

Apolipoprotein E and Tau phosphorylation in human neuroblastoma cells

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Abstract

Phosphorylation is the major post-translational modification of Tau proteins and it plays an important role in Tau biological functions. Hyperphosphorylation of these proteins occurs during neurodegenerative disorders such as Alzheimer's disease. It was hypothesized that some variants of apolipoprotein E (apo E) may have a protective effect against the normal or pathological phosphorylation of Tau proteins. We have recently shown that apo E synthesis occurs in human SY 5Y and Kelly neuroblastoma cell lines which express different isoforms (E3 for SY 5Y; E3 and E4 for Kelly) [Dupont-Wallois, L., Soulié, C., Sergeant, N., Wavrant-de Wrieze, F., Chartier-Harlin, M.C., Delacourte, A. and Caillet-Boudin, M.L., *Neurobiol. Dis.*, 4 (1997) 356–364]. Therefore, this cellular model makes it possible to study the differential influence, if any, of apo E3 and E4 on Tau phosphorylation. Using a large panel of Tau phosphorylation-dependent antibodies, we were not able to detect a significant difference in Tau immunoreactivity linked to the different apo E genotypes, even when the hyperphosphorylation of Tau proteins was induced by treating cells with Okadaic acid (OA), an inhibitor of phosphatase 1 and 2A proteins. Thus, a difference in apo E isoforms had no dramatic effect upon Tau phosphorylation in native or OA treated cells. © 1998 Elsevier Science Ireland Ltd. All rights reserved

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Tau proteins belong to the microtubule-associated proteins (MAP) family. In the central nervous system (CNS), they have an apparent molecular weight in the 50–64-kDa range on sodium dodecyl sulfate polyacrylamide gel.

Phosphorylation is the major post-translational modification of Tau proteins and plays an important role in Tau biological functions. In Alzheimer's disease (AD), all six Tau proteins are hyperphosphorylated [13] compared to normally-phosphorylated Tau proteins [15,17,22] and they are resolved on an SDS-PAGE as a triplet of Tau proteins referred to as tau 55, tau 64 and tau 69 [8]. During AD, these hyperphosphorylated Tau proteins are aggregated into paired helical filaments (PHFs) which are themselves assembled in neurofibrillary tangles (NTFs), characteristic of degenerating neurons [2,7].

A recent hypothesis suggested that, according to its isoforms, apolipoprotein E (apo E) may have a protective

effect against the phosphorylation of Tau proteins. Indeed, apo E exists under three major isoforms which differ from each other by the presence of a Cys or an Arg at the 112 and 158 amino-acid positions. These isoforms are named E2 (Cys 112–Cys 158), E3 (Cys 112–Arg 158) and E4 (Arg 112–Arg 158). Studies of apo E phenotype prevalence showed that $\epsilon 3/\epsilon 3$ and $\epsilon 3/\epsilon 4$ were the two most-represented phenotypes [6,18,21]. In vitro studies of gel-shift experiments show a specific binding between apo E3, apo E2 variant and Tau proteins, which disappears when Tau proteins are phosphorylated [24]. Thus, apo E3 and apo E2 variants, by their binding to Tau microtubule-binding repeat regions might protect the latter from hyperphosphorylation and self-assembly into paired helical filaments. The apo E4 variant was never bound to Tau proteins in such experiments [24]. These studies agree with the genetic studies which show allele $\epsilon 4$ is an important risk factor, and allele E2 a protective agent of AD [5,6,19]. Nevertheless, a weaker binding of apo E to Tau proteins is detected by overlay methods and this affinity seems to be independent of apo E isoform [11].

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Whereas in-vitro studies showed that apo E2 and E3 might act to protect tau proteins from phosphorylation, ambiguity about the role of apo E in Tau phosphorylation was encountered with in-vivo approaches. Indeed, Genis et al. [12] argued in favor of an increase of Tau phosphorylation in apo E knock-out mice, whereas Mercken and Brion [16] failed to detect it in a similar model. Therefore, the purpose of our study was to look for the consequences of apo E expression on Tau phosphorylation in a cell model. Indeed, former studies from our laboratory have shown that human neuroblastoma SY 5Y cells synthesize phosphorylated Tau proteins, mainly the fetal Tau isoform [9] and that an Alzheimer-type hyperphosphorylation of Tau proteins was induced by Okadaic acid cell treatment [4,9]. More recently, we show that these cells are able to synthesize

apo E [10]. SY 5Y cells, described by Biedler et al. [1] only synthesize apo E3 whereas Kelly cells, other human neuroblastoma cells [20], synthesize both apo E3 and apo E4 [10]. These cells have two distinct phenotypes which are the two most-represented ones. Thus, these neuronal-type cells constitute a suitable system to study the influence of the different apo E isoforms on Tau phosphorylation, the expression of both corresponding genes being regulated by their own promotor. In these conditions, the intracellular apo E represents 0.02% of total cellular proteins and the quantity of secreted apo E is 1000 times greater, as determined by dot-blot method with SY 5Y homogenates (unpublished data).

