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Interaction of amyloid binding alcohol dehydrogenase/Aβ mediates up-regulation of peroxiredoxin II in the brains of Alzheimer's disease patients and a transgenic Alzheimer's disease mouse model

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Alzheimer's patients have increased levels of both the 42 beta amyloid-β-peptide (Aβ) and amyloid binding alcohol dehydrogenase (ABAD) which is an intracellular binding site for Aβ. The over-expression of Aβ and ABAD in transgenic mice has shown that the binding of Aβ to ABAD results in exaggerating neuronal stress and impairment of learning and memory. From a proteomic analysis of the brains from these animals we identified that peroxiredoxin II levels increase in Alzheimer's diseased brain. This increase in peroxiredoxin II levels protects neurons against Aβ induced toxicity. We also demonstrate, for the first time in living animals, that the expression level of peroxiredoxin II is an indicator for the interaction of ABAD and Aβ as its expression levels return to normal if this interaction is perturbed. Therefore this indicates the possibility of reversing changes observed in Alzheimer's disease and that the Aβ–ABAD interaction is a suitable drug target.

Keywords: Proteomics; Neuronal stress; ABAD; Amyloid; Alzheimer's disease

Introduction

Little is known about the early stages of Alzheimer's disease before amyloid plaques are formed though studies have indicated that there is synaptic loss before the formation of plaques (Oddo et al., 2003). Previous investigations have demonstrated the intracellular production of Aβ, within the endoplasmic reticulum (ER) and trans-Golgi network of neurons (Hartmann et al., 1997). This production of Aβ may be the earliest event occurring in Alzheimer's disease and the identification of early markers for the presence of toxic Aβ would have strong implications for the prevention and treatment of Alzheimer's disease.

Amyloid-β binding Alcohol Dehydrogenase (ABAD) is a 27 kDa intracellular binding partner for Aβ at nM concentrations (Yan et al., 1997, 2007). In the absence of Aβ, ABAD can supply energy for the brain by facilitating utilization of ketone bodies (Yan et al., 2000) and is capable of utilizing fatty acids, alcohols and hydroxysteroids (Yan et al., 1999; He et al., 1999; Powell et al., 2000). Alzheimer's disease patients have increased expression of ABAD in Alzheimer's disease-affected regions as do transgenic animals which over-express Aβ (Yan et al., 1999; Lustbader et al., 2004). Indeed over-expression of both ABAD and Aβ leads to apoptosis only if the ABAD is catalytically active (Yan et al., 1997). The crystal structure of ABAD has been solved and provided the first three-dimensional structure of an internal binding site for Aβ. This region consists of a 20 amino acid region close to the active site of the enzyme. A 20 amino acid peptide of this Aβ binding domain of ABAD, fused to the cell membrane transduction domain of the HIV Tat protein was able to block Aβ induced toxicity (Lustbader et al., 2004).

ABAD is predominately localized in mitochondria (Yan et al., 1997; He et al., 1999; Lustbader et al., 2004) whose functions are impaired in Alzheimer's disease brains (Yan and Stern, 2005; Takuma et al., 2005). Previously we showed that ABAD and Aβ are both located within mitochondria and they can be immunoprecipitated from the brains of Alzheimer's disease patients and transgenic animals over-expressing Aβ. Also transgenic animals expressing both ABAD and a mutated form of the amyloid precursor protein (Tg mAPP/ABAD), resulting in an increase in intracellular Aβ have an enhanced neuronal cytotoxicity with subsequent changes in spatial learning memory (Lustbader et al., 2004). This is via mitochondrial dysfunction because the neurons from these animals show spontaneous generation of reactive oxygen species, a decrease in ATP levels and the release of cytochrome *c* from mitochondria (Takuma et al., 2005).

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Here we identified peroxiredoxin II as a protein whose expression increased within the brains isolated from Tg mAPP, Tg mAPP/ABAD animals and Alzheimer's disease patients. We show that the physiological role of this increase appears to be the protection of neurons from Aβ induced toxicity. Significantly, we also show that if the interaction of Aβ with ABAD is perturbed then these molecular changes are reversed in living animals, thus indicating that ABAD is a suitable target for the treatment of Alzheimer's disease.

Results

Proteomics analysis of transgenic brains

Five-month-old Tg mAPP/ABAD mice show significant behavioral changes and deficits in mitochondrial function, as compared with single transgenic animals (Lustbader et al., 2004; Caspersen et al., 2005). To maximize the potential changes in protein levels, we performed our initial proteomic analysis on animals of 8 months of age. Whole brains were analyzed from three mice of each genotype (matched for age and sex). The four genotypes analyzed were animals expressing either solely mAPP (Tg mAPP), ABAD (Tg ABAD), both mAPP and ABAD (Tg mAPP/ABAD), and non-transgenic litter mates (non-Tg) and have been previously described (Lustbader et al., 2004; Caspersen et al., 2005). Proteins were extracted and analyzed as detailed in the Experimental methods. All samples were run in duplicate. The two dimensional gels of the separated proteins from the brains of Tg mAPP, Tg ABAD, Tg mAPP/ABAD and non-Tg controls (Fig. 1A), led to the identification of a protein spot that was consistently up-regulated in the Tg mAPP and Tg mAPP/ABAD transgenic animals as compared to the Tg ABAD transgenic animals or the non-transgenic littermate controls (Fig. 1B). By mass spectrometry, this protein was identified as peroxiredoxin II (gene: PRDX2) (Swiss Prot Accession No. Q61171). Importantly, the predicted molecular weight and pI values (MWt 21,779, pI 5.2) of peroxiredoxin II corresponded favorably to the observed molecular weights (MWt 22,000, pI 5.0).

