The Role of Protein Phosphatase 2A in Alzheimer's disease pathogenesis

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I hereby certify that the work embodied in the thesis is my own work, conducted under normal supervision. The thesis contains no material which has been accepted, or is being examined, for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no previously published or written by another person, except where due reference has been made in the text. I give consent to the final version of my thesis being made available worldwide when deposited in the University's Digital Repository, subject to the provision of the Copyright Act 1968 and any approved embargo.

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I hereby certify that the work embodied in this thesis contains published papers of which I am a joint author. I have included as part of the thesis a written declaration endorsed in writing by my supervisor, attesting to my contribution to the joint publications.

ALEXANDER HOFFMAN

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Abbreviations

3R/4R	**Three Repeat / Four Repeat Tau**	**5HT**	**5-Hydroxy-Triptamine (Serotonin)**
ACh	Acetylcholine	**AD**	Alzheimer's Disease
AICD	Amyloid Intracellular Domain	**AKAP**	A-Kinase Anchoring Protein
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid	**APP**	Amyloid-β Precursor Protein
Aβ	Amyloid-β	**BACE**	B-Amyloid Cleaving Enzyme
BDNF	Brain-Derived Neurotrophic Factor	**BH4**	Tetrahydrobiopterin
CA	Cornu Ammonis	**cAMP**	Cyclic Adenosine Monophosphate
CBD	Corticobasal Degeneration	**CBS**	Cystathionine-β-Synthase
cdk5	Cyclin-dependent protein kinase 5	**CREB**	cAMP-responsive element binding protein
CTF	Carboxy-Terminal Fragment	**D_xR**	Dopamine (1-5) Receptor
ERK	Extracellular Regulated Protein Kinase	**FTD**	Fronto-temporal dementia
GAP	Guanine triphosphate-hydrolysing enzyme Activating protein	**GSK-3β**	Glycogen Synthase Kinase 3β
HA	Hemagglutinin	**Hcy**	Homocysteine
HHcy	Hyper-homocysteinemia	**JNK**	c-Jun N-terminal Kinase
LCMT-1	Leucine Carboxyl-methyltransferase 1	**LTD**	Long-term depression

LTP	Long-term potentiation	**mAChR**	Muscarinic Acetylcholine receptor
MAP2	Microtubule associated protein 2	**mGluR**	Metabotropic glutamate receptor
Mid1	Midline 1 protein	**MT**	Microtubule
MTHFR	5,10- methylene tetrahydrofolate reductase	**NFT**	Neufibrillary Tangle
NGF	Neurotrophic Growth factor	**NMDA**	N-Methyl-D-aspartate
OA	Okadaic Acid	**PDE**	Phospho-diesterase
PHF	Paired Helical Filaments	**Pin1**	Peptidyl- prolyl cis-trans isomerase NIMA-interacting 1
PKA	cAMP-dependent protein kinase A	**PME-1**	Protein Phosphatase Methylesterase 1
PP2A	Protein Phosphatase 2A	**SAH**	S-Adenosyl-Homocysteine
SAM	S-Adenosyl- Methionine	**sAPP**	Secreted Amyloid-β Precursor Protein
SH(x)	Src Homology Domain	**tHcy**	Total Plasma Homocysteine
TIPRL	Target of rapamycin signalling pathway regulator	**TrkA**	Tropomyosin Receptor Kinase A

Thesis Abstract

Sporadic Alzheimer's disease (AD) is the most prevalent form of dementia in Australia and worldwide. Studies on the Familial form of AD have identified many of the molecular participants in the pathophysiology of AD; included in these is the Amyloid-β (Aβ) Precursor Protein (APP), which undergoes proteolytic processing to yield one of the pathological hallmarks of AD, Aβ. Another major hallmark of AD is hyper-phosphorylated, oligomerised Tau protein, which aggregates into Paired Helical Filaments (PHFs), which are then liable to form Neurofibrillary Tangles (NFTs). In this Thesis, we aimed to interrogate potential links between these two major contributors to AD in order to untangle the disease process.

