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Decreased phosphatidylethanolamine binding protein expression correlates with Aβ accumulation in the Tg2576 mouse model of Alzheimer's disease

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Abstract

Phosphatidylethanolamine binding protein (PEBP) is a multifunctional protein, with proposed roles as the precursor protein of hippocampal cholinergic neurostimulating peptide (HCNP), and as the Raf kinase inhibitor protein (RKIP). Previous studies have demonstrated a decrease in PEBP mRNA in CA1 region of AD hippocampus. The current study demonstrates that PEBP is decreased in the hippocampus of 11 month Tg2576 mice, in the absence of change in mRNA levels compared to non-transgenic littermates. The level of PEBP in transgenic mouse hippocampus significantly decreases at 11 months (a time point when Aβ begins accumulating) and 15 months (when Aβ plaques have formed). There was a significant correlation between decreased PEBP expression and accumulation of Aβ. Immunohistochemical studies on Tg2576 and AD brain sections demonstrate that PEBP immunoreactivities are present at the periphery of dense multicore Aβ plaques, and in selective astrocytes, primarily surrounding plaques. These findings suggest that PEBP expression may be influenced by accumulation of Aβ. Down-regulation of PEBP may result in lower levels of HCNP or altered coordination of signal transduction pathways that may contribute to neuronal dysfunction and pathogenesis in AD.

Keywords: Alzheimer's disease; Phosphatidylethanolamine binding protein; Aβ amyloid; Amyloid precursor protein; Hippocampus; Transgenic mouse; Tg2576

1. Introduction

Alzheimer's disease (AD) is the most common form of diagnosed dementia in the ageing population, accounting for 50–70% of late-onset dementia cases [19], with approximately 10% of individuals over the age of 65, and 40–50% over the age of 75 affected by the disease [20]. AD is clinically characterised by a progressive decline in multiple cognitive functions, with memory impairment and the presence of aphasia, apraxia, agnosia and/or the loss of the ability to plan and organise normal activities [3]. AD is currently classified as either early-onset (<65 years) or late-onset (>65 years) [13]. Early-onset or familial AD (FAD) has been linked with mutations in three key genes that are suggested to be causative. These genes include the amyloid precursor protein (APP) [19], Presenilin 1 (PS1) [48] and Presenilin 2 (PS2) genes [29]. Mutations in

Abbreviations: Aβ, amyloid β peptide; AD, Alzheimer's disease; APP, amyloid protein precursor; BCIP, 5-bromo-4-chloro-3-indolyl-phosphate; CT, C-terminal; DAB, diaminobenzidine; HCNP, hippocampal cholinergic neurostimulating peptide; NBT, nitroblue tetrazolium chloride; NTg, non-transgenic; NT, N-terminal; PEBP, phosphatidylethanolamine binding protein; RKIP, Raf kinase inhibitor protein; SDS, sodium dodecyl sulfate; qRT-PCR, quantitative real-time PCR

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these genes are very rare, highly penetrant and transmitted through the family in an autosomal dominant manner. FAD accounts for approximately 10% of all AD cases, with mutations in APP, PS1 and PS2 accounting for ∼30% of this component [52].

At present, no causative genes have been associated with late-onset, or sporadic AD, although a positive correlation between a family history, and development of dementia in later life has been observed [14]. Genetic linkage studies of AD have identified the Apolipoprotein E (Apoe) gene as a risk factor. Studies have shown that inheritance of the Apoe ε4 allele may increase the risk of developing AD, while presence of the ε2 allele may be protective against developing the disease [12,45,50]. In searching for novel AD genes, complete genome screens have been performed to identify regions of genetic linkage that may be involved in its pathogenesis. At present, linkage studies have identified regions on chromosomes 9, 10 and 12 to be of some importance in AD [28,41]. However, the quest to identify potential genes in these 'hotspots' continues.

The use of high-throughput gene expression studies has helped elucidate different pathways of gene expression in humans [10,16,33,40] as well as mouse models of AD [14,51]. However, the heterogeneity observed between each of the different studies highlights the fact that AD is a genetically complex disease in which the expression of many different genes may play a role in pathogenesis. The current challenge is to identify gene targets that show some homogeneity in their expression amongst patients, as this may help further the understanding of the molecular pathogenesis, particularly in sporadic AD.

