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<th>&lt;Symposium III&gt; Pathology of neuro-glial α-synucleinopathy (Lewy body disease and multiple system atrophy)</th>
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<tr>
<td>Citation</td>
<td>弘前医学 61(Suppl.), 2010, p.S80-S88</td>
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<td>Issue Date</td>
<td>2010-07-08</td>
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PATHOLOGY OF NEURO-GLIAL α-SYNUCLEINOPATHY (LEWY BODY DISEASE AND MULTIPLE SYSTEM ATROPHY)

Koichi Wakabayashi, Yasuo Miki, Kunikazu Tanji, Fumiaki Mori

Abstract α-Synucleinopathies comprise a group of neurodegenerative disorders that share α-synuclein (αS) accumulation in selected vulnerable neurons and glia, i.e. Parkinson’s disease (PD), dementia with Lewy bodies and multiple system atrophy (MSA). The histological hallmark of PD is neuronal αS aggregates called Lewy bodies (LBs). LB formation can be considered to be a marker for neuronal degeneration, because neuronal loss is found in the predilection sites for LBs. However, recent studies have suggested that oligomers and protofibrils of αS are cytotoxic, and that LBs may represent a cytoprotective mechanism in PD. The histological hallmark of MSA is αS aggregates in the oligodendrocytes referred to as glial cytoplasmic inclusions (GCIls). αS inclusions are also found in the neuronal somata, axons and nucleus. At present, two degenerative processes have been considered in this disease; one is due to the widespread occurrence of GClls associated with oligodendrogia-myelin degeneration, and the other is due to the accumulation of αS in neurons in several brain regions. These two processes might synergistically cause neuronal depletion in MSA.

Key words: Parkinson’s disease; Lewy body; multiple system atrophy; glial cytoplasmic inclusion; α-synuclein.

Introduction

α-Synuclein (αS) was originally identified as a presynaptic nerve terminal protein. A direct role for αS in the pathogenesis of Parkinson’s disease (PD) was demonstrated by genetic evidence. A mutation was identified in the αS gene in a kindred with autosomal dominant PD. Two additional missense mutations and a triplication of the αS gene are also associated with the development of PD. It is now known that αS is a major component of Lewy bodies (LBs) in both sporadic and hereditary PD and dementia with Lewy bodies (DLB) 1,2 . Furthermore, αS immunoreactivity is also present in the neuronal and glial cytoplasmic inclusions found consistently in multiple system atrophy (MSA) 3,4 . Thus, LB diseases and MSA comprise a new disease concept, namely that of “α-synucleinopathies”. This review deals with the cellular pathology of α-synucleinopathies, with special reference to the relation of αS accumulation to neuronal degeneration.

What are Lewy bodies?

Morphology of Lewy bodies

Friederich H. Lewy first described eosinophilic neuronal inclusions in the nucleus basalis of Meynert and the dorsal vagal nucleus in patients with PD. The inclusions were named Lewy bodies (LBs) in his honour by Tretiakoff who confirmed their presence in the substantia nigra.

There are two types of LBs, the brainstem (classical) type and the cortical type 5 . Brainstem-type LBs are intracytoplasmic, single or multiple, spherical or elongated, eosinophilic masses possessing a dense core and a peripheral halo (Fig. 1A). Cortical LBs are also eosinophilic, but
are somewhat irregular structures often without a conspicuous halo or central core (Fig. 1B). Ultrastructurally, both brainstem-type and cortical LBs are composed of filamentous structures.\(^5\) The filaments resemble neurofilament, but are somewhat thicker than neurofilament and showed no side-arms, which are a characteristic feature of neurofilament. In the core of brainstem-type LBs, vesicular or circular structures are seen in addition to abnormal filaments.

In the substantia nigra and locus ceruleus, distinct neuronal inclusions called pale bodies are seen in the cytoplasm of pigmented neurons, showing well-defined, less eosinophilic, somewhat glassy areas without halo (Fig. 1C). Pale bodies frequently co-occur with LBs in the same neurons (Fig. 1C). Ultrastructurally, pale bodies contain sparse granular and vesicular structures, and filaments and are often found in close association with true LBs. These filaments are identical to those seen in LBs. Pale bodies are weakly positive for ubiquitin and are intensely immunolabeled with anti-αS\(^4\). Immunoelectron microscopy reveals that abnormal filaments constituting LBs and pale bodies are clearly recognized by anti-αS antibodies, whereas normal neurofilaments show no αS immunoreactivity\(^4\). The number of pale bodies is larger than that of LBs in the early stage of PD. There is a strong correlation between numbers of pale bodies and LBs. These findings suggest that pale bodies are closely associated with LB formation.

