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Original Article

Optineurin in neurodegenerative diseases

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Optineurin is a gene associated with normal tension glaucoma and primary open-angle glaucoma, one of the major causes of irreversible bilateral blindness. Recently, mutations in the gene encoding optineurin were found in patients with amyotrophic lateral sclerosis (ALS). Immunohistochemical analysis showed aggregation of optineurin in skein-like inclusions and round hyaline inclusions in the spinal cord, suggesting that optineurin appears to be a more general marker for ALS. However, our detailed examinations demonstrated that optineurin was found not only in ALS-associated pathological structures, but also in ubiquitin-positive intraneuronal inclusions in ALS with dementia, basophilic inclusions in the basophilic type of ALS, neurofibrillary tangles and dystrophic neurites in Alzheimer's disease, Lewy bodies and Lewy neurites in Parkinson's disease, ballooned neurons in Creutzfeldt-Jakob disease, glial cytoplasmic inclusions in multiple system atrophy, and Pick bodies in Pick disease. With respect to optineurin-positive basophilic inclusions, these structures showed variable immunoreactivities for ubiquitin; some structures were obviously ubiquitin-positive, while others were negative for the protein, suggesting that optineurin expression was not always associated with the expression of ubiquitin. This study indicates that optineurin is widely distributed in neurodegenerative conditions; however, its significance is obscure.

Key words: amyotrophic lateral sclerosis, glaucoma, neurodegenerative diseases, optineruin, ubiquitin.

INTRODUCTION

Optineurin is a cytoplasmic protein ubiquitously expressed in retina, brain, heart, skeletal muscle, placenta and kidney, although it was initially shown to be a Golgi-localized protein.[1,2] Its gene spans a ~37 kb genomic region and is located on chromosome 10p15-14. The gene consists of a total of 16 exons containing three non-coding exons in the 5′ region and 13 exons coding for 577 amino acid proteins. Optineurin contains several putative domains including one bZIP motif, two leucine zippers, multiple coiled-coil motifs, ubiquitin-binding domain, and a C-terminal C2H2 type of zinc finger.[1,2]

Several interacting partners with optineurin are identified such as GTPase molecule Rab8,[3] transcription factor III[4], metabotropic glutamate receptor 1a,[5] Huntingtin,[6] myosin VI[7], ring finger protein 11,[8] and serine/threonine kinase receptor-interacting protein 1 (RIP1),[1] indicating multiple cellular functions for optineruin. In addition, optineurin translocates to the nucleus on apoptotic stimuli, which is mediated by Rab8. The interaction of optineurin with myosin VI would be implicative of a role in vesicular trafficking between Golgi apparatus and plasma membrane.[7] Optineurin was recently shown to negatively regulate tumor necrosis factor α (TNF-α)-induced activation of transcriptional factor NF-kB via binding with polyubiquitinated RIP[9]. NF-kB is involved in protecting cells from apoptosis by inducing many anti-apoptotic genes.[10]

Glaucoma is one of the leading causes of irreversible blindness characterized by progressive loss of retinal ganglion cells, degeneration in the optic nerve head, and defect of visual field. The most common form of glaucoma is primary open angle glaucoma (POAG)[11] and is frequently associated with elevated intraocular pressure. However, there is a subtype of POAG without elevated intraocular pressure, normal tension glaucoma (NTG). According to Iwase *et al.*, > 90% of POAG patients were diagnosed as NTG.[12] Glaucoma is genetically heterogeneous and caused by several susceptible genes and environmental factors.

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At present, a total of 14 chromosomal loci designated as GLC1A to GLC1N have been reported as POAG,[13,14] one of which includes optineurin (GLC1E).[11,15,16] Mutation of the gene for optineurin is associated with some forms of glaucoma.

