INTRODUCTION

Lewy bodies are neuronal inclusions that are the pathological hallmark for several neurodegenerative disorders, including Parkinson disease (PD) and diffuse Lewy body disease (DLBD) (1). PD is characterized clinically by the progressive development of the extrapyramidal signs consisting of a resting tremor, muscular rigidity, bradykinesia, and postural instability (2). In the brains of PD patients, neuronal loss associated with Lewy bodies is observed in relatively restricted areas, such as the substantia nigra and locus ceruleus (3). DLBD is the second most common form of degenerative dementia after Alzheimer disease in the aged (4–6), and is characterized pathologically by an abundance of Lewy bodies in the cerebral cortex, as well as in the subcortical regions (6, 7). In addition to progressive dementia, patients with DLBD present with parkinsonism, fluctuating cognitive impairment, recurrent visual hallucinations, and neuropsychiatric symptoms (6, 7).

According to their morphological characteristics, 2 types of Lewy bodies have been documented in PD and DLBD brains (1, 9). Classical Lewy bodies, which are typically observed in monoaminergic and cholinergic neurons of the brainstem and basal forebrain, are eosinophilic inclusions with a hyaline core and a pale halo. Cortical Lewy bodies are uniformly eosinophilic structures without a clear halo and are observed exclusively in small- to medium-sized neurons in the deep cortical layers, particularly in the cingulate, insular, and temporal regions. Immunohistochemical studies have demonstrated that Lewy bodies are ubiquitinated (10) and contain some cytoskeletal elements such as neurofilament proteins (11, 12); however, the precise mechanism of Lewy body formation is as yet undetermined.

Synuclein was originally isolated from the electric lobes of Torpedo californica (13), and human α-synuclein has been identified as the precursor of the non-Aβ component of Alzheimer disease amyloid protein (14, 15). Recently, missense mutations of the α-synuclein gene have been demonstrated to be the cause of some pedigrees of autosomal dominant familial PD (16, 17), and α-synuclein has been confirmed to be a major component of Lewy bodies (18–21). α-Synuclein shares structural homology with 14-3-3 proteins (22), which are abundant in the brain (23), and an interaction between α-synuclein and 14-3-3 proteins has been reported (22).

The 14-3-3 protein family consists of highly conserved proteins that are expressed in a wide range of eukaryotic cells (24). 14-3-3 proteins form dimeric structures with a monomeric molecular weight of approximately 30 kDa (25, 26). There are at least 7 mammalian isoforms of 14-3-3, named with Greek letters (β, γ, ε, ζ, η, σ, τ) (26, 27). 14-3-3 is now known to be a novel adaptor protein that binds to the phosphoserine-containing motifs of target proteins (28, 29). 14-3-3 was first implicated as a kinase-dependent activator of tyrosine and tryptophan hydroxylases (30, 31). Subsequently, several target proteins for 14-3-3 have been identified. 14-3-3 binds to phosphorylated Cdc25C and maintains it in an inactive state (32). By binding to phosphorylated Raf-1, 14-3-3 regulates Raf-1 activity by keeping it in active or inactive...
forms (33, 34). Bad, a mediator of cell death, dimerizes with Bcl-X₁ or Bcl-2 and restores apoptosis (35). Through its interaction with phosphorylated Bad, 14-3-3 prevents it from bonding to Bcl-X₁ and controls the apoptotic process (36). Thus, 14-3-3 proteins modulate various types of signal transduction pathways. To elucidate the role of 14-3-3 in Lewy body-associated diseases, we performed immunohistochemical studies on brain tissue samples from normal subjects and patients with PD or DLBD using a specific anti-14-3-3 antibody. We found strong 14-3-3-like immunoreactivity in both classical and cortical Lewy bodies in the brains affected by PD and DLBD.

