Protein Phosphatase 2A and Its Methylation Modulating Enzymes LCMT-1 and PME-1 Are Dysregulated in Tauopathies of Progressive Supranuclear Palsy and Alzheimer Disease

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INTRODUCTION

Protein phosphorylation at serine and threonine residues plays a central role in the regulation of virtually all cellular activities. It has been estimated that about one-third of the proteins in vertebrate cells are subject to phospho-regulation at one or more specific serine or threonine hydroxyl side chains. The phosphorylation status of proteins depends on a precise balance between the activities of protein kinases and phosphoprotein phosphatases. Abnormal protein phosphorylation as well as mutations in particular protein kinases and phosphatases have frequently been implicated as a cause or consequence of human disease (1, 2).

In contrast to kinases, the removal of phosphoryl groups depends on the activities of a relatively limited set of phosphoprotein phosphatases, with most of phosphoserine/threonine phosphatase activity provided by just 2 enzyme families: protein phosphatase 1 and 2A (PP1 and PP2A). In addition to this, protein phosphatase 2A (PP2A), in PSP and AD. The assembly and activity of this PP2A isoform are regulated by reversible carboxyl methylation of its catalytic C subunit, while the B subunit confers substrate specificity. We sought to address whether the decreases in PP2A methylation and its methylating enzyme, leucine carboxyl methyltransferase (LCMT-1), which are reported in AD, relate to tau pathology or to concomitant amyloid pathology by comparing them in the relatively pure tauopathy PSP. Immunohistochemical analysis of frontal cortices showed that methyl-PP2A is reduced while demethyl-PP2A is increased, with no changes in total PP2A or B55α subunit, resulting in a reduction in the methyl/demethyl PP2A ratio of 63% in PSP and 75% in AD compared to controls. Similarly, Western blot analyses showed a decrease of methyl-PP2A and an increase of demethyl-PP2A with a concomitant reduction in the methyl/demethyl PP2A ratio in both PSP (74%) and AD (76%) brains. This was associated with a decrease in LCMT-1 and an increase in the demethylating enzyme, protein phosphatase methylesterase (PME-1), in both diseases. These findings suggest that PP2A dysregulation in tauopathies may contribute to the accumulation of hyperphosphorylated tau and to neurodegeneration.

Key Words: Alzheimer disease, Phosphatase, Phosphorylation, PP2A, Progressive supranuclear palsy, Protein aggregation, Tauopathy.
PP2A is a family of serine/threonine phosphatases that is highly expressed in the brain, comprising up to 1% of total cellular proteins, and accounting for the majority of serine/threonine phosphatase activity depending on the tissue origins and cell types (3, 4). Most of the PP2A in cells is found in heterotrimeric holoenzymes consisting of a highly conserved dimeric core of a catalytic C subunit and a scaffold-like A subunit associated with one of a family of regulatory B subunits, each of which directs PP2A to different spectra of substrate phosphoproteins (4, 5). Regulation of PP2A activity is a complex process involving autoregulation, subunit diversity, protein-protein interactions and posttranslational modifications. Carboxyl methylation of the C subunit at Leucine-309 is a key posttranslational modification that is critical for enhancing the binding affinity of the AC dimer with certain regulatory B subunits and, therefore, regulates selective phosphatase activity toward specific phosphoproteins (6–8). This methylation is governed by 2 specific enzymes with opposing activities: PP2A-specific leucine carboxyl methyltransferase (LCMT-1) and a PP2A-specific methylesterase (PME-1) (9–11).

The formation of characteristic inclusions of misfolded protein aggregates is a common neuropathological feature in many neurodegenerative disorders. Among these disease-associated proteins, tau deposits in the form of intraneuronal neurofibrillary tangles (NFTs) are a defining feature of tauopathies. Within these aggregates, tau is abnormally hyperphosphorylated, and this is believed to contribute to NFT formation and disease pathogenesis (12, 13). The most prevalent tauopathy is Alzheimer disease (AD), which also characteristically includes extracellular amyloid-beta (Aβ) containing plaques. NFTs, as well as glial tauopathy, may be observed in other neurodegenerative disorders as well, including progressive supranuclear palsy (PSP) (14–17). Widespread neocortical tauopathy may occur in PSP without Aβ plaques, allowing the study of tauopathy without this confounder.

