The role of neurofibrillary tangles in Alzheimer disease

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Abstract

The neuropathological diagnosis of Alzheimer disease relies on the presence of both neurofibrillary tangles and senile plaques. The number of neurofibrillary tangles is tightly linked to the degree of dementia, suggesting that the formation of neurofibrillary tangles more directly correlates with neuronal dysfunction. The regional pattern of areas affected by neurofibrillary tangle formation during the course of the disease is relatively stereotyped. Neurofibrillary tangles are composed of highly phosphorylated forms of the microtubule-associated protein tau. Phosphorylated tau proteins accumulate early in neurones, even before formation of neurofibrillary tangles, suggesting that an imbalance between the activities of protein kinases and phosphatases acting on tau is an early phenomenon. The latter might be related to changes in signalling through transduction cascades, since many of the protein kinases generating phosphorylated tau species participate in signalling pathways. The accumulation of neurofibrillary tangles and phosphorylated tau species is associated with disturbances of the microtubule network, and, as a consequence of the latter, of axoplasmic flows. The mechanistic relationship between the formation of neurofibrillary tangles and senile plaques is still poorly understood and in vivo formation of neurofibrillary tangles in experimental models has not yet been achieved. Future animal models, e.g. transgenic animals expressing combined key human proteins, will hopefully faithfully reproduce all the major cellular lesions of the disease.

Keywords: Neurofibrillary tangles; Alzheimer disease; Microtubule-associated protein tau; phosphorylation; microtubule.

Introduction

Alzheimer disease is the most frequent cause of dementing conditions and, with the population aging, it has become manifest that Alzheimer disease will constitute an increasing medical and economic problem.

The genetic analysis of Alzheimer disease has made major progress in the recent past. Familial Alzheimer disease, an autosomal dominant condition, has been estimated to represent up to 10% of all Alzheimer disease cases. Mutations of presenilin 1, presenilin 2, and of the amyloid precursor genes account for many of these familial cases. The e4 allele of apolipoprotein E gene is now known to be a susceptibility gene for both familial and sporadic cases of Alzheimer disease, acting in a dose-related manner to increase the risk and decrease the age of onset of the disease (Roses, 1996).

The characteristic neuropathological lesions of the disease, senile plaques and neurofibrillary tangles, are present in sporadic cases as well as in the familial forms due to various mutations, indicating that they constitute a kind of “final common pathway”, responsible for the clinical expression of the disease. Particularly, the formation of neurofibrillary tangles is thought to be closely linked to neuronal dysfunction and dementia. The study of the molecular composition and mechanisms of formation of neurofibrillary tangles is thus believed to be essential for our understanding of the pathogenesis of Alzheimer disease.

Numerous data have now been gathered on the molecular composition of neurofibrillary tangles and on the pathways regulating some aspects of the metabolism of tau proteins, the main component of neurofibrillary tangles.

This discussion is devoted to a general overview of the structural and molecular characteristics of neurofibrillary tangles and how these data can at the present time explain the molecular pathogenesis of this lesion and its effects on neuronal function.

The neuropathological lesions of Alzheimer disease

Neurofibrillary lesions

Neurofibrillary tangles (NFT) are composed of bundles of abnormal filaments accumulating in neurones (fig. 1). The abnormal filaments composing NFT have been observed in perikarya, in dendrites, and in axons. Ultrastructurally, these filaments appear straight or show regular constric-
Immunolabelling with an anti-tau antibody (no counterstaining) on tissue sections of the hippocampus of a patient with Alzheimer disease. A: The strong labelling is due to the detection of abnormal PHF-tau proteins associated with NFT, dystrophic neurites of senile plaques, and neuropil threads. In this advanced case, NFT are abundant in the Ammon's horn, the subicular areas, and the temporal neocortex (T). Some regions, e.g. the CA4 sector of the Ammon's horn are relatively spared. B: CA1 sector. Numerous neurofibrillary tangles are detected. Many fill the neuronal perikarya and extend into apical dendrites. The neuropil is crippled with small tau-immunoreactive neurites (neuropil threads). C: Two strongly tau-immunoreactive NFT are adjacent to two neurones exhibiting a fainter and granular tau-immunoreactivity in their perikarya and dendrites (“pretangle” stage, arrows). A: ×16; B: ×250; C: ×500.

