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Review

Aberrant protein phosphorylation in Alzheimer disease brain disturbs pro-survival and cell death pathways

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ABSTRACT

Protein phosphorylation of serine, threonine, and tyrosine residues is one of the most prevalent post-translational modifications fundamental in mediating diverse cellular functions in living cells. Aberrant protein phosphorylation is currently recognized as a critical step in the pathogenesis and progression of Alzheimer disease (AD). Changes in the pattern of protein phosphorylation of different brain regions are suggested to promote AD transition from a presymptomatic to a symptomatic state in response to accumulating amyloid β-peptide (Aβ).

Several experimental approaches have been utilized to profile alteration of protein phosphorylation in the brain, including proteomics. Among central pathways regulated by kinases/phosphatases those involved in the activation/inhibition of both pro survival and cell death pathways play a central role in AD pathology.

We discuss in detail how aberrant phosphorylation could contribute to dysregulate p53 activity and insulin-mediated signaling. Taken together these results highlight that targeted therapeutic intervention, which can restore phosphorylation homeostasis, either acting on kinases and phosphatases, conceivably may prove to be beneficial to prevent or slow the development and progression of AD.

1. Introduction

Alzheimer disease (AD) is an irreversible cognitive disorder that affects the integrity of the central nervous system and is the leading cause of dementia in the elderly. Slow and progressive degeneration of neurons in brain regions involved in learning and memory results in gradual cognitive decline, loss of memory, personality changes, and impairment of normal social and emotional behaviours. Extensive synapse and neuron loss in selective brain regions are key hallmarks of AD along with two other main histological lesions, amyloid plaques and neurofibrillary tangles.

Senile plaques are extracellular deposits resulting from the aggregation of amyloid-β (Aβ) peptides surrounded by dying neuritis [1], while neurofibrillary tangles [2] result from the intracellular accumulation of hyperphosphorylated tau, a microtubule-associated protein that is involved in stabilizing microtubules, necessary for axonal transport and other functions [3].

Growing evidence indicates that the progressive atrophy of the AD brain is due to cell and synaptic loss, however the precise mechanisms/types of cell death have to be clarified yet. Considering that AD is an age dependent neurodegenerative disease it is reasonable to speculate that neuronal death is the result of the accumulation of multiple "chronic" insults that alone are insufficient to lead to disease onset [4]. Among putative pathways, it is likely that disturbance of cell cycle machinery and/or increasing oxidative stress conditions make neurons vulnerable to further insults. If from one side low levels of ROS can activate physiological intracellular signaling and induce compensatory changes, from the other side with "chronic" progressive stress the majority of neurons become irreversibly damaged and may undergo neuronal loss [4]. Indeed, increased levels of oxidative stress are associated with mitochondrial dysfunction, sustained inflammation, axonal degeneration and impairment of synaptic transmission that contribute to neuronal death, possibly by apoptosis. Neuronal loss is particularly evident in vulnerable brain areas such as entorhinal cortex, hippocampus and association regions of the neocortex.

Cell survival and cell death pathways are mainly regulated by orchestrated signaling cascades due to a number of post-translational modifications (PTMs) of selected protein targets, among which phosphorylation/de-phosphorylation is the most prominent. The addition of phosphate groups to proteins is a major modification for initiating, coordinating and controlling complex cellular functions

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such as energy production, cell growth and survival. Phosphorylation can have profound effects on protein activity, function and localization in the cell, both in health and disease.

Because phosphorylation is fast, reversible, and highly specific, it is the most efficient way to temporarily modulate protein function, i.e. induce or inhibit enzyme activity, facilitate or disrupt protein interactions, alter protein conformations, or target proteins for clearance. Protein phosphorylation and dephosphorylation are catalyzed by over 500 kinases and 100 phosphatases and are themselves regulated by phosphorylation, revealing a complex crosstalk among multiple signaling pathways [5].

However, despite this great number of kinases and phosphatases in the brain, it is probably the aberrant regulation of a few that represent the triggering events leading to the spread of an aberrant signaling in AD. In particular, the role of the kinases regulating learning and memory is noteworthy of mention. In this context, the dysregulation of the calcium/calmodulin-dependent kinase II (CaMKII), the calcium/calmodulin kinase cascade, extracellular signal regulated kinase 1 and 2 (ERK1/2), cAMP-dependent protein kinase A (PKA), cGMP-dependent protein kinase G (PKG), the phosphatidylinositol 3-kinase (PI3K) pathway, and protein kinase M z (PKMz) could represent the starting signal, which promotes neurotoxic outcomes [6,7]. Indeed, these kinases have a main role in the regulation of a number of different molecular mechanisms involved either in the control of neuronal functions or in the maintenance of neuronal structures including: (1) regulation of ion channel density and/or conductivity, which impacts on synaptic transmission (e.g., regulation of AMPA receptor trafficking); and (2) regulation of gene transcription and/or local translation, which impact on structural growth of existing synapses and/or synaptogenesis (reviewed in [7]). This picture is further complicated by the fact that, a given kinase may regulate different processes. Furthermore, it should be noted that other than the kinases described above, a long list of other kinases play key roles in the regulation of neuronal plasticity and have been found to be altered in AD [8,9]. For example, PKMzeta and iota, another form of PKC, have specific roles in spine plasticity. The receptor tyrosine kinase TrkB, binding the neurotrophin BDNF, has been implicated in regulating spine plasticity through CREB-dependent transcription and local protein synthesis. Cdk5, a predominantly neural-specific serine/threonine kinase, regulates spine plasticity through phosphorylating NR2A subunits of NMDA receptors, and seems pivotal in the regulation of spine morphology by inhibiting, indirectly, actin polymerization and reducing the number of stubby-shaped spines [6,7].