Tau is highly enriched in neurons where it functions to maintain microtubule stability (18). Neuronal tau is subject to phosphorylation at over 40 different serine and threonine residues. At low levels of phosphorylation, tau binds and stabilizes microtubules, whereas hyperphosphorylated tau dissociates from microtubules and aggregates to form NFTs (19, 20). Hyperphosphorylation of tau is believed to result largely from an imbalance between tau-related kinases such as GSK3 and phosphatases such as the B55α-containing PP2A holoenzyme (21–23). It has previously been shown that levels of PP2A carboxyl methylation and B55α-containing holoenzyme are dramatically reduced in AD brains, along with decreased phosphatase activity, compared to normal controls (24–27). Considering that AD is a mixed proteinopathy involving prominent tau and amyloid aggregates, we sought to determine whether the PP2A aberrations observed in AD are related to tau pathology or to Aβ amyloid pathology. In contrast to AD, PSP is a classic tauopathy with hyperphosphorylated tau protein in NFTs distributed throughout the neocortex, basal ganglia, and brainstem, while amyloid plaques may be present or absent (28–31). Knowing that phospho-tau is a substrate for B55α-containing PP2A (21–23), we hypothesized that PP2A, and particularly its methylation state, is also dysregulated in PSP such that its phosphatase activity is reduced, contributing to the accumulation of hyperphosphorylated pathologic tau.

The present study attempts to address this hypothesis in order to gain insight into factors that contribute to the pathogenesis of PSP. We examined the state of PP2A methylation as well as the levels of the 2 enzymes that control this methylation, LCMT-1 and PME-1, in postmortem brains of patients with PSP whose plaque pathology was not greater than that of nonneurological controls and compared them to AD and age-matched controls. The results show a similar profile of PP2A dysregulation in both PSP and AD cases compared to controls. In addition to robust reduction in methylated PP2A levels, changes in the concentrations of its regulatory enzymes set the stage for compromised phosphatase activity that can contribute to the accumulation of abnormally hyperphosphorylated tau in tauopathies.

**MATERIALS AND METHODS**

**Human Brains**

Human postmortem brain tissue sections from patients with PSP (32), AD, as well as neurologically normal controls (Controls) were obtained from the Banner Sun Health Research Institute Brain and Body Donation Program of Sun City, Arizona. Full characterization of associated clinical and neuropathological information was also obtained. Cases with acute infarcts, extensive white matter rarefaction with history of hypertension, brain tumors, hippocampal sclerosis or other major neurodegenerative disease diagnoses, other than AD or PSP, were excluded. Cases with AD met NIA-Reagan intermediate (33) or high probability for AD and did not also have PSP. Cases with PSP did not also meet NIA-Reagan intermediate or high probability for AD. Cognitive status including dementia assessment was established through a consensus committee that reviewed the results of a neuropsychological test battery, neurological examination, functional tests, and review of medical records. Frontal cortex samples from 8 pathologically confirmed patients per disease and 8 age-matched control subjects were used to examine PP2A status in the study. Demographic and neuropathological characteristics of subjects in the three diagnostic groups are shown in the Table. The gender ratios of patients were equally distributed for both PSP (4:4) and AD (4:4) cases, while the Control group included 5 males and 3 females. Average age at death was comparable across the groups: 85 ± 4.4 years for PSP, 84 ± 2.2 years for AD cases, and 84 ± 2.1 years for Controls. All cases have postmortem intervals of <3 hours: Average postmortem interval (PMI) was 2.4 ± 0.1 hours for PSP, 2.6 ± 0.2 hours for AD, and 2.5 ± 0.2 hours for Controls. The degree of cognitive impairment assessed using Mean Mini-Mental State Exam showed scores of 23.3 ± 4.0 for PSP, 18.9 ± 3.7 for AD, and 27.1 ± 0.8 for Controls (p<0.05 for difference between AD and Controls).