The main component of PHF has been identified as the microtubule-associated protein tau (see below) and immunocytochemical labelling with antibody antibodies is a robust and reproducible method for the detection of neurofibrillary lesions (neurofibrillary tangles, neuropil threads, and the neuritic crown of senile plaques).

Several morphological types of NFT can be distinguished, most probably corresponding to different steps in an evolutionary process. “Pretangle” stages are characterised by the accumulation of phosphorylated tau proteins in the somatodendritic compartment, without formation of PHF (Bancher et al., 1989; Braak et al., 1994), and are only identified with anti-tau antibodies. At a following stage, a few immunoreactive rods appear in soma and dendrites. These inclusions are also detected by silver staining and seem to correspond to early NFT and neuropil threads. Fully developed NFT are made of tightly packed bundles filling a more or less important part of the cell body and extending into dendrites, eventually displacing the nucleus and cell organelles. Neuronal death is accompanied by a partial disaggregation of NFT, exhibiting a more loose aspect. After cell death, an extracellular fibrillar material, termed extracellular tangles, persists seemingly for a long period, presumably as a result of their partial resistance to proteolysis.

Extracellular tangles, which lack the N-terminal domain of tau proteins, are not labelled with anti-tau antibodies specific for epitopes localised in this domain (Brion et al., 1991a; Bondareff et al., 1994).
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Senile plaques and other lesions

The senile plaques are composed of an extracellular deposit of amyloid fibrils surrounded or not by a neuritic crown of dystrophic neurites. The amyloid fibrils are composed of the Aβ amyloid peptide (Aβ), a 39-43 amino acids peptide derived by proteolysis from the larger amyloid peptide precursor (APP) (Octave, 1995). Depositions of the Aβ are also observed in the walls of cerebral vessels (amyloid angiopathy) and in the form of diffuse, non-fibrillar deposits in the neuropil (diffuse plaques), considered as early stages of senile plaques.

In neuritic plaques, the amyloid deposit is surrounded by a corona of dystrophic neurites. Some of them contain PHF and are labelled by anti-tau antibodies. Only a proportion of plaques shows these tau-immunoreactive neurites, and this proportion is more important in the most demented patients (Delaère et al., 1989; Probst et al., 1989).

A detailed account of other molecular and cellular components of senile plaques can be found in some recent reviews (Dickson, 1997). Other important features of the disease are the neuronal loss and the synaptic loss. These losses are correlated with the number of NFT (Gómez-Isla et al., 1997; Terry et al., 1991).

Neuropathological criteria

The neuropathological diagnosis of Alzheimer disease relies on the presence of both NFT and senile plaques. Many anatomical-clinical studies have indicated that the densities of NFT are more tightly linked than senile plaques to the degree of dementia in Alzheimer disease (Duyckaerts et al., 1987; Delaère et al., 1989; Duyckaerts et al., 1990; Arriagada et al., 1992), in line with the suggestion that the formation of NFT more closely correlates with neuronal dysfunction. Recently, consensus recommendations for the post-mortem diagnosis of Alzheimer disease, which take into account semiquantitative estimates of both NFT and senile plaques and their topography, have been proposed (The National Institute on Aging, 1997). The regional pattern of areas affected by NFT formation during the course of the disease is relatively stereotyped and a hierarchical order of area involvement has been demonstrated (Braak and Braak, 1991; Price et al., 1991; Duyckaerts et al., 1997). A neuropathological staging of Alzheimer disease in 6 stages, based