Cell pellets were collected, resuspended in Laemmli sample buffer, boiled for 10 min and laid onto a 10% SDS-

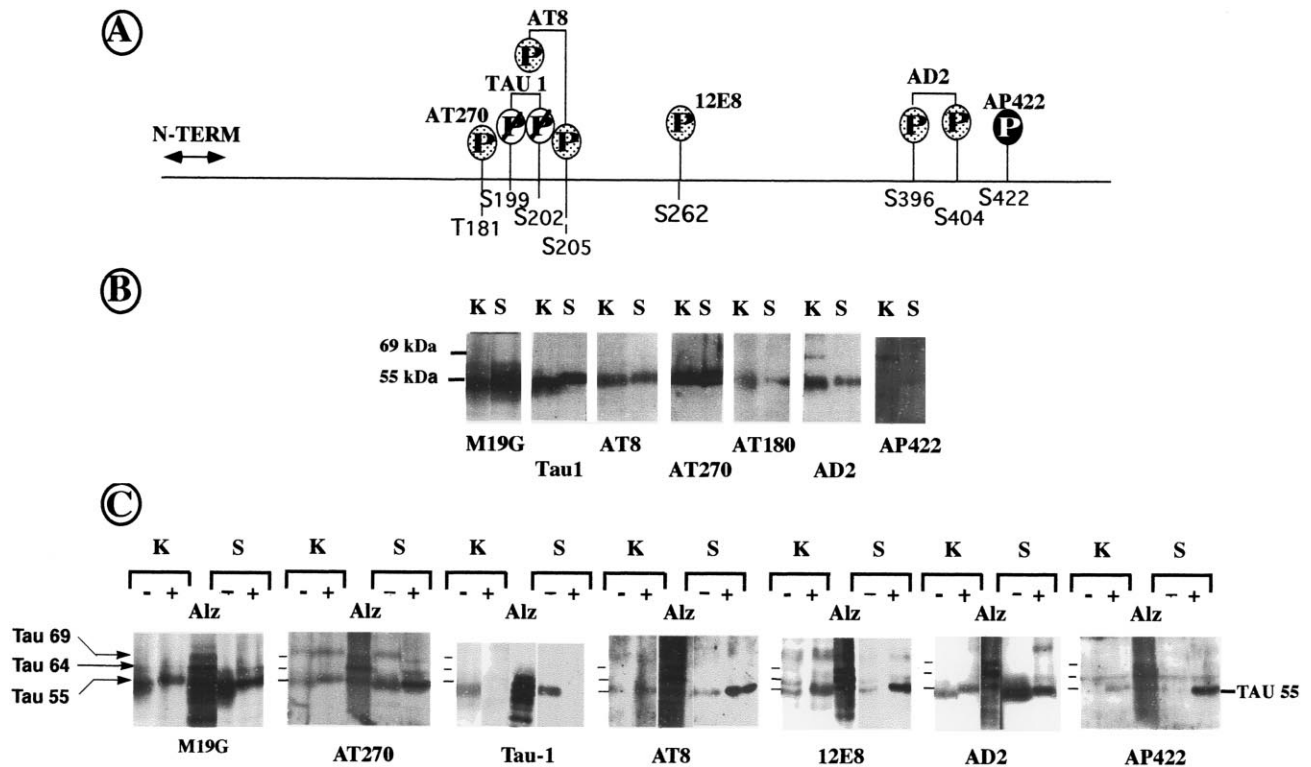


Fig. 1. Phosphorylation of tau proteins. (A) Schematic drawing of Tau proteins. The nature of epitope (phosphorylated (P) or unphosphorylated (U)) is indicated above the drawing with the name of the corresponding antibody. Under the drawing, the nature and the number of unphosphorylated or phosphorylated amino acids of each epitope is named. The circle background was respectively white when the epitope detection was specific only for normal Tau proteins, gray when the epitope was detected on both normal biopsy Tau and PHF-Tau proteins and black when the epitope detection was characteristic of only PHF-Tau proteins. (B) Analysis of Tau phosphorylation in Kelly (K) and SY 5Y (S) cellular extracts with different antibodies. Note that all the antibodies used in this study bound to Tau proteins of both Kelly and SY 5Y cells except the AP422 serum for which the epitope was absent on Tau proteins of both cellular types. (C) OA treatment of SY 5Y and Kelly cells. The Kelly (K) and SY 5Y (S) cells were treated (+) or not (-) with 250 nM OA for 6 h. The modification of electrophoretic Tau migration is clearly seen with M19G and AD2, AT270 antibodies. The OA-modified Tau proteins co-migrated with the Tau 55 band of the PHF-Tau. The electrophoretic migration of Tau proteins from the control cells is just a little faster. Tau 1 epitope disappeared in Kelly and SY 5Y after OA treatment whereas AP422 epitope was induced. The arrows on the left-hand side indicate the molecular weight of the PHF-Tau triplet and the three short lines on the left of each antibody panel make it possible to locate its migration. Alzheimer homogenates (Alz) were used as control for antibody detection: PHF-Tau triplet was effectively immunodetected with AT270, AT8, 12E8, AD2 and AP422 antibodies whereas, using Tau-1 antibody, normal Tau proteins but not the PHF-Tau triplet were revealed as already reported in several articles as in [15]. Note that in some lanes, non-specific bands can be seen (AT8, AT270, 12E8, AP422). This was probably due to the fact that we analyzed the total homogenates and not a purified fraction of Tau proteins and that most of these antibodies have a specific but faint capacity of binding to Tau epitope and were used with a low dilution. Furthermore, Tau proteins are known to cross-react with some cytoskeleton proteins such as MAP and neurofilaments. (B,C) Experiments performed at least three times.

