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Decreased expression of GGA3 Protein in Alzheimer's disease frontal cortex and increased co-distribution of BACE with the amyloid precursor protein

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ABSTRACT

BACE initiates the amyloidogenic processing of the amyloid precursor protein (APP) that results in the production of Aβ peptides associated with Alzheimer's disease (AD). Previous studies have indicated that BACE is elevated in the frontal cortex of AD patients. Golgi-localized γ-ear containing ADP ribosylation factor-binding proteins (GGA) control the cellular trafficking of BACE and may alter its levels. To investigate a link between BACE and GGA expression in AD, frontal cortex samples from AD (N = 20) and healthy, age-matched controls (HC, N = 17) were analyzed by immunoblotting. After normalization to the neuronal marker β-tubulin III, the data indicate an average two-fold increase of BACE protein ($p = 0.01$) and a 64% decrease of GGA3 in the AD group compared to the HC ($p = 0.006$). GGA1 levels were also decreased in AD, but a statistical significance was not achieved. qRT-PCR analysis of GGA3 mRNA showed no difference between AD and HC. There was a strong correlation between GGA1 and GGA3 in both AD and HC, but no correlation between BACE and GGA levels. Subcellular fractionation of AD cortex with low levels of GGA proteins showed an alteration of BACE distribution and extensive co-localization with APP. These data suggest that altered compartmentalization of BACE in AD promotes the amyloidogenic processing of APP.

Introduction

Alzheimer's disease is the most common form of dementia in the elderly. Pathology diagnosis is established at autopsy by the presence of abundant amyloid plaques in frontal and temporal cortices and in the hippocampus, and by the presence of intraneuronal fibrillary tangles due to aggregation of hyperphosphorylated Tau. A decrease of brain volume is also observed that is attributed to the loss of gray matter due to extensive neurodegeneration (reviewed by Blennow et al., 2008). The amyloid plaques mainly consist of Aβ amyloid protein, which derives from the proteolytic processing of the amyloid precursor protein (APP). Aβ forms toxic oligomers that are believed to trigger a cascade of cellular events resulting in neuronal death and AD pathogenesis (McLean et al., 1999; Selkoe, 2008). The production of Aβ involves sequential cleavages of APP by β-APP cleaving enzyme (BACE) and γ-secretase (reviewed by Evin et al., 2003).

Our previous studies, and reports by several independent groups have shown that the levels of BACE protein and activity are increased in the brain cortex of patients with AD (Borghi et al., 2007; Fukumoto et al., 2002; Holsinger et al., 2002; Li et al., 2004; Tesco et al., 2007; Yang et al., 2003; Zhao et al., 2007). Except for the study by Li and colleagues, which has reported an increase in BACE mRNA in AD, no change in BACE mRNA expression was usually observed (Holsinger et al., 2002, 2004; Johnston et al., 2005). However, three studies have recently described some alterations in the translational regulation of BACE in AD. Decreased levels of miRNA-29a and -29b were shown to be associated with increased BACE protein expression (Hébert et al., 2008; Wang et al., 2008). Increase in a non-coding antisense BACE RNA has also been associated with the disease (Faghihi et al., 2008).

Another plausible reason for elevated levels of BACE in AD may be the impairment of its metabolism due to abnormal subcellular distribution. The Golgi-localized γ-ear containing ADP ribosylation factor-binding (GGA) proteins control the trafficking of BACE between trans-Golgi network (TGN) and endosomes (He et al., 2004; von Arnim et al., 2006; Wahle et al., 2005) by interaction with a DXXLL consensus motif in BACE cytoplasmic tail (DISLL sequence, at residues 496–500). GGAs represent a family of Golgi-associated monomeric

Abbreviations: AD, Alzheimer's disease; APP, amyloid precursor protein; BACE, beta-APP Cleaving Enzyme; GGA, Golgi-localized γ-ear containing ADP ribosylation factor-binding protein; HC, healthy control; NC, neurological control; SD, standard deviation; TGN, trans-Golgi network.

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adaptors involved in endosomal sorting of a subset of membrane proteins such as the mannose-6-phosphate receptors (Bonifacino, 2004). The four human GGA homologues comprise three conserved structural domains (as illustrated in Fig. 2A): a vps, Hrs, and STAM (VHS) domain that binds to the acidic-cluster-dileucine motif of cargo proteins; a GGA and TOM (GAT) domain that mediates association with Golgi Arf-GTP receptors; a γ-adaptin ear (GAE) domain, involved in recruiting adaptin and other proteins for packaging of transport vesicles (reviewed in Boman, 2001). GGAs mostly differ within their hinge region, a flexible domain connecting the GAT and GAE domains and containing a clathrin-binding site, which may confer each GGA with a specific role besides endosomal sorting. GGA1 and GGA3 hinge regions contain a DXXLL motif for auto-inhibition and their function is regulated by cycles of phosphorylation and dephosphorylation (Doray et al., 2002). GGA1 has been involved in retrograde transport of internalized phosphorylated BACE from endosomes to TGN (He et al., 2004; von Arnim et al., 2004; Wahle et al., 2005), and thus may serve a specific role in the recycling from endosomes to TGN. GGA3 contains two binding sites for ubiquitin in its hinge region and has been shown to target ubiquitinated cargo proteins for lysosomal degradation (Puertollano and Bonifacino, 2004). BACE can be ubiquitinated at its C-terminal 501 lysine residue (Qing et al., 2004), therefore GGA3 has been proposed to play a role in the transport of ubiquitinated BACE to the lysosomes for degradation (Tesco et al., 2007).

