Abstract  There are two different types of Alzheimer’s disease, familial Alzheimer’s disease (FAD) and Sporadic Alzheimer’s disease (SAD), and the origin of the disease could be different in both familial and sporadic cases. In terms of FAD, mutations in three different genes are likely to promote the onset of the disease whereas for SAD, different risk factors might be involved. Nevertheless, downstream of the initial causes of the disease some common factors may be involved. In this review, rather than the differences, we have focused on some of the common features shared by Alzheimer’s patients, irrespective of the origin of their disorder. Among these common features, at the molecular level, the activation of the protein kinase GSK3 may be relevant. © 2008 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.

Keywords: GSK3; Alzheimer disease; Tau protein

1. Introduction

The autopsy of an Alzheimer’s disease (AD) patient indicates that a huge amount of cell death occurs in different regions of the brain that is associated with the presence of two aberrant structures: senile plaques (SP), composed by amyloid peptide, and neurofibrillary tangles (NFT), which main component is tau protein. This is the final picture of the disease, but what happens in the patients’ brain that provokes the formation of these aberrant structures and cell death is not properly known. At present, there are some hypotheses that try to explain why neuron dies, and how SP and NFT accumulate in the different types of AD such as the familial and sporadic types. Although, there are differences between these two different types of AD, we will concentrate here on some of the common features. Indeed, we will mainly discuss the role of the protein kinase glycogen synthase kinase 3 (GSK3) in the different types of this disease.

2. GSK3 and its regulation

GSK3 is a protein kinase encoded, in mammals, by two genes gsk3α and β. The proteins expressed by these genes mainly differ at their N terminal regions [1]. It is assumed that GSK3 is constitutively active in a cell and that it could be negatively regulated by phosphorylation at its N terminal region [2]. This phosphorylation may be regulated by insulin, through the activation of Akt [3]. On the other hand, GSK3 activity could be regulated by wnt proteins. GSK3 is present in a polyprotein complex formed by axin, APC and β-catenin together with GSK3 [4]. In the presence of wnt proteins this complex is disrupted and GSK3 activity inhibited [4]. Once GSK3 is activated it may modify some substrates, including the amyloid precursor protein, mainly by GSK3α [5], and tau protein mainly by GSK3β [6,7]. The modification of these substrates and others like β-catenin [4] or Bax [8] could result in neurodegeneration. Tau protein is also substrate for other kinases like cdk5 [9–11], MARK [12] or p38 [13,14], among other ones, but in this review, we will focus on its modification by GSK3. For further reading or tau phosphorylation and its inhibition we suggest the excellent review quoted in Ref. [15].

3. Familial Alzheimer disease

One particular class of AD, familial AD (FAD), is associated with mutations in the app, psl and ps2 genes [16] responsible for the expression of the APP, PS-1 and PS-2 proteins. APP is the protein precursor of the amyloid peptide (Aβ), the main component of SP, whereas PS-1/PS-2 are the catalytic components of a protein complex that displays gamma secretase (proteolytic) activity [17,18]. Significantly, cleavage of APP by beta (BACE-1) and gamma secretases yields the beta amyloid peptide [16]. Mutations of APP that result in AD facilitate its cleavage to Aβ. In addition, mutations in PS-1/PS-2 may produce a gain of (γ secretase) activity that may also facilitate the production of Aβ [19]. However, no mutations have been found in the gene expressing BACE-1. Alzheimer’s disease is a form of senile dementia and thus, aging is one of the principal risk factors associated with this disease. Since β-secretase activity in the brain increases with age, in relation to conditions of oxidative stress [20–22], at the ages at which β-secretase activity augments, any additional increase in γ-secretase due to mutations will also augment Aβ production. This is the basis of the Aβ cascade hypothesis [19] in which Aβ formation is the first step in the initiation of the disease, leading to the formation of senile plaques (aggregates of Aβ) and, afterwards, the hyperphosphorylation of tau, by kinases like
GSK3, which is capable of modifying several sites in the tau protein present in NFT.