on this pattern of development, has been proposed (Braak and Braak, 1991). NFT are first found in the transentorhinal cortex, a transition area between the adjacent entorhinal cortex and the temporal neocortex (stage I); NFT appear then in layer pre-α of the entorhinal cortex (stage II); at these stages, patients do not exhibit any cognitive deficit. At the following stages (III and IV), NFT become abundant in the entorhinal cortex and appear in hippocampus; these stages correspond to clinically incipient Alzheimer disease. At the final stages (V and VI), NFT are abundant in neocortical association areas (where they are predominantly found in layers III and V) and this stage corresponds to full-blown Alzheimer disease. The molecular reason underlying this relative specificity in the spreading and the distribution of NFT remains a still unresolved question in Alzheimer disease.

Strikingly, the development of abundant NFT in brain is a pathological process, mainly restricted to the humans. Occasional NFT have been described in some species, e.g. in aged sheep (Nelson et al., 1994). On the contrary, Aβ deposits and senile plaques are frequently observed in several species of aged mammals, e.g. in aged dogs (Giaccone et al., 1990).

Tau proteins

Tau proteins were originally discovered as factors promoting assembly of microtubules in vitro (Weingarten et al., 1975). Microtubules are one of the three main fibre systems (microfilaments, neurofilaments, and microtubules) which form the cellular cytoskeleton. Microtubules are essential for the maintenance of the shape of the neurone and its extensions, and play a fundamental role in targeted intracellular transport of various molecules and organelles through axoplasmic transport.

In neurones, microtubules are composed of the globular α- and β-tubulins proteins, and of a set of microtubule-associated proteins (e.g. the high molecular weight MAP1a, MAP1b, MAP2, and the low molecular weight tau proteins). As other microtubule-associated proteins, tau proteins co-polymerise with tubulin during microtubule assembly. In the adult human brain, tau exists as a set of six isoforms ranging from 352 to 441 amino acids (fig. 3) (Goedert et al., 1992), generated by alternative splicing of a single mRNA, transcribed from a gene localised on chromosome 17 in the human species. A higher molecular weight tau isoform has also been identified in the peripheral nervous system (Couchie et al., 1992). Tau proteins are abundantly expressed in neurones but are also expressed at lower level in oligodendrocytes and astrocytes. In developing neurones, tau proteins are present in cell bodies, dendrites and axons (Brion et al., 1994) but are concentrated in axons in mature neurones (Binder et al., 1985;
Flc. 3. Immunoblotting of purified human tau proteins and PHF-tau proteins. The blots were incubated with an anti-tau antibody insensitive to the phosphorylation status of tau (A) or with an anti-tau antibody recognising a non-phosphorylated epitope on tau (tau-l antibody) (B). The normal tau proteins are composed of a set of six isoforms (bracket: the bands with lower molecular weight correspond to degradation products). The PHF-tau proteins run as three major bands with slower electrophoretic mobilities, as a consequence of their high phosphorylation. The epitope of tau-l antibody is highly phosphorylated in PHF-tau proteins, which are consequently unlabelled by the tau-l antibody. Numbers on the right indicate the position of molecular weight markers (in kDa).

Brion et al., 1988), although a pool of phosphorylated tau species has been detected in the somatodendritic domain (Papassozomenos and Binder, 1987).

Introduction of tau proteins in cells that do not express them by microinjection or transfection induces binding of tau to tubulin and stabilises microtubules against depolymerising agents. Tau proteins also seem to play a role in the establishment of neuronal polarity during development.

Phosphorylation is thought to play a major role in the function of tau proteins: highly phosphorylated tau proteins are less efficient in their ability to promote microtubule polymerisation and stabilisation. Fetal tau proteins show a higher phosphorylation level than adult tau proteins (Brion et al., 1993), a situation believed to confer a more plastic and dynamic microtubule network to developing neurones.

