



Proteomic Identification of Oxidatively Modified Golgi-Enzyme Glucuronyltransferase and Potential Consequences Related to the Modulation of Synaptic Plasticity in a Mouse Model of Alzheimer's Disease

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I. Background

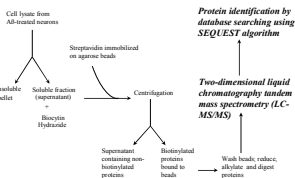
Oxidative stress imparted by reactive oxygen species (ROS) is implicated in the pathogenesis of Alzheimer's disease (AD). Given that amyloid β (AB) itself generates ROS that can directly damage proteins, elucidating the functional consequences of protein oxidation can enhance the understanding of the process of AB-mediated neurodegeneration. In the present study, the identification of targets of AB-induced oxidative stress was carried out by employing a biocytin hydrazide/streptavidin affinity purification methodology followed by two-dimensional liquid chromatography tandem mass spectrometry (LC-MS/MS) coupled with SEQUEST bioinformatics. The Golgi-resident enzyme glucuronyltransferase (GlcAT-P) was identified as a carbonylated target which was further investigated due to its involvement in the biosynthesis of HNK-1, a carbohydrate epitope expressed on cell adhesion molecules and implicated in modulating the effectiveness of synaptic transmission in the brain.

Our results suggest that the carbonylation of GlcAT-P is a result of AB-induced oxidative stress since the expression of HNK-1/NCAM is inversely related to the concentration of AB added to the culture media. Although the expression of HNK-1/NCAM was reduced, the level of NCAM expression was not found to vary with respect to AB concentration. Assessment of the tissue-level expression of HNK-1 via immunohistochemical methods revealed decreased HNK-1 immunoreactivity in hippocampal and cortical brain regions exhibiting AB plaque deposition in 12 month-old Tg2576 mice.

It has been demonstrated that GlcAT-P^{-/-} mice deficient in the HNK-1 carbohydrate epitope exhibit defects in functions of the nervous system such as aberrant long term potentiation (LTP) and hippocampus-dependent spatial learning (Yamamoto et al., *J Biol Chem*, 277:27227-31, 2002). Considering that AD is partly characterized by progressive memory impairment and disordered cognitive function, our data can be reconciled with results from other *in vivo* studies (Senn et al., *Mol Cell Neurosci*, 20:712-29, 2002; Strickova et al., *Mol Cell Neurosci*, 17:1102-13, 2001) which have demonstrated that HNK-1 modulates synaptic plasticity and is critically involved in memory consolidation.

II. Methods

Affinity purification of carbonylated proteins by biocytin hydrazide and streptavidin methodology



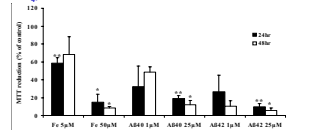
MTT reduction assay

Western blot

Immunohistochemistry

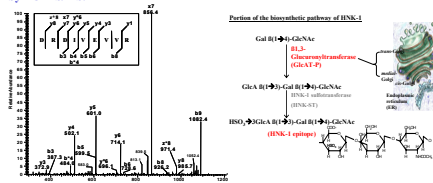
III. Results

1. Impaired MTT reduction in neuronal cultures treated with FeSO₄, Aβ1-40 and Aβ1-42



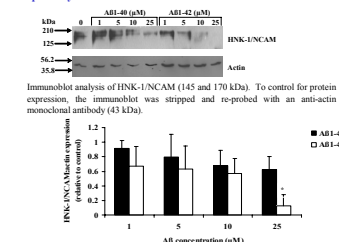
Levels of MTT reduction were quantified and are expressed as percentages of control values (means \pm SEM, n = 3). *p<0.01 compared with control value; **p<0.005 compared with control value (Student's t-test).

