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Relationship between ubiquilin-1 and BACE1 in human Alzheimer's disease and APdE9 transgenic mouse brain and cell-based models

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ABSTRACT

Accumulation of β-amyloid (Aβ) and phosphorylated tau in the brain are central events underlying Alzheimer's disease (AD) pathogenesis. Aβ is generated from amyloid precursor protein (APP) by β-site APP-cleaving enzyme 1 (BACE1) and γ-secretase-mediated cleavages. Ubiquilin-1, a ubiquitin-like protein, genetically associates with AD and affects APP trafficking, processing and degradation. Here, we have investigated ubiquilin-1 expression in human brain in relation to AD-related neurofibrillary pathology and the effects of ubiquilin-1 overexpression on BACE1, tau, neuroinflammation, and neuronal viability in vitro in co-cultures of mouse embryonic primary cortical neurons and microglial cells under acute neuroinflammation as well as neuronal cell lines, and in vivo in the brain of APdE9 transgenic mice at the early phase of the development of Aβ pathology. Ubiquilin-1 expression was decreased in human temporal cortex in relation to the early stages of AD-related neurofibrillary pathology (Braak stages 0–II vs. III–IV). There was a trend towards a positive correlation between ubiquilin-1 and BACE1 protein levels. Consistent with this, ubiquilin-1 overexpression in the neuron-microglia co-cultures with or without the induction of neuroinflammation resulted in a significant increase in endogenously expressed BACE1 levels. Sustained ubiquilin-1 overexpression in the brain of APdE9 mice resulted in a moderate, but insignificant increase in endogenous BACE1 levels and activity, coinciding with increased levels of soluble Aβ40 and Aβ42. BACE1 levels were also significantly increased in neuronal cells co-overexpressing ubiquilin-1 and BACE1. Ubiquilin-1 overexpression led to the stabilization of BACE1 protein levels, potentially through a mechanism involving decreased degradation in the lysosomal compartment. Ubiquilin-1 overexpression did not significantly affect the neuroinflammation response, but decreased neuronal viability in the neuron-microglia co-cultures under neuroinflammation. Taken together, these results suggest that ubiquilin-1 may mechanistically participate in AD molecular pathogenesis by affecting BACE1 and thereby APP processing and Aβ accumulation.

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1. Introduction

Accumulation of the toxic and aggregation-prone β-amyloid (Aβ) peptides has been postulated as a central underlying pathogenic event in Alzheimer's disease (AD) brain. Aβ may subsequently cause synaptic dysfunction, activation of microglia and astrocytes, oxidative and inflammatory stress, and formation of intraneuronal neurofibrillary tangles (NFTs) consisting of hyperphosphorylated tau (Hardy and Selkoe, 2002). Aβ is produced from amyloid precursor protein (APP) as a result of sequential cleavages by *β*-site-*A*PP-*c*leaving-*e*nzyme-1 (BACE1) and γ-secretase (De Strooper and Annaert, 2000). Depending on the site of the γ-secretase cleavage, Aβ peptides of different lengths, such as Aβ40 and Aβ42, can be generated. Of these, especially Aβ42 is prone to aggregate (Selkoe, 1994).

BACE1 is the initial and rate-limiting enzyme in Aβ production (Cole and Vassar, 2007). Previous studies have indicated that BACE1 protein levels are increased in AD brain as compared to age-matched controls (Fukumoto et al., 2002; Holsinger et al., 2002; Tyler et al., 2002; Yang et al., 2003; Li et al., 2004). BACE1 is a transmembrane aspartyl protease, which is synthesized in the endoplasmic reticulum (ER) and post-translationally modified by *N*-glycosylation and furin processing in the Golgi apparatus (Vassar et al., 2009). Mature BACE1 is predominantly localized in acidic intracellular compartments, such as endosomes and *trans*-Golgi network (TGN). APP and BACE1 are internalized from the plasma membrane to endosomes through clathrin-dependent and -independent endocytosis, respectively (Sannerud et al., 2011). Internalized APP- and BACE1-containing vesicles eventually come together and β-secretase cleavage of APP occurs in endosomes, where the low pH provides optimal conditions for BACE1 activity. BACE1 cycles between the cell surface, endosomes, and TGN, until it is degraded in the lysosomes (Koh et al., 2005). Several factors, including Golgi-localized γ-ear-containing ARF-binding proteins (GGAs), small GTPase ADP ribosylation factor 6 (ARF6), and ubiquitination, have been suggested to regulate the intracellular trafficking of BACE1 and thus BACE1-mediated APP cleavage (Greeve et al., 2000; Bonifacino, 2004, Tesco et al., 2007; Lauwers et al., 2009; Sarajärvi et al., 2009; Kang et al., 2010,).

Ubiquilin-1 (also referred to as Plic-1) is a ubiquitin-like protein, encoded by the *UBQLN1* gene. Ubiquilin-1 has been shown to genetically associate with AD and regulate APP trafficking, processing and degradation, and cellular stress responses (Bertram et al., 2005; Hiltunen et al., 2006; Lu et al., 2009). Our previous in vitro studies have demonstrated that the down-regulation of ubiquilin-1 increases the maturation and intracellular trafficking of APP and the levels of secreted Aβ40 and Aβ42 (Hiltunen et al., 2006). Furthermore, we and others have shown that ubiquilin-1 affects APP and presenilin-1 (PS1) degradation and PS1 accumulation into intracellular aggresomes (Massey et al., 2004; Hiltunen et al., 2006; Thomas et al., 2006; Lu et al., 2009; Su and Lau, 2009; Viswanathan et al., 2011; El Ayadi et al., 2012; Viswanathan et al., 2013).