**Molecular components of Lewy bodies**

To date, more than 70 molecules have been identified in LBs\(^6\). The list of these molecules can be divided into several groups: (1) structural elements of the LB fibril; (2) αS-binding proteins\(^7\); (3) synphilin-1-binding proteins\(^8\); (4) components of the ubiquitin-proteasome system\(^9\); (5) proteins implicated in cellular responses; (6) proteins associated with phosphorylation and signal

![Figure 1](image-url)  
**Figure 1** Histopathological features in Lewy body disease. (A) Lewy body in the substantia nigra. (B) Lewy bodies in the cerebral cortex (arrowheads). (C) Pale body in the substantia nigra (asterisk). Lewy body is seen in the peripheral portion of pale body (arrowhead). (D) Lewy body in the substantia nigra. (E) Lewy neurites in the sympathetic ganglia. (F) Glial inclusions in the substantia nigra. (A-C) H&E stain, (D-F) phosphorylated α-synuclein immunostain.
transduction; (7) cytoskeletal proteins; (8) cell cycle proteins; (9) cytosolic proteins that passively diffuse into LBs; and (10) others. The composition of LBs may constitute an important clue about the mechanisms of formation and degradation of the inclusions.

**Distribution of Lewy bodies**

LBs are widely distributed in the central nervous system, including the hypothalamus, nucleus basalis of Meynert, substantia nigra, locus ceruleus, dorsal raphe nucleus, dorsal vagal nucleus, intermediolateral nucleus of the spinal cord, and sacral autonomic nucleus.4,5,11-13 LBs are also seen in the neurons of the amygdaloid nucleus and cerebral cortex, particularly in deep layers of the limbic system.14,15 Similar inclusions are also found in the peripheral autonomic nervous system, including the sympathetic ganglia, enteric nervous system of the alimentary tract, cardiac and pelvic plexuses, adrenal medulla, salivary gland and skin.4-7,16-21 The widespread distribution of LB pathology may correspond to a variety of motor and nonmotor symptoms of PD.

**α-Synuclein and Lewy body formation**

αS immunohistochemistry reveals that the process of classical LB formation consists of several stages.2 Under normal conditions, αS immunoreactivity is not seen in the neuronal cytoplasm. Stage 1 is observed as a diffuse, pale cytoplasmic staining often seen in morphologically normal-looking neurons. This pattern is the earliest immunohistochemically observable abnormality of αS accumulation. Stage 2 is observed as an irregularly shaped, uneven staining of moderate intensity in neurons that are often poorly pigmented. Stage 3 is a discrete staining corresponding to pale bodies. Pale bodies often display a peripheral condensation. One or more, small LBs are occasionally located within the periphery of the pale bodies. These "early LBs" develop into typical LBs, whereas the remains of the pale bodies appear to eventually disappear. Stage 4 is a ring-like staining of a typical LB with a central core and a surrounding halo. Thus, αS is involved even in the early stage of LB formation and pale bodies may serve the material for that LBs continue to expand. Recently, Mori et al. have reported that 10% of pigmented neurons in the substantia nigra in PD contained abnormal αS aggregates; diffuse cytoplasmic staining (5.8%) was detected more often than pale bodies (2.5%) or LBs (1.7%).22 In the locus ceruleus, 54.9% of pigmented neurons contained αS aggregates; diffuse cytoplasmic staining (32.6%) was seen more frequently than pale bodies (9.5%) or LBs (12.8%).22

Synphilin-1 was initially identified as an αS-interacting protein. In PD and DLB, synphilin-1 is mainly localized in LBs.7,8 NUB1 is a potent down-regulator of the ubiquitin-like protein NEDD8. Tanji et al. found that NUB1 physically interacts with synphilin-1 through its NEDD8-binding site, implying that NUB1 targets synphilin-1 to the proteasome for degradation.9 Moreover, NUB1 is accumulated in LBs and suppresses the formation of LB-like inclusions by proteasomal degradation of synphilin-1.9 NUB1 could be a potential therapeutic target for α-synucleinopathies.