Neuropathological stage of tauopathy was graded according to Braak’s NFT staging (34), while neuritic plaque density was classified semiquantitatively according to the criteria established by the Consortium to Establish a Registry for
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Alzheimer’s Disease (CERAD) (35). Histopathological scoring was performed blinded to clinical and neuropathological diagnosis (36). All pathologic features were graded and staged at standard sites in frontal, temporal, parietal, and occipital cortices as well as hippocampus and entorhinal cortex. The total plaque score, considering all types of plaques (cored, neuritic, and diffuse) together, is predominantly derived from the Campbell-Switzer stain, while the Gallyas and thioflavin S stains were used for estimating neuritic plaque densities. As all 3 stains show neurofibrillary changes, the plaque score was estimated after viewing slides stained with all three. Both total and neuritic plaque densities are rated as none, sparse, moderate, and frequent using CERAD criteria (35). Conversion of the descriptive terms to numerical values gives scores of 0–3 for each area, with a maximum for total plaque score of 15 for all 5 areas combined. For neuritic plaque density, the scores range from 0 to 3, defined by the highest density seen in any cortical region. Neurofibrillary tangle abundance and distribution is also graded, again using the CERAD templates for this, while the original Braak protocol (34) is used for estimating the topographical distribution of neurofibrillary change. The tauopathy of PSP was assessed on the Gallyas-stained sections.

Braak stage analysis showed PSP cases ranged from stages II to IV. AD cases ranged from stages II to VI, whereas Controls were in stage II to III. Concomitantly, total tangle density score (34) (determined by the summation of means of semiquantitative 0–3 scores across frontal, temporal, and parietal lobes as well as hippocampus and entorhinal cortex, with a maximum score of 15) was: 5.4 ± 0.9 for PSP, 10.0 ± 2.1 for AD, and 4.1 ± 0.4 for Controls (p < 0.05 for difference between AD and Controls). Neuritic plaque density range was 0–2 for PSP, 2–3 for AD, and 1 for all Controls (p < 0.01 for difference between AD and Controls, and p < 0.0001 for AD vs PSP). Total plaque density score (which includes all plaque types and is calculated as for the total tangle density score) in PSP was 4.0 ± 2.0, in AD 12.3 ± 0.9, and in Controls 6.0 ± 1.3 (p < 0.05 for AD vs Controls, and p < 0.01 for AD vs PSP). Apolipoprotein E genotype determined using a DNA polymerase based assay showed one of the PSP patients and 3 of the AD patients were heterozygous for the ApoE ε4 allele. Of the Controls, 2 were heterozygous for the ε4 allele. None of the patients in this study was homozygous for the ApoE ε4 allele. Six patients with PSP had depigmentation of the substantia nigra ranging from mild to severe, while 5 AD and 4 Controls had no depigmentation.

Immunohistochemistry of Human Brain Sections

Formalin-fixed brain tissue free-floating sections, 40 μm in thickness, from middle frontal gyrus of the cerebral cortex were used for immunohistochemical analysis. The left cerebral hemisphere was sliced in the fresh state into 1-cm coronal segments and fixed in cold 10% formalin for no more than 48 hours. Subsequently, slices were dissected into standard blocks for paraffin embedding or sectioning on the sliding freezing microtome. For the latter, prior to sectioning, the fixative was poured off and replaced with cryoprotectant, composed of 2% dimethyl sulfoxide/20% glycerol. All staining procedures across the 3 diagnostic groups were performed identically under the same conditions. After washing with phosphate buffered saline (PBS), tissue samples were immersed in 3% hydrogen peroxide for 10 minutes to quench endogenous peroxidase activity. Sections were then processes for heat-induced antigen retrieval by placing them in sodium citrate (pH 6.0) for 30 minutes at 75°C to reduce nonspecific background staining and enhance epitope antigenicity. Following several washes in PBS, sections were blocked in PBS containing 5% BSA for 1 hour at room temperature, then incubated overnight at 4°C in blocking buffer with primary antibodies against methylated-PP2A (1:200, clone 4D9 generated at Princeton University) (7), demethylated-PP2A (1:1000; clone 1D6 from Millipore) (37), PP2A C subunit (1:500; clone GC-1E1 from Abnova) (38, 39), PP2A B55α subunit (1:500, clone 2G9 from Millipore) (27, 40), LCMT-1 (also known as PPMT) (1:100, a kind gift from Egon Ogris) (26), and PME-1 (1:500, 07-095 from Millipore) (11, 41).
washed with PBS-Tween (PBS-T) three times and incubated with the respective biotinylated secondary antibodies for 1 hour, followed by further incubation in biotinylated HRP complex (Vector Laboratories, Burlingame, CA) for 1 hour at room temperature. After washing again with PBS-T, the bounded peroxidase was visualized as a brownish color by immersing the sections in 3,3'-diaminobenzidine (DAB) solution. Water-washed sections were mounted on slides, dehydrated, and cleared in xylene before being coverslipped with permount.

