

Invited Mini Review

Emerging roles of 14-3-3 γ in the brain disorder

Eunsil Cho & Jae-Yong Park*

School of Biosystem and Biomedical Science, College of Health Science, Korea University, Seoul 02708, Korea

14-3-3 proteins are mostly expressed in the brain and are closely involved in numerous brain functions and various brain disorders. Among the isotypes of the 14-3-3 proteins, 14-3-3 γ is mainly expressed in neurons and is highly produced during brain development, which could indicate that it has a significance in neural development. Furthermore, the distinctive levels of temporally and locally regulated 14-3-3 γ expression in various brain disorders suggest that it could play a substantial role in brain plasticity of the diseased states. In this review, we introduce the various brain disorders reported to be involved with 14-3-3 γ , and summarize the changes of 14-3-3 γ expression in each brain disease. We also discuss the potential of 14-3-3 γ for treatment and the importance of research on specific 14-3-3 isotypes for an effective therapeutic approach. [BMB Reports 2020; 53(10): 500-511]

INTRODUCTION

The 14-3-3 proteins were first extracted from the bovine brain in the 1960s (1) and were identified as activators of tyrosine and tryptophan hydroxylases involved in the biosynthesis of neurotransmitters (2). Subsequently, intensive follow-up studies revealed that 14-3-3 proteins are involved in diverse cellular functions, including cell survival, growth, differentiation, migration, and signaling. The naming the protein '14-3-3' was based on the initial discovery that the proteins were extracted from the 14th fraction in a diethylaminoethyl-cellulose chromatography and were located at the 3.3 position on a starch gel electrophoresis (1). The 14-3-3 proteins are highly conserved, approximately 30-kDa acidic molecules that form dimers (3) and bind to various intracellular proteins. These partners are transmembrane receptors, cytoskeletal proteins, and signal-transducing proteins, such as kinases and phosphatases (4). They participate in the regulation of transcription, cell-cycle control,

protein trafficking, metabolism, signal transduction, stress response, and apoptosis (5).

Isotypes and general functions of 14-3-3 proteins

The 14-3-3 proteins are a family of highly homologous molecules expressed in all eukaryotic cells. About fifteen isotypes have been reported in plants, two in yeast, and two in *C. elegans* and *Drosophila* (6-8). In mammals, seven isotypes (β , ϵ , γ , η , τ/θ , ξ , σ) have been identified, and each of them is encoded by different genes (YWHAB, YWHAE, YWHAG, YWHAH, YWHAQ, YWHAZ and SFN, arranged in the order listed above). The previously named α and δ are phospho- τ -ylated β and ξ , respectively (Table 1) (9).

The 14-3-3 proteins regulate the activity of binding proteins by blocking or promoting protein-protein interactions with other proteins (10-12). In addition, the 14-3-3 proteins can regulate stability of binding proteins by occluding or opening the ubiquitination site and also control their trafficking and localization (13). In addition, 14-3-3 proteins interact with the chaperone proteins, such as the heat shock protein (HSP), and has the chaperone-like activity itself (13, 14). Moreover, the 14-3-3 proteins have a nuclear localization sequence (NLS), which can regulate the gene expression by transporting target proteins to the nucleus (Fig. 1) (15, 16).

Structural features of 14-3-3 proteins for dimerization and binding motif

The 14-3-3 proteins form dimers, which make a U-shaped binding groove (17, 18). One monomer has a bundle of nine α -helices; especially the first four helices are essential for dimer formation. First two helices at the N-terminal of one monomer are connected to the fourth helix of the other monomer by the salt bridge formation (Fig. 2). The dimer interface buries several hydrophobic and polar residues (L13, A17, S58, V62, I66, and Y85), with the salt bridge including Arg-Glu (R19 and E92) and the side chain containing Asp-Lys (D21 and K88). All residues are based on human 14-3-3 γ .

Most isotypes show a similar preference for either homodimer or heterodimer formation, but σ prefers homodimer (19), while ϵ prefers heterodimer formation (20). Different preferences for homo- or heterodimer formation of each 14-3-3 isotype appears to result from their detailed structural differences (21). For example, the side chains of Asp-Lys (D21 and K88) are preserved in most isotypes except for ϵ , which has Glu-Met (E22 and M88) instead. Given this difference, ϵ can easily form

*Corresponding author. Tel: +82-2-3290-5637; Fax: +82-2-921-7207; E-mail: jaeyong68@korea.ac.kr

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a heterodimer instead of a homodimer (22). Meanwhile, most 14-3-3 proteins generally act as a dimer, but sometimes they function as a monomer. For example, 14-3-3 ξ can regulate the activity of Raf kinase when they are dimers (23), but the activity of the *Drosophila* calcium-dependent potassium channel can be controlled by a monomeric form (24).

