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Perspectives

Alzheimer's disease, a multifactorial disorder seeking multitherapies

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Abstract Alzheimer's disease (AD) is multifactorial and apparently involves several different etiopathogenic mechanisms. There are at least five subgroups of AD based on cerebrospinal fluid levels of $A\beta_{1-42}$, a marker of beta-amyloid (Aβ) plaques, and tau and ubiquitin, two markers of neurofibrillary tangles. These different AD subgroups may respond differently to a given disease-modifying drug, and hence, different therapeutic drugs for different disease subgroups might be required. Stratification of AD patients by disease subgroups in clinical trials is critical to the successful development of potent disease-modifying drugs. Levels of disease markers in the cerebrospinal fluid are promising, both in identifying various subgroups of AD and in monitoring the response to therapeutic drugs.

Keywords: Alzheimer disease subgroups; Cerebrospinal fluid; CSF biomarkers; $A\beta_{1-42}$; Tau; Ubiquitin; Alzheimer disease therapeutics; Neurofibrillary degeneration; β-amyloid

1. Introduction

Alzheimer's disease (AD), the single major cause of dementia in middle- and old-age individuals, is histopathologically characterized by brain β-amyloidosis and neurofibrillary degeneration. The former is seen as plaques of extracellular deposits of beta-amyloid (Aβ) in the brain parenchyma and in the cerebral blood vessels, and is known as congophilic angiopathy. The neurofibrillary degeneration is a slow and progressive retrograde neuronal degeneration that is observed as neurofibrillary tangles of paired helical filaments (PHF)/straight filaments (SF) in the cell soma, surrounding the plaque core β-amyloid in dystrophic neurites, and as neuropil threads in the neuropil [1]. Aβ plaques identical to those in AD but lacking the dystrophic neurites with neurofibrillary pathology are also seen in the neocortex of the cognitively normal elderly individuals [2]. In contrast, neurofibrillary pathology of the AD type, which is made up of PHF/SF of abnormally hyperphosphorylated tau [3,4], is a hallmark of several related neurodegenerative diseases called tauopathies [5]. These tauopathies include frontotemporal dementia with Parkinsonism linked to chromosome 17 caused by tau mutations, corticobasal degeneration, Pick disease, dementia pugilistica, and progressive nuclear palsy. The occurrence of neurofibrillary degeneration in the neocortex in the absence of Aβ deposits in tauopathies is associated with dementia. In progressive supranuclear palsy, where the hyperphosphorylated tau lesions occur in the brain stem, motor dysfunction instead of dementia is observed. Unlike aging and tauopathies, AD is characterized by the presence of both numerous β-amyloid plaques and neurofibrillary tangles of abnormally hyperphosphorylated tau filaments in the neocortex, especially the hippocampus. The abnormally hyperphosphorylated tau in neurofibrillary tangles becomes ubiquitinated [6,7]. However, this ubiquitination occurs late, that is, when the pathological tau is in β-pleated sheets, and is mostly unsuccessful; neurons with ubiquitinated neurofibrillary tangles survive for up to several years [8] and then on cell death are seen in the extracellular space in the brain as ghost tangles, also called tombstones.

2. Multifactorial nature and a lack of apparent temporal relationship between plaques and tangles

AD is multifactorial and heterogeneous (Fig. 1). Less than 1% of AD cases are caused by certain mutations in the following three different transmembrane proteins: amyloid precursor protein (APP), presenilin 1, and presenilin 2 [9]. Furthermore, prion protein, the self-replication of the pronase resistant form of which causes the prion disease, is also

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a transmembrane protein, and a missense mutation in this protein in Gerstmann Straussler Syndrome in a family in Indiana, referred to as Indiana kindred, has been found to be associated with numerous neurons with neurofibrillary tangles of abnormally hyperphosphorylated tau along with prion plaques [10]. More than 99% of AD cases represent the so-called sporadic form of the disease which is not associated with any known mutation. The sporadic form of AD itself probably involves several different etiopathogenic mechanisms. Neuroinflammation, head trauma, and diabetes have been implicated as risk factors for AD. In the sporadic AD, the presence of one or two alleles of apolipoprotein (*APOE*) ε4 as opposed to *APOE* ε2 or *APOE* ε3 increases the disease risk by several folds [11].