**Progression of α-synuclein pathology in PD**

It is now known that αS is a major component of LBs and LB-related neuritic structures (Lewy neurites) in patients with PD and DLB. In addition, αS accumulated in LBs and Lewy neurites is phosphorylated.23 Phosphorylated αS immunohistochemistry is a useful tool to evaluate the cytoplasmic and neuritic pathology in α-synucleinopathies (Fig. 1D, E).24,25 αS-immunoreactive glial inclusions are also found in the brain of patients with PD and DLB (Fig. 1F).26 Braak et al. proposed an excellent staging
procedure for brain pathology related to sporadic PD\textsuperscript{27}. They devised a staging system for αS pathology, with six stages that characterize a progression from the dorsal vagal nucleus (stage 1), through the pontine tegmentum (stage 2), into the midbrain (stage 3), and then the basal procencephalon and mesocortex (stage 4), and finally though the neocortex (stages 5 and 6). Mori et al. have reported that abnormal αS begins to accumulate in the neostriatal neurons and glial cells in the relatively early stage of PD and that the severity of αS pathology correlates with the PD stage\textsuperscript{28}. Recently, Miki et al. reported a non-parkinsonian young adult who was proved at autopsy to have LBs and Lewy neurites in only the cardiac sympathetic nerve and stellate ganglia, suggesting that the pathological process of PD targets the peripheral autonomic nervous system at the same time or even before lower brainstem nuclei become involved\textsuperscript{29}.

**Lewy bodies and neurodegeneration**

Although the cause of sporadic PD remains unknown, aging, environmental factors, oxidative stress, mitochondrial dysfunction, genetic factors and dysfunction of ubiquitin-proteasome system may be involved in PD pathogenesis. Before the discovery of α-synuclein as the major component of LBs, the inclusions have been considered to be related to neurodegeneration by the following observations. (1) Significant loss of neurons is found in the predilection sites for LBs, particularly in the substantia nigra and locus ceruleus. (2) The number of LBs in patients with mild to moderate loss of neurons in the substantia nigra is higher than in patients with severe neuronal depletion, suggesting that LB-containing neurons may be dying neurons. (3) Cortical LB density could be one of the major correlates of cognitive impairment in PD and DLB. However, that LBs are related to neuronal loss does not imply that the inclusions are the cause of cell death.

Recent studies have suggested that oligomers and protofibrils of αS are cytotoxic, and that fibrillar aggregates of αS may represent a cytoprotective mechanism in PD. Lansbury and coworkers have shown that annular α-synuclein protofibrils bind to lipid bilayers and increase the membrane permeability by forming pore-like structures\textsuperscript{30,31}. McNaught and coworkers have hypothesized that LB formation is an aggresome-related process\textsuperscript{32}. Aggresomes are proteinaceous inclusions formed at the centrosome that segregate and facilitate the degradation of excess amounts of damaged, mutant and cytotoxic proteins. These findings suggest that LBs could be cytoprotective. However, another interpretation may be possible. If LB formation represents a response to sequester and degrade the toxic proteins, LBs continue to expand as long as the host cells produce toxic proteins. Such conditions cause excessive build-up of protein aggregates in the host cells, finally leading to cell death.

**Glial cytoplasmic inclusions in MSA**

**Discovery of glial cytoplasmic inclusions**

Glial cytoplasmic inclusion (GCI) is a histopathological hallmark of MSA, a sporadic neurodegenerative disorder characterized clinically by combinations of parkinsonism, cerebellar signs, autonomic failure and pyramidal signs. MSA was previously thought to be three separate diseases known as striatonigral degeneration, olivopontocerebellar atrophy and Shy-Drager syndrome. Graham and Oppenheimer first proposed the term MSA based on the finding that sporadic striatonigral degeneration, olivopontocerebellar atrophy and Shy-Drager syndrome can co-exist both clinically and pathologically\textsuperscript{33}. Papp et al.\textsuperscript{34} and Nakazato et al.\textsuperscript{35} demonstrated that GCIs are consistently found in the brain of patients with MSA regardless of clinical presentation. The discovery of GCIs confirmed that MSA is a single clinicopathological entity.
Morphology of GCIs

GCIs are argyrophilic and oligodendroglial in origin. They are triangular, sickle, half-moon, oval, or conical in shape. The nuclei of the GCI-bearing cells appear to be slightly larger and lighter than those of normal-looking oligodendrocytes. Immunohistochemical studies have shown that GCIs contain many substances, including αS, ubiquitin, tubulin and αB-crystallin. The cells containing GCIs also give positive staining with oligodendroglial markers, including carbonic anhydrase isoenzyme II, transferrin, Leu-7 and heat-stable tubulin polymerization promoting protein. Gallyas-Braak impregnation method and αS immunohistochemistry are the most sensitive techniques to demonstrate GCIs (Fig. 2A). Ultrastructurally, GCIs consist of randomly arranged, loosely packed, granule-coated fibrils approximately 25-40 nm in diameter. Filamentous inclusions are also found in the nuclei of GCI-bearing oligodendrocytes.