Table 1. The isotypes of the 14-3-3 proteins

| Gene | Chromosome | Isotypes |
|-------|------------|--|
| YWHAH | 20q13.1 | Beta/Alpha β/α α is a phosphorylated form of β |
| YWHAE | 17p13.3 | Epsilon ϵ |
| YWHAG | 7q11.23 | Gamma γ |
| YWHAH | 22q12.3 | Eta η |
| YWHAQ | 2p25.1 | Tau/Theta τ/θ θ is an alternative name for τ |
| YWHAZ | 8q23.1 | Zeta/Delta ξ/δ δ is a phosphorylated form of ξ |
| SFN | 1p35.3 | Sigma σ |

Highly conserved seven isoforms of the 14-3-3 proteins have been identified. The α and δ are phosphorylated forms of the β and ξ , respectively and theta is an alternative name for tau. Genes encoding these isotypes are located at different positions of the human chromosomes.

For binding with the target proteins, the first, third, fifth, and seventh helices are located inside the cup, and the third, fifth, seventh, and ninth helices form a conserved amphipathic binding groove, which has a hydrophobic patch on one side and a cluster of charged and polar residues on the other side (Fig. 2) (25). On one side of the groove, the seventh and ninth helices form a hydrophobic surface which includes four leucine side chains (L173, L211, L221, and L226). On the other side, the third helix has three basic side chains (K49, R56, and R61) and the fifth helix has polar and charged groups (K120, D129, Y130, and R132) (26). The phosphate group of the interacting protein binds to the binding site formed by the conserved Lys-Arg-Arg-Tyr (K50, R57, R132, and Y133) in the third and fifth helices inside the cup (Fig. 2) (17).

The 14-3-3 proteins recognize the high-affinity binding motifs containing phosphoserine or phosphothreonine (pSer/pThr) (18, 27), including RSX[pS/pT]XP and RXXX[pS/pT] XP, or the C-terminal phosphorylation motif (28, 29), such as pS/TX-COOH of target proteins and bind them in a phosphorylation-dependent or -independent manner. The X represents any residue and the p indicates phosphorylation. Furthermore, other nonphosphorylated binding motifs have also been found (30-32). The basic cluster of binding grooves consisting of K49, R56, and R127 in 14-3-3 proteins mediates the interaction

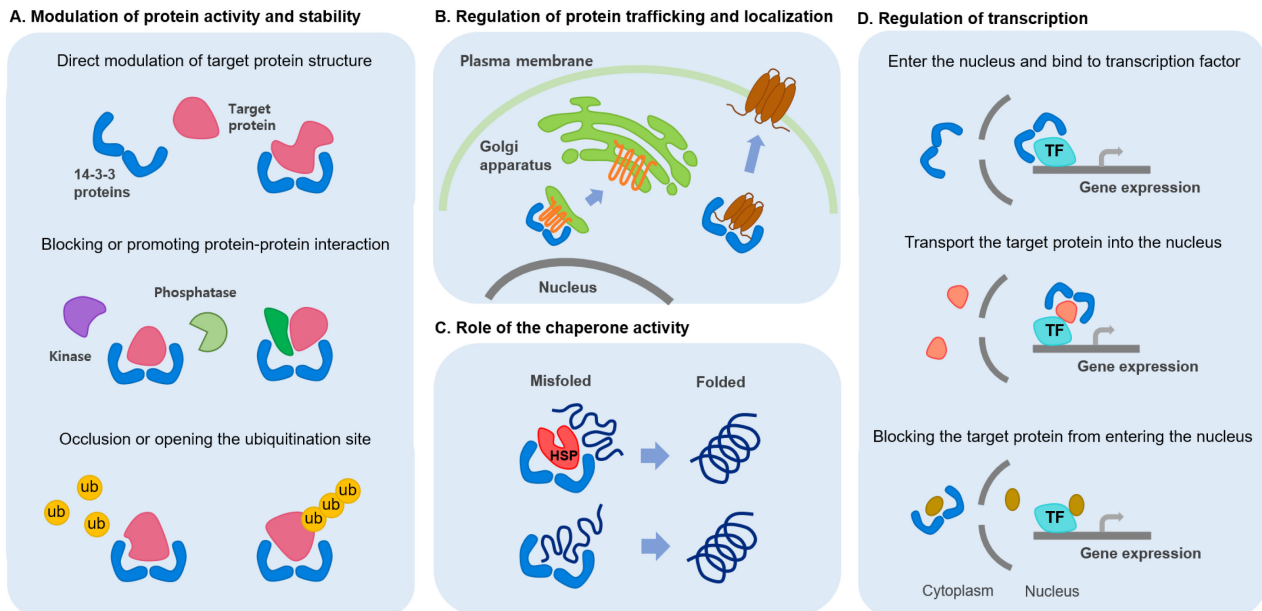


Fig. 1. Functional characteristics of the 14-3-3 proteins. The 14-3-3 proteins form dimers and bind to various proteins in the cell to regulate the function of the target proteins. (A) The target proteins can be structurally modified by binding to the 14-3-3 proteins. The 14-3-3 proteins bind to the target proteins and modulate the activity of the binding protein by blocking or promoting protein-protein interaction with other proteins. In addition, by blocking or opening sites where ubiquitin (ub) is attached to the target protein, 14-3-3 proteins can be involved in protein degradation to regulate stability. (B) The 14-3-3 proteins can regulate the trafficking and localization of binding proteins. (C) The 14-3-3 proteins interact with chaperone proteins such as heat shock proteins (HSP) and have chaperone-like activity themselves. (D) The 14-3-3 proteins have a nuclear localization sequence (NLS), can enter the nucleus, and can bind to transcription factors (TF). Moreover, the 14-3-3 proteins can regulate gene expression by transporting the target protein to the nucleus, or by blocking the target protein from entering the nucleus.

