IMMUNOHISTOCHEMICAL AND IMMUNOELECTRON MICROSCOPICAL INVESTIGATION OF TAU-POSITIVE NEURONS LACKING TANGLE-FORMATION IN PROGRESSIVE SUPRANUCLEAR PALSY

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ABSTRACT

Tau-positive neurons lacking tangle-formation (TNLT) show diffuse or fine granular cytoplasmic staining with the tau-immunohistochemistry, and are more frequently found in progressive supranuclear palsy (PSP) and corticobasal degeneration (CBD) than in Alzheimer's disease (AD). In this study, the regional incidence and ultrastructure of TNLT in 9 PSP cases were examined. The regional proportion of TNLT in tau-positive neurons was especially high in the hippocampus and amygdala, although the incidence of TNLT was dependent on the case and region. Ultrastructurally, numerous tau-positive ribosome-like granules were found in the perikarya and dendrites of TNLT, but paired helical filaments (PHF) and straight tubules were not identified. These findings suggest that TNLT in PSP or CBD are not the early stage of neurofibrillary tangles (NFT) unlike TNLT in AD, but are another form of abnormally phosphorylated tau.

Tau-positive neurons lacking tangle-formation (TNLT) have been so far described under various nomenclatures such as pretangle neurons (12, 25), neurons lacking neurofibrillary tangles (NFT) (23), stage 0 tangles (2) and diffuse tau staining (22), and have been identified in Alzheimer's disease (AD) (2, 9-12, 18, 19, 21, 22, 27), progressive supranuclear palsy (PSP) (6, 13, 15, 21, 23), corticobasal degeneration (CBD) (8, 16, 17, 25, 26), parkinsonism-dementia complex of Guam (11) and aged non-demented individuals (2, 14, 22). TNLT show diffuse or fine granular cytoplasmic staining with the Gallyas-Braak silver stain or the tau-immunohistochemistry (2, 12, 23), although they are morphologically normal-shaped neurons. Ultrastructurally, TNLT in AD have many ribosomes and diffusely scattered abnormal fibers within the neuronal perikarya. Some of these abnormal fibers are presented as paired helical filaments (PHF) and

others as straight fibers with granular materials (2, 19, 23). Bancher *et al.* (2) suggested that accumulation of abnormally phosphorylated tau is one of the earliest cytoskeletal changes in the process of tangle-formation in AD, and that TNLT represented an early stage of NFT formation. TNLT are more frequently found in CBD and PSP than in AD, and are particularly characteristic of CBD (6, 15-17, 25, 26). Nevertheless, it remains unclear whether TNLT are the early stage of NFT in CBD and PSP, because the ultrastructure of TNLT in CBD and PSP is largely unknown.

In this study, we investigated the regional incidence of TNLT with various anti-cytoskeletal protein antibodies and the ultrastructure of TNLT using tau-immunoelectron microscopy in PSP, and discussed the relationship between TNLT and NFT in PSP.

MATERIALS AND METHODS

In this study, 9 pathologically verified PSP cases

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Table 1 *Semiquantitative Analysis of the Regional Incidence of TNLT*

Case	Age (y)/Sex	Duration (y)	B.W. (g)	SFC	PC	EC	TEC	Hipp	Amy	Mey	Hypo	Pal	Sub
1	57/M	6	1,280	+	+	+	—	—	+	+	+	—	—
2	62/F	9	1,015	++	NA	++	++	+++	++	+	++	+	+
3	66/M	5	970	—	+	+	+	—	+	+	+	+	NA
4 ^a	67/M	3	1,160	++	++	++	++	+++	++	++	++	+	+
5	67/M	8	1,350	+	+	—	+	+	++	+	+	+	+
6	69/M	4	1,280	—	+	+	+	++	+	+	+	—	+
7	71/M	4	1,250	++	++	+	++	+++	++	+	+	+	++
8	76/M	10	1,200	+	+	+	+	++	++	+	+	+	++
9	80/F	5	1,150	—	—	+	+	++	NA	+	+	+	+

TNLT, tau-positive neurons lacking tangle-formation; B.W., brain weight; SFC, superior frontal cortex; PC, precentral cortex; EC, entorhinal cortex; TEC, transentorhinal cortex; Hipp, hippocampus; Amy, amygdala; Mey, Meynert nucleus; Hypo, hypothalamus; Pal, pallidum; Sub, subthalamus. —, none; +, a few; ++, moderate; +++, frequent; NA, not available

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(5) were examined (Table 1). The brains were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4. The cerebral hemispheric slices were embedded in paraffin, cut into 8 μ m-thick sections and stained with the hematoxylin-eosin, Klüver-Barrera, Holzer, methenamine-silver and Gallyas-Braak methods for pathological examination.

For the immunohistochemistry, 9 kinds of primary antibodies were used in this study (Table 2). Alz-50 and Human-tau, TF-11, and Ab71 are the antibodies for the amino half of tau, the middle region of tau, and the carboxy-terminal region of tau, respectively. Ab39 is the antibody for AD-NFT. SMI 31 and SMI 32 are the antibodies for phosphorylated and non-phosphorylated neurofilaments, respectively. MAB1510 and MAP2 are the antibodies for ubiquitin and microtubule-associated protein 2, respectively. These primary antibodies were detected using the avidin-biotinylated HRP complex (ABC) method (Elite Kit, Vector, U.S.A.) and visualized with DAB (Sigma, U.S.A.).