Increased expression of peroxiredoxin II in Tg mAPP/ABAD and Alzheimer's disease brains

Peroxiredoxin II has been previously reported to have an increased expression in the frontal cortex of patients with Alzheimer's disease, Parkinson's disease, Pick's disease and Down syndrome (Kim et al., 2001; Krapfenbauer et al., 2003). We confirmed that this also occurred in the cerebral cortex of the double transgenic animals by immunocytochemistry (Fig. 2A); thus indicating that the Tg mAPP/ABAD animals at 8 months of age show a molecular sign of neurodegeneration that occurs in humans. We also confirmed the previous human studies by demonstrating an increase in peroxiredoxin II expression in the cerebral cortex of Alzheimer's patients, as compared to non-demented age matched controls, by both Western blot analysis (Fig. 2B) and immunocytochemistry (Fig. 2C).

Increased peroxiredoxin II expression protects neurons from Aβ induced toxicity

The role of peroxiredoxin II is as an anti-oxidant protein (Wood et al., 2003) and so we surmised that its increased expression was symptomatic of cells attempting to protect themselves against the increased Aβ levels. Indeed previous studies have shown that an

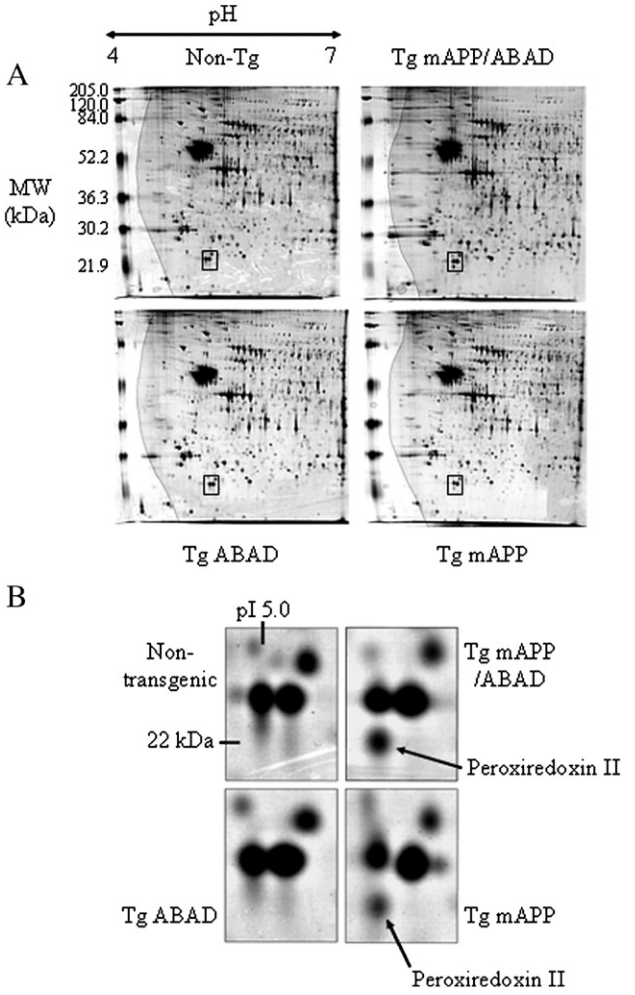

Fig. 1. Two-dimensional separation of proteins from (A) the total brain extract and (B) the identified peroxiredoxin II protein isolated from transgenic animals expressing solely mAPP (Tg mAPP), ABAD (Tg ABAD), both mAPP and ABAD (Tg mAPP/ABAD) or non-transgenic littermate controls (non-Tg).

increase in peroxiredoxin II can protect cells against increased levels of reactive oxygen species (Simzar et al., 2000; Han et al., 2005). Therefore we tested directly for the ability of an increased expression of peroxiredoxin II to protect neurons in a high Aβ concentration environment. Primary cortical neurons were either mock transfected or transfected with a DNA plasmid expressing peroxiredoxin II or a non-related protein PSD95. These neurons were then tested for their capability of surviving the addition of toxic levels of Aβ (Morishima et al., 2001). Significantly, only in those experiments where neurons were transfected with the peroxiredoxin II expressing plasmid were the cortical neurons protected against toxic levels of Aβ (Fig. 3). Therefore this indicates that an increased expression of this anti-oxidant enzyme in Alzheimer's diseased brain is symptomatic of the neurons attempting to protect themselves in a high Aβ concentration environment.

Perturbing the interaction of Aβ with ABAD results in a reversal in expression of peroxiredoxin II in Tg mAPP animals

Analyzing the Aβ–ABAD crystal structure suggests that residues 94–114 of ABAD interact with Aβ. A peptide spanning