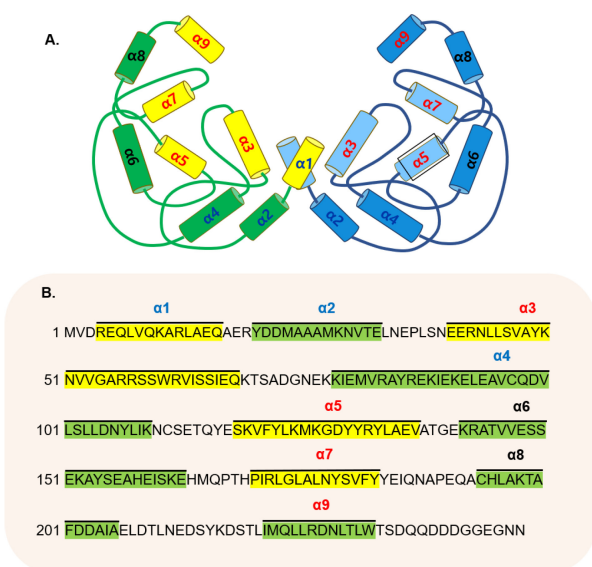


Fig. 2. Structural features of the 14-3-3 γ . (A) The 14-3-3 proteins consist of nine α -helical bundles to form a dimer for making a U-shaped groove. The first and second helices of the N-terminus of one monomer are connected to the fourth helix of the other monomer by the salt bridge. The first, third, fifth and seventh helices of one monomer are located inside the cup, and the third, fifth, seventh and ninth helices form a conserved amphiphilic groove that binds to the target protein. (B) The 14-3-3 γ is a highly conserved protein that has the same protein sequence in humans, rat, and mice. Like most isoforms, the 14-3-3 γ consists of nine helical structures.

with the phosphoamino acid of target proteins, and R19 is involved in the binding of the non-phosphorylated proteins (33, 34).

Binding specificity of 14-3-3 proteins

Since most residues involved in dimer formation and protein-binding sites are conserved in most isoforms of 14-3-3 proteins, it could be speculated that the 14-3-3 proteins can randomly form dimers and interact with numerous binding proteins. However, 14-3-3 isoforms can specifically select their target proteins by forming a complex tertiary structure via dimerization (18, 21, 22). Homo- or heterodimers of 14-3-3 isoforms can create various structural differences in position, angle, and specificity of binding groove (21, 22, 35). In addition to the conserved amphipathic binding groove, the C-terminal region of 14-3-3 proteins can be involved in regulation of binding specificity with target proteins (36, 37). Since C-terminal region of 14-3-3 proteins is the most flexible region, relatively freely rotating C-terminus of 14-3-3 proteins can help to permit isoform-specific interactions with target proteins. It has been reported that various factors such as stress, drugs, and disease can regulate gene expression of 14-3-3 isoforms (16, 38). Altered gene expression of specific 14-3-3 isoform could contribute to the changes in the ratio of 14-3-3 isoforms and

the contents of 14-3-3 dimers. Therefore, regulated gene expression of specific 14-3-3 isoform also can affect binding specificity of other 14-3-3 proteins.

14-3-3 γ in brain function

The 14-3-3 proteins are ubiquitously expressed in almost all organs (39, 40), and are most expressed in the brain, except for σ , also known as stratifin, which is predominantly expressed in epithelial cells (41). It is not surprising that 14-3-3 proteins are involved in numerous brain disorders, considering that 14-3-3 proteins bind to several critical proteins that have cellular and molecular functions in the brain. Among the 14-3-3 proteins, the 14-3-3 γ is encoded by the gene YWHAG, located on the 7th chromosome (7q11.23) (Table 1) and is most frequently expressed in neurons at the mRNA level (42), unlike other isoforms that were relatively evenly expressed in various brain cells. Thus, the 14-3-3 γ is expected to be importantly involved in neuronal functions by binding to several signaling proteins, such as β -catenin (43), RGS14 (44) and LRRK2 (45). Moreover, the 14-3-3 γ participates in neural processes such as ion channel regulation and receptor trafficking (46–48). Although 14-3-3 γ studies on brain and nerve function are still lacking, the possibility of 14-3-3 γ playing a significant role is emerging.