A crucial kinase in AD pathology is FYN kinase, that belongs to the Src family of nonreceptor tyrosine kinases [15]. Fyn activity, like that of other Src family kinases, is regulated by intramolecular interactions upon tyrosine phosphorylation and de-phosphorylation [16]. Fyn possesses diverse biological functions such as T-cell receptor signaling, cell division and adhesion, platelet function, synaptic function and plasticity, and central nervous system myelination [17]. Of particular interest for AD is the link between Fyn kinase and synaptic function. Shirazi and Wood reported that a subset of neurons from AD brain exhibited strong Fyn immunoreactivity compared with control brains, and that these neurons were also positive for abnormally phosphorylated Tau protein [18]. Conversely, Fyn negative cells are protected against Aβ-induced neurotoxicity [19]. Further, the finding that tau-negative cells are also protected against Aβ-induced neurotoxicity leads to speculation that the interaction between Fyn and tau has a crucial role in the neurodegenerative process [15]. Thus, Fyn represents a promising therapeutic target in AD as it is involved both in Aβ signal transduction and also has major functional interactions with Tau, thereby unifying the two pathological hallmarks of AD.

In addition, dysregulation of protein phosphatases is of extreme importance due to the fact that: i) their activity is often regulated by kinase-dependent phosphorylation; and ii) they are responsible of protein de-phosphorylation. Although tau-associated phosphatases are widely investigated in AD [10], other phosphates could be of relevance to AD, especially because they regulate the activity of the kinase cited above. These include, e.g., CD45 [11], PTP1B [12] PTEN [13], PP2A and PP2B [14], which have been also identified as possible target for AD treatment.

Aberrant phosphorylation of several proteins occurs in the brain of AD patients and also in its prodromal phase, amnestic mild cognitive impairment (MCI) [20,21]. Several experimental approaches have been utilized to profile alteration of protein phosphorylation in the brain, including proteomics platforms and top-down approaches, suggesting that the protein phosphorylation/dephosphorylation system might be dysregulated in AD brain. Among central pathways regulated by kinases/phosphatases those involved in the activation/inhibition of either pro-survival or cell death pathways play a central role in disease pathology, both in cancer and in neurodegeneration.

Emerging evidence obtained from brain tissue derived from patients with chronic neurodegenerative diseases and animal models implicates the p53 tumor suppressor protein in the regulation of neuronal cell death. Recent observations suggest that p53 plays a crucial role in aging and in neurodegenerative disorders. However, there is still an open debate whether brain aging is due to a programmed process or is the consequence of failed mechanisms for regeneration and/or repair. Although p53 promotes longevity by decreasing the risk of cancer through activation of apoptosis or cellular senescence, several findings suggest that the uncontrolled increase of its activity may have deleterious effects leading to “abnormal” aging phenotypes [22–24].

Phosphorylation cascades also are crucial in transducing pro-survival responses. Among these, key survival pathways mediated by several kinases are controlled by insulin and insulin growth factor (IGF1). In the brain, insulin contributes to synaptic maintenance, neuronal outgrowth and survival, learning and memory, as well as weight and sexual maintenance and regulation [25]. Increasing studies report that aberrant insulin signaling contributes to neurodegeneration in AD by affecting all the above-mentioned pathways [26].

In the sections below, we report proteomics results showing aberrant phosphorylation of specific proteins in AD brain that suggest the impairment of several neuronal functions. In addition, we will discuss in detail how aberrant phosphorylation could contribute to dysregulation of p53 activity and insulin-mediated signaling. Taken together, these results highlight that therapeutic intervention that can restore phosphorylation homeostasis, either acting on kinases and phosphatases, may prove to be beneficial to prevent or slow the development and progression of AD.

2. Phosphoproteomics: a tool to identify aberrant signaling in Alzheimer brain

Protein phosphorylations is one of the most prevalent PTMs that mediates diverse cellular functions in living cells [27]. It is estimated that at least one-third of eukaryotic proteins are phosphorylated, however only a subset these are modified by any given stimulus [28–31]. A highly dynamic network of about 500 kinases and 100 phosphatases regulates protein phosphorylation. The complex cooperation between the different classes of kinases and phosphatases triggers the dynamics of phosphorylation cycles regulating major cellular processes like proliferation, differentiation, apoptosis, protein subcellular localization and degradation [32,33]. Aberrant protein phosphorylation is generally accepted as a critical step in the onset or progression of neurodegenerative disorders such as AD [34,35]. Changes in the pattern of protein phosphorylation of different brain regions is likely crucial to promote AD transition from a presymptomatic to a symptomatic state in response to accumulating amyloid β-peptide (Aβ) [36]. Indeed, phosphorylation changes contribute to disturbance of multiple signaling pathways and, in part, contribute to the transition to a pathological state postulated to be necessary for cognitive decline [37]. The hyperphosphorylation of tau, which leads to the formation of