Neurobiology of Aging 49 (2017) 145–153

Contents lists available at ScienceDirect

Neurobiology of Aging

journal homepage: www.elsevier.com/locate/neuaging

Presenilin 1 mutations influence processing and trafficking of the ApoE receptor apoER2

Wei Wang [a,1], Andrea M. Moerman-Herzog [a,2], Arthur Slaton [a], Steven W. Barger [a,b,c,*]

[a] Department of Geriatrics, University of Arkansas for Medical Sciences, Little Rock, AR, USA
[b] Department of Neurobiology & Developmental Sciences, University of Arkansas for Medical Sciences, Little Rock, AR, USA
[c] Geriatrics Research, Education and Clinical Center, Central Arkansas Veterans Healthcare System, Little Rock, AR, USA

ARTICLE INFO

Article history:
Received 4 August 2016
Received in revised form 29 September 2016
Accepted 1 October 2016
Available online 11 October 2016

Keywords:
Alzheimer's disease
ApoER2
γ-Secretase
Lipoprotein receptor
Presenilin
Protein processing
Protein trafficking

ABSTRACT

Presenilin (PS)-1 is an intramembrane protease serving as the catalytic component of γ-secretase. Mutations in the PS1 gene are the most common cause of familial Alzheimer's disease (FAD). The low-density lipoprotein (LDL)-receptor family member apoER2 is a γ-secretase substrate that has been associated with AD in several ways, including acting as a receptor for apolipoprotein E (ApoE). ApoER2 is processed by γ-secretase into a C-terminal fragment (γ-CTF) that appears to regulate gene expression. FAD PS1 mutations were tested for effects on apoER2. PS1 mutation R278I showed impaired γ-secretase activity for apoER2 in the basal state or after exposure to Reelin. PS1 M146V mutation permitted accumulation of apoER2 CTFs after Reelin treatment, whereas no difference was seen between wild-type (WT) and M146V in the basal state. PS1 L282V mutation, combined with the γ-secretase inhibitor N-(N-[3,5-Difluorophenacetyl]-L-alanyl)-S-phenylglycine t-butyl ester, greatly reduced the cell-surface levels of apoER2 without affecting total apoER2 levels, suggesting a defect in receptor trafficking. These findings indicate that impaired processing or localization of apoER2 may contribute to the pathogenic effects of FAD mutations in PS1.

Published by Elsevier Inc.

1. Introduction

Several aspects of Alzheimer's disease (AD) pathology and genetics are associated with the low-density lipoprotein (LDL) receptor family of lipoprotein receptors. An important link is conferred by apolipoprotein E (ApoE), which binds LDL receptors alone or as a component of lipoprotein complexes; polymorphisms in the *APOE* gene are responsible for the most prevalent genetic risk factor for late-onset AD. In the LDL receptor family, 2 members have garnered considerable attention from neuroscientists: apoER2 (a.k.a. LRP8) and VLDLR. Unlike LDL receptor–related protein 1 (LRP1) and most other members of the family, these are signal-transducing receptors. Binding of reelin, a potent developmental paracrine factor, to apoER2 and VLDL activates a well-established signal transduction pathway (Ballif et al., 2003). It can lead to inhibition of the activity of glycogen synthase kinase 3β, preventing hyperphosphorylation of tau and stabilizing microtubules (Beffert et al., 2002). In addition, the interaction of apoER2 and post-synaptic density protein 95 couples Reelin signals to the N-methyl-D-aspartate receptor (Chen et al., 2005), which is critical for long-term potentiation (LTP) and synaptic plasticity required for memory formation and maintenance. Neuronal apoER2 and VLDLR participate in critical regulation of neuroblast migration and the neurophysiology of intact adult circuits (Reddy et al., 2011). Genetic ablation of either produces a diminution of LTP and disrupts migration of new dentate granule cells into their proper position during adult neurogenesis, resulting in seizures (Forster et al., 2006). These critical roles in LTP and dentate maintenance provide additional links between the LRPs and AD; LTP is a biochemical component of memory, and emerging data is extending the proposed role for epileptiform activity in AD (Vossel et al., 2013).