14-3-3 γ IN BRAIN DISORDERS

The 14-3-3 γ protein has been implicated in neurodegenerative diseases as well as neurodevelopmental and neuropsychiatric disorders (Table 2). Moreover, the 14-3-3 γ has also been reported in several neurological disorders, such as neuroinflammatory disease and glioma (Table 2).

14-3-3 γ in neurodevelopmental disorders

It has been reported that 14-3-3 proteins are a key regulator in neurodevelopmental processes, including neuronal differentiation, migration, and morphogenesis including synaptogenesis (49). Among the studies of each 14-3-3 isoform associated with neuronal development, those on 14-3-3 ϵ and ξ are relatively advanced, but studies on other isoforms are still lacking.

The 14-3-3 γ is abundantly expressed in the cerebral cortex of developing mice. The mRNA of 14-3-3 γ is highly expressed in the embryonic state and decreases from 30 days after birth. (50). Therefore, 14-3-3 γ is expected to play an important role in cortical development. Indeed, 14-3-3 γ directly binds to Copine1, known as a calcium-dependent membrane-binding protein, and overexpression of 14-3-3 γ and Copine1 causes an increase in neuronal differentiation via protein kinase B (AKT) phosphorylation in the hippocampal progenitor cell line (51). But to date, research into the molecular mechanisms of 14-3-3 γ in neuronal development and differentiation during cortical development is still lacking. Interestingly, there was a report of 14-3-3 γ playing an important role in neuronal migration (50). In the cerebral cortex of postnatal P3 mice in

Table 2. The 14-3-3 γ in brain disorders

| Category of disease | Name of disease | Expression pattern or potential role in disease | References |
|--|----------------------------------|---|------------|
| Neurodevelopmental disorders | Williams syndrome | Associated with epilepsy phenotypes | 54-57 |
| | Down syndrome (DS) | Decreased in the cortex of fetal DS patients | 63 |
| | | Increased in the cortex of aged DS patients | 62 |
| Neurodegenerative disorders | Parkinson's disease (PD) | Colocalized with the Lewy body of PD | 70-72 |
| | | Decreased in the transgenic α -syn mouse | 73 |
| | | Neuroprotection against the rotenone or MPTP induced cytotoxicity | 74 |
| | | Promote the α -syn aggregation | 75, 76 |
| | Alzheimer's disease (AD) | Colocalized with the NFT in hippocampus of AD patients | 83 |
| | | Increased in overall cortical regions of aged AD patients | 84 |
| | | Decreased in the frontal cortex of postmortem AD patients | 85 |
| Neuroinflammatory disease | Creutzfeldt-Jakob disease (CJD) | Useful marker for CJD diagnosis using CSF | 88-95 |
| | Inflammatory joint disease (IJD) | Highly observed in SF and serum of patients with IJD | 96 |
| | Cruciate ligament rupture (CCLR) | A marker for osteoarthritis caused by CCLR | 97 |
| | Multiple sclerosis (MS) | Increased in the spinal cord of chronic MOG-EAE | 98 |
| | | A protective factor for OL against autoimmune dehydration | 98 |
| | Ischemic damaged brain | Increased in infarct lesions of ischemic brain | 102, 103 |
| | | Detected in CSF of MELAS patients | 104 |
| A survival factor for ischemic-induced cell death in neurons | | 106-108 | |
| CNS cancer | Glioma | A protectant against ischemic cortical astrocytes | 109-115 |
| | | Promote glioblastoma progression | 126-128 |

The 14-3-3 γ is implicated in neurodegenerative and neurodevelopmental disorders, and is also associated with neuroinflammatory diseases and CNS cancer such as glioma. The 14-3-3 γ is likely to be involved in the development of various brain diseases, and is useful as a biomarker for diagnosis and a potential therapeutic target for treatment. (α -syn: α -synuclein; NFT: neurofibrillary tangles; SF: synovial fluid; CSF: cerebrospinal fluid; CCLR: cranial cruciate ligament rupture; MOG-EAE: murine myelin oligodendrocyte-induced experimental autoimmune encephalomyelitis; OL: oligodendrocyte; MELAS: mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes).

which 14-3-3 γ knockdown was induced at fetal-stage embryonic day (E)14.5 or E16.5 in utero, 14-3-3 γ deficient neurons generated from the ventricular zone and subventricular zone did not properly migrate to the cortical plate but rather stayed in the ventricular zone or the intermediate zone (50). Therefore, it has been suggested that 14-3-3 γ knockdown leads to the migration delay of pyramidal neurons in the cerebral cortex and plays an important role in the late developmental stage. Interestingly, the delay of neuronal migration was also observed in the 14-3-3 γ overexpression model using the same strategy (52), which suggests the importance of the balance of 14-3-3 γ . However, the detailed molecular mechanisms how 14-3-3 γ regulates neuronal migration are still unknown.

