Neurobiology of Aging 35 (2014) 1801–1812

Contents lists available at ScienceDirect

Neurobiology of Aging

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

Altered lipid composition in cortical lipid rafts occurs at early stages of sporadic Alzheimer's disease and facilitates APP/BACE1 interactions

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ARTICLE INFO

Article history:
Received 2 October 2013
Received in revised form 18 January 2014
Accepted 5 February 2014
Available online 11 February 2014

Keywords:
Lipid rafts
Membrane lipids
Polyunsaturated fatty acids
Docosahexaenoic acid
Membrane viscosity
Anisotropy
β-secretase
Amyloid precursor protein
Alzheimer's disease
Frontal cortex
Entorhinal cortex

ABSTRACT

The presence of lipid alterations in lipid rafts from the frontal cortex in late stages of Alzheimer's disease (AD) has been recently demonstrated. Here, we have isolated and analyzed the lipid composition of lipid rafts from different brain areas from control and AD subjects at initial neuropathologic stages. We have observed that frontal cortex lipid rafts are profoundly altered in AD brains from the earliest stages of AD, namely AD I/II. These changes in the lipid matrix of lipid rafts affected both lipid classes and fatty acids and were also detected in the entorhinal cortex, but not in the cerebellum from the same subjects. Paralleling these changes, lipid rafts from AD frontal and entorhinal cortices displayed higher anisotropy for environment-sensitive probes, indicating that lipid changes in AD lipid rafts increased membrane order and viscosity in these domains. The pathophysiological consequences of these alterations in the development and progression of AD were strengthened by the significant, and specific, accumulation of β-secretase within the lipid rafts of AD subjects even at the earliest stages. Our results provide a mechanistic connection between lipid alterations in these microdomains and amyloidogenic processing of amyloid precursor protein.

1. Introduction

Alzheimer's disease (AD) is a neurodegeneration characterized by the presence of senile plaques (SP) and neurofibrillary tangles (NFT) in the brain, leading to a progressive neuronal loss accompanied by dramatic impairment of cognitive functions (Duyckaerts and Dickson, 2003; Ferrer, 2012; Serrano-Pozo et al., 2011). It is widely accepted that the formation of SP occurs as a consequence of amyloid-beta peptide (Aβ) aggregation, a peptide formed by the amyloidogenic cleavage of the amyloid precursor protein (APP). During this process, APP is initially cleaved by β-secretase (BACE) followed by the subsequent intramembrane proteolysis of the membrane bound C-terminal fragment catalyzed by γ-secretase to generate Aβ40 and Aβ42 peptides. A great deal of evidence has shown that the amyloidogenic processing of APP occurs primarily in membrane signaling platforms designated as lipid rafts (Cordy et al., 2006; Schengrund, 2010). Lipid rafts are membrane microdomains enriched in cholesterol, glycosphingolipids, and sphingomyelin into which specific subsets of proteins and lipids partition, to build up signaling platforms that are essential for a number of neuronal functions (Rushworth and Hooper, 2011; Tsui-Pierchala et al., 2002). APP is localized in 2 cellular pools, one associated with lipid rafts, in which Aß is generated. Both β and γ-secretases subunits undergo post-translational S-palmitoylation, which aids their targeting to lipid raft domains (Hicks et al., 2012; Vetrivel and Thinakaran, 2010). BACE cleavage of APP has been proposed to occur primarily in membrane rafts of the plasma membrane and endosomes, whereas γ-secretase cleavage of C99 appears to occur primarily in rafts located in the endosomes (Beel et al., 2010; Cordy et al.,

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http://dx.doi.org/10.1016/j.neurobiolaging.2014.02.005

2006; Dislich and Lichtenthaler, 2012; Hicks et al., 2012; Vetrivel and Thinakaran, 2010).

Systematic anatomic study of cases with AD-related pathology has prompted a staging classification of sporadic AD. Stages I and II are defined by the presence of NFTs in the entorhinal cortex (stage I) and progression to the transentorhinal cortex and mild involvement of the CA1 region (stage II). Limbic stages III and IV implicate the presence of NFTs in the upper and inner layers of the entorhinal cortex, transentorhinal cortex, CA1 region of the hippocampus, subiculum, anterodorsal thalamic nucleus, amygdala, magnocellular nuclei of the basal forebrain (including Meynert nucleus), tuberomammillary nucleus (stage III), plus associated areas of the temporal cortex, striatal neurons, raphe nucleus, and locus ceruleus (stage IV). Neocortical stages V and VI require, in addition, NFTs in cortical association areas, claustrum, reticular nucleus of the thalamus, and substantia nigra (stage V), plus primary sensory areas (stage VI). Similarly, SP burden and distribution allows the classification of SP pathology into stages A (basal neocortex including orbitary and temporal cortices), B (isocortical involvement covering associated cortices), and C (involving in addition the primary cortical areas) (Braak and Braak, 1991; Braak et al., 2006; Ferrer, 2012; Serrano-Pozo et al., 2011).