Recently Maruyama *et al.* showed that patients with amyotrophic lateral sclerosis (ALS) have mutations of the gene encoding for optineurin: a homozygous exon 5 deletion, a homozygous Q398X nonsense mutation, and a heterozygous E478G missense mutation.[17] Furthermore, the pathological structures of skein-like inclusions and round hyaline inclusions immunoreactive for ubiquitin and TDP-43 were also optineurin-positive.[17] They suggested that optineurin was a more general marker for inclusions in various types of ALS and was different from SOD1 and TDP-43.[17]

In this study, we examined the unique structures in several neurodegenerative diseases such as ALS and ALS with dementia and basophilic inclusions, Alzheimer's disease (AD), Parkinson's disease (PD), Creutzfeldt-Jakob disease (CJD), multiple system atrophy (MSA) and Pick disease, to see if comparatively disease-specific bodies or inclusions were immunoreactive to optineurin.

METHODS

We examined a total of 18 patients with neurodegenerative diseases, including three sporadic ALS cases, three sporadic ALS cases with dementia, one sporadic ALS case with basophilic inclusions, two AD cases, four PD cases, two CJD cases, two MSA cases and one case of Pick disease. Brain tissues were all obtained from the Geriatrics Research Institute and Gunma University, Japan. In all cases, the autopsies were performed in accordance with established procedures and the samples were used in this study after obtaining informed consent from the family of each patient. All patients were definitively diagnosed based on clinical and light microscopic findings. Tissues were fixed with 4% paraformaldehyde in PBS (pH 7.4) and embedded in paraffin. Five-μm-thick paraffin sections were prepared for immunohistochemistry, which was carried out using a polyclonal anti-human optineurin antibody (1:200; code no. 10837-1-AP, ProteinTech Group, Chicago, IL, US) or a rabbit polyclonal anti-ubiquitin antibody (1:2000; code no. Z0458, DAKO, Glostrup, Denmark). For enhancement, autoclave treatment for 5 min was performed before reaction with the antibody for optineurin. Sections were blocked in a solution supplied by Histofine SAB-PO kit (Nichirei, Tokyo, Japan) for 30 min at room temperature, then labeled with the first antibody at 4°C overnight, washed in PBS for 30 min, incubated with the second antibody provided by Histofine SAB-PO kit, washed in PBS for 30 min, and finally visualized by the avidin-biotin-peroxidase method. Observation was performed using an Olympus BX50 microscope (Olympus, Tokyo, Japan).

Since basophilic inclusions are demonstrated by HE staining, this process was initially performed to observe where these structures were present. After we photographed the sections, we removed the cover glasses from the slides in xylene, decolorized the specimens in alcohol, and then performed immunohistochemistry with anti-optineurin antibody.

RESULTS

We started by examining anterior horn cells of spinal cords in patients with ALS (Fig. 1). Although Bunina bodies were optineurin-negative (data not shown), skein-like inclusions were clearly immunostained with antibody for optineurin (Fig. 1A), as previously reported by Maruyama *et al.*[17] Concerning the round hyaline inclusions, the peripheral portions of these inclusions showed patchy reaction with anti-optineurin antibody, while the center was weakly immunostained (Fig. 1B). Some of the neurons in the hippocampal granular cell layer in patients with ALS with dementia were immunoreactive for optineurin (Fig. 1C), which are often immunostained with anti-ubiquitin[18] and anti-vacuole-creating protein[19] antibodies forming crescent or circular immunoreactivities. The cortical neurons in those cases also contained cytoplasmic optineurin-positive inclusions (data not shown). In another type of ALS demonstrating basophilic inclusions, the aggregates were markedly immunostained by the antibody (Fig. 1D) and some of the glia also included optineurin-positive cytoplasmic aggregates (insert in Fig. 1D).