For the tau-immunoelectron microscopy, the tissue sample including the CA2–3 of the hippocampus from case 4 was cut into 40 μ m-thick vibratome sections and immunostained with Alz-50 (dilution, 1:50) by the ABC procedure. The sections were post-fixed with 2.5% glutaraldehyde and 2% OsO₄, embedded in epon, dissected in ultrathin sections and stained with lead citrate.

RESULTS

The immunohistochemical staining properties of

tau-positive neurons in the examined cases are summarized in Table 3. Tau-positive neurons consisted of NFT and TNLT. NFT demonstrated tangle-formation, but TNLT showed normal morphological appearance and diffusely granular cytoplasmic staining without tangle-formation (Fig. 1A). Alz-50 and Human-tau immunostained both NFT and TNLT, while Ab71 and Ab39 immunostained only a part of NFT. MAB1510 immunostained a part of NFT and a small number of TNLT.

Semiquantitative regional incidence of TNLT immunostained by Human-tau is shown in Table 1. The incidence of TNLT was dependent on the case and region. The regional proportion of TNLT in tau-positive neurons was especially high in the hippocampus and amygdala.

In the tau-immunoelectron microscopic findings of TNLT in the hippocampal CA2–3 (Fig. 1B), numerous tau-positive ribosome-like granules were

Fig. 1 A: NFT (arrow) and TNLT (arrowhead) in the amygdala (Human-tau immunostaining). B: TNLT and neuropil threads in the hippocampal CA2–3 (Alz-50 immunostaining)

Fig. 2 A: Immunoelectron microscopic appearance of a TNLT. $\times 2,200$. B: A higher magnification; numerous tau-positive ribosome-like granules are found in the perikaryon, mixed with the organelles. $\times 30,800$. C: Immunoelectron microscopic appearance of a neuropil thread, corresponding to the dendrite of TNLT. Arrow shows a presynaptic terminal. $\times 8,500$. D: A higher magnification; Many tau-positive ribosome-like granules and some tau-negative neurofilaments of 10–15 nm in diameter (arrows) are found. $\times 33,700$

Figs. 1 and 2

Table 2 *Antibodies Used for Immunohistochemical Studies*

Antibody	Species	Type	Isotype	Source	Dilution
Alz-50	Mouse	Monoclonal	IgM	Dr Davies	1:200
Human-tau	Rabbit	Polyclonal		Dr Ihara	1:2,000
TF-11	Mouse	Monoclonal		Dr Mori	1:30
Ab71	Mouse	Monoclonal	IgG ₁	Dr Yen	1:2,000
Ab39	Mouse	Monoclonal	IgG ₁	Dr Yen	1:2,000
MAB1510	Mouse	Monoclonal	IgG ₁	Chemicon	1:8,000
SMI31	Mouse	Monoclonal	IgG ₁	Sternberger	1:16,000
SMI32	Mouse	Monoclonal	IgG ₁	Sternberger	1:8,000
MAP2	Mouse	Monoclonal	IgG ₁	Chemicon	1:1,000

Table 3 *Immunohistochemical Staining Properties of Tau-Positive Neurons*

Antibody	NFT	TNLT
Alz-50	++	+/++
Human-tau	++	+/++
TF-11	+	±
Ab71	+	—
Ab39	+	—
MAB1510	+	±
SMI31	±	—
SMI32	—	—
MAP2	±	—

NFT, neurofibrillary tangles; TNLT, tau-positive neurons lacking tangle-formation; —, negative; ±, weakly positive; +, positive; ++, strongly positive

found in the perikarya of TNLT, mixed with the organelles and lipofuscin granules. A few neurofilaments of 10–15 nm in diameter were tau-negative, although they were partly coated with tau-positive granules (Fig. 2, A and B). PHF and straight tubules were not identified. Many tau-positive ribosome-like granules and some tau-negative neurofilaments were also found in the neuropil threads around TNLT, corresponding to the dendrites of TNLT (Fig. 2, C and D).

DISCUSSION

In this study, TNLT were identified in the cerebral cortex and subcortical nuclei of all the examined cases, although their incidence was dependent on the case and region. The distribution of TNLT was different from that of NFT in PSP (3, 4, 6, 7) and their proportion in tau-positive neurons was especially high in the hippocampus and amygdala.

With the tau-immunoelectron microscopy, numerous tau-positive ribosome-like granules were found in the perikarya and dendrites of TNLT, but tau-positive fibrillary structures including PHF and straight tubules were not identified in TNLT. These ultrastructural appearances are different from those of PSP- or CBD-NFT (1, 20, 24, 26, 28), suggesting that there is no transitional form between TNLT and NFT in PSP. The ultrastructure of TNLT in CBD is considered to be identical to that in PSP, due to the cytopathological similarity between PSP and CBD.

In this study, it is suggested from the distribution, antigenicity and ultrastructure of TNLT that TNLT in PSP or CBD are not the early stage of NFT unlike TNLT in AD (2, 19), but are another form of abnormally phosphorylated tau. In the future, the cytoskeletal abnormality should include the cytoskeletal protein abnormality without abnormal filaments.

This study was partly supported by a grant-in-aid for scientific research from the Ministry of Education, Science and Culture, Japan. We are grateful to Drs P. Davies, Y. Ihara, K. Mori and S.-H. Yen for their generous supply of antibodies.

Received 27 January 1998; and accepted 16 February 1998

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