Abnormal patterns of microtubule-associated protein-2 (MAP-2) immunolabeling in neuronal nuclei and Lewy bodies in Parkinson’s disease substantia nigra brain tissues

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Abstract

Parkinson’s disease (PD) is a neurodegenerative disorder associated with the appearance of cytoplasmic Lewy bodies (LBs) in dopaminergic neurons of the substantia nigra and the progressive loss of these neurons. Cytoskeleton alterations and associated impairments of neuronal transport may contribute to neuronal death. Microtubule-associated protein-2 (MAP-2), a cytoskeleton protein is localized primarily in neuronal dendrites and is known to stabilize microtubule assembly and mediate their interactions with other neuronal cell components. To determine if alterations in MAP-2 morphology are present in PD neurons, we used single and double immunohistochemical and immunofluorescent techniques to characterize MAP-2 in PD neuronal tissues. We report abnormal MAP-2 immunolabeling in some neurons of the substantia nigra of PD brain tissues, which were not observed in the normal, age-matched, control brain tissues. Furthermore, MAP-2 was co-localized with α-synuclein and ubiquitin in cytoplasmic LBs of neurons. Surprisingly, MAP-2 was also found to form fibrous aggregates and crystal-like structures within neuronal nuclei. These PD-associated alterations in MAP-2 morphology and distribution suggest that impaired neuronal transport may contribute to the progression of neuronal loss in the brains of PD patients. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

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Parkinson’s disease (PD) is a neurodegenerative disorder associated with the appearance of cytoplasmic Lewy bodies (LBs) in dopaminergic neurons of the substantia nigra and the progressive loss of these neurons [5,8,10]. Studies have indicated that these LBs consist largely of α-synuclein [5,8,10] and ubiquitin [7] and that they may be involved in the pathophysiology of PD. Since abnormal cytoskeletal proteins such as tau, microtubule-associated protein-2 (MAP-2) and neurofilaments are known to be associated with neuronal death, we used immunohistochemical techniques to examine the distribution of MAP-2 in substantia nigra of patients with PD. MAP-2 is a 280 kDa protein located primarily in neuronal dendrites that stabilizes microtubule assembly as well as mediating interactions with other neuronal cell components. We observed abnormal MAP-2 morphology in some of the neurons of the PD substantia nigra.

Substantia nigra tissues from PD (n = 5, mean age, 73.9 ± 1.5, mean postmortem time interval, 11.7 ± 1.4 h) and normal age-matched control brains (n = 2, mean age, 71.6 ± 1.4, mean postmortem time interval, 10 ± 2.0 h) were obtained from the Harvard Brain Tissue Resource Center (HBTRC, Belmont, MA, USA) and fixed in a 10% neutral-buffered formalin solution. The diagnosis of PD (encephalopathy, degenerative, marked, consistent with PD) had been made by established criteria [3,4,6,12] of the HBTRC, which is supported in part by a Public Health Service grant #MH/NS 31862. Tissues were embedded in paraffin and serial sections were cut (5 μm) and mounted onto SuperFrost slides (Fisher Scientific, Pittsburgh, PA). Sections were deparaffinized, hydrated and processed for single and double immunohistochemistry (IHC) [1] as well as double immunofluorescence (IF) [2] protocols. Primary antibodies included two commercially obtained anti-MAP-2 antibodies (monoclonal, 1:100, Zymed Laboratories Inc., South San Francisco, CA; monoclonal, 1:500, Sigma, St. Louis, MO), anti-α-synuclein (polyclonal, 1:1000, Chemicon, Temecula, CA) and anti-ubiquitin (poly-
Antibodies were detected as follows: α-synuclein was detected using the HRP: avidin-biotin complex (ABC, Vector Labs, Burlington, CA); 3,3′-diaminobenzidine (DAB, Biomeda, Foster City, CA, USA) detection system. Double IHC antibodies were detected as follows: α-synuclein was detected with a Texas red-conjugated secondary antibody (Vector Labs); MAP-2 was detected using a FITC-conjugated secondary antibody (Vector Labs). Slides for double IF were coverslipped using DAPI (4′, 6 diamino-2-phenylindole) aqueous coverslipping media (Vector Labs).