polyacrylamide gel. After electrophoresis and transfer onto nitrocellulose, the analysis of SY 5Y and Kelly Tau proteins was performed by western blotting, using the following specific antibodies: M19G [9], a rabbit polyclonal antiserum raised against the amino-terminal part of Tau proteins, used at 1/2000; AT 8 (1/100), AT 270 (1/500) [15], 12E8 (1/2000) [23] and AD2 (1/2000) [3]: monoclonal antibodies which recognize phosphorylated epitopes present on both pathological or normal Tau proteins; Tau-1 (1/1000): a monoclonal antibody specific of unphosphorylated epitope present only on normal Tau proteins [15]; AP422 (1/500), polyclonal antibodies which bind to phosphorylated Ser422, specific of PHF-Tau [4,14]. The sites recognized by all these antibodies are mentioned in Fig. 1A. To compare the phosphorylation state of Tau proteins from SY 5Y and Kelly cells, we checked that, for a given antibody, similar quantities of Tau proteins from each cell, samples had been transferred by performing a second blotting using M19G serum after stripping off the first specific antibody by overnight incubation of the nitrocellulose sheet in 3 M guanidine hydrochloride and 50 mM dithiothreitol buffer. All the immunorevelations were performed using the ECL kit from Amersham.

The Tau patterns revealed with M19G serum are similar for Tau proteins from SY 5Y or Kelly cells. This probably means that these two cell lines mainly synthesized the same Tau isoforms, i.e. the fetal Tau isoform. This result was confirmed by RT-PCR analysis (not shown). When looking for normal Tau phosphorylation, no significant difference was detected in the two human neuroblastoma cell lines (Fig. 1B). AT8, AT230, AD2 and 12E8 antibodies recognize phosphorylated sites encountered in both normally-phosphorylated Tau proteins, i.e. fetal Tau and biopsy Tau, and Tau-PHF [3,15,23]. These antibodies bound to Tau proteins from both cell lines, even if the detection was weak for some of them. On the contrary, AP422 epitopes which are almost absent from biopsy and fetal Tau [4,14] were not detected on native cellular Tau proteins but a faint cross-immunoreactivity was observed for some bands different from Tau proteins, especially in the control cells [4]. Finally, Tau-1 which bound to the Tau unphosphorylated 199–202 region present on Tau from biopsy and autopsy Tau samples was also effective for the detection of Tau proteins from both cell lines [15]. These results may mean that a normal expression of various apo E isoforms (apo E3, apo E4) is not sufficient to influence, in a drastic and significant manner, the phosphorylation of native Tau proteins.

Then, in order to check whether apo E variants could influence the hyperphosphorylation of Tau, each cell line was treated with 250 nM OA for 6 h (Fig. 1C). OA treatment induced a similar shift in the SY 5Y and Kelly Tau electrophoretic mobility as was revealed using M19G serum, which binds to the N-extremity of Tau whatever its phosphorylation state. This corresponded to an increase of the Tau phosphorylation as was confirmed by using specific

phosphorylation-dependent antibodies. Tau 1 unphosphorylated epitope, characteristic of normal Tau, disappeared upon OA treatment while AP422 epitope, specific of Alzheimer PHF-Tau, was induced. An increase of phosphorylation of normally-phosphorylated sites in biopsy Tau (AT8, AT230, 12E8 and AD2) was observed after OA treatment, in the same way as it was observed in PHF-Tau compared to biopsy Tau. These results mean that a significant phosphorylation of the region 199–204 (Tau-1 site), Thr181 (AT270), Ser202/205 (AT8), Ser262 (12E8), Ser396/404 (AD2), and Ser422 (AP422) occurred on Tau proteins during cell OA treatment. Thus, we did not detect a clear difference in phosphorylation of the SY 5Y and Kelly Tau proteins with the different antibodies used.

In conclusion, the identity of the variants of apo E did not seem to influence in drastic manner the regulation of Tau phosphorylation or hyperphosphorylation on the studied sites in a cell system.

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