In AD cases (Fig. 2), senile plaque, neurofibrillary tangles and granulovacuolar degeneration are the major structures and these were immunohistochemically examined. Anti-optineurin antibody labeled neurofibrillary tangles (Fig. 2A). In addition, some dystrophic neurites in senile plaque, which are observed as swollen processes, were positive for optineurin (Fig. 2B). However, granulovacuolar degeneration frequently detected in hippocampal pyramidal neurons of Sommer's sector was not stained with anti-optineurin antibody (data not shown). Lewy bodies and Lewy neurites in the midbrain were also examined in patients with PD (Fig. 2). The peripheral portion of Lewy bodies showed a strong reaction to anti-optineurin antibody, while the center was weakly immunostained (Fig. 2C). Lewy neurites were also positive for optineurin (Fig. 2D).

Examination of the ballooned neurons in the temporal lobe of patients with CJD showed the disappearance of Nissl bodies as well as swollen cytoplasm with peripheral

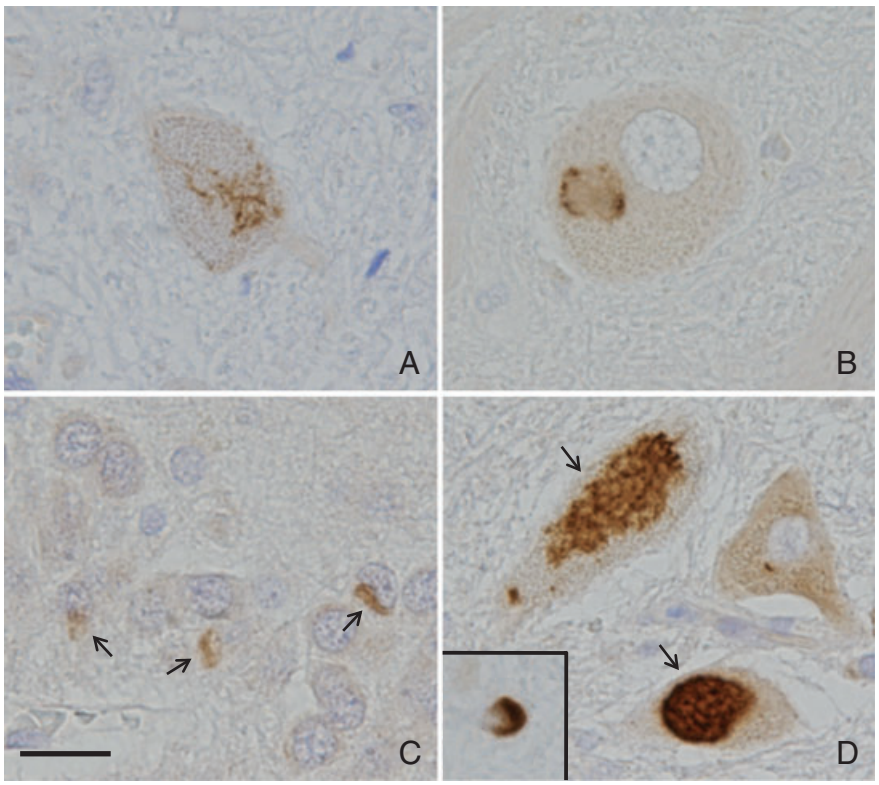

Fig. 1 Optineurin immunoreactivitiy for several pathological structures in patients with different types of amyotrophic lateral sclerosis (ALS). A–D. Immunostaining for optineurin. Skein-like inclusions were optineurin-positive (A) and round hyaline inclusions were irregularly immunoreactive for optineurin around the rim (B) with the center being weakly immunostained. In patients with ALS with dementia, some of the intraneuronal inclusions of the hippocampal granular cell layer were positively immunostained (C; arrows). In patients with ALS with basophilic inclusions, these aggregates showed obvious immunostaining (D; arrows) and some of the glia, probably oligodendrocytes, also included optineurin-positive aggregates (D; insert). Scale bar: 20 μm.

