with photodynamic therapy for glioblastoma treatment. *Biophys Rev* 9: 761-773, 2017.
84. Sontheimer H: Ion channels and amino acid transporters support the growth and invasion of primary brain tumors. *Mol Neurobiol* 29: 61-71, 2004.
85. Sontheimer H: An unexpected role for ion channels in brain tumor metastasis. *Exp Biol Med* (Maywood) 233: 779-791, 2008.
86. Garzon-Muvdi T, Schiapparelli P, apRhys C, Guerrero-Cazares H, Smith C, Kim DH, Kone L, Farber H, Lee DY, An SS, *et al*: Regulation of brain tumor dispersal by NKCC1 through a novel role in focal adhesion regulation. *PLoS Biol* 10: e1001320, 2012.
87. Zhou B, Lu X, Hao Y and Yang P: Real-time monitoring of the regulatory volume decrease of cancer cells: A model for the evaluation of cell migration. *Anal Chem* 91: 8078-8084, 2019.
88. Algharabil J, Kintner DB, Wang Q, Begum G, Clark PA, Yang SS, Lin SH, Kahle KT, Kuo JS and Sun D: Inhibition of Na(+)-K(+)-2Cl(-) cotransporter isoform 1 accelerates temozolomide-mediated apoptosis in glioblastoma cancer cells. *Cell Physiol Biochem* 30: 33-48, 2012.
89. Ernest NJ and Sontheimer H: Extracellular glutamine is a critical modulator for regulatory volume increase in human glioma cells. *Brain Res* 1144: 231-238, 2007.
90. Haas BR and Sontheimer H: Inhibition of the sodium-potassium-chloride cotransporter isoform-1 reduces glioma invasion. *Cancer Res* 70: 5597-5606, 2010.
91. Hamann S, Herrera-Perez JJ, Zeuthen T and Alvarez-Leefmans FJ: Cotransport of water by the Na⁺-K⁺-2Cl(-) cotransporter NKCC1 in mammalian epithelial cells. *J Physiol* 588: 4089-4101, 2010.
92. Mach C and Dollfus S: Scale for assessing negative symptoms in schizophrenia: A systematic review. *Encephale* 42: 165-171, 2016 (In French).
93. Tandon R, Gaebel W, Barch DM, Bustillo J, Gur RE, Heckers S, Malaspina D, Owen MJ, Schultz S, Tsuang M, *et al*: Definition and description of schizophrenia in the DSM-5. *Schizophr Res* 150: 3-10, 2013.
94. Guessoum SB, Le Strat Y, Dubertret C and Mallet J: A transnosographic approach of negative symptoms pathophysiology in schizophrenia and depressive disorders. *Prog Neuropsychopharmacol Biol Psychiatry* 99: 109862, 2020.
95. Gonzalez-Burgos G and Lewis DA: GABA neurons and the mechanisms of network oscillations: implications for understanding cortical dysfunction in schizophrenia. *Schizophr Bull* 34: 944-961, 2008.
96. Lewis DA, Hashimoto T and Volk DW: Cortical inhibitory neurons and schizophrenia. *Nat Rev Neurosci* 6: 312-324, 2005.
97. Arion D and Lewis DA: Altered expression of regulators of the cortical chloride transporters NKCC1 and KCC2 in schizophrenia. *Arch Gen Psychiatry* 68: 21-31, 2011.
98. Blanquie O, Liebmann L, Hubner CA, Luhmann HJ and Sinning A: NKCC1-mediated GABAergic signaling promotes postnatal cell death in neocortical Cajal-Retzius cells. *Cereb Cortex* 27: 1644-1659, 2017.
99. Lewis DA and Sweet RA: Schizophrenia from a neural circuitry perspective: Advancing toward rational pharmacological therapies. *J Clin Invest* 119: 706-716, 2009.
100. de Los Heros P, Kahle KT, Rinehart J, Bobadilla NA, Vázquez N, San Cristobal P, Mount DB, Lifton RP, Hebert SC and Gamba G: WNK3 bypasses the tonicity requirement for K-Cl cotransporter activation via a phosphatase-dependent pathway. *Proc Natl Acad Sci USA* 103: 1976-1981, 2006.
101. de Los Heros P, Alessi DR, Gourlay R, Campbell DG, Deak M, Macartney TJ, Kahle KT and Zhang J: The WNK-regulated SPAK/OSR1 kinases directly phosphorylate and inhibit the K⁺-Cl⁻ co-transporters. *Biochem J* 458: 559-573, 2014.
102. Vorontsova I, Donaldson PJ, Kong Z, Wickremesinghe C, Lam L and Lim JC: The modulation of the phosphorylation status of NKCC1 in organ cultured bovine lenses: Implications for the regulation of fiber cell and overall lens volume. *Exp Eye Res* 165: 164-174, 2017.
103. Alessi DR, Zhang J, Khanna A, Hochdorfer T, Shang Y and Kahle KT: The WNK-SPAK/OSR1 pathway: Master regulator of cation-chloride cotransporters. *Sci Signal* 7: re3, 2014.
104. Conway LC, Cardarelli RA, Moore YE, Jones K, McWilliams LJ, Baker DJ, Burnham MP, Bürling RW, Wang Q, Brandon NJ, *et al*: N-Ethylmaleimide increases KCC2 cotransporter activity by modulating transporter phosphorylation. *J Biol Chem* 292: 21253-21263, 2017.
105. Glover M, Zuber AM and O'Shaughnessy KM: Renal and brain isoforms of WNK3 have opposite effects on NCCT expression. *J Am Soc Nephrol* 20: 1314-1322, 2009.
106. Lu DC, Hannemann A, Wadud R, Rees DC, Brewin JN, Low PS and Gibson JS: The role of WNK in modulation of KCl cotransport activity in red cells from normal individuals and patients with sickle cell anemia. *Pflügers Arch* 471: 1539-1549, 2019.