Microtubule-associated protein tau in human fibroblasts with the Swedish Alzheimer mutation

Martin Ingelson a, Eugen Vanmechelen b, Lars Lannfelt a,*

aKarolinska Institute, Alzheimer's Disease Research Centre, Department of Clinical Neuroscience, Novum, KFC, S-141 86 Huddinge, Sweden
bIntergenetics N.V. Industriepark Zwijnaarde 7, Box 4, B-9052, Ghent, Belgium

Received 16 September 1996; revised version received 21 October 1996; accepted 30 October 1996

Abstract

The principal neuropathological hallmarks of Alzheimer's disease (AD) are plaques containing amyloid β peptide (Aβ) and tangles with hyperphosphorylated tau. Tau is predominantly found in the nervous system but has been reported in fibroblasts from individuals with and without AD. Aβ is also found outside the nervous system and is released three times more from cultured fibroblasts carrying the Swedish Alzheimer mutation in the amyloid precursor protein (APP) gene. In the present study, we determined tau levels in fibroblasts from carriers of the Swedish Alzheimer mutation and controls. We also characterized the expression of tau in these cells. Primary fibroblast cell lines from six individuals with and six without the mutation were investigated. ELISA measurements showed no statistically significant difference in tau levels between mutation-carrying cell lines and controls. On Western blot, four bands in the range of 47–67 kDa, corresponding to traditional tau isoforms, were detected with the Tau-1 and AT 120 antibodies. Furthermore, four bands between 110–125 kDa were detected. We thus conclude that increased levels of Aβ do not seem to increase the levels of tau in human fibroblasts. We also suggest that several of the traditional tau isoforms as well as isoforms of higher molecular weights, big tau, are expressed in this cell type. Copyright © 1996 Elsevier Science Ireland Ltd.

Keywords: Alzheimer's disease; Tau; The Swedish Alzheimer mutation; Fibroblasts; Amyloid β peptide; Peripheral marker

The microtubule-associated protein tau is the major component of the neurofibrillary tangle, one of the neuropathological hallmarks of Alzheimer's disease (AD). The normal function of tau is to promote the assembly and stability of microtubules by binding to tubulin. In AD, tau is found in an abnormally phosphorylated form [10] with a weaker affinity for the microtubule, leading to a destabilized cell [18]. Tau is present in increased amounts in cerebrospinal fluid (CSF) from patients with AD compared to controls [11,25].

Tau is expressed as six different isoforms of 46–63 kDa. It has been demonstrated that tau is expressed not only in neurons but also in astrocytes [21], oligodendrocytes [15] and in various peripheral tissues of different species [1,13,16]. Recently, tau-like immunoreactivity was detected in human fibroblasts [3,19]. Matsuyama et al. found no difference in levels of tau between AD fibroblasts and controls, using immunocytochemical methods [19]. However, Blass et al. reported higher tau-immunoreactivity in fibroblasts from patients with AD compared to controls [3].

An isoform of approximately 110–125 kDa, known as big tau, was first demonstrated in the peripheral nervous system [8]. This larger isoform, caused by alternative splicing, was also detected in myocytes from individuals with different muscle diseases [16].

The other neuropathological hallmark of AD, the senile plaque, contains amyloid β peptide (Aβ) in an aggregated state. Aβ is derived by proteolytic cleavage from the amyloid precursor protein (APP) and in a large Swedish pedigree, a disease-causing double mutation in the APP gene at codons 670/671 has been found [2,14,20]. The Swedish Alzheimer mutation causes an increased release of Aβ, which is believed to be the mechanism leading to AD in these individuals [23]. Like tau, Aβ is also expressed in...
non neural cells. In conditioned media from cultured human fibroblasts with the Swedish Alzheimer mutation, the level of Aβ is increased three times compared to controls [5,12].

A definite relation between tau and Aβ in AD has not been established. However, the formation of both plaques and tangles seem to be important early events in the development of the disease and are probably interrelated. An interesting feature is that also presymptomatic carriers of the Swedish mutation display elevated CSF-tau levels [11]. Furthermore, both APP and aggregated Aβ have been shown to increase the rate of tau phosphorylation [4,9]. We reasoned that if APP/Aβ metabolism is linked to tau metabolism, then it could be possible that fibroblasts with the Swedish Alzheimer mutation have increased intracellular levels of tau.

