Hypothesis

Protein phosphatase 2A methylation: a link between elevated plasma homocysteine and Alzheimer's Disease

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Abstract Tau hyperphosphorylation is a central event in the development of Alzheimer's Disease (AD). Protein phosphatase 2A (PP2A) heterotrimer formation is necessary for efficient dephosphorylation of the tau protein. *S*-Adenosylmethionine-dependent carboxyl methylation is essential for the assembly of PP2A heterotrimers. Epidemiological evidence indicates that elevated plasma homocysteine is an independent risk factor for AD. Homocysteine is a key intermediate in the methyl cycle and elevated plasma homocysteine results in a global decrease in cellular methylation. We propose that the PP2A methylation system is the link relating elevated plasma homocysteine to AD. © **2002 Published by Elsevier Science B.V. on behalf of the Federation of European Biochemical Societies.**

Key words: Alzheimer's Disease; Homocysteine; Protein phosphatase 2A methylation; Methyl cycle; Tau phosphorylation

1. Protein phosphatase 2A (PP2A) and Alzheimer's Disease (AD)

In this paper, we will use evidence from the basic and the clinical literature to formulate a hypothesis regarding the etiology of AD that provides an explanation for the recent observation that elevated plasma homocysteine (Hcy) is an independent risk factor for AD [1]. Our proposition that elevated plasma Hcy is causally involved in AD pathogenesis has strong implications for the role that nutrition may play in the development of the disease. AD is a progressive neurodegenerative disease associated clinically with memory impairment and decreased cognitive function (see [2] for a recent review of AD). Post-mortem brains of AD patients display two pathological hallmarks: neuritic plaques and neurofibrillary tangles (NFTs). The plaques are composed of extracellular deposits of the amyloid β-protein (Aβ), a polypeptide derived from proteolytic cleavage of the β-amyloid precursor protein (βAPP) [3]. NFTs are found primarily within the cell and essentially comprise filaments of tau protein [4].

Normally, tau is found predominantly in the axons of neurons [5] where it stabilizes microtubules (MTs) and promotes their polymerization [6]. MTs play a major role in maintaining the cellular architecture of neurons and are largely responsible for axonal transport (see [7] for a recent review of MT-based transport in neurons). The integrity of MT structure is critical for proper neuronal function and synaptic transmission. The tau found in NFTs is abnormally hyperphosphorylated [8]. This high level of phosphorylation appears to precede and promote NFT formation [9]. Hyperphosphorylated tau is also found in the cytosol of NFT-containing neurons [10]. Phosphorylation inhibits tau's ability to bind and stabilize MTs [11,12]. Hyperphosphorylated tau has a dominant negative effect on MTs insofar as it binds normal tau as well as MT-associated proteins 1 and 2, thereby interfering with the ability of these three proteins to stabilize MTs [13]. These data help account for the observation that neurons that contain NFTs lack MTs [14]. Thus, the cytoskeletal disruption brought about by hyperphosphorylated tau provides an explanation for the neurodegeneration associated with AD.

Genetic evidence supports the conclusion that tau hyperphosphorylation is a critical event in the development of dementia [15]. Mutations in the tau gene underlie several familial neurodegenerative diseases where filamentous deposits of hyperphosphorylated tau have been observed in the absence of amyloid plaques, most notably frontotemporal dementia and Parkinsonism linked to chromosome 17 [15]. Though under some conditions Aβ accumulation has been shown to promote NFT formation [16,17], plaque formation is not essential for NFT-associated dementias, the so-called 'tauopathies' (see [18] for a recent review of these neurodegenerative diseases).

Tau hyperphosphorylation results from an imbalance between kinase and phosphatase activities (for a recent review see [19]). Phosphorylation is catalyzed by the neuronally enriched serine/threonine kinases glycogen synthase kinase 3β (GSK-3β) [20] and cyclin-dependent kinase 5 (CDK5) [21]. Tau dephosphorylation is mediated by PP2A [22]. Recent results suggest that a decrease in PP2A activity, rather than an increase in kinase activity, is crucial for the elevated levels of tau phosphorylation associated with NFT formation [23]. Work in mouse brain indicates a general decrease in PP2A expression levels with age [24], and PP2A expression has been found to be significantly reduced in the hippocampus of AD

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Abbreviations: AD, Alzheimer's Disease; MT, microtubule; NFT, neurofibrillary tangle; Hcy, homocysteine; Aβ, amyloid β-protein; βAPP, β-amyloid precursor protein; SAM, *S*-adenosylmethionine; SAH, *S*-adenosylhomocysteine; PP2A, protein phosphatase 2A; GSK-3β, glycogen synthase kinase 3β; CDK5, cyclin-dependent kinase 5

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brains relative to control brains [25]. Treatment of cultured human neurons with the PP2A inhibitor okadaic acid results in tau hyperphosphorylation, reduced binding of tau to MTs, MT depolymerization, and axonal degeneration [26]. Moreover, starved mice display a pattern of tau hyperphosphorylation similar to that found in AD brains, and this hyperphosphorylation appears to result from decreased PP2A activity towards tau rather than increased kinase activity [23]. In fact, the tau phosphorylating activities of CDK5 and GSK-3β decreased under these conditions. Thus, reduced PP2A activity towards tau must be part of any model accounting for NFT formation during the progression of AD.

