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The complex relationship between soluble and insoluble tau in tauopathies revealed by efficient dephosphorylation and specific antibodies

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Abstract Phosphorylated tau is deposited as insoluble inclusion bodies in the tauopathies. We have used a new efficient method to dephosphorylate tau extracted from control and tauopathy brain. In some tauopathies, including Alzheimer's disease and progressive supranuclear palsy, the pattern of insoluble tau isoforms reflected that of soluble tau. In contrast, in corticobasal degeneration, Pick's disease, and some forms of fronto-temporal dementia, specific tau isoforms were selectively sequestered into insoluble inclusion-forming tau. Therefore the overall expression of individual tau isoforms does not predict which tau isoforms are deposited in all tauopathies and different mechanisms must operate that result in the deposition of specific tau isoforms.

Key words: Tau; Alzheimer's disease; Tauopathy; Neurodegeneration; Paired helical filament; Lambda protein phosphatase

1. Introduction

Tau protein in human brain exists as a series of six isoforms generated by alternative splicing from a single gene. Amino-terminal inserts of 0 (0N), 29 (1N), or 58 (2N) amino acids, in combination with three (3R) or four (4R) microtubule-binding repeat regions distinguishes the tau isoforms [1].

The neurodegenerative diseases including Alzheimer's disease (AD), Pick's disease (PiD), progressive supranuclear palsy (PSP), corticobasal degeneration (CBD) and fronto-temporal dementia with Parkinsonism linked to chromosome 17 (FTDP-17) have been collectively termed the tauopathies due to the presence of pathological deposits of tau in affected brain regions. Insoluble tau deposited in AD was found to differ from soluble tau by increased phosphorylation that resulted in reduced electrophoretic mobility of insoluble tau [2,3]. AD is the best characterised of the tauopathies, and in this case the paired helical filaments (PHF) that form characteristic neurofibrillary tangles are comprised of insoluble tau (PHF-tau). On Western blots, PHF-tau appears as three major bands and a fourth minor tau species [4]. The different patterns of insoluble tau obtained on blots of tauopathy brains have been suggested to be the result of differential tau isoform expression [5,6]; we report here data showing that this is probably an over-simplification.

Dephosphorylation of tau with alkaline phosphatase has been used to show that, compared to soluble tau from control brain, PHF-tau and insoluble tau from other tauopathies is abnormally phosphorylated [3,5,7–11]. Dephosphorylation of PHF-tau using *Escherichia coli* alkaline phosphatase is currently the most widely reported method [12]. However, this method can result in incomplete dephosphorylation of PHF-tau and poor yield, possibly due to the need for incubation of solubilised and denatured PHF-tau at elevated temperature.

Bacteriophage lambda protein phosphatase is a dual-specificity protein serine–threonine and tyrosine phosphatase that is also active on phosphorylated histidine residues [13,14] and has been reported to dephosphorylate tau extracted from neuroblastoma cells [15]. We found that lambda protein phosphatase dephosphorylates soluble and insoluble tau extracted from tauopathy brain more efficiently than alkaline phosphatase. We have used this viral enzyme in combination with a panel of tau isoform-specific antibodies to investigate phosphorylation patterns of individual tau isoforms extracted from human brain.

2. Materials and methods

2.1. Human brain tissue

Tau was extracted from neurologically and neuropathologically confirmed cases of control, AD, FTDP-17 (+16 intronic and G389R tau mutations), PiD, PSP and CBD brain. All tissues were taken from frontal lobe with the exception of PSP (pons) and CBD (amygdala). Tissue was obtained from the MRC London Neurodegenerative Diseases Brain Bank at the Institute of Psychiatry, King's College London, UK and the Queen Square Brain Bank for Neurological Disorders at the Institute of Neurology, London, UK.