MATERIALS AND METHODS
Characterization of the Antibody

To localize 14-3-3 proteins in the human brain, we used a mouse monoclonal antibody raised against a peptide mapping to the amino terminus of human 14-3-3 β (H-8; Santa Cruz Biotechnology, Santa Cruz, CA). The specificity of this antibody was verified by Western blotting using human brain homogenates and purified human 14-3-3 proteins. Fresh brain tissues obtained from the middle frontal gyrus of a normal autopsied case (68-yr-old male) were homogenized in 3 volumes of ice-cold 10 mM phosphate-buffered saline (PBS) containing 1% Nonidet P-40 (Nacalai Tesque, Kyoto, Japan), 0.5% sodium deoxycholate (Difco, Detroit, MI), 0.1% sodium dodecyl sulfate (Nacalai Tesque), 0.01% phenylmethylsulfonyl fluoride (Nacalai Tesque), 3% aprotinin (Sigma, St. Louis, MO) and 1 mM sodium orthovanadate (Sigma). The homogenates were centrifuged at 15,000 rpm for 15 min at 4°C, and the supernatants were then mixed with an equivalent volume of electrophoresis sample buffer containing 10% glycerol (Nacalai Tesque), 2% sodium dodecyl sulfate, 5% 2-mercaptoethanol (Nacalai Tesque), and 0.00125% bromophenol blue (Nacalai Tesque) in 62.5 mM Tris-HCl (pH 6.8). 14-3-3 proteins purified from a normal human brain (Biogenesis, Poole, England) were dissolved in the same electrophoresis sample buffer at a concentration of 2 μg/ml. All of the samples were heated for 3 min at 100°C and then cooled to room temperature. A 10-μl aliquot of the samples was loaded onto each lane of Mini-Protein II Ready Gels J (Bio-Rad, Hercules, CA), electrophoresed at a constant voltage of 200 V and then transferred to polyvinylidene difluoride membranes (Bio-Rad) at a constant voltage of 100 V. After blocking non-specific reactions with 3% nonfat milk and 3% normal horse serum in 25 mM Tris-buffered saline (TBS), the membranes were incubated with the anti-14-3-3 antibody (1:1,000) in 25 mM TBS containing 3% nonfat milk for 4 hours (h) at room temperature. After washing with 25 mM TBS containing 0.1% Tween-20 (TBST), the membranes were reacted with alkaline phosphatase-labeled anti-mouse IgG (1:1,000, Vector, Burlingame, CA) in 25 mM TBS with 3% nonfat milk for 4 hours (h) at room temperature. After rinsing with 25 mM TBST, the primary antibody was visualized with 0.033% nitroblue tetrazolium (Life Technologies, Gaithersburg, MD) and 0.0017% 5-bromo-4-chloro-3-indolyl-phosphate (Life Technologies) in 100 mM Tris-HCl (pH 9.5) containing 100 mM NaCl and 50 mM MgCl₂. For the immunoabsorption test, the primary antibody (0.2 μg/ml) was pretreated with an excess amount of the antigenic peptide (10 μg/ml; H-8P, Santa Cruz Biotechnology) and the blotted membranes were immunostained as described above.

Tissue Preparation

We examined the autopsied brains from 7 patients with PD (mean age: 77.4 yr, range: 66–90 yr) and 3 patients with DLBD (mean age: 73.0 yr, range: 69–81 yr). Five autopsy cases without any neurological abnormalities (mean age: 69.2 yr, range: 62–75 yr) served as controls. The clinical profiles from all cases are summarized in Table 1. All brains were cut through the midsagittal plane and the left hemispheres were fixed in 10% neutral formalin for 2 wk. Representative tissue samples from the neocortices, hippocampus, amygdala, basal forebrain, midbrain, pons, and medulla were embedded in paraffin and then