PHF-tau proteins

PHF in Alzheimer disease have been demonstrated to be composed of the microtubule-associated protein tau (Brion et al., 1985; Delacourte and Defossez, 1986; Grundke-Iqbal et al., 1986a; Kosik et al., 1986; Nukina and Ihara, 1986; Wood et al., 1986; Goedert et al., 1988) and self-assembly of tau proteins into PHF-like filaments has been performed in vitro (Wille et al., 1992).

Tau proteins composing NFT are generally referred to as PHF-tau proteins and differ from normal tau proteins by several types of posttranslational modifications; the best documented of these modifications is a high state of phosphorylation (Grundke-Iqbal et al., 1986b; Lee et al., 1991; Brion et al., 1991b; Morishima-Kawashima et al., 1995). PHF-tau proteins are more heavily phosphorylated than fetal and adult tau proteins. However, it has been demonstrated that biopsy-derived human tau is much more phosphorylated than autopsy-derived tau, and that a significant dephosphorylation of normal tau occurs during the postmortem period (Matsuo et al., 1994). Nevertheless, PHF-tau can be differentiated from biopsy-derived tau by its more acidic isoelectric point (Sergeant et al., 1995), and the existence of phosphorylated sites unique to PHF-tau proteins (Morishima-Kawashima et al., 1995). In Alzheimer disease, PHF-tau proteins run as three major bands of 55, 64 and 69 kDa (composed of the six tau isoforms) on one-dimensional polyacrylamide gels (fig. 3) (Lee et al., 1991; Flamant et al., 1989). The accumulation of NFT in brain tissue is correlated with a decrease in the levels of normal tau and increase in the PHF-tau proteins (Bramlett et al., 1992; Mukaetova-Ladinska et al., 1993).

It is not known whether phosphorylation of tau proteins per se is needed for their assembly in the form of PHF, although phosphorylation of tau in vitro has been observed to promote the formation of tau protein dimers (Paudel, 1997). In addition, the accumulation of phosphorylated tau proteins in the somatodendritic compartment of neurones, before the formation of NFT, is an early event (Baner et al., 1989; Braak et al., 1994). This accumulation of tau proteins could favour their secondary assembly into PHF: e.g. by inducing the formation of disulphide cross-linked tau dimers, suggested to be a key step in assembly of PHF (Schweers et al., 1995).

Other posttranslational modifications of PHF-tau proteins have been identified: e.g. ubiquitination (Perry et al., 1987), glycation (Ledesma et al., 1995), and glycosylation (Sparkman et al., 1991). In situ, other molecules than tau proteins have been identified in NFT, e.g. MAP2 (Brion
et al., 1990), APP (Smith et al., 1995), heparan sulphate (Snow et al., 1989), presenilin in a subset of NFT (Murphy et al., 1996). Antibodies to phosphorylated epitopes shared between neurofilaments and PHF-tau also label NFT (Miller et al., 1986). The association of some of these molecules with NFT might have a physiopathological meaning: e.g. heparan sulphate (Goedert et al., 1996) has been reported to induce the assembly of tau in PHF-like filaments. Glycation of PHF-tau proteins could be the result of oxidative stress and induces itself an additional oxidative stress in neurones (Yen et al., 1995).

Microtubules in Alzheimer disease

This high state of phosphorylation of PHF-tau is believed to play a critical role in the physiopathology of Alzheimer disease by directly affecting the stability of the microtubule network in neurones. This in turn would lead to disturbances of cellular functions performed by microtubules such as axoplasmic transport (Terry, 1996). In ultrastructural studies, it was reported that NFT-bearing neurones are devoid of normal microtubules (Flament-Durand and Couch, 1979; Gray et al., 1987) and show accumulation of membranous organelles, suggesting the existence of disturbances in axoplasmic flow in these cells (Terry et al., 1964; Dustin and Flament-Durand, 1982; Richard et al., 1989). A decrease in tubulin expression (Hempen and Brion, 1996) and in the ability of tubulin to polymerise into microtubules (Iqbal et al., 1986) has been observed in Alzheimer disease. In vitro, PHF-tau proteins are highly inefficient in promoting microtubule assembly (Lu and Wood, 1993). PHF-tau proteins can bind to normal tau proteins, and could thus sequester the latter in a non-functional form and even directly disassemble MT (Alonso et al., 1996). The selective binding of apolipoprotein E3 to tau, but not apolipoprotein E4, would protect tau from becoming highly phosphorylated and would play a role in the control of microtubule stability (Roses et al., 1996). Tau phosphorylation was however not observed to be affected in apolipoprotein E deficient mice (Mercken and Brion, 1995).