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Abbreviations: AD, Alzheimer's disease; CBD, corticobasal degeneration; FTDP-17, fronto-temporal dementia with Parkinsonism linked to chromosome 17; PHF-tau, paired helical filament-tau; PiD, Pick's disease; PSP, progressive supranuclear palsy; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis

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2.2. Preparation of soluble tau

Human brain tissue was homogenised in 4 vol. 100 mM MES buffer, pH 6.5, containing 1 M NaCl and complete protease inhibitor (Roche) and centrifuged at 27 000×*g* for 30 min at 4°C. The supernatant was centrifuged at 100 000×*g* for 1 h at 4°C, the 100 000×*g* pellet containing insoluble tau was retained (see below) and the 100 000×*g* supernatant contained soluble tau. In the case of control brain, soluble tau was enriched further by heating for 10 min at 100°C and centrifuging at 15 000×*g* for 30 min at 4°C. The supernatant, containing heat-stable soluble proteins, was made to 45% saturation with ammonium sulphate, maintained in ice for 15 min, and centrifuged at 15 000×*g* for 30 min at 4°C. Precipitated proteins were resuspended in 0.1 vol. (relative to weight of starting brain material) 50 mM Tris–HCl, pH 7.5, and dialysed against the same buffer overnight at 4°C. The dialysate was clarified by centrifugation at 15 000×*g* for 30 min at 4°C, and the supernatant, containing soluble heat-stable control brain tau, was retained.

2.3. Preparation of guanidine-solubilised tau

Guanidine-solubilised tau was extracted from the 100 000×*g* pellet of the same brain tissue specimens used for the preparation of soluble tau as described previously [16]. Guanidine-solubilised tau was dialysed against 50 mM Tris–HCl, pH 7.5, overnight at 4°C and centrifuged at 100 000×*g* for 1 h at 4°C to remove precipitating material.

2.4. Preparation of recombinant human tau

The six isoforms of human tau were expressed individually as recombinant proteins in *E. coli* and purified as described previously [17].

2.5. Dephosphorylation of tau with lambda protein phosphatase

Human tau preparations in 50 mM Tris–HCl, pH 7.5, were dephosphorylated with 20 U/μl lambda protein phosphatase (New England Biolabs) for 3 h at 30°C. Reactions were stopped by the addition of 2× sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) sample buffer and heating for 5 min at 100°C.

2.6. Tau antibodies

TP70 and B19 are polyclonal antibodies recognising all isoforms of tau [18,19]; RD3 and RD4 are monoclonal antibodies that label tau isoforms containing three or four microtubule-binding repeats, respectively (de Silva et al., submitted) [20]; BR10 is a rabbit polyclonal antibody that labels only tau isoforms containing an amino-terminal insert of 58 residues (2N tau), raised to the peptide C-APGKQAAAQPHTEIPEGT, corresponding to residues 84–101 in human tau (numbering of the largest human tau isoform) with the addition of an amino-terminal cysteine [21]. The epitopes recognised by the monoclonal antibodies to tau, Tau.1 and PHF-1, have been described previously [22,23].

2.7. SDS–PAGE and Western blotting

Tau preparations were resolved on 7.5% or 10% (w/v) polyacrylamide gels, electroblotted onto polyvinylidene fluoride or nitrocellulose membranes and probed with antibodies to tau. Blots were developed using horseradish peroxidase or alkaline phosphatase-conjugated anti-rabbit or anti-mouse secondary antibodies.