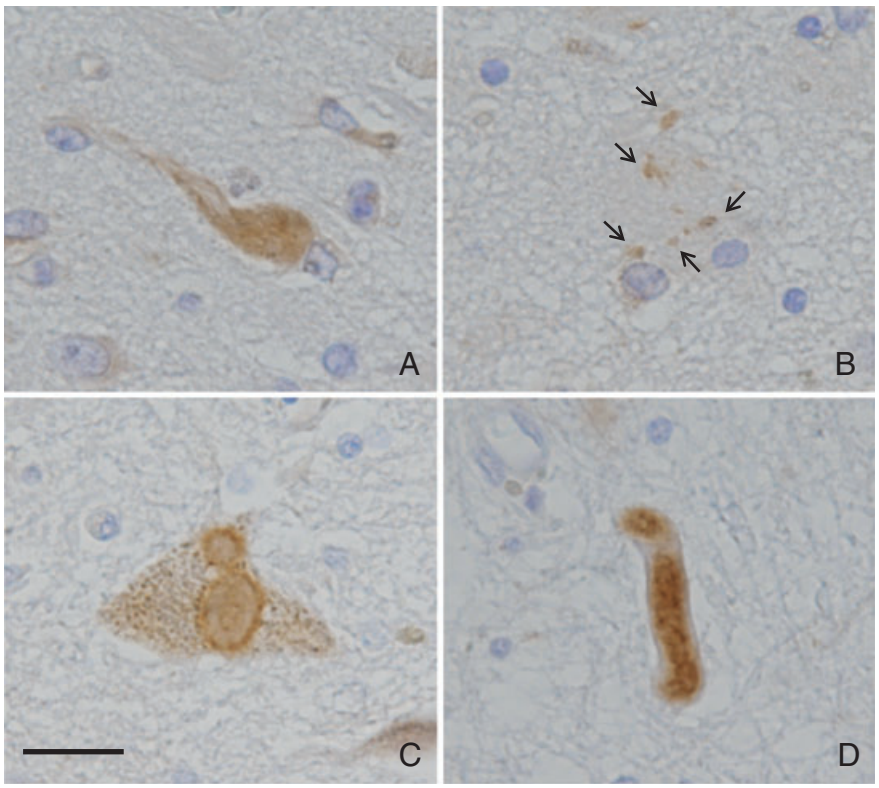

Fig. 2 Optineurin immunoreactivity for several pathological structures in patients with Alzheimer's disease (AD) and Parkinson's disease (PD). A–D. Immunostaining for optineurin. A, B: AD. C, D: PD. Neurofibrillary tangles (A) and dystrophic neurites (B; arrows) were optineurin-positive, and Lewy bodies (especially periphery) (C) and Lewy neurites (D) were positive for optineurin. Scale bar: 20 μm.

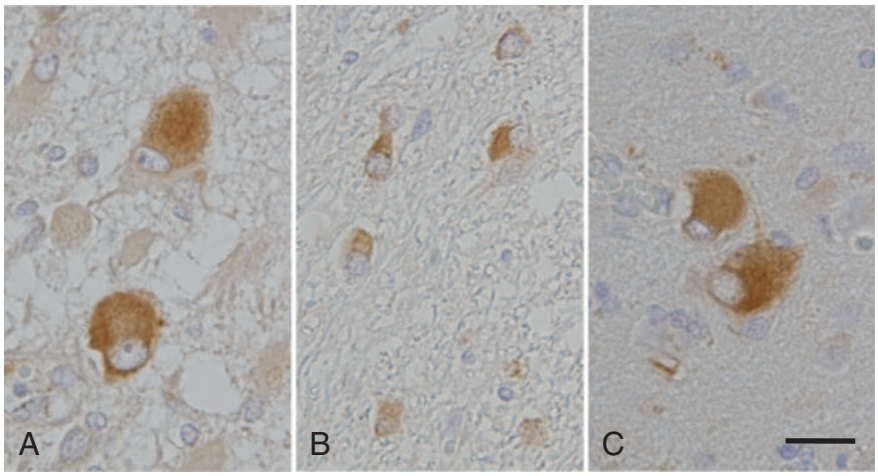

Fig. 3 Optineurin immunoreactivities of other pathological structures. A: Ballooned neurons in Creutzfeldt-Jakob disease. B: Glial cytoplasmic inclusions in multiple system atrophy. C: Pick bodies. Scale bar: 20 μm.