GGA3 is a substrate for caspase cleavage and, under apoptotic conditions, its cleavage by caspase-3 prevents its sorting function and prolongs BACE cellular half-life (Tesco et al., 2007). Tesco and colleagues reported a decrease of GGA3 in AD brain cortex that is inversely correlated to BACE expression, suggesting that GGA3 regulates BACE cellular levels. Two separate studies have described the expression of GGA1 in human temporal cortex and reported its decrease in AD (von Arnim et al., 2006; Wahle et al., 2006). In the present study we have examined simultaneously and compared the expression of GGA1, GGA3, and BACE proteins in a series of frontal cortex samples from AD and control patients, including other neurodegenerative conditions.

Materials and methods

Human brain samples

The following study was approved by an institutional human ethics committee, at the University of Melbourne. Frontal cortex samples (~100 mg) were obtained from the National Tissue Resource Centre, Victorian Brain Bank Network (Melbourne, Australia). Alzheimer's disease (AD) pathological diagnostic was established according to the CERAD criteria. The sample cohort (Table 1) included twenty AD (16 males and 4 females; mean age 75 ± SD 6.8 years), seventeen healthy controls (HC: 12 males and 5 females; mean age 74 ± SD 7 years), and ten controls with neurological disorders other than AD (NC: 6 males and 4 females; mean age 74 ± SD 6.9 years). AD severity, according to the Braak and Braak criteria, was at stage 5 for 15 cases, and stage 4 for the other 5 cases. The NC group was comprised of patients with Huntington's disease (2 cases), Pick's disease (1 case), Parkinson's disease (1 case), frontotemporal dementia (2 cases), corticobasal neurodegeneration (1 case), and dementia with Lewy bodies (3 cases). Post-mortem interval ranged from 9–72 h, with an average of 18 h and was not statistically different between the three groups.

Tissue processing and immunoblotting

Frontal cortex samples were suspended in TRIzol (Invitrogen) at a ratio of 1 mL per 100 mg and homogenized by repeated passages through needles of increasing gauge (19–26 G). RNA and protein were isolated according to the TRIzol manufacturer's protocol. The protein precipitates were dried and solubilized in 1% SDS (0.2 mL/100 mg sample), with heating at 37 °C for 20 min. Protein concentration was determined using Pierce BCA kit (Thermo Scientific). Protein was separated in 8.5% acrylamide gels (25 μg/lane for BACE detection; or 30 μg/lane for GGA detection) and transferred to nitrocellulose (Transblot, BioRad). The blots were incubated with, anti-BACE1 [486–501] rabbit polyclonal antibody (1:1500 in 5% skim milk plus 1% BSA in PBS), anti-BACE1 [46–62] (Sigma EE-17; 1:1000 in 0.5% hydrolysed casein in PBS), anti-GGA1 [1–50] (Abcam; 1: 200 in TBS-T [Tris buffer saline, pH 8.0, plus 0.2% Tween-20]), or anti-GGA3 [400–415] (Rockland; 1:1000 in TBS-T). Secondary antibodies were anti-rabbit HRP conjugate from Dako (1:2000 in blocking buffer) for BACE detection, and Pierce anti-rabbit HRP (1:5000 in TBS-T) for GGA detection. The blots were developed with West DURA Super Signal (Pierce) and chemiluminescence signals were captured with a GeneGnome cold camera digital instrument (Syngene, Cambridge, UK). Blots were stripped with 1% hydrochloric acid for 10 min and reprobed with anti-β-tubulin III

Table 1
Demographics and pathology of the cases.

Case	Age (years)	Gender	Diagnosis
HC1	79	Male	Normal
HC2	75	Female	Normal
HC3	73	Male	Normal
HC4	59	Male	Normal
HC5	82	Male	Normal
HC6	81	Male	Normal
HC7	82	Female	Normal
HC8	78	Female	Normal
HC9	78	Male	Normal
HC10	77	Male	Normal
HC11	73	Male	Normal
HC12	69	Male	Normal
HC13	63	Male	Normal
HC14	72	Male	Normal
HC15	77	Male	Normal
HC16	80	Female	Normal
HC17	63	Female	Normal
HC18	60	Male	Normal
HC19	72	Female	Normal
HC20	72	Male	Normal
AD1	60	Male	AD (5)A
AD2	72	Female	AD (5)
AD3	72	Male	AD (4)
AD4	76	Female	AD (4)
AD5	78	Female	AD (4)
AD6	78	Male	AD (4)
AD7	79	Male	AD (5)
AD8	81	Male	AD (4)
AD9	82	Male	AD (5)
AD10	83	Female	AD (5)
AD11	65	Male	AD (5)
AD12	80	Male	AD (5)
AD13	68	Male	AD (5)
AD14	71	Male	AD (5)
AD15	83	Male	AD (5)
AD16	84	Male	AD (5)
AD17	71	Male	AD (5)
AD18	80	Male	AD (5)
AD19	70	Male	AD (5)
AD20	67	Male	AD (5)
NC1	72	Female	CBD[b]
NC2	76	Female	Picks
NC3	69	Male	FTD[c]
NC4	79	Male	DLB[d]
NC5	65	Male	Huntington
NC6	75	Male	DLB
NC7	64	Female	Huntington
NC8	85	Male	DLB
NC9	73	Male	FTD
NC10	82	Female	PD[e]

[a]AD severity stage according to the Braak and Braak scale; [b]corticobasal degeneration; [c]frontotemporal dementia; [d]dementia with Lewy bodies; [e]Parkinson's disease.