3. Results and discussion

Soluble tau from control human brain is dephosphorylated readily by lambda protein phosphatase and co-migrates with the six recombinant human tau isoforms (Fig. 1A, TP70, +). RD3 and RD4, the antibodies recognising tau with either three (3R) or four (4R) microtubule-binding repeat domains, respectively, each labelled different triplets of dephosphorylated control brain tau with electrophoretic mobilities co-migrating precisely with recombinant tau isoforms corresponding to 3R and 4R tau (Fig. 1A, RD3 and RD4, +). RD3 and RD4 strongly labelled the 0N and 1N tau isoforms and more weakly labelled the 2N tau isoforms, confirming the lower level of expression of 2N tau isoforms in human brain [24]. Surprisingly however, RD3 and RD4 each labelled more than three bands of untreated control brain tau (Fig. 1A, RD3 and RD4). RD3 labelled two major bands of untreated soluble tau, apparently co-migrating with 1N3R and 1N4R tau, as well as several more diffuse bands, some of which did not co-migrate with recombinant tau isoforms. RD4 labelled four bands of approximately equal intensity that did not co-migrate with the recombinant human tau isoforms. The increase in electrophoretic mobility of soluble tau following dephosphorylation was much more marked with RD3 and RD4 than with TP70 labelling as the individual sets of dephosphorylated three- and four-repeat tau isoforms were well discriminated by the isoform-specific antibodies. The results with RD3 and RD4 show that individual tau isoforms extracted from post-mortem human brain exist as species with multiple electrophoretic mobilities due to differences in their patterns or degrees of phosphorylation.

Antibody BR10 is a polyclonal antibody specific for 2N tau species as shown by the labelling of only the two uppermost bands in the mixture of all six recombinant human tau isoforms (Fig. 1A, BR10, R). Antibody labelling of dephosphorylated soluble tau from adult mouse brain, containing predominantly 4R tau (Fig. 1B, B19), confirms the specificity of BR10 for 2N tau and the lack of labelling of the smaller 1N and 0N tau isoforms by this antibody (Fig. 1B, BR10). In human brain, BR10 also labels a more diffuse band migrating close to the position of the smallest tau isoform in human brain (Fig. 1A, BR10). This species may be a degradation product of 2N tau as its mobility is slightly increased following dephosphorylation and minor degradation products of 4R tau are visible following dephosphorylation and labelling with RD4. This species is not due to a cross-reaction of BR10 with

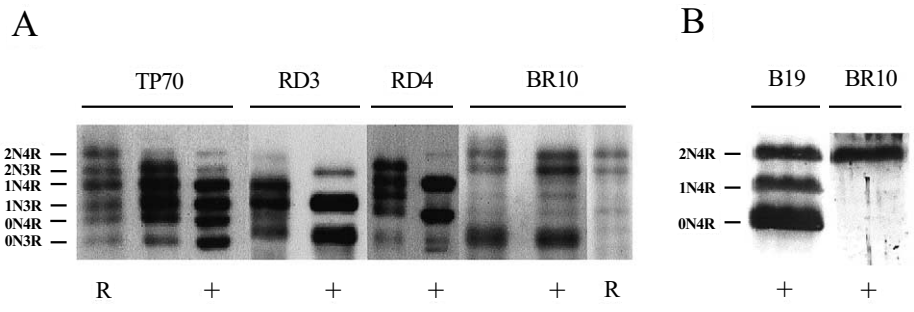

Fig. 1. Isoforms of soluble tau in control human brain exist in multiple phosphorylation states. Soluble tau from control human (A) or mouse (B) brain was untreated or dephosphorylated (+) and proteins were separated on 10% (w/v) SDS–PAGE. Western blots were probed with the pan-tau antibodies, TP70 or B19, or with the tau isoform-specific antibodies, RD3, RD4, or BR10. Alignment of dephosphorylated tau with the mixture of recombinant tau isoforms (R) shows the specificity of the tau isoform-specific antibodies.