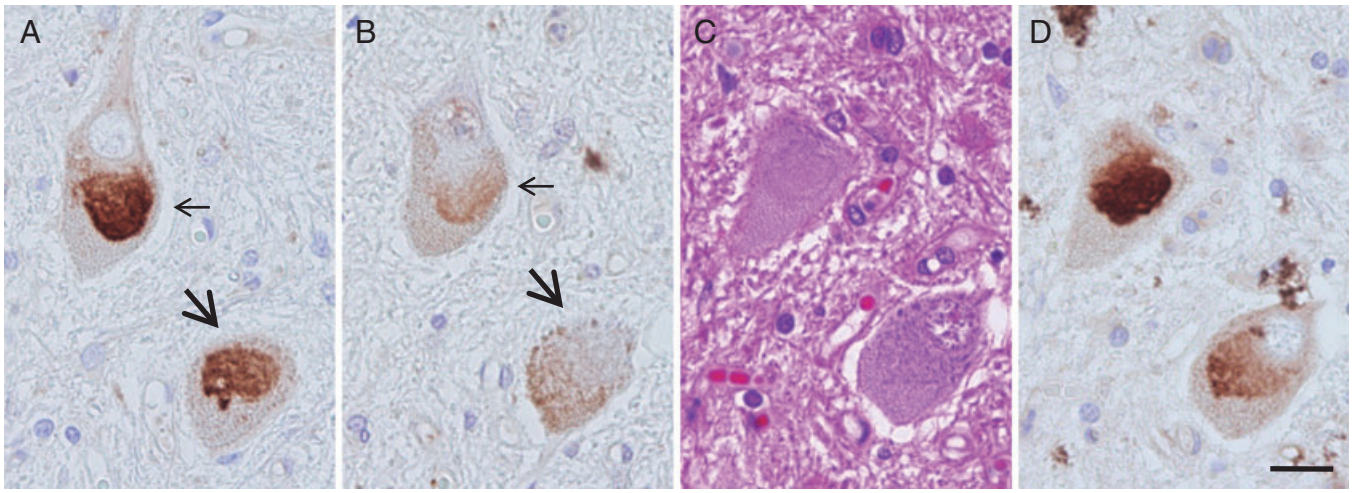

Fig. 4 Optineurin immunoreactivity of basophilic inclusions. A, D: Immunostaining for optineurin. B: Immunostaining for ubiquitin. C: HE staining. A–C: Serial sections. C, D: Same section. Some of the basophilic inclusions (C) showed obvious immunostaining for both optineurin and ubiquitin (A, B; small arrow), while others were optineurin-positive but ubiquitin-negative (A, B; large arrow). Panel C shows a serial section of B stained with HE to confirm the presence of basophilic inclusions, while Panel D shows positive immunoreactivities for optineurin in the same section (D). Scale bar: 20 μm.

nuclei. The cytoplasm of ballooned neurons was well immunostained with anti-optineurin antibody (Fig. 3A). Furthermore, the cytoplasm of the oligodendroglia in the brainstem from patients with MSA was immunoreactive with antibody for optineurin, indicating that glial cytoplasmic inclusions were optineurin-positive (Fig. 3B). In Pick diseases, Pick bodies characterized by slightly basophilic staining by HE were positive for optineurin (Fig. 3C).