Distribution of GCIs

GCIs are widely distributed throughout the central nervous system. Although GCIs are more numerous in the areas showing neuronal loss and gliosis, as well as their targets, they are distributed even in regions in which neuropathological changes (neuronal loss, myelin loss, or astrocytosis) seem minimal or negligible.

High GCI density is seen in the motor and supplementary motor cortical areas, white matter subjacent to the motor cortical areas, caudate nucleus, putamen, globus pallidus, internal capsule, external capsule, pontine base, middle cerebellar peduncle, cerebellar white matter, and reticular formation of the brainstem.

Neuronal cytoplasmic inclusions in MSA

In addition to GCIs, neuronal inclusions have also been observed in MSA. Neuronal cytoplasmic inclusions (NCIs) were first described by Kato and Nakamura in the pontine and arcuate nuclei of patients with OPCA, using silver impregnation techniques such as Bielschowsky and Bodian staining. Subsequently, several investigators revealed that NCIs were also found in the putamen, substantia nigra, inferior olivary nucleus, motor cortex and dentate gyrus. The NCIs in the pontine nuclei are visible as round or ovoid inclusions (Fig. 2B), whereas those in the inferior olivary nucleus are reniform, crescent-shaped or coarse granular in shape. The NCIs in the dentate granule cells are ring-like or C-shaped inclusions. Moreover, neuronal nuclear inclusions (NNIs) are found in the same populations as the NCIs. The NNIs consist of fibrillary or thread-like structures, irregularly arranged near to the nuclear membrane, and are occasionally seen to fill the whole karyoplasm (Fig. 2C). Recently, Nishie et

Figure 2  Phosphorylated α-synuclein-immunoreactive inclusions in multiple system atrophy. (A) Glial cytoplasmic inclusions in the pontine base. (B) Neuronal cytoplasmic inclusion in the pontine nucleus. (C) Neuronal nuclear inclusion in the pontine nucleus.
al. have shown that NCIs were present in both the pontine and inferior olivary nuclei in all 14 patients with MSA. The incidence of NCIs in the inferior olivary nucleus (average 25.8%) was much higher than in the pontine nucleus (average 9.1%). In addition, NNIs were also found in all of the cases of MSA; the average incidence of NNIs in the pontine and inferior olivary nuclei was 9.2% and 9.0%, respectively. Thus, the frequent occurrence of NCIs and NNIs in the pontine and inferior olivary nuclei is a consistent histopathological finding in MSA.

Neurodegenerative mechanism in MSA

Distribution of neuronal loss in MSA

The most severe neuronal loss was found in the putamen, the substantia nigra, the pontine nuclei, the inferior olivary nuclei, the Purkinje cells, and the intermediolateral nuclei of the spinal cord. However, the neuropathology of MSA is more extensive than previously thought. Atrophy of the cerebrum, if any, is usually mild in MSA. However, some cases of MSA have been reported to show severe atrophy of the frontal or temporal lobes. Primary motor cortex and anterior horn of the spinal cord are also involved in MSA. Neuronal depletion has also been reported in some nuclei of the medulla oblongata.

GCI and neuronal degeneration

The occurrence of GCIs precedes neuronal degeneration in MSA. This notion is supported by some cases of “minimal change” MSA in which neuronal loss was restricted to the striatonigral or olivopontocerebellar system but GCIs were widely distributed in the central nervous system. The incidence of GCIs is correlated with the severity of neuronal loss in the olivopontocerebellar system as well as in the striatonigral system. Several investigators have reported that apoptosis occurs almost exclusively in oligodendrocytes and that extensive myelin damage is present in the MSA brain. Moreover, loss of myelin is much more severe than that of axons in the pontine base (transverse fibers), suggesting that loss of myelin precedes loss of axons in the disease process of MSA. Thus, widespread occurrence of GCIs may cause oligodendroglia- myelin degeneration (oligodendrogliopathy) in the central nervous system in MSA.

NCI and neuronal degeneration

The number of NCIs in the pontine nucleus of patients with moderate MSA is higher than in patients with mild MSA, and the number is decreased in patients with severe MSA. These findings suggest that in MSA, αS accumulation in the neuronal somata is accelerated by the progression of the disease and that NCI-containing neurons may be dying neurons. Moreover, transgenic mice expressing wild-type and mutant human αS develop motor impairments and exhibit α-synuclein aggregates in neuronal somata and processes, but not in oligodendrocytes. These findings suggest that NCIs also play a significant role in the neurodegenerative changes associated with MSA.

Acknowledgements

This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology, Japan and a Grant for Priority Research Designated by the President of Hirosaki University (to K.W.). The authors wish to express their gratitude to M. Nakata for her technical assistance.

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