<table>
<thead>
<tr>
<th>Case</th>
<th>Age (years)/Gender</th>
<th>Diagnosis</th>
<th>Duration of illness (years)</th>
<th>Postmortem delay (hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD1</td>
<td>90/F</td>
<td>Parkinson disease</td>
<td>9/2.0</td>
<td></td>
</tr>
<tr>
<td>PD2</td>
<td>76/M</td>
<td>Parkinson disease</td>
<td>14/2.5</td>
<td></td>
</tr>
<tr>
<td>PD3</td>
<td>79/F</td>
<td>Parkinson disease</td>
<td>13/1.5</td>
<td></td>
</tr>
<tr>
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<td>81/F</td>
<td>Parkinson disease</td>
<td>8/2.5</td>
<td></td>
</tr>
<tr>
<td>PD5</td>
<td>74/M</td>
<td>Parkinson disease</td>
<td>20/UD</td>
<td></td>
</tr>
<tr>
<td>PD6</td>
<td>66/M</td>
<td>Parkinson disease</td>
<td>10/2.3</td>
<td></td>
</tr>
<tr>
<td>PD7</td>
<td>76/M</td>
<td>Parkinson disease</td>
<td>8/1.3</td>
<td></td>
</tr>
<tr>
<td>DLBD 1</td>
<td>81/M</td>
<td>Diffuse Lewy body disease</td>
<td>UD/9.0</td>
<td></td>
</tr>
<tr>
<td>DLBD 2</td>
<td>69/M</td>
<td>Diffuse Lewy body disease</td>
<td>9/11.5</td>
<td></td>
</tr>
<tr>
<td>DLBD 3</td>
<td>69/F</td>
<td>Diffuse Lewy body disease</td>
<td>27/1.0</td>
<td></td>
</tr>
<tr>
<td>Control 1</td>
<td>62/M</td>
<td>Pancreatic carcinoma</td>
<td>NA/3.0</td>
<td></td>
</tr>
<tr>
<td>Control 2</td>
<td>68/M</td>
<td>Rheumatoid arthritis</td>
<td>NA/2.0</td>
<td></td>
</tr>
<tr>
<td>Control 3</td>
<td>73/M</td>
<td>Hepatocellular carcinoma</td>
<td>NA/4.5</td>
<td></td>
</tr>
<tr>
<td>Control 4</td>
<td>68/F</td>
<td>Breast cancer</td>
<td>NA/2.5</td>
<td></td>
</tr>
<tr>
<td>Control 5</td>
<td>75/M</td>
<td>Pulmonary emphysema</td>
<td>NA/2.0</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: F, female; M, male; NA, not applicable; UD, undetermined.
cut into 6-μm-thick sections on a microtome. Routine pathological studies were performed on deparaffinized sections stained with hematoxylin and eosin, Klüver-Barrera, and modified Bielschowsky stains. No histological abnormalities were detected in the sections from any of the control cases. The diagnostic assessment of all PD and DLBD cases was carried out in concordance with standard pathological criteria (6, 9). The pathological findings in the brains from all PD patients and 2 DLBD patients were compatible with their clinical diagnoses. The remaining patient (DLBD 2) was clinically diagnosed with PD but was proven to be DLBD on the basis of the pathological criteria (6).

### Immunohistochemistry

Deparaffinized sections were pretreated with 0.3% hydrogen peroxide (Santoku, Tokyo, Japan) in 0.1 M PBS for 30 min at room temperature to inhibit endogenous peroxidase activity. After washing with 0.1 M PBS, these sections were blocked with 0.1 M PBS plus 3% nonfat milk and 3% normal horse serum for 2 h at room temperature. After rinsing with 0.1 M PBS, the sections were incubated with the anti-14-3-3 antibody in 0.1 M PBS (1:1,000) at room temperature overnight in a humidified chamber. After washing with 0.1 M PBS, the sections were reacted with biotinylated anti-mouse IgG (Vector) diluted in 0.1 M PBS (1:200) for 1 h at room temperature, followed by an incubation with an avidin-biotin-peroxidase complex kit (Vector) diluted in 0.1 M PBS (1:400) for 1 h at room temperature. After rinsing with 0.1 M PBS and then 0.05 M Tris-HCl (pH 7.6), the sections were developed in a staining solution containing 0.02% diaminobenzidine tetrahydrochloride (Dojin, Kumamoto, Japan), 0.6% ammonium nickel (II) sulfate (Wako, Osaka, Japan), and 0.005% hydrogen peroxide in 0.05 M Tris-HCl (pH 7.6) for 10 min at room temperature. As negative immunohistochemical controls, some sections were incubated with normal mouse serum or the primary antibody (0.2 μg/ml) preabsorbed with an excess amount of the antigenic peptide (10 μg/ml; H-8P). No specific immunopositive staining was detected in these control sections.