The activity of GSK3β increases through deregulation of the insulin and wnt pathways. Indeed, in its oligomerized form Aβ binds to frizzled, the receptor of wnt, preventing wnt from downregulating GSK3 [23]. On the other hand, Aβ is thought to act as an antagonist of the insulin receptor [24–27], its presence augmenting GSK3 activity. Indeed, chronic exposure of mice to Aβ produced a down regulation of Akt phosphorylation [28] that in turn, provokes GSK3 activation. GSK3 may promote tau phosphorylation, and it has been suggested that phosphotau can be assembled into filaments similar to those that make up the NFT [29]. In addition, Aβ aggregates may reactivate microglia inducing associated inflammatory processes [30]. Thus, the Aβ cascade hypothesis suggests that the first step in AD development could be the formation of Aβ oligomers. This event would explain, in a rather general way, how SP and NFT form together, as well as the appearance of others features found in the brain of Alzheimer’s disease patients, such as events associated with inflammation.

It is significant that the development of the disease is more closely related to neuron death or the appearance of NFT than to the development of SP [31]. The tau and the disease pathology are thought to commence in the entorhinal/hippocampal region [32] and, from there, it spreads before it finally affects the different cortical regions where many SP can ultimately be found. At earlier stages (in the hippocampus), there is no evidence of amyloid pathology even though NFT can be observed. Curiously, in transgenic mouse models that are thought to mimic amyloid or tau pathologies, it seems that cortical neurons could be more sensitive to the amyloid pathology than hippocampal neurons, whereas the opposite could occur in tau pathologies [33]. However, the amyloid pathology appears earlier than the tau pathology in the cortex [32]. Thus, it is not clear whether the first steps involve the appearance of beta amyloid peptide aggregates, at least in the entorhinal cortex–hippocampal region. This issue might be clarified by taking into account the appearance of smaller beta peptide oligomers [34], which might be present but less easy to visualize in that region. Alternatively, since the most prominent FAD is that provoked by the presence mutations in PS-1, the pathological consequences of these PS-1 mutations could be unrelated to Aβ formation in some cases, and more directly related to other aberrant PS-1 activity. Indeed, it has been suggested that PS-1 activates PI3 kinase, an enzyme that inhibits GSK3 activity [35], and that mutated PS-1 fails to inhibit GSK3 [36]. Such alterations might promote GSK3 activity in regions like the hippocampus and this active GSK3 may then phosphorylate tau protein, causing its accumulation in NFT.

4. Sporadic Alzheimer’s disease

Several risk factors have been associated with sporadic AD, the most prevalent being aging and the presence of specific ApoE isoforms [37]. In addition, oxidative damage has long been related to aging [38], and it has been proposed as a primary cause for the initiation of the AD pathology [39,40]. LRP6 is one of the cell receptors for ApoE and it acts as a co-receptor in the wnt pathway [41,42]. The risk of expressing a specific ApoE isofom may be related with GSK3 inactiva-

tion and indeed, a common genetic variant of LRP6 was recently associated with late onset AD [43]. One possible consequence of the appearance of this variant, that should be tested, is the activation of GSK3 and the ensuing phosphorylation of the tau protein. Little is known about the molecular mechanisms underlying Aβ generation in sporadic AD, but there are some indications that the activation of beta secretase might be involved. It is known that oxidative stress increases beta secretase activity [22] and it has recently been reported that the loss of a microRNA cluster (miR-29 ab-1), or of other micro RNAs, is correlated with an increased BACE1 expression and of sporadic AD [44,45]. In addition, a non-coding antisense RNA for BACE1 may stabilize BACE mRNA, thereby increasing the amount of BACE1 protein available [46]. The increase in β secretase will facilitate Aβ production, which together with a deficiency in Aβ clearance (possibly due to the presence of a specific ApoE isoform) will result in the appearance of Aβ aggregates such as senile plaques. Indeed, ApoE has been implicated in Aβ clearance [47], whereby ApoE stimulates the degradation of soluble Aβ and the presence of ApoE4 isoforms makes this process less effective [48]. About the role of GSK3 in the regulation of the production of beta amyloid peptide, it was indicated that GSK3 (mainly GSK3β) regulates APP cleavage and, concomitantly, the production of Aβ [5]. About cdk5 and Aβ, has been shown a transcriptional regulation of BACE by cdk5 that leads to enhance amyloidogenic processing [49].

5. Oxidative stress and Alzheimer’s disease

Oxidative stress has also been proposed to participate in the initiation of sporadic AD. Indeed, like the amyloid cascade hypothesis [19] to explain the onset of FAD, the oxidation damage hypothesis [40,50,51] offers an attractive explanation of SAD based on the premise that oxidative stress precedes SP and NFT formation in AD [40]. Oxidative stress is defined as the imbalance between biochemical processes leading to the production of reactive oxygen species (ROS) and those responsible for their removal [52]. Neurons from the central nervous system (CNS) are particularly vulnerable to oxidative insults due to their high rate of oxygen consumption.