2. Identification of a glucuronyltransferase (GlcAT-P) peptide by LC-MS/MS.



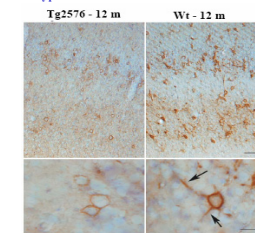
Identified b-, x-, y-, and z-ions resulting from fragmentation of the peptide with the charge retained on the N-terminus (b-ions) or C-terminus (x-, y- and z-ions) are indicated. * indicates an ion that has lost an ammonia molecule (NH₃). ^o indicates an ion that has lost a water molecule (H₂O).

3. AB leads to the down-regulation of HNK-1/NCAM expression in primary neurons.



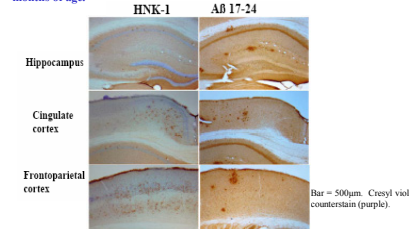
Bands from immunoblots were quantified by densitometric analysis and are expressed as values relative to control (no peptide added). Data represent means \pm SEM for three independent cell preparations. Statistical significance was assessed using Student's t-test. *p<0.01 compared with control.

4. HNK-1 protein immunoreactivity is reduced in frontoparietal cortex brain region of 12 month-old Tg2576 mice, relative to age-matched wild-type control mice.

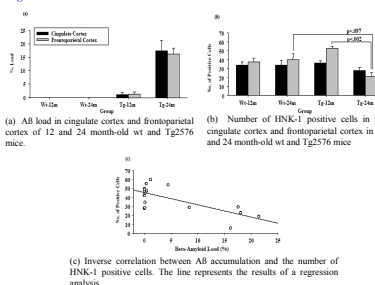


Anti-HNK-1 antibody (brown label) and cresyl violet counter stain (purple). Wt animals showed HNK-1 immunostaining on neuronal membranes and dendrites (arrows) whereas immunolabeling was more limited to the plasma membrane in Tg2576 animals. Magnification: top panel bar = 100μm, bottom panel bar = 20μm.

5. HNK-1 immunoreactivity is reduced in cortical and hippocampal brain regions exhibiting AB plaque deposition in Tg2576 mice at 12 months of age.

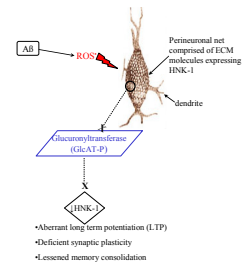


6. HNK-1 immunoreactivity decreases with increasing AB deposition in Tg2576 mice.



(c) Inverse correlation between AB accumulation and the number of HNK-1 positive cells. The line represents the results of a regression analysis.

IV. Conclusions



Glucuronyltransferase (GlcAT-P), an enzyme involved in the biosynthesis of HNK-1, was one of the carbonylated proteins identified by our biocytin hydrazide/streptavidin affinity methodology and LC-MS/MS.

Increasing amounts of AB, added exogenously to the culture media of primary cortical neurons, significantly decreased HNK-1 expression.

HNK-1 immunoreactivity was decreased in cortical and hippocampal brain tissue of a transgenic mouse model of AD.

A potential consequence of AB-mediated oxidation of GlcAT-P is the impairment of its enzymatic function, thereby disrupting HNK-1 biosynthesis and possibly adversely affecting synaptic plasticity.

Considering that AD is partly characterized by progressive memory impairment and disordered cognitive function, the data from our *in vitro* studies can be reconciled with results from other *in vivo* studies which have demonstrated that HNK-1 modulates synaptic plasticity and is critically involved in memory consolidation.

>Portions of this work will appear as a manuscript entitled "Reduced neuronal expression of synaptic transmission modulator HNK-1/Neural cell adhesion molecule (NCAM) as a potential consequence of AB-mediated oxidative stress: a proteomic approach" - *Journal of Neurochemistry* in press

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