### Comparison of 14-3-3- and α-Synuclein-Immunopositive Lewy Bodies

To compare the Lewy body immunolabeling pattern between 14-3-3 and α-synuclein, serial sections of the midbrain from 7 PD patients and the frontal cortex from 3 DLBD patients were immunostained alternately with antibodies raised against 14-3-3 and α-synuclein (1:500; goat polyclonal, C-20, Santa Cruz Biotechnology). For the quantitative evaluation, we selected half of the midbrain from 1 cross-section of the rostral midbrain at the level of the red nucleus and superior colliculus from each PD patient, and the superior frontal gyrus from 1 coronal section of the frontal lobe through the genu of the corpus callosum from each DLBD patient. On this condition, the frontal gyrus was surrounded by the interhemispheric fissure, superior frontal sulcus, and cingulate sulcus. The total number of immunopositive Lewy bodies in the designated areas was counted manually on the 14-3-3- and α-synuclein-stained sections in all PD and DLBD cases; the results on the Lewy body frequency are summarized in Table 2. Differences in the number of immunopositive Lewy bodies between 14-3-3 and α-synuclein were analyzed statistically using a Mann–Whitney U-test (p < 0.05).

### Results

#### Western Blot Analysis

In human brain homogenates, the anti-14-3-3 antibody recognized 1 major band at approximately 30 kDa and an additional minor band with a slightly larger molecular weight (Fig. 1a). These immunopositive bands were abolished when we pretreated the primary antibody with the antigenic peptide (Fig. 1b). Two immunolabeled bands with slightly different molecular masses were detected in the lane containing purified human 14-3-3 proteins (Fig. 1c), which was consistent with the manufacturer’s statement that these human 14-3-3 proteins were composed of 2 subunits of 26 and 29 kDa.

### 14-3-3-like Immunoreactivity in the Control and Disease Brains

In agreement with a previous report (25), 14-3-3-like immunoreactivity was mainly observed in the neuronal somata, dendrites, and axons in various cortical and subcortical areas of normal brains (Fig. 2A). In the PD and DLBD brains, a similar immunostaining pattern was found and immunoreactivity in the surviving neurons was

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**Table 2**

<table>
<thead>
<tr>
<th>Case</th>
<th>Area</th>
<th>14-3-3</th>
<th>α-Synuclein</th>
<th>14-3-3/α-Synuclein (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD 1</td>
<td>Midbrain</td>
<td>5</td>
<td>8</td>
<td>62.5</td>
</tr>
<tr>
<td>PD 2</td>
<td>Midbrain</td>
<td>11</td>
<td>14</td>
<td>78.6</td>
</tr>
<tr>
<td>PD 3</td>
<td>Midbrain</td>
<td>18</td>
<td>25</td>
<td>72.0</td>
</tr>
<tr>
<td>PD 4</td>
<td>Midbrain</td>
<td>10</td>
<td>21</td>
<td>47.6</td>
</tr>
<tr>
<td>PD 5</td>
<td>Midbrain</td>
<td>12</td>
<td>11</td>
<td>109.1</td>
</tr>
<tr>
<td>PD 6</td>
<td>Midbrain</td>
<td>32</td>
<td>36</td>
<td>88.9</td>
</tr>
<tr>
<td>PD 7</td>
<td>Midbrain</td>
<td>22</td>
<td>30</td>
<td>73.3</td>
</tr>
<tr>
<td>DLBD 1</td>
<td>Frontal cortex</td>
<td>113</td>
<td>145</td>
<td>77.9</td>
</tr>
<tr>
<td>DLBD 2</td>
<td>Frontal cortex</td>
<td>85</td>
<td>116</td>
<td>73.3</td>
</tr>
<tr>
<td>DLBD 3</td>
<td>Frontal cortex</td>
<td>66</td>
<td>92</td>
<td>71.7</td>
</tr>
</tbody>
</table>

Midbrain: total number of immunopositive Lewy bodies in one half of the midbrain per 1 section; Frontal cortex: total number of immunopositive Lewy bodies in the superior frontal gyrus per 1 section.

Furthermore, we performed double-labeling immunohistochemistry for 14-3-3 and α-synuclein. Some sections were incubated with the H-8 (1:1,000) and C-20 (1:500) antibodies in 0.1 M PBS at room temperature overnight. After washing with 0.01 M PBS, the sections were reacted with secondary antibodies consisting of rhodamine-conjugated donkey anti-goat IgG (Chemicon International, Temecula, CA) and fluorescein isothiocyanate (FITC)-labeled rabbit anti-mouse IgG (DAKO, Glostrup, Denmark). After rinsing with 0.01 M PBS, the slides were coverslipped with Vectashield (Vector) and viewed with an Olympus fluorescent microscope.