The γ-secretase is an integral membrane protein complex which has been chiefly characterized as a complex of 4 subunits: nicastrin, Pen-2, Aph-1, and presenilin (PS) 1 or 2. PS is the catalytic core of γ-secretase, and it may be sufficient (Ahn et al., 2010). The PSs are nonhelical transmembrane proteins with 2 aspartyl residues that are critical for proteolysis (Edbauer et al., 2003). Mutations in the genes for either PS1 or PS2 can convey autosomal dominant inheritance of FAD. The mechanistic explanation of PS's genetic contribution to AD

* Corresponding author at: Department of Geriatrics, University of Arkansas for Medical Sciences, 4301 W. Markham, #807, Little Rock, AR 72205, USA. Tel.: +1 501 526 5811; fax: +1 501 526 5830.
E-mail address: bargerstevenw@uams.edu (S.W. Barger).
[1] Present address: Department of Physiology and Biophysics, University of Arkansas for Medical Sciences, Little Rock, AR 72205, USA. Email: wangwei@uams.edu
[2] Present address: Department of Dermatology, University of Arkansas for Medical Sciences, Little Rock, AR 72205, USA. Email: moermanandream@uams.edu

0197-4580/$ – see front matter Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.neurobiolaging.2016.10.005

has focused primarily on the tendency of FAD mutations to elevate the ratio of $A\beta_{42}$:$A\beta_{40}$ produced, and Takami et al. (2009) have proposed that this reflects a consistent loss of processivity in PSs bearing FAD mutations. However, it is dissatisfying that certain FAD mutations do not manifest this shift and others do so within the context of a diminished overall production of total Aβ (Page et al., 2008; Shioi et al., 2007; Walker et al., 2005; Xia et al., 2016). In addition, some PS1 mutations result in γ-secretase processivity that is higher than wild type (Quintero-Monzon et al., 2011). As the most aggressive PS1 mutations result in loss of function toward several substrates, Chavez-Gutierrez et al. (2012) concluded that "it is not unlikely that 'partial loss' of γ-secretase function at Notch or other γ-secretase substrates acts as an aggravating factor in FAD."

ApoER2 has been identified as one of the substrates of γ-secretase (Hoe and Rebeck, 2005; May et al., 2003), but how FAD-linked mutations in PS1 impact its processing is heretofore unknown. Moreover, some PS mutations have been connected to Reelin-signaling elements such as PI3-kinase and Crk/Dock1/Rac (Chen et al., 2004; Parent and Thinakaran, 2010; Qiu et al., 2006), which suggests that part of the effect of PS mutation on synaptic processes could be related to Reelin receptors such as apoER2. We have initiated studies of the impact of FAD *PSEN1* mutations on the processing and trafficking of apoER2. These have used primary embryonic fibroblasts and astrocytes from mice "knocked in" with 2 different FAD PS1s, as well as mouse embryonic fibroblasts (MEFs) from mice genetically ablated of both *Psen1* and *2*. Our results indicate differential effects of wild-type and mutated PS1s on apoER2 γ-cleavage and intracellular trafficking, suggesting the possibility that this receptor and its coupled Reelin signaling pathway could contribute to the pathological mechanism of the development of AD conferred by PS1 mutations.

2. Materials and methods

2.1. Plasmid constructs

Mouse apoER2 cDNA with deletion of exon 5 (apoER2-ΔEX5) was subcloned into pcDNA3.1 (Invitrogen), which was then digested with XhoI and KpnI. The excised fragment of DNA was ligated into the corresponding sites of the p1005+ expression vector with eGFP (generously provided by Yuzhi Chen, University of Arkansas for Medical Sciences). Human PS1 wild-type (WT) and L282V open reading frames were subcloned into pcDNA3.1. All plasmid constructs were made using standard techniques and were verified by sequence analysis.

2.2. Packaging of replication-defective HSV-1 virus

All herpes simplex virus-1 (HSV-1) viruses were packaged using the 2-2 cell line (generously provided by Yuzhi Chen, University of Arkansas for Medical Sciences) according to Neve et al. (2005). Briefly, 2-2 cells were transfected with empty p1005+ vector or p1005-apoER2-ΔEX5. The cells were superinfected with helper virus 27 hours after transfection. Virus was amplified by 3 passages of cells and purified from crude cell lysate by sucrose-gradient centrifugation. The infectious units of virus stock were determined by infecting NTera2 human neuronal cells for 12 hours with serial dilutions. After fixation with 4% paraformaldehyde, eGFP-positive cells were counted with fluorescence microscopy. All the experimental cell cultures were infected with the appropriate viruses at a multiplicity of infection of 1.