The generation of ROS can be modulated by the presence of certain metals, like iron, that might accumulate in the brain with age [53]. In addition, metal-catalyzed oxidation of Aβ induces its aggregation [54]. As previously indicated, oxidative stress may also provoke an increase in BACE1 activity [21,22], facilitating the formation of Aβ [55]. In addition, hydrogen peroxidase can activate γ-secretase [56] and indeed, γ-secretase may play a role in the oxidative-stress-induced BACE1 expression in AD [57]. Alternatively, oxidative stress could produce the oxidation of polyunsaturated fatty acids yielding products like the aldehyde hydroxynonenal (HNE) [58,59]. It has been proposed that 4-hydroxynonenal (HNE), produced as a result of lipid peroxidation, could be sufficient to induce an AD like pathology in mice [51]. HNE binds to tau protein inducing a conformational change, that is defined by the appearance of the Aiz-50 epitope found in the aggregated tau present in the brain of AD patients [60]. HNE also facilitates the formation of tau aggregates “in vitro” [61].
Finally, oxidative stress could be related to GSK3 activation. Insulin (or IGF-1) has been shown to protect against neural oxidative stress [62] and thus, when oxidative stress develops in conjunction with a decrease in the availability of these growth factors, activation of GSK3 will be provoked. On the other hand, the association of aging, the most important risk factor for Alzheimer disease, with oxidative stress is probably associated with impaired mitochondrial activities [63]. Defects in mitochondrial complex I facilitate the formation of oxidized ubiquinone (coenzyme Q), and this compound may favor the formation of Aβ aggregates [64]. In addition, tau hyperphosphorylation occurs in mice with reduced levels of superoxide dismutase and that are therefore susceptible to an increase in oxidative damage [65]. Defects in the mitochondrial complex IV also contribute to oxidative stress and could result in the accumulation of Aβ, possibly due to beta secretase activity [22,63].

By contrast, an increase in GSK3 activity could inhibit the antioxidant cell response through the phosphorylation of the Nrf2 transcription factor [66]. It can not be overlooked that phosphorylation by p38 kinase could also be activated in response to oxidative stress and that this may regulate GSK3 activity [67]. Also, other proteins like cdk5 may regulate GSK3 activity [68].

6. Aging and GSK3 activation

As previously indicated, aging is one of the main risk factors for AD. Almost all CNS neurons are of the same age as the organism in which they are found, and aged neurons are more susceptible than younger ones to increasing concentrations of glutamate. Hence, attempts are being made to slow down the progression of AD with a weak NMDA antagonist, memantine [69]. It was recently proposed [70] that glutamate toxicity could facilitate the activation of NMDA receptors, an increase in intracellular calcium and calpain activation, as well as the cleavage of GSK3 that could activate this kinase [70]. Alternatively, an increase in GSK3 immunoreactivity with age has been reported in the CNS of rats [71]. Thus, GSK3 activation could be a common feature of familial and sporadic AD, implicated in the formation of SP and NFT, as well as in neuron death, particularly since GSK3 overexpression may result in neuron death [7].

7. The spreading of tau pathology

The development of AD in different brain regions appears to somehow follow the spreading of the tau pathology [32,72] (evident through the aggregation of tau and its phosphorylation at sites modified by GSK3), and cell death. These pathological changes commence in the transentorhinal/entorhinal region, and they spread to the hippocampus and the limbic regions and finally, similar damage is found in different cortical regions. It is well known, that there are memory deficits at earlier stages of the disease, which could be related to the damage in the hippocampus, while the dementia that appears at later stages, could be related to damage in the cortex. In terms of the initial damage found in the entorhinal cortex (upper layers), expression of a FAD-linked presenilin 1 variant induces neuronal loss in these layers [73]. A decrease in the number of cells expressing reelin in regions like the entorhinal cortex (layers I and II) and hippocampus is also associated with aging, and this decrease could be due to the formation of amyloid-like reelin aggregates that may further stimulate the formation of Aβ oligomers [74]. Reelin signaling can down regulate GSK3 activity [75–77] and thus, the formation of these oligomers or the decrease in reelin could augment GSK3 activation. To mimic this situation, a GSK3 transgenic mouse was generated that mainly overexpresses the kinase in the hippocampus and cortex [7]. One consequence of GSK3 overexpression in this model was a decrease in the volume of the dentate gyrus (DG) as a result of its degeneration [78]. One important characteristic of the DG is the existence of neurogenesis, a process that diminishes during aging [79,80]. Neurogenesis in the DG produces newborn neurons that are integrated into functional neural networks [81] through the formation of synapses [82]. These new neurons may be important in hippocampal-dependent learning [82]. Thus, degeneration in that area could be correlated with memory deficits and such effects may possibly be corrected with transplants of neuronal precursor cells [83]. In addition, it has been suggested [8] that up regulation of GSK3 activity could provoke the death of neuronal precursors (S. Siresol, P. Gómez-Ramos, F. H., M. Pérez, M.A. Morán, J.J. Lucas, J. A., J.M. García Verdugo to be published) and microglia activation, which in turn may induce the aberrant proliferation and differentiation of those precursors [84], a phenomenon thought to be associated with AD [85,86].