Cytoplasmic LBs with α-synuclein immunolabeling were observed in neurons of the substantia nigra in PD brains (Fig. 1A, large arrowhead), but were not present in the same neurons of control brains (data not presented), consistent with previous reports [5,8,10]. Hematoxylin-stained (blue) nuclei were often observed compressed or displaced by large, α-synuclein-labeled LBs (Fig. 1A, small arrowhead). The aggregates of dark blue nuclear chromatin as well as the unusually light staining of most of the nucleoplasm was consistent with nuclear changes associated with cell stress or impending degeneration [9,13] as cell nuclei can break down in three ways: it can slowly lose its affinity for basic dyes and fade away (karyolysis); it can shrivel into a dense, highly basophilic mass (pyknosis) or it can become pyknotic and then break up into small pieces (karyorrhexis). Pyknosis and karyokenexis are important because they suggest that the cell may have committed suicide or apoptosis [9,13]. In any case, these nuclear observations in the cells with LBs suggest a relationship between the presence of cytoplasmic α-synuclein-positive LBs and abnormal nuclear morphology.

The LBs were also strongly ubiquitin-positive (Fig. 1B, large arrowhead), which is consistent with previous reports [7]. No detectable ubiquitin immunolabeling was observed in cells of the substantia nigra tissues of control brains (data not shown). In some cases, abnormal nuclear morphology was also observed in cells possessing cytoplasmic ubiquitin-positive LBs (Fig. 1B, small arrowhead). The presence of a nearby, blue hematoxylin-stained, normally appearing euchromatic neuronal nucleus (Fig. 1B, open arrow) rules out the possibility that these nuclear changes are artifacts due to post-mortem tissue changes from autolysis or fixation associated factors.

MAP-2 antibodies immunolabeled the neuronal dendrites in normal, age-matched control tissues as reported (data not presented). In serial sections of substantia nigra tissue from PD brains, the MAP-2 labeling (top, arrowheads) appeared co-localized with α-synuclein (bottom, arrowheads) in the dendrites of neurons (Fig. 1C). In addition to the normal MAP-2 localization in dendrites, serial section analysis also showed the presence of MAP-2 immunolabeling (top, large arrowhead) in α-synuclein LBs (bottom, large arrowhead) (Fig. 1D). Additional examples of large, spherically shaped, extracellular, MAP-2-positive LBs (arrowheads) are presented in Fig. 1E. Most of these LB-like structures were fairly uniform in size and remarkably uniform in shape, and were either homogeneously MAP-2-positive throughout most of their volume (Fig. 1E, top panels) or showed a core region that was almost completely devoid of MAP-2 immunoreactivity (Fig. 1E, bottom panels), which may represent grazing histological sections of a somewhat thick MAP-2 shell. Using double IHC (Fig. 1F) and double IF (Fig. 1G) methods, we confirmed the localization of MAP-2 in α-synuclein positive LBs. Although these MAP-2 LBs (Fig. 1E,F) were generally located extracellularly, we observed clear examples of MAP-2 positive structures intracellularly as well (Fig. 1H, arrowheads). Surprisingly, peculiar irregular, crystal-like or circular LB-like structures of MAP-2 immunolabeling observed in the nuclei of some neurons of the substantia nigra in the PD tissues (Fig. 1I, arrowheads), which were not observed in other areas of the PD brain neuronal nuclei (Fig. 1J, arrowheads). Although it is possible that these structures may be present within cytoplasmic infoldings that protrude into the space formerly occupied by the nucleoplasm and not in the nuclei, our histological sections suggest these structures are present in the nuclei as the nuclei in these affected cells did not possess obvious hematoxylin, basophilic staining.

Immunohistochemistry has been a useful tool for identifying the elements of LBs of the substantia nigra in PD, especially since attempts to isolate LBs from the substantia nigra using biochemical methods have not been successful [11]. Our study is the first to demonstrate MAP-2 in the α-synuclein-, ubiquitin-positive LBs in neurons of the substantia nigra in PD brains. Furthermore, we also observed crystal-like or spherical LB-like MAP-2 labeling in some of the neuronal nuclei. The significance of MAP-2 in these structures is unknown, but it may be a consequence of one or more global alterations in cytoskeletal structure that may contribute to neurodegeneration in PD.