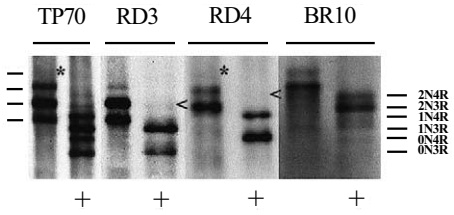

Fig. 2. Dephosphorylation of PHF-tau with lambda protein phosphatase. PHF-tau from AD was dephosphorylated (+), separated on 10% (w/v) SDS–PAGE and blots were probed with tau antibodies, TP70, RD3, RD4 or BR10. Asterisks indicate the minor PHF-tau isoform which is difficult to discern. Arrowheads indicate the weak bands labelled by RD3 and RD4 after dephosphorylation of PHF-tau. The positions of the four PHF-tau bands are indicated on the left and the six recombinant human tau isoforms on the right.

other tau isoforms as shown by its selective labelling of 2N tau in the recombinant human tau mixture. Thus, BR10 is specific for 2N tau isoforms and the minor increase in electrophoretic mobility following dephosphorylation of 2N tau, compared to that of 0N and 1N tau observed with RD3 and RD4, implies that the phosphorylation of 2N tau may be less heterogeneous or different to that of the smaller 0N and 1N tau isoforms.

It has previously been assumed that for soluble tau from control brain, the six bands observed on Coomassie Blue-stained polyacrylamide gels and Western blots labelled with pan-tau antibodies correspond to the six tau isoforms [25–29]. Our results show instead that the tau species visible on gels of soluble tau from control brain consist of a heterogeneous mixture of individual tau isoforms in multiple states of phosphorylation. Because electrophoresis of untreated tau does not separate the individual tau isoforms, correct identification of the isoform composition of soluble tau therefore requires an efficient dephosphorylation reaction, followed by labelling with tau isoform-specific antibodies.

Guanidine-solubilised PHF-tau from AD brain was dephosphorylated with lambda protein phosphatase and compared with untreated PHF-tau. Dephosphorylated PHF-tau labelled with TP70 appeared as a pattern of six bands with electrophoretic mobilities and intensities essentially indistinguishable from dephosphorylated soluble tau extracted from control brain (compare Fig. 1, control and Fig. 2, AD, TP70, +). This result indicates that, in the case of AD, aggregation of tau does not depend on altered isoform composition but instead particular phosphorylation profiles may regulate the propensity of tau to aggregate into PHF. The overall amount of PHF-tau immunoreactivity visible on blots following dephosphorylation was equal to that of untreated PHF-tau incubated for the same time at the same temperature, indicating that PHF-tau is not degraded by contaminating proteases during incubation with this phosphatase. Probing with the monoclonal antibodies to tau, Tau.1 and PHF-1 showed that dephosphorylated PHF-tau was labelled by Tau.1, but not by PHF-1, consistent with the dephosphorylation of tau at these two epitopes by lambda protein phosphatase (not shown). Thus, dephosphorylation of PHF-tau by lambda protein phosphatase is efficient and the recovery of tau is improved, at least in our hands, compared to previously reported methods using *E. coli* or calf intestinal alkaline phosphatases [2,3,12].

Antibody RD3 labelled the three lower bands of untreated PHF-tau that contain the 3R tau isoforms (Fig. 2, RD3) but not the minor 72-kDa band of PHF-tau faintly labelled by TP70 (asterisk), as this is comprised of 2N4R tau. After dephosphorylation, RD3 labelled a triplet of tau species similar to that seen in soluble tau from control brain, the uppermost species, corresponding to 2N3R tau, exhibiting the weakest labelling (Fig. 2, RD3, +, arrowhead). RD4 labelled the upper two major bands of untreated PHF-tau as well as the minor 72-kDa species (Fig. 2, RD4, asterisk). Dephosphorylated PHF-tau resulted in RD4 labelling bands that aligned with 0N4R and 1N4R tau with weak labelling of 2N4R (Fig. 2, RD4, +, arrowhead). BR10 labelled the upper major band of untreated PHF-tau and the 72-kDa species (Fig. 2, BR10). After dephosphorylation BR10 labelled two bands corresponding to 2N3R and 2N4R tau as well as a few minor degradation products. Probing untreated PHF-tau with tau isoform-specific antibodies shows that the lower and middle major bands of PHF-tau contain 3R tau, with a small contribution to the upper major band; the middle and upper major bands contain 4R tau, with an additional minor band (72 kDa) running above this, and the upper major and the 72-kDa band both contain 2N tau. This result confirms previous work from our laboratory identifying the isoform composition of PHF-tau [17].