**Image Capture and Quantification**

Immunostained tissue sections were examined under a Nikon Eclipse 55i light microscope, and the images were acquired using NIS-Elements D software (Nikon). Quantification of the immunostaining intensity for each marker was achieved by means of optical densitometry using the thresholding tool in ImageJ (NIH, Bethesda, MD). Briefly, immunostained discrete elements in each image were densitometrically quantified in arbitrary units where a value of 255 is a complete transparency and a value of 0 is complete darkness. Immunostained discrete elements in each image were measured by adjusting parameters in thresholding tool to a point at which only positively stained cells (area), excluding background, were selected. Identical densitometric analysis method was applied for all immunostains. The densitometric value of the immunostained area in each image was averaged for each disease group and compared to the average densitometric value of the Control group.

**Tissue Homogenization and Western Blotting**

Frontal cortex tissue samples dissected from each subject’s brain and stored at −80°C were used for Western blot analyses. After thawing, the exact wet mass of each sample was measured. The volume of lysis buffer for each sample was then determined in relation to the amount of tissue used (10 mL of buffer per 1 g of tissue). Tissue samples were homogenized with a handheld motor grinder in 2% SDS lysis buffer containing protease inhibitor cocktail with the addition of 2 µM of okadaic acid and 5 mM of PMSF to block the activity of PP2A methylesterases, followed by brief sonication (26). Equal amount of protein from each sample was separated in 4%–20% SDS-PAGE and transferred onto polyvinylidene difluoride (PVDF) membranes. Membranes were then blocked for 1 hour at room temperature with 5% nonfat dry milk and probed overnight with primary antibodies specific for methylated-PP2A (1:2000, ab32104 from Abcam) (42, 43), total-PP2A (1:2000, 07-095 from Millipore) (11, 41), -Actin (1:10 000, A5441 from Sigma) was used as a protein loading control. After washing, membranes were probed with HRP-conjugated secondary antibodies (1:5000) for 1 hour, and the immunocomplex signals were detected using enhanced chemiluminescence detection system (ECL, PerkinElmer). Band intensities for each blot were determined by ImageJ, normalized to the level of either β-actin or total PP2A, and expressed as fold changes compared with their respective control.

**Statistical Analysis**

Statistical analysis was performed using GraphPad Prism 7 (GraphPad Software, San Diego, CA). Densitometric values for each case and marker were converted to percentages (for immunohistochemistry) or fold (for Western blots) of the mean of the Control group, and these percent values were used for statistical analyses to compare the 3 groups. One-way analysis of variance (one-way ANOVA) with Bonferroni’s multiple comparison test was used when analyzing differences among the 3 groups in the levels of methylated-PP2A, demethylated-PP2A, total PP2A, B55α, LCMT-1, and PME-1 from immunohistochemical and Western blot analyses. Differences in demographic characteristics (age, postmortem interval), total tangles, and total plaques were also analyzed using one-way ANOVA followed by Bonferroni’s multiple comparison test. Mini-Mental State Examination, Braak NFT density scores, and neuritic plaque density scores were analyzed using nonparametric Kruskal-Wallis test. All data are presented as means ± SEM except for Braak NFT density scores and neuritic plaque density scores, which are shown as ranges and medians. Differences between values were considered statistically significant when p < 0.05. In addition, power analysis was performed with G*Power software (45) using the observed means and SDs, and alpha error = 0.05, and confirmed a power of 98% for Methyl-PP2A, 91% for Demethyl-PP2A, 98% for LCMT-1, and 88% for PME-1.