With respect to optineurin-positive basophilic inclusions, three serial sections were prepared and then stained with anti-optineurin antibody (Fig. 4A), anti-ubiquitin antibody (Fig. 4B), and HE (Fig. 4C) to determine whether basophilic inclusions showed ubiquitin expression. First, when a structure showing basophilic inclusions could be confirmed with HE staining (Fig. 4C), the same section was immunostained with the antibody for optineurin to determine whether basophilic inclusions showed positive immunoreaction for optineurin (Fig. 4D). Second, the next serial section was examined using anti-ubiquitin antibody to determine whether basophilic inclusions were ubiquitin-positive (Fig. 4B). The third serial section was immunostained with anti-optineurin antibody (Fig. 4A) to confirm whether basophilic inclusions were still present. Our findings indicated that basophilic inclusions were immunoreactive for optineurin (Fig. 4A,D), while basophilic inclusions showed variable immunostainings for ubiquitin, ranging from negative (large arrow in Fig. 4B) to positive (small arrow in Fig. 4B) and from weak to strong (data now shown). In total, five of nine basophilic inclusions were ubiquitin-negative but optineurin-positive and four were positive for both.

DISCUSSION

In this study, we confirmed that optineurin immunoreactivities are more widely distributed among other neurodegenerative diseases than previously reported.[17] Since both intraneurocytoplasmic and intraglial inclusions/bodies are common pathological features among these neurodegenerative diseases, we focused on disease-characteristic pathologies, such as senile plaques and neurofibrillary tangles in AD, Lewy bodies and Lewy neurites in PD, ballooned neurons in CJD, glial cytoplasmic inclusions in MSA, and Pick bodies in Pick disease, in addition to skein-like inclusions and round hyaline inclusions in ALS, neurons in the hippocampal granular cell layer from ALS with dementia, and basophilic inclusions in basophilic type of ALS. Our findings showed that optineurin immunoreactivities were more widely detected in a variety of neurodegenerative diseases in addition to ALS cases and that optineurin was not only specific for ALS but also for other diseases. It remains unclear why optineurin was found in the variety of pathological structures in neurodegenerative diseases. We could not deny that optineurin might be just bystanders to the various inclusions. The aggregation of optineurin, which is generally found to be ubiquitin-positive, may be the common process involved in neurodegeneration and cell death. It is possible that optineurin is just secondarily entrapped in the pathological structures or in ubiquitin. To investigate this possibility, we focused on the basophilic inclusions. Since variable levels of ubiquitin were present in the basophilic inclusions, we investigated whether there was some relationship between the expression of optineurin and ubiquitin. The results indicated that nine of the basophilic inclusions we examined were all optineurin-positive, and four of these were ubiquitin-positive, while the others were negative for ubiquitin, showing a wider presence of optineurin than of ubiquitin. Although the role of optineurin remains unknown, our study obviously showed that optineurin could be an aggregation-prone protein in the affected neurons and glia.

The level of optineurin is variable under certain conditions of stress. High levels of oxidative stress lead to overexpression of optineurin in NIH 3T3 cells, protecting these cells from cell death.[20] However, overexpression of normal optineurin sensitizes RGC-5 cells to TNF-α-induced cell death. In contrast, normally overexpressed optineurin reduces TNF-α-induced cell death in HeLa cells.[21] Our findings suggest that the expression of optineurin could be upregulated in pathological conditions such as ALS, AD, PD, CJD, MSA and Pick diseases. There are lots of associated factors modulating expression of optineurin, making interpretation more complicated. However, understanding the molecular mechanism that regulates the level of optineurin is a very important step in the detailed investigation of optineurin.

In conclusion, we demonstrated that optineurin-positive structures are more common to various neurodegenerative diseases, including cases of not only ALS but also AD, PD, CJD, MSA and Pick disease. As such aggregates with optineurin are generally shown to be ubiquitin-, p62-[22] and TDP-43-positive, elucidation of the relevance to these proteins would be the next step. Detailed examination of how the inclusions are formed, how these cells survive by forming the inclusions, and what effects the inclusions have on the remaining neurons, could contribute to clarifying the pathogenesis of neurodegenerative diseases.

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