Guanidine-solubilised PHF-tau has been dephosphorylated successfully using *E. coli* alkaline phosphatase at 67°C [12]; however, at least in our hands, this method often results in poor yields, possibly due to the activity of contaminating proteases and the increased temperature. We have found previously that the specific activity of *E. coli* and calf intestinal phosphatases and relative resistance of PHF-tau to dephosphorylation is such that significant amounts of phosphatase protein are required and these are present on gels following electrophoresis. These phosphatases have similar electrophoretic mobilities to untreated PHF-tau (calf intestinal phosphatase) and dephosphorylated tau (*E. coli* phosphatase) and thus they interfere with the resolution of tau species. It has proved difficult therefore to obtain well-resolved bands of untreated and dephosphorylated PHF-tau treated with these two phosphatases. However, using lambda protein phosphatase, dephosphorylated PHF-tau is obtained in good yield and appears to be dephosphorylated efficiently using the conditions described. Furthermore, lambda protein phosphatase (25 kDa) on gels does not interfere with the tau pattern obtained after dephosphorylation. The degree of dephosphorylation of PHF-tau achieved following incubation with lambda protein phosphatase appears to be comparable to that of hydrogen fluoride treatment [8]. Since hydrogen fluoride is a hazardous reagent that cannot be used routinely in many laboratories, dephosphorylation of tau by lambda protein phosphatase represents a significant improvement on methods in current use. Lambda protein phosphatase has been used previously to dephosphorylate tau in extracts from a neuroblastoma cell line but, to our knowledge this is the first report of this enzyme being used to dephosphorylate tau extracted from human brain [15]. The successful dephosphorylation by lambda protein phosphatase of guanidine-solubilised tau suggests that this enzyme might also be used to dephosphorylate other proteins that are relatively resistant to alkaline phosphatases.

Lambda protein phosphatase dephosphorylated tau from the different tauopathies and the isoform composition of soluble and guanidine-solubilised fractions in these brains was

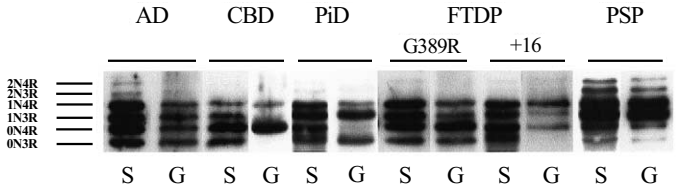

Fig. 3. Isoform composition of tau extracted from soluble and guanidine-solubilised fractions from different tauopathies. Soluble (S) and guanidine-solubilised (G) tau extracted from AD, CBD, PiD, FTDP-17 (G389R and +16 intronic mutations), and PSP brain tissue was dephosphorylated and separated on 7.5% (w/v) polyacrylamide gels. Western blots were probed with TP70. The positions of the six recombinant human tau isoforms are indicated on the left.