**RESULTS**

To study the state of PP2A regulation in taurapathies, we first examined the levels of methylated and demethylated PP2A in the brain, since the former is the active form of the enzyme. Using immunohistochemical analyses, methylated PP2A level was found to be down-regulated in both PSP and AD compared with Controls, but the decline was significant only in AD brains (Fig. 1A–D). The immunoreactive signal of methyl-PP2A was lower in AD by 44% compared to Controls (p < 0.001), and by 24% in PSP brains (p < 0.05). On the other hand, a marked increase of demethylated PP2A immunoreactivity was observed in both PSP and AD brain samples. Demethyl-PP2A level was increased in PSP by 104% (p < 0.05) and in AD by 119% (p < 0.01) compared to Controls (Fig. 1F–J). As total PP2A and total B55α subunit were no different among the 3 groups (Fig. 1K–N, P–S), differences in methyl- and demethyl-PP2A levels were also reflected in their ratios to total PP2A levels. The proportion of methyl-PP2A to total PP2A was lower by 16% in PSP (p < 0.05) and by 45% in AD (p < 0.001), and that of demethyl-PP2A to total PP2A were higher by 124% in PSP (p < 0.01) and by 115% in AD (p < 0.05) compared to Controls (Fig. 1E, J). As a result, the pathologic imbalance between methylated and demethylated PP2A is evident from the marked decrease in the ratio between methylated and demethylated PP2A in both PSP (63%, p < 0.0001) and AD (75%, p < 0.0001) brains (Fig. 1O).
Consistent with these immunohistochemical findings, Western blot analyses showed similar changes in PP2A methylation level in both PSP and AD brains compared with Controls (Fig. 2). The expression of methyl-PP2A (normalized against total PP2A) tended to be decreased in PSP by 54% (p = 0.10) and in AD by 57% (p = 0.08), while the expression of demethyl-PP2A is increased significantly in PSP by 78% (p < 0.05) and in AD by 77% (p < 0.05) compared to Controls (Fig. 2A–C). Consequently, the ratio between methylated and demethylated PP2A is markedly reduced in PSP by 74% (p < 0.01) and in AD by 76% (p < 0.01) compared to Controls (Fig. 2D). Total PP2A was no different among the 3 groups (Fig. 2E).

To determine if these changes in PP2A methylation state in tauopathies are associated with alterations in the enzymes that control this posttranslational modification, we next assessed levels of the PP2A methylating enzyme LCMT-1 and demethylating enzyme PME-1. In agreement with the decrease in methyl-PP2A level, immunohistochemical analysis revealed that LCMT-1 concentration was reduced by 44% in PSP (p < 0.01) and by 41% in AD brains (p < 0.01) compared to Controls (Fig. 3A–D). This finding was confirmed by Western blot analysis showing reduced level of LCMT-1 in both PSP (25%, p < 0.001) and AD (38%, p < 0.0001) brains (Fig. 3E, F). Conversely, PME-1 level was up-regulated in both conditions compared to Controls, with an increase of 100% in PSP (p < 0.05) and 106% in AD brains (p < 0.05) (Fig. 4A–D). Similar increases in PME-1 expression in PSP (72%, p < 0.05) and AD brains (148%, p < 0.0001) were found by Western blotting (Fig. 4E, F). These findings support the notion that proportionally less active methylated PP2A relative to the inactive demethylated form in cortical tissue of...
both PSP and AD is due to a decrease in the level of the methylating enzyme LCMT-1, with contribution from increased level of the demethylating enzyme PME-1.

**DISCUSSION**

The present findings demonstrate that in the tauopathies PSP and AD, PP2A C subunit is in a hypomethylated state that does not promote the assembly of the B55α-containing heterotrimeric PP2A isoform needed for dephosphorylating tau. This is associated with a marked reduction in the methylating enzyme LCMT-1 and increase in the demethylating enzyme PME-1, creating conditions that shift the balance in favor of demethylated PP2A in the brain. The lower levels of LCMT-1 and associated reductions in PP2A methylation in AD brains are consistent with a previous report (26). Our study links for the first time dysregulated PP2A methylation and the pure tauopathy PSP and suggests that compromised PP2A activity is likely to contribute generally to the pathogenesis of tauopathies.