Ubiquilin-1 contains an N-terminal ubiquitin-like domain (UBL), which mediates interaction with the proteasome, and a C-terminal ubiquitin-associated domain (UBA) mediating binding to polyubiquitinated proteins (Kleijnen et al., 2000; Mah et al., 2000). Thus, ubiquilin-1 is suggested to regulate protein targeting to different cellular compartments, such as the proteasome and autophagosomes. The conserved asparagine- and proline-rich repeats in ubiquilin-1, may mediate interaction with specific domains in proteins involved in endocytosis and vesicle sorting, suggesting that ubiquilin-1 might also control vesicular trafficking (Mah et al., 2000). Ubiquilin-1 is upregulated during ER-stress and it regulates ER-associated protein degradation. Ubiquilin-1 also protects mice from oxidative stress and ischemic stroke-induced neuronal damage (Ko et al., 2002; Lim et al., 2009; Lu et al., 2009; Liu et al., 2014), suggesting that ubiquilin-1 is a stress-related protein. Neuroinflammation is a central stress condition involved in the pathogenesis of AD (Morales et al., 2014), but the role of ubiquilin-1 has not been previously studied in this context.

In spite of several studies suggesting the link between ubiquilin-1 and neurodegenerative diseases, only a few previous studies have provided data on ubiquilin-1 in human brain. Ubiquilin-1 mRNA splicing was found altered in the brain of individuals with the genetic *UBQLN1-8i* variation (Bertram et al., 2005). Accordingly, another study showed that ubiquilin-1 protein levels are decreased in the frontal cortex in the early phase of AD (Stieren et al., 2011). Ubiquilin-1 has also been shown to associate with NFTs, Hirano bodies, and dystrophic neurites in the hippocampus of AD brain (Mah et al., 2000; Satoh et al., 2013; Mizukami et al., 2014), but the interrelationship of ubiquilin-1 and tau has not been further characterized. In addition to the limited data on ubiquilin-1 in the brain of individuals without or with a neurodegenerative disease, there are no previous reports describing the effects of ubiquilin-1 in vivo in vertebrate animal models of AD. Studies in *Drosophila melanogaster* suggest that ubiquilin-1 affects APP processing and neurodegeneration, but these data are so far incoherent (Li et al., 2007; Ganguly et al., 2008; Gross et al., 2008).

The aim of the current study was to elucidate ubiquilin-1 expression and its relationship with AD-related cellular processes and targets, such as BACE1 in human brain samples and in well-established in vitro and in vivo models of AD overexpressing ubiquilin-1. Consequently, we have examined ubiquilin-1 expression in human temporal cortex in relation to AD-related neurofibrillary pathology. In addition, the effects of transient and stable ubiquilin-1 overexpression on BACE1, tau, neuroinflammation, and neuronal viability in co-cultures of mouse embryonic primary cortical neurons and microglial cells and in different neuronal cell lines in vitro as well as sustained ubiquilin-1 overexpression in the brain of APdE9 transgenic mice in vivo have been investigated. Our results provide evidence for the first time on the interrelationship between ubiquilin-1 and BACE1, which may play a role in AD pathogenesis.

2. Materials and methods

2.1. Plasmids and siRNAs

Plasmids encoding ubiquilin-1 TV1 (the full-length human ubiquilin-1 variant containing all 11 exons), myc-TV1-mRFP (human ubiquilin-1 TV1 containing a 3′-myc-tag and a 5′-mRFP-tag), 5′3′UTR-BACE1 (human BACE1 containing endogenous 5′- and 3′-UTRs), 5′ UTR-BACE1 (human BACE1 containing endogenous 5′UTR), BACE1-Myc (human BACE1 with a CMV promoter and a 3′-myc-tag sequence producing BACE1 protein containing a myc tag in the C-terminus), 0N4R-tau (human wild type 0N4R-tau isoform; GeneCopoeia), mRFP, and pEGFP-F (Clontech) were used in the transfections. pcDNA3.1 plasmid was used as a control. Silencer™ Pre-designed siRNA targeted to exon 5 of the human ubiquilin-1 gene (GGCGCATGTACACAGATAT) was used for downregulation of ubiquilin-1 expression (Ambion) in H4 TV1 clone-13 cells. Silencer™ Negative control #1 siRNA was used as a control in RNA interference experiments (Ambion).

2.2. Lentiviral vectors

Genes encoding human ubiquilin-1-TV1 and GFP under chicken beta actin promoter (CAG) were cloned into lentivirus transfer (HIV) plasmid using PCR-cloning method. The constructs were verified by sequencing. Third-generation self-inactivating lentiviruses expressing ubiquilin-TV1 (TV1) and GFP (GFP) were prepared in triple flasks by a calcium phosphate transfection method in 293T cells as described previously (Follenzi and Naldini, 2002) and concentrated by ultracentrifugation. CAG-promoter in empty HIV-plasmid was used as a transduction control in primary embryonic cortical cells.