compared (representative cases in Fig. 3). We found that none of the tauopathy cases examined showed guanidine-solubilised tau consisting exclusively of either 3R or 4R tau isoforms, consistent with recent reports [10,30] and conflicting with previous studies on untreated insoluble tau [31,32]. AD tau contained all six isoforms and there was no difference in patterns between soluble and guanidine-solubilised fractions (Fig. 3, AD). Guanidine-solubilised tau from CBD brain consisted predominantly of 0N4R with a lesser amount of 1N4R tau and negligible amounts of the other tau isoforms (Fig. 3, CBD). This was in contrast to soluble CBD tau that appeared to contain slightly more 0N4R tau but otherwise similar tau isoform ratios to control brain. PiD showed a soluble tau pattern that was similar to that of control brain, with a slight reduction in 0N3R tau, whereas the guanidine-solubilised fraction of PiD contained increased 3R isoforms (Fig. 3, PiD). This finding implicates primarily 1N3R tau, as well as lesser amounts of 0N3R, 1N4R, and 2N3R tau in the formation of tau-positive Pick bodies and confirms recent reports of 4R tau in PiD [11,33]. Soluble FTDP-17 (G389R) tau did not contain increased 4R tau despite the guanidine-solubilised tau from this brain having a marked increase in 0N4R tau, whereas tau from FTDP-17 (+16) brain exhibited an increased 4R/3R tau ratio in both soluble and guanidine-solubilised fractions (Fig. 3, FTDP lanes). Soluble and guanidine-solubilised tau from PSP brain gave similar isoform patterns after dephosphorylation, with a predominance of 1N3R and 1N4R isoforms, and a reduced amount of 0N3R tau, but no overall increase in 4R tau isoforms (Fig. 3, PSP). The consistent finding of reduced or absent 0N3R tau in PSP is interesting and mirrors a previous report in CBD [34], however, we found differences in the overall compositions of the tau isoforms in PSP and CBD.

Therefore only in CBD and FTDP-17(+16) did we detect overt alterations of soluble tau isoform expression that were reflected in guanidine-solubilised tau. It appears that, at least in the brain regions examined here, there may be sequestration of specific tau isoforms into insoluble (and hence inclusion-forming) tau in CBD, PiD and FTDP-17, but in AD and PSP such a selective sequestration may not occur. Therefore, further work is required to identify the mechanisms giving rise to the deposition of tau inclusions in the tauopathies, such mechanisms may include subtle changes in isoform expression, post-translational modification, and/or selective incorporation of isoforms into insoluble tau.

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References

[1] Goedert, M., Spillantini, M.G., Jakes, R., Rutherford, D. and Crowther, R.A. (1989) Neuron 3, 519–526.
[2] Flament, S., Delacourte, A., Hemon, B. and Defossez, A. (1989) C. R. Acad. Sci. III 308, 77–82.
[3] Hanger, D.P., Brion, J.-P., Gallo, J.-M., Cairns, N.J., Luthert, P.J. and Anderton, B.H. (1991) Biochem. J. 275, 99–104.
[4] Brion, J.-P., Hanger, D.P., Couck, A.M. and Anderton, B.H. (1991) Biochem. J. 279, 831–836.
[5] Hong, M., Zhukareva, V., Vogelsberg-Ragaglia, V., Wszolek, Z., Reed, L., Miller, B.I., Geschwind, D.H., Bird, T.D., McKeel, D., Goate, A., Morris, J.C., Wilhelmsen, K.C., Schellenberg, G.D., Trojanowski, J.Q. and Lee, V.M. (1998) Science 282, 1914–1917.
[6] Buee, L. and Delacourte, A. (1999) Brain Pathol. 9, 681–693.
[7] Flament, S., Delacourte, A., Hemon, B. and Defossez, A. (1989) J. Neurol. Sci. 92, 133–141.
[8] Greenberg, S.G., Davies, P., Schein, J.D. and Binder, L.I. (1992) J. Biol. Chem. 267, 564–569.
[9] Hasegawa, M., Morishima-Kawashima, M., Takio, K., Suzuki, M., Titani, K. and Ihara, Y. (1992) J. Biol. Chem. 267, 17047–17054.
[10] Liu, W.K., Le, T.V., Adamson, J., Baker, M., Cookson, N., Hardy, J., Hutton, M., Yen, S.H. and Dickson, D.W. (2001) Ann. Neurol. 50, 494–502.
[11] Arai, T., Ikeda, K., Akiyama, H., Shikamoto, Y., Tsuchiya, K., Yagishita, S., Beach, T., Rogers, J., Schwab, C. and McGeer, P.L. (2001) Acta Neuropathol. 101, 167–173.
[12] Goedert, M., Spillantini, M.G., Cairns, N.J. and Crowther, R.A. (1992) Neuron 8, 159–168.
[13] Cohen, P.T. and Cohen, P. (1989) Biochem. J. 260, 931–934.
[14] Zhuo, S., Clemens, J.C., Hakes, D.J., Barford, D. and Dixon, J.E. (1993) J. Biol. Chem. 268, 17754–17761.
[15] Davis, P.K. and Johnson, G.V. (1999) J. Biol. Chem. 274, 35686–35692.
[16] Hanger, D.P., Betts, J.C., Loviny, T.L., Blackstock, W.P. and Anderton, B.H. (1998) J. Neurochem. 71, 2465–2476.
[17] Mulot, S.F.C., Hughes, K., Woodgett, J.R., Anderton, B.H. and Hanger, D.P. (1994) FEBS Lett. 349, 359–364.
[18] Brion, J.-P., Couck, A.M., Robertson, J., Loviny, T.L. and Anderton, B.H. (1993) J. Neurochem. 60, 1372–1382.
[19] Brion, J.-P., Hanger, D.P., Bruce, M.T., Couck, A.M., Flament-Durand, J. and Anderton, B.H. (1991) Biochem. J. 273, 127–133.
[20] Togo, T., Sahara, N., Yen, S.H., Cookson, N., Ishizawa, T., Hutton, M., De Silva, R., Lees, A. and Dickson, D.W. (2002) J. Neuropathol. Exp. Neurol. 61, 547–556.
[21] Brion, J.P., Tremp, G. and Octave, J.N. (1999) Am. J. Pathol. 154, 255–270.
[22] Binder, L.I., Frankfurter, A. and Rebhun, L.I. (1985) J. Cell Biol. 101, 1371–1378.
[23] Otvos Jr., L., Feiner, L., Lang, E., Szendrei, G.I., Goedert, M. and Lee, V.M. (1994) J. Neurosci. Res. 39, 669–673.
[24] Goedert, M. and Jakes, R. (1990) EMBO J. 9, 4225–4230.
[25] Ksiezak-Reding, H., Shafit-Zagardo, B. and Yen, S.-H. (1995) J. Neurosci. Res. 41, 583–593.