Among the various posttranslational modifications of tau, including acetylation, glycosylation, methylation, and sumoylation, its hyperphosphorylation has been investigated the most, as this is a major biochemical abnormality and is a consistent feature of all tauopathies (46, 47). Hyperphosphorylation promotes the dissociation of tau from microtubules (15, 48–51) and increases its propensity to form fibrils that are seen predominantly in intraneuronal inclusions known as NFTs and neuritic threads (15, 52, 53). There is also considerable evidence that tau hyperphosphorylation and subsequent high molecular weight aggregates lead to neuronal degeneration. One hypothesis is that reduced binding of hyperphosphorylated tau to microtubules results in altered structure or function of microtubules ultimately leading to neuronal degeneration.

**FIGURE 2.** Expression of methylated-PP2A, demethylated-PP2A, and total PP2A in the brains of patients with PSP, AD, and age-matched Controls. Tissue samples from frontal cortices were used for immunoblotting. (A) Representative image of Western blot analyses showing the expression of methylated-PP2A, demethylated PP2A, and total PP2A in each group. (B, C) Densitometric analyses of the expression level of methylated-PP2A and demethylated-PP2A normalized to total-PP2A levels. (D) Ratio between methylated-PP2A and demethylated-PP2A expression. (E) Total PP2A levels normalized to β-actin. n = 8 in each group and presented as fold change relative to the Control group. *ANOVA, p < 0.05; **p < 0.01 compared to Controls.
FIGURE 3. Analysis of LCMT-1 concentration in the brains of patients with PSP, AD, and age-matched Controls. Tissue sections from the middle frontal gyrus of the cerebral cortex were immunohistochemically stained for LCMT-1. (A–C) Representative images show the relative immunoreactivity of LCMT-1 in each group. (D) Optical density values for LCMT-1 staining intensity in each group. n = 8 in each group and presented as percent of the Control group. (E) Representative image of Western blot analysis showing the expression of LCMT-1 in each group. (F) Densitometric analysis of LCMT-1 expression, which was normalized to β-actin and presented as fold change relative to the Control group. **ANOVA, p < 0.01; ***p < 0.001; ****p < 0.0001 compared to Controls. Scale bar = 100 μM.

FIGURE 4. Analysis of PME-1 concentration in the brains of patients with PSP, AD, and age-matched Controls. Tissue sections from the middle frontal gyrus of the cerebral cortex were immunohistochemically stained for PME-1. (A–C) Representative images show the relative immunoreactivity of PME-1 in each group. (D) Optical density values for PME-1 staining intensity in each group. n = 8 in each group and presented as percent of the Control group. (E) Representative image of Western blot analysis showing the expression of PME-1 in each group. (F) Densitometric analysis of PME-1 expression, which was normalized to β-actin and presented as fold change relative to the Control group. *ANOVA, p < 0.05; ****p < 0.0001 compared to Controls. Scale bar = 100 μM.
dysfunction and death. The pathogenetic link between hyperphosphorylated tau and neurodegeneration is supported by reports of a positive correlation between the number of NFTs in the cortex and severity of dementia in AD (54).

PP2A is a major phosphatase controlling key neuronal signaling pathways and accounts for 70% of the total tau phosphatase activity. Most of the PP2A in the healthy brain is associated with carboxyl methylated forms of the phosphatase—principally heterotrimeric complexes containing the B55α regulatory subunit (24, 25). The present findings show that methylated PP2A is decreased particularly in AD, while the demethylated form, which has relatively little activity toward phospho-tau, is increased in both PSP and AD brains. This results in a dramatic decrease in the ratio between methylated and demethylated PP2A in both tauopathies, a change that likely contributes significantly to NFT formation as well as a plethora of other dysregulations associated with the progression of these neurodegenerative diseases. The dramatic shift in PP2A carboxyl methylation appears to derive from an imbalance in the levels of the 2 highly specific PP2A modifying enzymes that control methylation, LCMT-1 and PME-1. These findings do not exclude the possibility that other changes in kinase or phosphatase activities may work to alter the phosphorylation status of PSP-tau. Other phosphatases such as PP1, PP2B, and PP5 have been reported to act on tau at several different serine and threonine residues that are highly phosphorylated in PSP (55–58).