Williams Syndrome (WS) is a neurodevelopmental disorder caused by a deletion on chromosome 7q11.23 and is accompanied by symptoms of delayed developmental and intellectual disabilities (53). As previously reported, typical WS patients showed deletions of 1.5-1.8 Mb in size, whereas atypical patients showed deletions greater than 1.8 Mb; the deletions of the atypical patients include the YWHAG gene that encoded the 14-3-3 γ (54-56). Interestingly, atypical patients have epilepsy symptoms that do not occur in typical patients, and epilepsy has also been reported in WS patients with a reciprocal duplication syndrome of the 7q11.23 gene (57). These reports

suggest that changes in the level of 14-3-3 γ may be associated with epilepsy phenotypes in WS patients.

Also, a more direct link between 14-3-3 γ and seizures was observed in temporal lobe epilepsy (58-60). The 14-3-3 γ and ξ were detected in mitochondrial and microsome-enriched fractions, whereas other isoforms are expressed only in the cytoplasmic compartment of the hippocampus. Especially, the expression of 14-3-3 γ was significantly reduced in the microsome-enriched compartment of the rat hippocampus, when experienced the kainic acid (KA)-induced acute seizure (58). However, in the human hippocampus of chronic temporal lobe epilepsy, there was no difference in 14-3-3 γ expression, but the levels of 14-3-3 ϵ and ξ were increased in the microsomal-rich fraction. Thus, this report identified microsomal-rich fractions in the hippocampus of epileptic rat as major sites for altered 14-3-3 protein levels, which suggests changes in isotype distribution in acute and chronic seizures (58). Recently in a KA-induced rat model, it has been shown that 14-3-3 γ , ϵ , η , σ and τ/θ were abundantly detected in the frontal cortex, whereas levels of β and ξ isoforms were relatively low, and the down-regulation of total 14-3-3 proteins was also observed (59). Both BAX and phosphorylated ERK bind to 14-3-3 proteins, showing increased levels after KA intraperitoneal injection (59). Recently, high enrichment of *de novo*

variants in the YWHAG gene of the developmental and epileptic encephalopathies have been identified by *in silico* modeling, suggesting that 14-3-3 γ *de novo* mutations are implicated in early-onset epilepsy (60). Taken together, 14-3-3 γ appears to be involved in early events related to acute epileptic seizures and excitatory toxicity.

Down syndrome (DS), also known as trisomy 21, is another genetic neurodevelopmental disorder characterized by a third copy on chromosome 21, which has symptoms of delayed physical growth and intellectual disability, along with characteristic facial features (61). Notably, compared to healthy controls, the aged DS patients displayed an increase in expression of 14-3-3 γ and ϵ in the cortical regions (62). In contrast, by subsequent matrix-assisted laser/desorption ionization (MALDI) identification, the decreased 14-3-3 γ expression was detected in the fetal brain cortex of DS (63). These results suggest that 14-3-3 γ might be involved in the pathology of DS, but that the expression of 14-3-3 γ may differ depending on the age and brain regions involved.

14-3-3 γ in neurodegenerative disorders

Parkinson's disease (PD) is an age-related neurodegenerative disease with symptoms of motor dysfunction, such as progressive rigidity, bradykinesia, tremor, and postural instability (64). Some PD patients have symptoms of dementia and mood problems such as depression and anxiety (65, 66). PD is characterized by a loss of dopaminergic neurons and Lewy body formation in the substantia nigra *pars compacta* as well as the cortical regions and brainstem (67). The Lewy body is mainly composed of α -synuclein (α -syn), which contributes to PD pathogenesis (68, 69). The interaction of the 14-3-3 proteins and α -syn was confirmed by co-immunoprecipitation in the rat brain (70), and the 14-3-3 proteins were also observed by immunohistochemistry in Lewy bodies of PD and the Diffuse Lewy body disease (LBD) (71). Among the 14-3-3 family, 14-3-3 γ , ϵ , ξ , and τ/θ were observed to be colocalized with the Lewy body of PD (72). In addition, a decrease in 14-3-3 γ , ϵ and τ/θ was detected in transgenic α -syn mouse, an animal model of PD (73). It was also observed that overexpression of 14-3-3 τ/θ reduced cytotoxicity of dopaminergic neurons in MPTP mouse model of PD (74). Mechanistically, it has been suggested that 14-3-3 γ , ϵ , η , and ξ promote the formation of α -syn aggregates and participate in oligomer degradation and aggregation (75, 76). Furthermore, it was proposed that 14-3-3 η affects α -syn aggregation by binding with the α -syn oligomer, and that overexpression of 14-3-3 η reduces α -syn toxicity and binds with the parkin, which is another factor of PD pathogenesis (77). According to other studies, 14-3-3 ξ reportedly inhibits the ubiquitin-ligase activity of Parkin (78) and activates the tyrosine hydroxylase (TH), a rate-limiting enzyme in catecholamine biosynthesis (79). Thus, the 14-3-3 proteins generally appear to influence aggregation and neurotoxicity of α -syn and may be involved in the dopamine synthesis by interacting with α -syn. Since 14-3-3 γ , along with

η and ξ , is responsible for the α -syn aggregates (75), it will likely play a critical role and be the potential target for neuroprotective effects on dopaminergic neurons (77-79).