In order to extricate this pathological cascade, we consulted some of the risk factors associated with AD. Among some others, AD risk is heightened by impaired folate metabolism and elevated plasma homocysteine levels; these two conditions may arise through inadequate dietary consumption of folate and associated micronutrients, or genetic impairments in the processing of folate and methyl groups referred to as "One-Carbon Metabolism". A major intracellular signalling mediator which is dependent on the proper cycling of one-carbon groups is the enzymatic family known as Protein Phosphatase 2A (PP2A). Methylated PP2A is widely recognised as the major Tau phosphatase, and deregulated PP2A enzymes have been found to co-exist in AD pathology with hyperphosphorylated Tau and degenerated brain regions most affected in AD. *In vivo* models of One-Carbon metabolism also show that Tau phosphorylation is also significantly elevated in the brain. In this study, we used the same model, mice with genetic deficiencies in the 5,10-methylenetetrahydrofolate reductase (MTHFR) enzyme, which were fed a normal folate or folate-deficient diet. We were able to show that the regulation of APP expression and post-translational modification is altered in major substructures on the brain, which helps affirm the link between a risk factor for AD (impaired folate metabolism), existing evidence for this risk in AD showing that PP2A and Tau are dysregulated in this model, and the perturbation of APP regulation.

Since it is evidently a major enzyme deregulated in AD, we endeavoured here to tease apart how PP2A can be precisely controlled by post-translational modification in neurons. The two major post-translational modifications of PP2A are leucine methylation and tyrosine phosphorylation of the catalytic subunit.

Currently, the only known source of the control of PP2A methylation arises from dietary supply of methyl groups, or One-Carbon metabolism, as briefly described above. Some groups have presented evidence suggesting that activation of cAMP signalling in non-neuronal cells affect the activity state of PP2A, while others show similar evidence for changes in PP2A methylation with the initiation of cAMP signalling. We thus deemed it necessary to delineate a more precise understanding of if, and how cAMP signalling affects PP2A methylation in neurons. To do this, we specifically investigated the activity and kinase targeting of cAMP-dependent protein kinase A (PKA) in cultured N2a cells. Indeed, we were able to show that activation of PKA with the cAMP-generating drug Forskolin led to time-sensitive demethylation of PP2A. We also observed that overexpression of the catalytic subunit of PP2A (PP2Ac) reduced the PKA-targeted phosphorylation sites of Tau and the transcription factor CREB, which is heavily involved in learning and memory consolidation. We thereby demonstrate a novel mode of PP2A regulation with direct consequences for both AD pathogenesis and regulation of learning and memory.

PKA-mediated changes in PP2A methylation appeared to have such important consequences, so we also used the MTHFR- and folate-deficient mouse models described above to

investigate a major neural target of PKA and PP2A, CREB. We observed an interesting array of effects of disturbed One-Carbon metabolism on CREB expression and phosphorylation, which is linked to its transcription factor activity state. In the cortex and midbrain regions of mice with genetic deficiency of the MTHFR enzyme, CREB expression was altered, and CREB activity was elevated in the midbrain of these mice. Hence, in this thesis, we demonstrate that disturbed One-Carbon metabolism, which is related to multiple AD risk factors, dysregulates both APP and CREB, both of which are implicated in the AD process. These are linked through previous results from our lab showing that PP2A methylation and Tau phosphorylation are concurrently affected by this model in the same way they are dysregulated in AD pathology.

In contrast to methylation of the catalytic subunit, tyrosine phosphorylation of PP2Ac was first reported to catalytically inactivate the enzyme. Unfortunately, these results have never been corroborated *in vivo* using independent methodologies. Since tyrosine phosphorylated PP2A has been reported to accumulate in close proximity to dystrophic neurites in AD, we deemed it important to substantiate the nature of PP2A tyrosine phosphorylation. If we could confirm that PP2A tyrosine phosphorylation and inactivation occurs in neurons, this may elucidate novel neurotoxic cascades in AD. In two distinct cell lines, we were able to show that the non-receptor tyrosine kinase, Src indeed phosphorylates PP2A, but at two previously unidentified tyrosine sites. We also demonstrate that phosphorylation of one of these sites can impair the Tau phosphatase activity of PP2A in N2a cells.

In the following body of work, we explore the importance of post-translational modifications of PP2A in the context of AD pathogenesis. Using cellular, *in vivo* and *ex vivo* techniques, we use this enzyme to navigate the role of dietary and genetic disturbances in the regulation of major proteins disturbed in AD, specifically Tau, APP and CREB. The novel findings we present provide the foundation for future study in the dysregulation of PP2A in memory disturbances and neurotoxicity in the molecular course of AD.