PP2A is a multimeric protein complex consisting of a 65-kDa A subunit that acts as a scaffold for the association of a 36-kDa catalytic C subunit and one of a variety of regulatory B subunits (see [27] for a general review of PP2A structure and function). B subunits control the substrate specificity and subcellular localization of PP2A. B_α, the major regulatory subunit in brain, causes PP2A to bind to MTs and tau and dramatically increases the enzyme's activity towards the tau protein [28]. The highly conserved carboxyl-terminal sequence of the PP2A C subunit is a focal point for phosphatase regulation [27]. This is the site of a reversible methyl esterification reaction that controls the formation of $AB_\alpha C$ heterotrimers [29].

2. Role of PP2A methylation and the methyl cycle in AD

PP2A methylation is controlled by a specific, *S*-adenosylmethionine (SAM)-dependent methyltransferase [30] and a specific methylesterase [31]. Methylation of AC dimers has been shown to dramatically increase their affinity for B_α regulatory subunits [29]. The assembly of $AB_\alpha C$ heterotrimers proceeds as a multistep process with AC dimer methylation followed by binding of regulatory B_α subunits (Fig. 1). Given the critical role of B_α in directing PP2A activity towards tau [28], one would expect that a decrease in PP2A methylation could lead to tau hyperphosphorylation, NFT formation, and neurodegeneration.

Over the last several years data have emerged in the clinical literature demonstrating a significant correlation between elevated plasma Hcy and the occurrence of AD [1,32,33]. Though elevated plasma Hcy has long been established as an independent, graded risk factor for cardiovascular disease [34,35], the correlation with AD is a fairly recent observation. An early study found that patients with pathologically confirmed AD had significantly elevated plasma Hcy levels relative to a control group [33]. Hcy levels in the AD patients remained stable over time even as the disease progressed, suggesting that the elevation was not a result of neurodegeneration or AD-associated behavioral changes. Furthermore, patients with high plasma Hcy displayed more rapid neural atrophy over the course of three years than did patients with lower levels. More recent data from a prospective study provides convincing evidence that a rise in plasma Hcy precedes the onset of AD and is an independent risk factor for the disease [1]. Baseline plasma Hcy levels were measured in 1092 non-demented patients and the occurrence of AD in this group was followed for several years. After adjusting for other AD risk factors, the authors found that plasma Hcy levels greater than 14 μM coincided with a roughly two-fold increased risk of developing AD. Further, elevated plasma Hcy appears to be a graded risk factor, with a 40% increased risk of developing AD associated with each 5 μM incremental rise. These studies indicate a connection between high plasma Hcy and AD. Clearly, insight into the mechanism underlying the Hcy–AD correlation could give important clues for treatment of the disease.

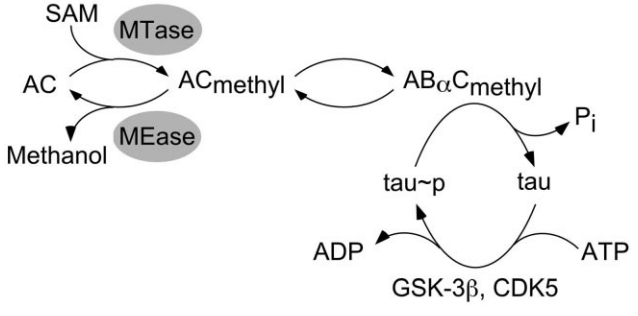

Fig. 1. Summary of PP2A methylation and tau phosphorylation. The PP2A methyltransferase, MTase, binds to and methylates AC dimers while the methylesterase, MEase, removes the modification. Methylation of AC dimers dramatically increases their affinity for B_α subunits. The $AB_\alpha C$ heterotrimer is the major tau dephosphorylating activity in vivo and phosphorylation appears to be controlled primarily by GSK-3β and CDK5.