2.3. Production of Reelin and ApoE

Reelin was partially purified from serum-free medium (50/50 MEM/F12) conditioned by a HEK293 line stably transfected with Reelin expression construct pCrl (D'Arcangelo et al., 1997) (courtesy of Tom Curran; Children's Hospital of Philadelphia Research Inst.) or, as a control, conditioned medium from untransfected HEK293 cells. Phenylmethylsulfonylfluoride (PMSF) was added to the conditioned medium to a final concentration of 0.5 mM, and the medium was chilled to 4 °C. A saturated solution of $(NH_4)_2SO_4$ was then added gradually, with continuous stirring, to a concentration of 45%. After stirring ~18 hours at 4 °C, the suspension was subjected to centrifugation at 20,000 × *g* for 1 hour at 4 °C. Pellets were dissolved in Dulbecco's phosphate-buffered saline (PBS), pH 7.4, 1-mM $CaCl_2$, and 0.5-mM $MgSO_4$. This protein preparation was dialyzed against the previously mentioned solution for 1 hour at 4 °C to remove excess ammonium sulfate and then subjected to sterile filtration (0.2 μm). Glycerol was then added to 25% before storage at −80 °C. Reelin concentrations were determined by densitometric comparison of the major bands to those produced by known quantities of bovine serum albumin in Coomassie-stained SDS-PAGE. The preparations of conditioned medium from untransfected HEK293 cells were tested in pilot experiments for stimulation of Dab1 phosphorylation and other indices of apoER2 activity; no activity was noted, thus this preparation was omitted in subsequent experiments.

ApoE proteins were expressed in clones of the T98G human astroglioma cell line stably transfected with expression plasmids encoding ApoE3 and ApoE4. Conditioned medium was collected from these lines as previously described. Rather than $(NH_4)_2SO_4$ precipitation, centrifugal filtration (Amicon filters, 10-kDa cutoff) was employed to concentrate the lipoprotein content of the conditioned medium. This concentrated fraction was then loaded onto a Pharmacia Superdex 200 (10/300) gel-filtration column resolved with phosphate buffer (20-mM $NaPO_4$, pH 7.5, 50-mM NaCl, 1-mM EDTA) at a flow rate of 0.5 mL/min. Elution fractions were monitored at A_{280}, and those with elution times shorter than that of free ApoE were subjected to detection of ApoE by Western blot analysis. Pools that contained high concentrations of ApoE within an elution time consistent with lipoprotein particles were pooled and sterilized by filtration. Serial dilutions of these samples were run on Western blots alongside dilutions of purified ApoE of known concentration; the antibody used for detection was verified to have equal reactivity with ApoE3 and ApoE4. Digital images of the Western blot results were scanned and compared for the determination of ApoE content within the lipoprotein preparations.

2.4. Cell culture and treatments

The primary glial cells generated from heterozygous *Psen1* knock-in mice carrying the M146V mutation were generously provided by Mark P. Mattson (National Institute on Aging); primary MEF generated from homozygous *Psen1* knock-in mice carrying the R278I mutation were generously provided by Takaomi C. Saido (RIKEN Brain Science Institute); the $Psen1^{-/-}$;$Psen2^{-/-}$ double-knockout (DKO) MEF cell line was generously provided by Dr. Bart De Strooper (Katholieke Universiteit Leuven) (Herreman et al., 2000). All cells were maintained in minimal essential medium with Earle's salts (MEM, Invitrogen) plus 10% fetal bovine serum (FBS, Invitrogen).

Primary glial cells and primary MEF cells were washed with PBS and then treated with 10-nM Reelin, 20-nM ApoE3, or 20-nM ApoE4 combined with 30-μM γ-secretase inhibitor DAPT (EMD Millipore) or vehicle (dimethyl sulfoxide [DMSO]) in serum-free MEM. Three hours later, cells were infected with an HSV-1 vector or the vector containing sequences encoding apoER2-ΔEX5. After 16 hours, cells were lysed in SDS lysis buffer (2.5% SDS, 78 mM Tris, pH 6.8).

$Psen1^{-/-}$; $Psen2^{-/-}$ DKO MEF cells were transiently cotransfected with empty pcDNA3.1 or pcDNA3.1-apoER2-ΔEX5 and