Methylation and Phosphorylation: A Tangled Relationship?

Many neurodegenerative diseases are associated with distinctive brain lesions. One particular group of heterogeneous dementias and movement disorders is characterized by intracellular accumulations of abnormal filaments called neurofibrillary tangles (NFT), formed by the microtubule-associated protein tau. These neurodegenerative tauopathies include Pick disease and Alzheimer disease (AD), but the term also encompasses a range of other clinical conditions that share a common end-point: neurodegeneration with pathological tau accumulation (1).

Tau occurs predominantly in neuronal axons, where it binds to microtubules and regulates their length and “treadmilling” dynamics. Tight regulation of microtubule activity is critical to cell viability, and fine regulation of tau is likely to be equally important (2).

Tau activity is modulated by phosphorylation, and the ability of tau to bind to and stabilize microtubules correlates inversely with its degree of phosphorylation. This relationship has led to the suggestion of a role for tau in the adaptive response of neurons to stress (3). Tau phosphorylation may represent a physiological and reversible process integral to the stress response system. For example, in animal models, tau phosphorylation occurs in response to ether anesthesia, cold-water stress, and starvation (4).

Tau is highly phosphorylated in several neurodegenerative diseases associated with NFT formation. Disordered phosphorylation disrupts the normal colocalization of tau with microtubules, leading to further phosphorylation at fibrillogenic sites and/or cleavage by caspases. This process increases the probability of tau-tau interactions leading to the formation of paired helical filaments, and their subsequent aggregation into NFTs (5).

What is the origin of tau hyperphosphorylation associated with the tauopathies? Protein phosphorylation is governed by the competing effects of kinases and phosphatases. Among several potential kinases, attention has focused on glycogen synthase kinase 3β and cyclin-dependent kinase 5, both of which are associated with NFTs in the brains of AD patients. The signaling mechanisms responsible for the regulation of these kinases are complex, but may be modulated, inter alia, by inflammation (6). Phosphoseryl/phosphothreonyl protein phosphatase-2A (PP2A), which is found in association with tau and microtubules in the brain, appears to be the most active enzyme in dephosphorylating abnormal tau to a normal-like state (7).

The processes regulating PP2A activity are still emerging, but activation via methylation seems to be an important step. Vafai and Stock (8) highlighted the importance of such methylation for PP2A activity, suggesting that decreased methylation could lead to tau hyperphosphorylation.

The effect of decreased methylation on tau is of particular interest in light of accumulating data demonstrating a relationship between increased plasma homocysteine and neurodegenerative diseases, including AD (9). Homocysteine is derived from dietary methionine, and increased concentrations are signs of disrupted cellular methylation. Homocysteine can be remethylated to methionine by the vitamin B₁₂–dependent enzyme methionine synthase, with methyl-folate providing the methyl group. The activated methionine derivative γ-adenosylmethionine (SAM) is essential for many cellular methylation reactions, and is converted to S-adenosylhomocysteine (SAH) in the process. SAH hydrolase converts SAH to homocysteine in a reversible reaction, the kinetics of which favor condensation of homocysteine and adenosine. Clearance of homocysteine by methionine synthase therefore maintains a favorable SAM:SAH ratio, an index of cellular methylation potential (10).

In this issue of Clinical Chemistry, Obeid et al. (11) provide in vivo evidence for an association between disrupted methylation and P-tau accumulation. These authors report a strong negative correlation, in the cerebrospinal fluid, of P-tau concentrations and the SAM:SAH ratio among 182 patients with various neurological disorders, including AD. Cerebrospinal fluid folate concentrations also correlated inversely with P-tau. Aging was associated with higher concentrations of homocysteine and SAH in cerebrospinal fluid, and with lower concentrations of folate and a lower SAM:SAH ratio. Nevertheless, the striking association between SAH and higher concentrations of P-tau was observed across 3 separate age groups (<41, 41–60, and >60 years). Given the elegant and plausible hypothesis that PP2A hypomethylation can lead to tau hyperphosphorylation, it is tempting to view these findings as providing strong evidence for a direct effect of impaired methylation on P-tau accumulation, but is there an alternative explanation?