The presence of α-synuclein, ubiquitin and MAP-2 in LBs may indicate that one or both of these proteins are ubiquinatated, and thus targeted for destruction. The presence of these LBs prompts us to speculate that affected neurons in PD brains display an abnormal or overactive ubiquination system, which results in the accumulation of these and other proteins within LBs. Similarly, in patents with myotonic dystrophy, moderately intense immunoreactivity for ubiquitin and MAP-2 were identified in cytoplasmic LBs of their substantia nigra neurons [11]. They suggested that an alteration of neuronal cytoskeleton metabolism is accompanied by changes in ubiquitin proteolytic systems [11]. However, in contrast to their observations, we observed displaced and invaded nuclei in the affected neurons.

It is possible that these unique, altered labeling patterns in the MAP-2 neuronal cytoskeleton in the substantia nigra neurons represent impaired transport associated with PD.
Fig. 1. (A) Immunohistochemical localization of intracytoplasmic α-synuclein-positive Lewy bodies (LBs) (large arrowhead) in a Parkinson’s disease (PD) substantia nigra neuron. Small arrowhead indicates that the nucleus of this cell with abnormal morphology. (B) Immunohistochemical localization of a cytoplasmic ubiquitin-positive LB (large arrowhead) in a PD substantia nigra neuron. Small arrowhead shows the associated nucleus with abnormal morphology. Below is a neighboring neuron (open arrow) with normal nuclear chromatin morphology. (C) Serial sections of PD substantia nigra tissues show co-expression of MAP-2 (top, arrowheads) and α-synuclein (bottom, arrowheads) in normally appearing dendritic processes. (D) Serial sections with same vessel (asterisk) and neuron (small arrowhead) show that the presence of MAP-2 (top, large arrowhead) may be co-localized in an α-synuclein LB (bottom, large arrowhead). (E) Examples of circular, LB-like MAP-2 labeling structures (arrowheads) in the substantia nigra of the PD tissues. (F) Co-localization of α-synuclein (blue) and MAP-2 (red) (large arrowheads) labeling in LBs using double IHC. Note a nearby area of pigmented lipofuscin, which was not affected by the double IHC reagents (asterisk). Also note MAP-2 red processes around the double labeled LBs (small arrowheads). (G) Co-localization (bottom right, solid arrow) of red-fluorescent α-synuclein (top right, solid arrow) and green-fluorescent MAP-2 (top left, solid arrow) in a LB beside DAPI-positive (bottom left panel, open arrows) nuclei using double IF. (H) Representative examples of intracellular MAP-2 immunolabeling in PD substantia nigra neurons (arrowheads). (I) Presence of irregular, crystal-like, MAP-2-positive structures (left panels labeled with Sigma’s MAP-2 antibody, right panels labeled with Zymed’s MAP-2 antibody) in the cell nuclei (arrowheads) of PD substantia nigra neurons. (J) Normal neurons are observed in non-affected areas of the PD brain showing ordinary MAP-2 intracellular labeling (arrowheads). Note that the morphology of the affected PD neurons (I) contrast these ‘normally’ appearing neurons with features, such as clear nuclear membrane borders, prominent nucleoli, and centrally located circular nuclei. The presence of these features rule-out fixation and post-mortem artifacts. (Scale bars, 12 μm).
Whether nuclear MAP-2 immunoreactivity contributes to the manifestations of PD or is a consequence of neurodegeneration in the pathogenesis of PD remains to be determined. Since we observed MAP-2 labeled structures in the nuclei of substantia nigra neurons without intracytoplasmic α-synuclein-positive LBs, we speculate that either two distinct pathologies (abnormal α-synuclein and abnormal MAP-2 dependent) exist in these neurons or the bizarre nuclear MAP-2 labeled structures precede the formation of cytoplasmic LBs and that these observations are assembled over time. It is interesting to note that the average sizes of these extracellular MAP-2, α-synuclein-positive LBs (Fig. 1E) are roughly the same size of a substantia nigra neuronal nucleus (Fig. 1J), a feature not observed with the intracytoplasmic MAP-2 structures (Fig. 1H), which are generally smaller that the size of a neuronal nuclei (Fig. 1J). The connection between abnormal intranuclear crystal-like MAP-2 structures (Fig. 1I), the abnormal intracellular aggregations of MAP-2 LBs (Fig. 1H) and the presence of MAP-2 in α-synuclein LBs without a clear cellular structure (Fig. 1E) in the substantia nigra of PD brain tissues remains to be determined. Nevertheless, our data indicates that MAP-2 immunoreactivity may prove to be a useful immunohistochemical tool as an additional neuropathological marker for PD and may provide novel insight into the pathogenesis of neuronal cell loss in PD.

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