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In addition to the substantia nigra, classical Lewy bodies (Fig. 4A–D) and dystrophic neurites (Fig. 4E) were immunostained by the anti-14-3-3 antibody in other brainstem areas from both PD and DLBD patients. These Lewy bodies showed both the ring-like (Fig. 4A, D) and the homogenous immunolabeling patterns (Fig. 4B, C). Immunopositive classical Lewy body-containing neurons were also observed in the basal forebrain (Fig. 4F) and hypothalamus of both types of patients.

14-3-3-like immunolabeled cortical Lewy bodies were scattered in the deep layers of the cerebral cortex of patients with DLBD (Fig. 5A, C). Dense immunoreaction products accumulated in almost all of these cortical Lewy bodies (Fig. 5D–F). Immunopositive cortical Lewy bodies were also observed throughout the amygdaloid subnuclei of the DLBD cases and were particularly abundant in the medial parts of the basal and accessory basal nuclei (Fig. 5G).

14-3-3-Immunopositive Lewy Body Assessment

As previously reported (18–21), both classical and cortical Lewy bodies were intensely immunolabeled by the antibody raised against α-synuclein. When comparing consecutive sections immunostained with antibodies against 14-3-3 or α-synuclein, a similar distribution of immunopositive Lewy bodies was seen between 14-3-3 and α-synuclein. As shown in Table 2, the number of 14-3-3-positive Lewy bodies was comparable to the number of α-synuclein-positive Lewy bodies in all cases, except for 1 PD case (PD 4) in which the number of Lewy bodies immunoreactive for 14-3-3 was less than half of that of α-synuclein. Statistical analysis revealed that there were no significant differences in the number of immunopositive Lewy bodies between 14-3-3 and α-synuclein (Midbrain: p = 0.3711; Frontal cortex: p = 0.1266). Immunohistochemical double-staining showed that 14-3-3-like immunoreactivity in the pars compacta of the substantia nigra from control (A: Control 2) and Parkinson disease (B: PD 3) cases. In the normal and neurodegenerative conditions, 14-3-3-like immunoreactivity was observed mainly in the neuronal somata and proximal processes. The nerve fibers were also immunolabeled. Scale bar: A, B = 50 μm.
and α-synuclein were co-localized in most of classical (Fig. 6A–C) and cortical Lewy bodies.

DISCUSSION

Layfield et al reported that 14-3-3 was a component of the neurofibrillary tangles in Alzheimer disease brains (37). They also reported very low numbers of 14-3-3-immunopositive cortical Lewy bodies (37), but the exact relationship between the 14-3-3 proteins and the Lewy bodies remains to be elucidated. In the present study, we demonstrated strong 14-3-3-like immunoreactivity in both classical and cortical Lewy bodies in brains affected with PD and DLBD.

In accordance with a previous report (37), 2 types of bands were detected around 30 kDa on the Western blots from human brain homogenates in our studies. The minor band showed a slightly higher molecular weight than that of the major band. The 14-3-3 protein family consists of several homologous isoforms (26, 27), and the molecular weight of the 14-3-3 protein monomers has been reported
Fig. 4. 14-3-3-like immunoreactivity in other brainstem areas and the basal forebrain in patients with Parkinson disease (A, B, F: PD 3; C, D: PD 6) and diffuse Lewy body disease (E: DLBD 1). Strongly immunopositive classical Lewy bodies were observed in various brainstem areas, including the Edinger-Westphal nucleus (A), red nucleus (B), periaqueductal gray matter (C), and locus ceruleus (D). Intensely immunolabeled, swollen neurites were found in the dorsal motor nucleus of the vagus (E). Immunopositive Lewy body-containing neurons (arrowheads) were distributed in the basal nucleus of Meynert (F). Scale bars: 20 \( \mu m \).