Alzheimer's disease (AD) is also a neurodegenerative disease with the symptoms of dementia accompanied by memory loss and cognitive impairment (80). AD is characterized by the accumulation of amyloid plaques caused by neurotoxic extracellular β -amyloid (A β) aggregation (81) and intracellular neurofibrillary tangles (NFT) composed of the abnormally hyper-phosphorylated microtubule-associated protein tau (82). Although the 14-3-3 proteins were not observed in amyloid plaques, there is growing evidence of the link between NTF and the 14-3-3 proteins. The expression of the 14-3-3 proteins in hippocampal NFT of AD have been shown by immunohistochemistry (83), and the increased 14-3-3 γ and ϵ in overall cortical regions of aged AD patients were observed (84). However, in a recent report, the levels of 14-3-3 γ and η shown to be significantly decreased in the frontal cortex of postmortem AD patients (85). Therefore, it is possible that 14-3-3 γ expression can be differently regulated in the different stages of AD progression or the different cortical regions of AD patients. Research into the association of NFT with specific isoforms of the 14-3-3 protein is still missing.

Creutzfeldt-Jakob Disease (CJD) is a fatal degenerative brain disorder caused by a misfolded protein, PrP^{Sc} prion (scrapie isoform of the prion protein), with the symptoms of confusion, depression, abnormal body sensations, autonomic nervous system disorders, and dementia (86, 87). The importance of the 14-3-3 proteins as a biomarker for the diagnosis of CJD has been frequently discussed over the past decade. Since the 14-3-3 proteins were first proposed as a potential CJD marker for the rapid detection in cerebrospinal fluid (CSF) (88), many studies have confirmed that the 14-3-3 proteins are a useful diagnostic marker for CJD (89, 90). Interestingly, the 14-3-3 proteins were not observed in the CSF of patients with some brain destructions, such as Rasmussen's encephalitis, Schilder's disease, or diffuse large B-cell lymphoma (91). These results may indicate that the increased level of 14-3-3 proteins in CSF is not caused by protein leakage due to cell death, it is more likely that the 14-3-3 proteins are specifically involved in the pathological mechanism of CJD. Early-stage CJD diagnostic antibodies detected all 14-3-3 isoforms, but a recent study of the diagnostic accuracy of CJD reported that 14-3-3 γ and β were more reliable (92, 93). More recently, it has been suggested that 14-3-3 γ could be used as a specific marker for neuronal damage caused by CJD (94, 95). Therefore, this suggests that 14-3-3 γ is closely related to the pathogenesis of CJD.

14-3-3 γ in neuroinflammatory disease

In several studies, there is evidence that 14-3-3 γ is closely related to the inflammatory conditions (96, 97). High levels of 14-3-3 γ and η were observed in synovial fluid (SF) and serum obtained from patients with inflammatory joint disease, and their expression levels correlated strongly with the levels of

matrix metalloproteinase (MMP)-1 and MMP-3, two biomarkers for rheumatoid arthritis (96). A subsequent study confirmed that 14-3-3 γ and η were abundantly detected in the SF from dogs with cranial cruciate ligament rupture (CCLR). Thus, these results suggest that 14-3-3 γ and η are potent stimulators of MMPs and could be a marker for osteoarthritis caused by CCLR (97).

The 14-3-3 γ is also shown to be involved in inflammatory diseases in the central nervous system (CNS) (98). Multiple sclerosis (MS) is a demyelinating disease caused by damage to the insulating sheath of neurons in the brain and spinal cord. MS is the most common immune-mediated disorder with neurodegenerative symptoms, such as sensory nerve damage and movement disorder (99). Experimental autoimmune encephalomyelitis (EAE) is a model that mimics the pathological features of MS, showing oligodendrocyte (OL) damage, inflammatory demyelination, and astrocyte activation (100, 101). The mRNA expression of 14-3-3 γ was increased in the spinal cord of chronic murine myelin oligodendrocyte-induced EAE (98). Thus, the deficiency of 14-3-3 γ promotes demyelination and increases the sensitivity of OL to inflammatory attacks. Therefore, 14-3-3 γ is likely to act as a protective factor for OL against autoimmune demyelination by participating in the apoptotic mechanism (98).

The importance of 14-3-3 γ in the inflammatory conditions can be further explored in ischemic stroke (102-104). The intensity of 14-3-3 staining was increased in cortical neurons of rat brains with ischemic damage (102), and 14-3-3 γ , β , ϵ , σ , and τ/θ were detected in the CSF of ischemic patients (103). An increase of these isoforms was also observed in infarct lesions of the human brain obtained from an autopsy. This increase was proportional to the severity of the ischemia pathology, particularly abundant in the chronic phase (103). Furthermore, 14-3-3 γ and τ/θ were also detected in the CSF of patients with mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes (MELAS), such as severe mental regression and limb paralysis (104). The detection of these proteins was correlated with the degree of damage to brain observed by magnetic resonance imaging (104). Therefore, it has been suggested that some 14-3-3 isoforms may be potential biomarkers for ischemic CNS damage.