A consequence of cell death is that intracellular components are released into the extracellular space where they may be toxic to the surrounding cells. It has been proposed that extracellular tau may be toxic and that it could promote the death of neighboring neurons; after binding of extracellular tau to muscarinic receptors of neighboring neurons [8,87]. This mechanism has been suggested as a possible way by which the tau pathology can spread to the hippocampus [87,88], although whether it participates in the further spreading of the pathology to the cortex, where dementia commences, should be analyzed.

8. The GSK3 hypothesis of Alzheimers’ disease

Recently, in an article published with this very same title [89], it was proposed that the over activity of GSK3 accounts for memory impairment, tau phosphorylation, increased amyloid production and microglia-mediated inflammation, all features of AD. It was also suggested that GSK3 might be a link between the amyloid and tau pathology [90]. In a different but complementary way, we might consider whether changes in GSK3 activity could account for the changes in tau that are found in AD and tau pathologies [32,72]. Tau pathology first develops in the entorhinal cortex, probably in layer II where the stellate neurons are found [32] (Fig. 1A). In that layer, little is known about the possible differences in the GSK3 signaling pathways between these stellate cells and the pyramidal cells found in other layers or hippocampal regions. On the other hand, the expression of a FAD-linked presenilin 1 variant in layer II (that could provoke an increase in GSK3 activity) in-
duces neuronal loss in that layer [73]. Furthermore, an increase in GSK3 activity in layer II of the EC may be correlated with cell aging through the changes in reelin levels that take place specifically in that region [74]. Thus, GSK3 activation in layer II may provoke neurodegeneration. EC layer II neurons connect with the dentate gyrus through electrical signals, and electrical activity influences hippocampal neurogenesis in the subgranular zone of the adult dentate gyrus [91]. GSK3 may play a role in the neurogenesis in that region, since increased GSK3 activity could influence the proliferation and differentiation of newborn neurons (S. Siresol, P. Gómez-Ramos, F. H., M. Pérez, M. A. Morán, J. J. Lucas, A. J., J. M. García Verdugo
to be published) (see also [92,93]), and such an effect may provoke defects in the connections that are involved in memory formation (Fig. 1A) [94]. The consequences of any such defects will be an impairment of memory, a well known feature of AD.

Neurodegeneration may also be a consequence of altering the maturation of newborn neurons and the associated release of intracellular components might establish a sort of positive feedback loop that results in cell death and the spreading of tau pathology by the mechanisms indicated above (Fig. 1C) [87,88]. Alternatively, in brain regions like the cortex, mutations in APP or PS-1 may result in FAD, and they may provoke an increase in GSK3 activity that will facilitate tau phosphorylation and probably, cell toxicity (Fig. 1B) [72]. The death of cortical neurons will result in the appearance of dementia, being the definition of dementia: the loss of intellectual abilities that is severe enough to interfere with social or occupational functioning. It involves language and behavioral disturbances associated with frontal lobe atrophy, or with deficits in visuospatial function and performance of over-learned tasks, functions of the parietal lobe [95]. Much work should be done to analyze if there is a role for GSK3 in cortex neurons in this stage (dementia) of the disease, similar to that found in hippocampal neurons.

In summary, in addition to the well known hallmarks of Alzheimer's disease, such as SP, NFT or neuron death, there are some common features that are shared by Familial and Sporadic AD. These phenomena include the common localization of the origin of the disease and the activation of the GSK3 protein kinase, in the early stages of the disease. Although these features have yet to receive the attention given to the more classical hallmarks of AD, they could serve as useful markers for the early diagnosis of the disease or provide important novel therapeutic targets.

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References