The phosphatidylethanolamine binding protein (PEBP) gene has been associated with AD, where decreased mRNA expression was observed in the brain of AD patients, in particular, in the CA1 hippocampal field of patients with late-onset AD [34]. PEBP is also known as Raf kinase inhibitor protein (RKIP), prostatic binding protein, hippocampal cholinergic neurostimulating peptide precursor (HCNPp) and in humans specifically, as neuropolypeptide h3 [47]. PEBP is a small cytosolic protein that was initially purified from bovine brain [5], but was later shown to be expressed in a large range of tissues from mammalian species including humans [23,47], monkeys [42], rats [17] and mice [2]. PEBP is localised to oligodendrocytes and Schwann cells and was postulated to be involved in membrane biogenesis or lipid transfer [35]. Further studies have shown that PEBP is abundantly distributed throughout the cytoplasm, and its association with plasma membrane suggests that it has a potential role in signalling mechanisms between the membrane and cytoplasm of cells [21,46]. PEBP is phosphorylated at serine 153 [32], which may be an important feature in its ability to inhibit Raf kinase, and hence, its involvement in regulating the MAP kinase signalling pathway [11].

PEBP is the precursor of the hippocampal cholinergic neurostimulating peptide (HCNP), an 11 amino acid peptide that possesses cholinergic neuronal stimulatory activity. The HCNP is suggested to be able to act independently and also synergistically with nerve growth factor to enhance the production of choline acetyltransferase, which assists in cholinergic development of the medial septal nuclei of the brain [38]. As the cholinergic hypothesis suggests that cognitive decline observed in AD is primarily due to a cholinergic deficit [4], a decrease in overall levels of PEBP and/or HCNP in AD affected patients may help explain why cholinergic impairments are prevalent in the disease.

In this paper, we investigated the levels of PEBP at the transcriptional and post-transcriptional levels in hippocampus and cortex of the Tg2576 mouse model of AD, which develop Aβ plaques at approximately 9–12 months of age [24] and non-transgenic littermates. We also studied the cellular distribution of PEBP in the Tg2576 mouse and end-stage sporadic AD.

2. Materials and methods

2.1. Tissue samples

For the initial mouse study, 11-month-old female Tg2576 mice ($n = 5$) and non-transgenic (NTg) littermates ($n = 5$) were cervically dislocated and brain tissue was collected. For the age study, female Tg2576 mice that were 6 months ($n = 5$), 11 months ($n = 5$) and 15 months ($n = 4$), along with the same number of NTg littermates per group, were asphyxiated with CO_2 and the brain removed. Brain tissue taken from the mice was microdissected to obtain hippocampus and cortex for further analysis. Samples were snap-frozen in liquid nitrogen and stored at −80 °C until use. For immunohistochemical analysis, hemibrains from Tg2576 and NTg mice at 6, 12 and 18 months ($n = 3$ for each age group) were fixed in 10% neutral buffered formalin and embedded in paraffin for sectioning. To determine the absence/presence of the APP transgene, mice were genotyped from tail DNA collected at 4 weeks of age. Formalin fixed human cortical tissue from AD and control patients ($n = 3$ per group) were obtained from the Victorian Brain Bank Network (VBBN) (affiliation of the National Neural Tissue Resource Centre). The diagnosis of AD was confirmed using pathological and clinical criteria [18]. All experimentation (in both mice and humans) were carried out under ethical approval from The University of Melbourne Ethics Committees.

2.2. RNA isolation and cDNA synthesis

For qRT-PCR experiments, total RNA was extracted from mouse brain samples using the RNeasy Lipid Mini Kit (Qiagen, Doncaster, Australia), with DNase I treatment carried out on the spin-column using RNase-free DNase I (Qiagen), as per manufacturers' instructions. Each RNA extract was analysed spectrophotometrically to determine purity and concentration. RNA was stored in aliquots at −70 °C.