Moreover, several studies have reported that 14-3-3 γ acts as a survival factor for cortical neurons in ischemia (43, 105). The nuclear translocation of Akt-phosphorylated SRPK2 inactivates p53 in the ischemic-attacked brain and induces an increase in cyclin D1 to promote cell-cycle progression and apoptosis. The 14-3-3 proteins have been proposed to inhibit cell-cycle and neuronal cell death by interacting with and inhibiting SRPK2, a protein kinase for serine/arginine (SR)-rich splicing factor (106). In another study, an increase of 14-3-3 γ was observed in primary cortical neurons under oxygen-glucose deprivation (105). Moreover, 14-3-3 γ overexpression reduced oxygen glucose deprivation (OGD)-induced cell death in cultured neurons, whereas 14-3-3 γ knockdown increased the expres-

sion of BAX to promote neuronal cell death (105). It was confirmed that OGD promotes entry into nuclei of 14-3-3 γ , induces binding with increased nuclear phosphorylated- β -catenin, and reduces BAX expression, thereby suppressing neuronal apoptosis (105).

In inflammatory conditions, the 14-3-3 proteins have been reported to be involved in the activation of the NF κ B-signaling pathway for the regulation of immune responses and apoptosis (107). After ischemia-reperfusion (I/R) in transient middle cerebral artery occlusion, an animal model that mimics ischemic stroke, upregulation of 14-3-3 γ and increased translocation from the cytoplasm to the nucleus were observed in cortical neurons. In this process, 14-3-3 γ interacts with p65 to regulate its expression (107). Therefore, 14-3-3 γ may be a potential therapeutic target for stroke.

Recently, the role of 14-3-3 γ in starvation-activated neuronal autophagic influx signaling has been suggested (43). By immunostaining, the increased expression levels of Beclin-1 and 14-3-3 γ , and the colocalization of 14-3-3 γ , Beclin-1, and LC3, were detected in starvation-treated neuroblastoma (N2a) cells and cultured cortical neurons of ischemic brains. Inhibition of 14-3-3 γ reduced hunger-induced activation of Beclin-1 and LC3, and overexpression of 14-3-3 γ increase Beclin-1/LC3 signal transduction. Under starvation, 14-3-3 γ preferred binding to phosphorylated- β -catenin rather than Beclin-1. Thus, it is suggested that 14-3-3 γ overexpression reactivates Beclin-1-LC3 signaling because of the inhibition of β -catenin in starving neurons (43). It is also reported that pre-ischemic exercise increases the expression of 14-3-3 γ , thereby reducing ischemia-induced neuronal cell death through anti-apoptotic pathways including phosphorylated β -catenin, BAX, and caspase 3 (108). Therefore, 14-3-3 γ is proposed to act as an initial survival factor for ischemia-induced cell death in neurons.

In addition, a potential protective role of 14-3-3 γ has been proposed in astrocytes, which play an important regulatory role in the inflammatory and apoptotic conditions of stroke-induced ischemic attacks. The 14-3-3 γ is mainly expressed in neurons, but was also observed in primary cultures of mouse cortical astrocytes (109, 110). Moreover, the increased level of 14-3-3 γ was detected in ischemic cortical astrocytes (111) and only 14-3-3 γ was observed in the astrocytic nuclei in the ischemic human brain (112). Another study has confirmed that overexpression of 14-3-3 γ promotes the survival of astrocytes under ischemia, whereas 14-3-3 γ knockdown increases apoptosis of astrocytes, and suggested as a protective mechanism of 14-3-3 γ that 14-3-3 γ binds to a phosphorylated BAD, thereby preventing BAD from entering the mitochondria and consequently inhibiting the initiation of apoptosis (113). Moreover, 14-3-3 γ upregulation by ischemia in astrocytes was observed to be blocked by the inhibitions of c-Jun N-terminal kinase (JNK) and activator protein 1 (AP-1) (114). A subsequent study confirmed that an increase of 14-3-3 γ during ischemic preconditioning reduced astrocytic death caused by ischemic damage via the JNK pathway (115). Thus, 14-3-3 γ was selectively increased

during ischemia and has a protective role against astrocytes, which seem to be implicated in the JNK signaling pathway.

Taken together, 14-3-3 γ could be a biomarker for ischemic stroke and can act as a cell protectant in inflammatory conditions against ischemic damage. The protective mechanism of 14-3-3 γ appears to involve the pathways associated with apoptosis and immune responses. Therefore, it is worth considering 14-3-3 γ as a new strategic target to reduce cell damage in astrocytes as well as neurons and to improve the recovery of stroke.