In this work, we did not find any difference in intracellular tau levels between fibroblasts with and without the Swedish Alzheimer mutation. Furthermore, we suggest the presence of traditional tau isoforms as well as big tau isoforms in cultured human fibroblasts.

Fibroblasts were isolated from skin biopsies of six individuals carrying the Swedish Alzheimer mutation and of six age-matched non-mutation carriers from the same family as controls. The cells were grown in 75 cm² flasks (Costar) in Dulbecco’s modified Eagle medium (DMEM; Gibco) with glutamine and an addition of 10% of fetal bovine serum (FBS; Gibco). 1% penicillin/streptomycin (PEST; Gibco) and 1% anti-PPLO (Gibco). The cells were transferred to 140 mm Petri dishes (Falcon), harvested at confluency with ice-cold phosphate-buffered saline (PBS) and centrifuged in +4°C for 15 min at 3500 rev./min. Lysate buffer (200 μl; 35 mM Tris-HCl, 2% sodium dodecyl sulfate (SDS), pH 7.35) was added to the pellet before ultrasonicking and boiling for 3 min.

For ELISA measurements, lysates from one confluent Petri dish were used on a sandwich ELISA, with AT120 as primary antibody and HT7/BT2 as secondary antibodies (Innotest hTAU Antigen, Innogenetics Belgium) [25]. A triplet of 25 μl samples was analyzed with an automated plate reader (E_max/Molecular Devices) and the average concentrations were calculated. The total amount of protein was determined by spectrophotometry, using the method of Peterson [22]. The concentration of tau was related to the total protein concentration.

Statistical comparisons between mutation carriers and controls were made with the Mann–Whitney test.

In Western blot experiments four cell lines, two with and two without the mutation, were investigated and ten confluent Petri dishes were pooled for each cell line. Lysate (12 μl) was loaded on a 10% SDS-polyacrylamide gel electrophoresis (SDS-PAGE) gel and run for 2 h at 60 mA (Mini-Protein/Bio-Rad). The gel was transferred to a nitrocellulose membrane and immunoblotted according to standard procedures. As primary antibodies, we used the monoclonal antibodies Tau-1 (Boehringer Mannheim) [17] and AT 120 [25]. As secondary antibody an anti-mouse Ig (Amersham) was used. Blots were detected by chemiluminiscence (ECL; Amersham). As references, tau standards of the 46 kDa- and 62 kDa-isoform (htau 23, htau 40) [7] and lysates from the human neuroblastoma cell line SHSY5Y were used. The SHSY5Y cell line was chosen as reference since tau has been reported in neuroblastoma cell lines [24].

ELISA measurements were made on all 12 cell lines, six with and six without the mutation. Tau levels showed an almost four times difference between the lowest and the highest value (65 ng versus 246 ng tau/g total protein) (Table 1). There was no statistically significant difference in tau levels between mutation carrying cell lines and controls (P = 0.26). Furthermore, no correlation could be seen between tau levels in lysate and previously reported levels of Aβ in conditioned media from fibroblasts with and without the Swedish Alzheimer mutation [12]. The level of tau in lysate from the SHSY5Y cell line was found to be approximately 450 ng/g total protein.

Four bands in the range of 47–67 kDa were detected on Western blot after probing fibroblast lysates from two cell lines with and two without the mutation with the Tau-1 and AT 120 antibodies (Figs. 1 and 2). The intensity of the bands in the four cell lines correlated well to the tau levels measured by ELISA. Furthermore, four additional bands in the range of 110–125 kDa were detected with Tau-1 (Fig. 1) and AT 120, but with AT 120 the bands in this region were somewhat blurred (Fig. 2). The blot with Tau-1 was reproduced several times, each time giving an identical pattern of bands.