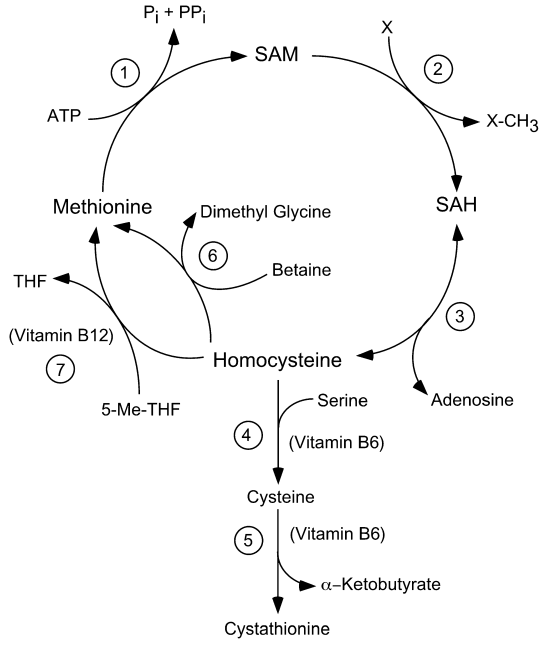

Fig. 2. Key to enzymes: 1: methionine adenosyl transferase; 2: SAM-dependent methyltransferases; 3: SAH hydrolase; 4: cystathionine β-synthase; 5: cystathionine γ lyase; 6: betaine homocysteine methyltransferase; 7: methionine synthase.

Hcy is central to the methyl cycle (Fig. 2) and plays an important role in cellular methylation (see [36] for a recent review of Hcy metabolism). SAM-dependent methylation reactions result in the production of *S*-adenosylhomocysteine (SAH) (reaction 2), which is subsequently cleaved by the enzyme SAH hydrolase to adenosine and Hcy (reaction 3). The SAH hydrolase reaction is reversible with the equilibrium actually favoring the condensation of Hcy and adenosine to form SAH [37]. SAH is a potent competitive inhibitor of

virtually all methyltransferase enzymes and accumulation of Hcy is associated, via an increase in SAH, with a global decrease in cellular methylation [38–40]. These results are consistent with the hypothesis that Hcy facilitates the progression of AD by inhibiting PP2A methylation in brain since decreased PP2A methylation will result in reduced $AB_{\alpha}C$ heterotrimer formation and thereby lead to tau hyperphosphorylation, NFT formation, neurodegeneration, and dementia. Thus, PP2A methylation provides the link between elevated plasma Hcy and AD.

Hcy is eliminated by two mechanisms. It can be methylated to regenerate methionine or it can be converted to cysteine via the transsulfuration pathway (Fig. 2). Hcy can be methylated by betaine Hcy methyltransferase (reaction 6), an enzyme present only in liver and kidney [41]. Virtually all tissues convert Hcy to methionine via a vitamin B_{12}-dependent enzyme that transfers a methyl group from 5-methyltetrahydrofolate to Hcy (reaction 7). Low levels of folate or vitamin B12 will decrease methionine synthesis from Hcy (reaction 7) in turn compromising the production of SAM from methionine (reaction 1). Thus, folate or vitamin B12 deficiency could reduce cellular methylation due to both a decrease in the availability of SAM as well as product inhibition by elevated SAH. Hcy is removed from the methyl cycle by a series of reactions catalyzed by vitamin B_6-dependent enzymes (reactions 4 and 5). Plasma Hcy levels tend to increase with age and the primary determinants appear to be plasma folate, vitamin B_{12} and vitamin B_6 [42]. Given the prominent role that vitamin B_{12}, vitamin B_6, and folate play in Hcy metabolism, it seems likely that deficiency for these vitamins underlies the observed elevation of Hcy.

Though our hypothesis clearly connects elevated plasma Hcy to tau hyperphosphorylation, NFT formation, and neurodegeneration, the role of Aβ plaque formation is not as clear. The proteolytic events that convert βAPP to toxic Aβ peptides occur in the trans-Golgi network [43]. βAPP can travel in axons along MT tracks carried in kinesin-driven vesicles [44]. Inhibition of anterograde axonal transport results in the accumulation of βAPP in the cell body [45], where conversion to Aβ takes place [43]. This would be expected to result in increased Aβ production, secretion, and plaque formation [45]. The MT depolymerization associated with tau hyperphosphorylation will also inhibit anterograde axonal transport resulting in βAPP accumulation in the cell body. Therefore, the loss of MT-based transport systems provides a potential link between tau hyperphoshorylation and Aβ plaque formation.

We propose that elevated Hcy causes tau hyperphosphorylation, NFT formation, neurodegeneration, dementia, and Aβ plaque formation via a decrease in PP2A methylation. Plasma Hcy levels are generally decreased by dietary supplements of folate, vitamin B_{12}, and vitamin B_6 [46]. By providing evidence for a causal involvement of Hcy in AD pathogenesis, our hypothesis suggests that lowering plasma Hcy by such dietary intervention may help to reduce occurrence of AD or slow the disease's progress. Grain products in the United States have been fortified with folate since 1998 and this is sufficient to decrease Hcy levels in some individuals [47]. It will be interesting to see if this policy alters the frequency with which AD occurs in the future. One can imagine that metabolic, genetic, and behavioral factors that lead to increased plasma Hcy are causal events in many instances of AD. Our hypothesis should serve as a foundation for understanding the molecular basis of these effects and it raises the possibility for the development of anti-AD pharmaceuticals by screening for agents that modulate methylation of PP2A.

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