AD is a typical tauopathy, in which NFT formation and amyloid beta peptide deposition are both key pathological features. Chronic inflammation, however, is now a recognized additional component of AD, and contributes to disease progression (6). Various neuroinflammatory mediators, including complement activators and inhibitors, chemokines, cytokines, radical oxygen species, and inflammatory enzymes are generated in the disease by microglia, astrocytes, and neurons. Although amyloid beta peptide probably plays a central role in the neurodegenerative process, inflammatory mediators also stimulate its deposition, thereby establishing a vicious cycle of inflammation (12).

It is likely that similar inflammatory cascades are a common feature of the neurodegenerative tauopathies. Neuroinflammation-associated redox changes might therefore independently drive both P-tau generation and a methylation disturbance. The peptidyl-prolyl isomerase Pin1 regulates the function and processing of tau and amyloid precursor protein. In particular, it ensures that P-tau is in the correct conformation for dephosphorylation. Pin1 activity is downregulated under conditions of oxidative stress (13). Folate depletion may also occur as
a consequence of oxidative stress associated with chronic inflammation. Tetrahydrofolate is very susceptible to oxidation; a resulting increase in homocysteine concentrations may become relevant under such conditions (14). Furthermore, methionine synthase activity is also exquisitively sensitive to cellular redox status, which affects oxidation of the cob(I)alamin form of its vitamin B\textsubscript{12} cofactor (15).

Chronic neuroinflammation, with its associated oxidative stress, may therefore be an alternative explanation for both the observed reduction in methylation capacity and also the increase in P-tau; hypomethylation and hyperphosphorylation may not necessarily be directly causally related.

Obeid et al. (11) cautiously speculate that restoration of methylation might exert a neuroprotective effect by preventing P-tau accumulation. In a folate-depleted and oxidatively-stressed mouse model, however, dietary supplementation with the methyl-donor SAM alleviated an increase in non–phospho-tau, but failed to attenuate increased P-tau (16). This finding lends support to the alternative suggestion that oxidative stress plays an additional contributory role. Similarly, B-vitamin supplementation can decrease serum homocysteine without necessarily affecting any underlying inflammation; treatment with B-vitamins reduces serum homocysteine in AD patients, but this reduction is not accompanied by a reduction in markers of immune activation or inflammation (17).

The findings of Obeid et al. (11) are certainly thought-provoking, and represent an important bridge between clinical observations of hyperhomocysteinemia-related neurological dysfunction and pathological observations of P-tau accumulation. In addition, given the increasing realization of the role of chronic inflammation in neurodegenerative disease, and of the contribution of redox regulation to homocysteine metabolism (10), these findings perhaps hint that oxidative stress is an important underlying common denominator. The challenge now is to determine exactly how disrupted methylation and aberrant phosphorylation are temporally related, and whether modification of these processes can make any meaningful impact in treating these neurodegenerative disorders.

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References

Andrew McCaddon*†
Peter R. Hudson‡†
1 Cardiff School of Medicine
Wrexham, United Kingdom
2 Department of Medicinal Biochemistry
Maelor Hospital
Wrexham, United Kingdom
† Honorary Senior Research Fellow, Cardiff School of Medicine, Gardden Road Surgery, Rhosllanerchrugog, Wrexham, United Kingdom
‡ Principal Biochemist, Department of Medical Biochemistry, Maelor Hospital, Croneswydd Road, Wrexham, United Kingdom

* Address correspondence to this author at: Cardiff School of Medicine, Gardden Road Surgery, Rhosllanerchrugog, Wrexham LL142EN, United Kingdom. Fax +44-1978-845782; e-mail andrew.mccaddon@pearlmedical.co.uk.
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