[26] Billingsley, M.L. and Kincaid, R.L. (1997) Biochem. J. 323, 577–591.
[27] Sergeant, N., Sablonniere, B., Schraen-Maschke, S., Ghestem, A., Maurage, C.A., Wattez, A., Vermersch, P. and Delacourte, A. (2001) Hum. Mol. Genet. 10, 2143–2155.
[28] Lee, V.M., Goedert, M. and Trojanowski, J.Q. (2001) Annu. Rev. Neurosci. 24, 1121–1159.
[29] Hayashi, S., Toyoshima, Y., Hasegawa, M., Umeda, Y., Wakabayashi, K., Tokiguchi, S., Iwatsubo, T. and Takahashi, H. (2002) Ann. Neurol. 51, 525–530.
[30] Arai, Y., Yamazaki, M., Mori, O., Muramatsu, H., Asano, G. and Katayama, Y. (2001) Brain Res. 888, 287–296.
[31] Mailliot, C., Sergeant, N., Bussiere, T., Caillet-Boudin, M.L., Delacourte, A. and Buee, L. (1998) FEBS Lett. 433, 201–204.
[32] Sergeant, N., Wattez, A. and Delacourte, A. (1999) J. Neurochem. 72, 1243–1249.
[33] Zhukareva, V., Mann, D., Pickering-Brown, S., Uryu, K., Shuck, T., Shah, K., Grossman, M., Miller, B.L., Hulette, C.M., Feinstein, S.C., Trojanowski, J.Q. and Lee, V.M. (2002) Ann. Neurol. 51, 730–739.
[34] Forman, M.S., Zhukareva, V., Bergeron, C., Chin, S.S., Grossman, M., Clark, C., Lee, V.M. and Trojanowski, J.Q. (2002) Am. J. Pathol. 160, 2045–2053.