Although numerous plaques and neurofibrillary tangles are seen in an AD brain, for reasons currently not understood, these two lesions occur in disproportionate numbers in different cases of the disease, especially in the plaque- and tangle-dominant AD subgroups [12,13]. This lack of direct relationship between the numbers of plaques and tangles in AD and the presence of numerous Aβ plaques without accompanying neurofibrillary degeneration in normal elderly human beings are inconsistent with the Amyloid Cascade Hypothesis [14,15], according to which Aβ, the metabolite of the β-amyloid precursor protein (βAPP) is the primary neurotoxic molecule which causes neurofibrillary degeneration and leads to dementia. In support of this hypothesis, infusion of $A\beta_{1-42}$ in P301L tau transgenic mice [16] as well as crossing of P301L tau transgenic mice with APP_{SWE} transgenic mice [17] has been shown to exacerbate the tau neurofibrillary pathology. In these studies, the infusion of $A\beta_{1-42}$ or overexpression of APP_{SWE} could have exacerbated the tau pathology by activating the stress-activated protein kinases which are known to phosphorylate tau at several proline-directed sites. However, no mutations in tau have been found to date in AD and the frontotemporal dementia with Parkinsonism linked to chromosome 17 tau mutation cases do not show any Aβ deposits. Furthermore, numerous Aβ plaques in the neocortex of normal elderly human beings in the absence of neurofibrillary pathology [2] and a very high Aβ load in hereditary cerebral hemorrhage with amyloidosis, Dutch type (HCHWA-D) but without accompanying neurofibrillary pathology [18] are also seen to occur. Thus, the Amyloid Cascade Hypothesis does not seem to hold in the human brain.

Two AD phase III clinical trials using an Aβ aggregation inhibitor Alzhemed, and another using a γ-secretase modulating nonsteroidal anti-inflammatory drug Flurazine (Myriad, Salt Lake City, UT) have failed terribly. A clinical trial using Aβ vaccine (AN1792; Elan Pharmaceutical, Dublin, Ireland) for direct removal and/or clearance of β-amyloid from the brains of patients with AD had to be interrupted because of the vaccine-induced meningoencephalitis in several participants. Nevertheless, the Aβ vaccine though successful in clearing β-amyloid from the brain parenchyma of the treated AD cases that were studied postmortem, it failed to reduce the number of neurofibrillary tangles and significantly alter the cognitive decline [19]. Lastly, a phase II clinical trial of patients with AD treated by passive immunization with monoclonal antibody to Aβ (Elan/Wyeth Pharma, Dublin, Ireland) failed to show any significant clinical improvement. Thus, it appears that either Aβ deposition is not the primary cause of dementia, or inhibition or clearance of Aβ without inhibition of neurofibrillary degeneration might not be sufficient to inhibit the progressive cognitive decline in patients with AD. Another possibility is that Aβ initiates neurofibrillary pathology, which then becomes self-propagating. In such a case, removal and/or clearance of Aβ from the brain of an individual suffering from AD could be too late to be of any clinical benefit. However, the presence of numerous Aβ plaques without accompanying neurofibrillary pathology in the neocortex of cognitively normal elderly human beings and in hereditary cerebral hemorrhage with amyloidosis, Dutch type cases does not support this scenario.

Failures with Aβ-based therapies have shifted interest to developing therapeutic drugs that can inhibit Alzheimer neurofibrillary degeneration. The development of drugs that can inhibit neurofibrillary degeneration has its own challenges as well as opportunities. Neurofibrillary degeneration of AD type can result from several different etiopathogenic mechanisms and, thus, offers many therapeutic targets.

Tau protein has 80 serines/threonines and five tyrosines as potential sites that can be phosphorylated by protein kinases [20]. Normal brain tau has 2–3 moles phosphate per mole of the protein and this stoichiometry is apparently optimal for its biological activity which is regulated by its degree of phosphorylation. By hyperphosphorylation of tau, a neuron can reduce the microtubule network and the axonal transport and other cellular activities that depend on microtubules. However, this is reversible and plays a physiologically significant role. It is important to distinguish this type of hyperphosphorylation of tau from that which occurs in AD and related tauopathies. Two main characteristics of the AD abnormally hyperphosphorylated tau (AD P-tau) that were discovered in our lab are (*i*) that instead of interacting with tubulin, the AD P-tau binds to normal tau, microtubule associated protein (MAP)1 and MAP2, and this sequestration of normal MAPs results in depolymerization of microtubules [21–24], and (*ii*) that the AD P-tau can self-assemble into bundles of PHF/SF [25,26].

Phosphorylation sites that lead to the AD type abnormal hyperphosphorylation of tau are phosphorylated by different combinations of non-proline-directed protein kinases like cyclic adenosine monophosphate-dependent protein kinase (PKA) and calcium and calmodulin-dependent protein kinase II (CaMKII) with the proline directed kinases like glycogen synthase kinase 3β, and cyclin-dependent protein kinase 5, and are dephosphorylated largely by protein phosphatase-2A [26]. Furthermore, phosphorylation of tau is also regulated by its glycosylation, which is altered in an AD brain [27,28]. These studies suggest that different etiopathogenic mechanisms could be involved in the abnormal hyperphosphorylation of tau.