14-3-3 γ in glioma

Research into the role of 14-3-3 γ in astrocytes is ongoing in glioma, a CNS tumor associated with glial cells. These studies have been linked to the function of the 14-3-3 proteins on the regulation of cell cycle and apoptosis, which contribute to cell growth and cancer progression (116-118). The increase of 14-3-3 γ expression has been observed in several cancers, and it has been suggested that the mechanism may promote cancer development via pathways associated with P53 and BAD (105, 119, 120). However, it is still unclear whether the role of 14-3-3 γ is pro-apoptotic or anti-apoptotic in glioma (40).

Analysis of the 14-3-3 isotype-specific expression in early glioma studies showed contradictory results. An increase of all 14-3-3 isotypes was observed in most primary nervous-system tumors (121), but 14-3-3 γ and σ were not detected in astrocytomas (122, 123). Moreover, in human glioma tissues and para-cancerous brain tissues, 14-3-3 γ expression was detected but no increase was reported (124). However, using Isotope-Coded Protein Label technology, an increase of 14-3-3 γ was observed in the tumor zone of glioblastoma patients and the overexpression of 14-3-3 γ was confirmed using immunoblotting and immunohistochemistry (125). Thus, the expression of 14-3-3 γ might be regulated under certain glioma cases.

Furthermore, 14-3-3 has been proposed as a potential molecular target for the development of anti-cancer drugs. It has been reported that inhibition of 14-3-3 by difopein or siRNA suppresses glioma growth and improves sensitivity of glioma cells to apoptosis (126). It has been also observed that 14-3-3 γ knockdown reduces the migration and invasion of glioblastoma cells, and a deficiency of 14-3-3 γ results in a decrease in surface expression and channel activity of ANO1, a calcium-activated chloride channel, which binds directly to 14-3-3 γ . Therefore, 14-3-3 γ may mediate the progression of glioblastoma by interacting with ANO1 (46), which has been shown to increase its expression in various cancer cells, including glioblastoma (127). More recently, it has been observed that Comp 5, a potential activator of sirtuin-1, which is a highly conserved NAD⁺-dependent protein deacetylase and an emerging tumor suppressor, down-regulates 14-3-3 γ to inhibit autophagy/mitophagy in glioblastomas (128). Therefore, the regulation of 14-3-3 γ may provide new opportunities for glioma treatment.

DISCUSSION

Alteration in the expression levels of the 14-3-3 proteins detected under disease are related to their function. Accumulating evidence on the signaling pathways involved in the 14-3-3 binding proteins reveals their contribution to the diseases and narrows the scope of the targets for the therapeutic approach. However, there are still pieces to be put together. The difference in 14-3-3 isotypes in various brain disorders makes it clear that they have different roles. Also, the expression of the same isotype is regulated temporally and regionally in different ways in the same disease, even with opposite expression patterns. For example, the level of 14-3-3 γ was reduced in the rat hippocampus of KA-induced acute seizure but there was no difference in the human hippocampus of chronic epilepsy (58). Similarly, the decreased 14-3-3 γ in the cortex of fetal DS (63) was observed to increase in aged DS patients (62). In addition, 14-3-3 γ was increased in the overall cortex of AD patients (84) but decreased in the frontal cortex (85). Therefore, further research into the multifaceted function of 14-3-3 γ is needed to overcome their structural similarity, reduce side effects, and enable accurate drug treatment for the diseases involving 14-3-3 γ .

As described above, based on evidence that 14-3-3 γ is involved in brain pathogenesis and is a useful biomarker of brain diseases, generating knockout mice of 14-3-3 γ is worthy. However, unexpectedly, previously generated 14-3-3 γ knockout mice showed normal behavior and no significant defects (129). In contrast, the recently generated 14-3-3 γ -deficient mice were lethal in the prenatal period, and the haploinsufficient heterozygous mice were smaller in body size and weight compared to the wild-type mice, which means that 14-3-3 γ may play an important role in the development (130). Moreover, these mice showed hyperactive locomotion and stress-sensitive behaviors, demonstrating that 14-3-3 γ can be implicated in various brain diseases (130). In fact, in the case of the ξ and ϵ , their deficient mice show abnormal behavioral patterns that show symptoms of disease. The 14-3-3 ξ -deficient mice exhibit neuropsychiatric behaviors, cognitive defects, and hyperactivity locomotion (131-133). The 14-3-3 ϵ -deficient mice showed schizophrenia-like behavior, hyperactive locomotion, and working memory defect (134, 135). Considering the behavioral patterns of mice lacking ξ or ϵ , and 14-3-3 γ -related brain diseases as described in this review, the abnormal phenotypes in recently produced 14-3-3 γ -deficient mice is very reliable. The 14-3-3 γ -deficient mice will be used as a powerful tool for identifying the functions of 14-3-3 γ and developing new therapeutic methods in studies including various brain disorders summarized in this review.

In conclusion, this review is focused on the involvement of 14-3-3 γ in various brain disorders. This review also can provide a helpful guidance for future studies about crucial roles of 14-3-3 γ in brain disorders.

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CONFLICTS OF INTEREST

The authors have no conflicting interests.

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