Tau in CSF

Many studies have concluded that the concentration of tau proteins, on average, is increased in the cerebrospinal fluid of Alzheimer disease patients when compared with nondemented controls (Vandermeeren et al., 1993; Vigo-Pelfrey et al., 1995), including in mildly demented patients (Galasko et al., 1997). Elevated tau in the cerebrospinal fluid has also been observed occasionally in patients with other neurological conditions, which can however often be distinguished from Alzheimer disease on clinical grounds.

The control of tau phosphorylation and signal transduction in Alzheimer disease

As a consequence of the high phosphorylation status of PHF-tau proteins, much efforts have been devoted to the analysis of metabolic pathways involved in the regulation of tau phosphorylation. The generation of highly phosphorylated tau proteins in Alzheimer disease must result from a disequilibrium between the activities of protein kinases and phosphatases using tau as a substrate.

Protein kinases

All identified phosphorylation sites in PHF-tau proteins are serine or threonine and many of these serine/threonine are followed by prolines. In vitro, tau proteins can be phosphorylated by the proline-directed kinases MAP kinase/extracellular regulated kinase 2, the glycogen synthase kinases-3α and 3β (GSK-3α and β), the cyclin-dependent kinases cdk2, cdk5, and the stress-activated protein kinase. Tau proteins are also phosphorylated in vitro by non-proline directed kinases, including protein kinase A, protein kinase C, calcmodulin-activated protein kinase, casein kinases, and the p110MARK kinase (Billingsley and Kincaid, 1997).

None of these individual kinases generate all the phosphorylated sites present in PHF-tau, suggesting that the action of several kinases might be necessary to generate fully phosphorylated PHF-tau proteins. Available data indicate that tau proteins phosphorylated in vitro by these kinases are less efficient in promoting microtubule assembly.

Protein phosphatases

As mentioned before, protein phosphatases might play an equally important role in the generation of PHF-tau proteins (Trojanowski and Lee, 1995). Phosphorylated tau proteins have been found to be an adequate substrate for phosphatase 1, 2A and 2B. The treatment of cultured neurones or tissue blocks with phosphatase inhibitors leads to the formation of highly phosphorylated tau species (Dupont-Wallois et al., 1995). It has also been observed that treatment of cultured neurones with glutamate or colchicine (Davis et al., 1995), hydrogen peroxide generating free radicals (Davis et al., 1997), and ionophores increasing intracellular calcium (Adamec et al., 1997) all induces tau dephosphorylation; tau dephosphorylation has also been observed in vivo after heatshock (Papassozomenos and Su, 1995) and ischaemia (Geddes et al., 1994). These effects are at least in part mediated through the activation of phosphatases.

Most of the above-mentioned kinases and phosphatases are expressed in human brain and some
have been detected in neurones containing NFT (Hanger et al., 1992; Trojanowski et al., 1993; Brion et al., 1995; Vincent et al., 1997).

Transduction cascades

Many of the candidates kinases (and phosphatases) acting on tau are key elements of signalling cascades involved in transduction of extracellular signals. An interesting hypothesis is that the generation of highly phosphorylated tau species could result from a disregulation of one or several of these cascades in Alzheimer disease.