14-3-3 proteins share amino acid sequence homology with \( \alpha \)-synuclein. Two regions with 43% and 36% sequence homology are seen between amino acids 45 and 30 kDa (25, 26) with the exception of 14-3-3E, which has a slightly larger monomeric molecular mass (31, 38). According to the manufacturer’s instructions, the anti-14-3-3 antibody is broadly reactive among all human 14-3-3 protein family members, suggesting that the minor band may correspond to 14-3-3E and the major band may correspond to the other 14-3-3 isoforms. Further Western blotting revealed that the antibody also recognized 14-3-3 proteins purified from a normal human brain. Moreover, the immunopositive staining on these sections was abolished by the preabsorption of the primary antibody with the antigenic peptide. Taken together, these data strongly indicate that the antibody is specific for 14-3-3 and that the immunopositive structures on the human brain tissue sections immunostained by the antibody represent the presence of 14-3-3 proteins.
102 of 14-3-3 and between amino acids 8 and 61 of α-synuclein (22). However, the N-terminal portions of human 14-3-3 have little in common with the amino acid sequence of human α-synuclein (14, 15, 39). Thus, any cross-reactivity of the anti-14-3-3 antibody with α-synuclein is very unlikely. This conclusion is supported by the fact that the molecular weight of the 14-3-3-immunolabeled bands was different from that of α-synuclein (14, 15).

After the discovery of missense mutations of the α-synuclein gene in a few related patients with autosomal dominant familial PD (16, 17), α-synuclein has been confirmed to be a major component of Lewy bodies (18–21); however, its pathogenetic role in PD is still unclear. Recently, synphilin-1 was identified as a novel protein that interacts with α-synuclein (40), and the presence of synphilin-1 in the Lewy bodies from PD patients was demonstrated immunohistochemically (41). In the present study, we
investigated 14-3-3 as another protein that interacts with α-synuclein and found strong 14-3-3-like immunoreactivity in both classical and cortical Lewy bodies. Synphilin-1 is localized to the central cores of classical Lewy bodies and Lewy neurites are immunonegative for synphilin-1 (41). In contrast, our immunohistochemical study showed that 14-3-3 often accumulated in the peripheral zones of classical Lewy bodies and some dystrophic neurites contained abundant 14-3-3. These results suggest that both synphilin-1 and 14-3-3 are components of Lewy bodies, but the roles of these α-synuclein-related proteins may be different in the pathogenesis of PD.

Although the function of synphilin-1 is unknown, co-transfection of synphilin-1 and the central part of α-synuclein into mammalian cells was shown to induce cytoplasmic eosinophilic inclusions resembling Lewy bodies (40). Both wild and mutant types of the α-synuclein protein have the property of forming insoluble fibrillar aggregates by themselves (42–44). Since relatively high concentrations of α-synuclein are required for self-aggregation in vitro, Wakabayashi et al proposed the hypothesis that synphilin-1 could accelerate the deposition of α-synuclein within the cells (41). On the other hand, 14-3-3 has been generally accepted as a novel type of chaperone protein that binds to the phosphorylated serine residues of target proteins and maintains them in an activated or inactivated state (28–34, 36). Recently, serine 129 within the C-terminal domain of α-synuclein was demonstrated to be phosphorylated by casein kinase 1 (CK-1) or CK-2 (45). An additional site that could be phosphorylated was detected at serine 87 of α-synuclein (45). These data suggest that 14-3-3 may interact with α-synuclein via constitutive phosphorylation and might play an important role in stabilizing α-synuclein so that it accumulates in Lewy bodies.

Lewy bodies contain several serine/threonine kinases, including Ca\(^{2+}\)/calmodulin-dependent protein kinase II (CaM kinase II) (46). After the phosphorylation of tyrosine hydroxylase by CaM kinase II, 14-3-3 binds to the phosphorylated tyrosine hydroxylase and activates it (30, 31). Tyrosine hydroxylase has also been reported to be localized in the Lewy bodies in catecholaminergic neurons of patients with PD (47). These findings suggest that 14-3-3 may promote the formation of Lewy bodies in catecholaminergic neurons by activating tyrosine hydroxylase.

In conclusion, our study has demonstrated the immunohistochemical localization of 14-3-3 in both classical and cortical Lewy bodies, suggesting that 14-3-3 may be associated with Lewy body formation. 14-3-3, a family of highly homologous proteins, has the ability to modulate the functions of diverse target proteins by binding to them, and over 50 proteins have been reported as 14-3-3 ligands to date (26). Thus, further research focused on the main 14-3-3 isoforms in Lewy bodies and on the main target proteins of 14-3-3 in Lewy bodies will be needed to clarify the pathogenetic role of 14-3-3 in PD and DLBD.

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