PP2A has previously been shown to dephosphorylate tau in vitro at S202, S262, and S356, sites (25, 59) that are hyperphosphorylated in AD (60). Considering that PSP is a classic tauopathy with prominent hyperphosphorylated tau aggregates in the brain, it is likely that deficiencies in the activity of B55α subunit containing PP2A play an important role in PSP tau pathology. This hypothesis is supported by a previous study that identified 8 phosphorylation sites in PSP-tau, at least one of which includes known dephosphorylation targets of PP2A (58). Notably, the similar profile of dysregulated PP2A between AD and PSP found in the present study appears to underlie the shared phospho-tau epitopes in tau filaments in both conditions (61). This is despite morphological differences in tau filaments between the 2 tauopathies whereby abnormal tau filaments in PSP are predominantly straight whereas the pathological tau filaments in AD are predominantly in the form of paired helical filaments (61, 62).

Considerable evidence suggests that B55α-containing PP2A is a primary tau phosphatase, and the carboxyl methylation state of PP2A AC dimers is known to affect the association of B55α to form the B55α-PP2A holoenzyme (6, 21, 63–66). It has previously been reported that decreased B55α-PP2A activity is responsible for increased phosphorylation of tau at the phosphoepitopes associated with AD, and that demethylation of PP2A decreases the level of B55α subunits and subsequently increases the phosphorylation of tau in AD brains (27). However, the present study did not reveal significant changes in the level of total B55α in AD or PSP brains although the relative methylation status of C subunit was significantly altered in both cases. It is worth noting that in vitro studies have shown that methylation significantly increases the phosphatase activity of AC dimers toward neurodegeneration-associated substrates such as phosphotau and phospho–α-synuclein in the absence of B55α (21, 67, 68).

In healthy brain tissue, most PP2A is methylated so that inhibition of LCMT-1 or activation of PME-1 tends to generate large changes in demethylated PP2A from relatively small changes in levels of PP2A methylation. This accounts for our present finding that demethylated PP2A level in both PSP and AD brains is more than double that of Controls, whereas methylated PP2A is reduced by only 24% in PSP and 44% in AD brains. It is also noted that the formation of PP2A heterotrimeric holoenzymes with other regulatory subunits such as B’56α may not require methylation (63, 65). These forms of PP2A would, therefore, be favored by PP2A demethylation, and evidence suggests that the B’56α PP2A holoenzyme activates neuronal tau kinases such as GSK3β (69). The latter would then further promote hyperphosphorylation of tau and subsequent misfolding. Though it is possible that tau phosphorylation can be indirectly affected by other PP2A isoforms through modulating key tau protein kinases, PP2A containing B55α subunit is considered as a primary tau phosphatase. The methylation state of PP2Ac affects only the assembly of PP2A-B55α holoenzyme as well as its activity as a tau phosphatase (23, 70).

The present study adds PSP to the list of neurodegenerative disorders that are associated with deficiencies in PP2A carboxyl methylation. These include the mixed Aβ amyloidosis plus tauopathy of AD as well as the α-synucleinopathies, Parkinson disease, and dementia with Lewy bodies (71, 72). Thus, it is apparent that a similar defect in PP2A regulation leads to a variety of clinical and pathologic outcomes involving protein misfolding disorders. Hypotheses concerning the pathogenesis of such age-associated neurodegenerative diseases generally fall into 2 broad categories: general defects such as mitochondrial dysregulation, oxidative stress, and neuroinflammation that are associated with aging, and disease-specific protein misfolding and accumulation such as amyloid Aβ, tau, and α-synuclein. This view is supported by the common occurrence of pathologic and clinical comorbidities between these different disease states. For example, commonalities in neurodegenerative diseases that are known to impact PP2A carboxyl methylation and activity include oxidative stress (73–78) and elements of methylation metabolism such as levels of the methyl donor S-adenosylmethionine (SAM), the competitive inhibitor, S-adenosyl homocysteine (SAH), or the methylation cycle intermediate intermediate homocysteine (71, 79–83). These shared features suggest that efforts to enhance PP2A activity may reduce the hyperphosphorylation of tau and α-synuclein in these disorders and mitigate the associated pathology, a notion that has been tested successfully in rodent models of both tauopathy and α-synucleinopathy (67, 84–86). This appears to be a potentially viable therapeutic approach particularly considering the multiple kinases involved in phosphorylating these pathogenic proteins (87–89).

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