Although the two highest tau levels were from mutation carriers, there was no statistically significant difference of tau levels between cells with and without the Swedish Alzheimer mutation, which is in agreement with the

Table 1

<table>
<thead>
<tr>
<th>Age</th>
<th>Tau (ng/g)</th>
<th>Aβ° (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutation carriers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>69</td>
<td>82</td>
</tr>
<tr>
<td>2</td>
<td>61</td>
<td>207</td>
</tr>
<tr>
<td>3</td>
<td>67</td>
<td>91</td>
</tr>
<tr>
<td>4</td>
<td>56</td>
<td>108</td>
</tr>
<tr>
<td>5</td>
<td>45</td>
<td>74</td>
</tr>
<tr>
<td>6</td>
<td>52</td>
<td>246</td>
</tr>
<tr>
<td>Non-mutation carriers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>73</td>
<td>65</td>
</tr>
<tr>
<td>8</td>
<td>65</td>
<td>77</td>
</tr>
<tr>
<td>9</td>
<td>60</td>
<td>130</td>
</tr>
<tr>
<td>10</td>
<td>62</td>
<td>69</td>
</tr>
<tr>
<td>11</td>
<td>58</td>
<td>105</td>
</tr>
<tr>
<td>12</td>
<td>47</td>
<td>102</td>
</tr>
</tbody>
</table>

*Values from Ref. [12].

*No value available.
work of Matsuyama et al. [19]. As mutation-carrying fibroblasts release three times more Aβ, we conclude that increased levels of Aβ do not seem to cause an increased level of intracellular tau, at least not in human fibroblasts.

In this study we detected tau isoforms in human fibroblast lysates with both Western blot and ELISA, using different tau antibodies. The possibility of cross-reactions with other homologous proteins should be low since the same pattern of bands was seen in the blots, using two different monoclonal antibodies (Figs. 1 and 2). Moreover, the correlation between the levels of tau, as measured by ELISA, and the intensity of bands, as detected by immunoblotting, indicates that the same proteins were detected with the two different methods.

Western blot with the monoclonal antibodies Tau-1 and AT 120 detected four isoforms with an apparent molecular weight between 47 and 67 kDa. When the tau isoforms were expressed as recombinants in E. coli, their apparent molecular weights on the blot were between 48 and 67 kDa [6]. Hence, the sizes of the different isoforms in the fibroblast lysates correspond very well to the known molecular forms of tau. Furthermore, four additional bands in the range of 110–125 kDa detected on Western blot with Tau-1 were interpreted as isoforms of big tau. The possibility that the bands consist of dimeric forms of tau can not be ruled out since we were not able to detect these bands in the same distinct manner using the AT 120 antibody. However, we obtained an identical pattern of bands in the 110–125 kDa region in blots with Tau-1 on lysates from different cell lines and on different lysates from the same cell line, which would favour the interpretation that big tau isoforms are present in these fibroblasts. In conclusion, we suggest that several of the traditional isoforms and different isoforms of big tau are expressed in human fibroblasts.

There is a large need for peripheral markers of AD to improve early diagnosis. Measurements of tau in CSF provide a promising antemortem diagnostic marker but it is unclear whether also other peripheral tissues could be used for this purpose. This study does not clearly support that tau metabolism is generally disturbed in AD. However, the ELISA used by us measures both phosphorylated and unphosphorylated tau. If raised levels of Aβ lead to excessive phosphorylation of tau, fibroblasts with the Swedish Alzheimer mutation could possibly have increased levels of phosphorylated tau. This could be investigated in future studies by quantifying phosphorylated tau specifically.

We thank Dr. Bengt Winblad for generous support and Richard Cowburn for helpful comments on the manuscript. The following foundations are acknowledged for their financial support: Gamla Tjänarinnor, Lars Hierta, Magnus Bergvall, Claes Groschinsky, Captain Artur Eriksson, Osterman, Old Servants Foundation, the Alzheimer Foundation, King Gustaf V’s and Queen Victoria’s Foundation, Sandoz Foundation for Gerontological Research and the Swedish Medical Research Council (project no. 10819). Parts of this work were presented at the Vth International Conference on Alzheimer’s Disease and Related Disorders, July 24–29, 1996, Osaka, Japan.