For instance, the kinase GSK-3β is negatively regulated by the wingless/wnt pathway, a signal transduction cascade involved in developmental patterning. Many elements of this cascade are expressed in the adult brain. Expression of GSK-3β in intact cells induces a tau phosphorylation and a loss of microtubule stability (Lovestone et al., 1996). In vitro phosphorylation of human brain tau by GSK-3β generates PHF-tau like proteins (Mulot et al., 1994). Inhibition of GSK-3β activity in neuronal cells by lithium treatment (Stambolic et al., 1996) induces a tau dephosphorylation. There is thus now good evidence that GSK-3β is a physiological kinase for tau and participates to the generation of PHF-tau proteins.

The MAP kinases are activated by growth factors, hormones and cytokines acting through tyrosine kinase receptors, and signalisation through this cascade leads to activation of transcription factors and mitosis in cycling cells. However, activation of MAPK in intact cells did not induce the tau phosphorylation changes characteristics of PHF-tau (Lovestone et al., 1994).

Some of the phosphoepitopes found in PHF are generated by kinases involved in mitotic mechanisms (Vincent et al., 1997). An abnormal stimulation in neurones of metabolic cascades involved in mitosis could engage these postmitotic cells in a programmed cell death program. Apoptotic-like cell death in Alzheimer disease has been reported (Su et al., 1994) and might be more frequent, although not systematic, in neurones containing NFT (Lassmann et al., 1995).

Neurofibrillary tangles and the amyloid “cascade”

The relationship between NFT and senile plaques remains a central question in Alzheimer research. Does one of the lesions precede and cause the other, or are they evolving independently? Their detailed relationship remains a controversial issue. A most dominant hypothesis, termed the amyloid cascade, advocates that the deposition of amyloid deposits, made of Aβ peptide, is the primary event leading to other cellular lesions (Hardy, 1997). There is now strong evidence that modification of APP metabolism leads to Aβ deposits, but no experimental evidence that Aβ deposits induce directly the formation of NFT. A link between the formation of NFT and senile plaques in Alzheimer disease might rely on alternative hypotheses, such as an event affecting both the metabolism of tau and APP at some steps upstream of Aβ peptide deposition. A direct interaction between tau and APP has also been documented (Smith et al., 1995; Philippe et al., 1996) and might play a role in the pathogenesis of NFT.

Perspectives

NFT clearly appear as an essential neuronal lesion directly linked to neuronal dysfunction and cognitive deterioration in Alzheimer disease. NFT formation contributes per se to this neuronal dysfunction but earlier events preceding massive NFT formation, i.e. the accumulation of highly phosphorylated tau proteins might play an equally important role, by disturbing the functions of the microtubular network in these cells. The generation of highly phosphorylated tau proteins probably also represents one aspect of a more general disturbance of metabolic pathways involving protein kinases and phosphatases, playing a role in signal transduction cascades. More studies will however be necessary to unravel the complexity of the control of tau phosphorylation in vivo, likely to be complex and involving a subtle equilibrium between the activities of phosphatases and kinases and the cross-talk between different cascades.

One important drawback in the study of cellular mechanisms of Alzheimer disease is the actual lack of an animal model faithfully reproducing all the major cellular lesions of the disease, i.e. senile plaques, neurofibrillary tangles, neuronal and synaptic loss, and behavioural deficits. Some transgenic mice, expressing mutant forms of APP and presenilins, exhibit amyloid deposits and/or evidence of neuronal loss and behavioural deficits but not yet NFT. The overexpression of human tau proteins in transgenic mice leads to a somatodendritic accumulation of phosphorylated tau proteins, mimicking early stages of neurofibrillary degeneration but PHF formation has not yet been observed in these animals (Götz et al., 1995; Brion et al., 1998). Future transgenic animals expressing a combination of these proteins and others (e.g. protein kinases) will hopefully constitute powerful models for the detailed analysis of cellular mechanisms of Alzheimer disease.

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