Recent studies published by our research group in lipid rafts purified from human frontal cortex from normal and advanced stages of AD, have demonstrated that lipid rafts from AD brains at stages V–VI, exhibit altered lipid profiles compared with control brains (Martín et al., 2010). Amongst other alterations, lipid rafts from AD frontal cortex displayed abnormally low levels of n-3 long-chain polyunsaturated fatty acids (LCPUFA) as well as reduced unsaturation and peroxidability indexes. LCPUFA, mainly docosahexaenoic acid (DHA; 22:6n-3), are particularly enriched in cell membrane phospholipids, especially in neural tissues (Chan et al., 2012; Fabelo et al., 2012a, 2012b; Uauy et al., 2001) and their deficiency has been associated with AD (Prasad et al., 1998; Soderberg et al., 1991, 1992; Young and Conquer, 2005). These fatty acids have the capacity to influence plasma membrane organization and activity of resident proteins by modulating the lipid composition and functionality and thermodynamics of lipid raft domains (Almansa et al., 2003; Shaikh et al., 2003, 2004; Stillwell and Wassall, 2003).

The aim of the present study was to examine the progression of the lipid changes in the composition of lipid rafts from different brain areas in AD at stages I–II and III–IV of NFT pathology with associated stages 0, A, and B of SP burden, to ascertain whether alterations in lipid rafts: (1) occur at initial stages of the neurodegenerative process; (2) occur in regions not already affected by NFTs as frontal cortex at stages III–IV; (3) are associated with β-amyloid deposition; (4) result in alterations of physicochemical properties of raft membranes; and (5) modify interactions between APP, BACE, and γ-secretase thus influencing β-amyloidogenesis.

2. Methods

2.1. Human brain tissue

Brain tissues were obtained from the Institute of Neuropathology Brain Bank (Bellvitge University Hospital, Spain) following the guidelines of the local ethics committee. Eleven cases were neurologically normal and had no lesions on the neuropathologic examination; these were considered the control (CTRL) group. Metabolic diseases, including metabolic syndrome were excluded. Another 16 cases had not suffered from apparent cognitive impairment and dementia but had lesions consistent with AD-related pathology at postmortem examination (Braak et al., 2006). Cases with combined pathology (i.e., associate vascular pathology, α-synucleinopathy, argyrophilic grain disease and other tauopathies, and metabolic syndrome) were excluded from the present series. The postmortem delay was between 1.5 and 7 hours. Selected cases were classified into 3 categories according to Braak and Braak (1991): AD stages I/II (AD I/II, average age 64.6 ± 8.7 years), AD stages III/IV (AD III/IV, average age 74.6 ± 10.4 years), and control group (CTRL, average age 74.0 ± 2.16 years). In every case, frontal cortex gray matter (area 8) was carefully dissected and separated from the subcortical white matter immediately at autopsy, frozen, and stored at −80 °C until use for lipid rafts isolation. In some cases, samples of entorhinal cortex and cerebellar vermis were dissected and processed in the same way for lipid raft isolation. A summary of data from all cases used in the present series are summarized in Table 1.

2.2. Isolation of lipid rafts and non-raft fractions

Lipid raft fractions were isolated following Mukherjee et al. (2003) with slight modifications (Martín et al., 2010; Ramírez et al., 2009). Briefly, 0.1 g samples of nerve tissues were homogenized in 1 mL buffer A (50 mM Tris-HCl, pH 8.0, 10 mM $MgCl_2$, 150 mM NaCl), containing 1% Triton X-100% and 5% glycerol, and supplemented with 20 mM NaF, 1 mM Na_3VO_4, 5 mM β-mercaptoethanol, 1 mM PMSF, and a cocktail of protease inhibitors (Roche Diagnostics, Barcelona, Spain). All steps in the protocol were performed on ice or in a cold room at 4 °C. Tissue was then centrifuged at 500*g* for 5 minutes and the supernatant was collected and mixed in an orbital rotor for 1 hour at 4 °C. About 800 μL of sample was mixed with an equal volume of 80% sucrose in buffer A and overlayed with 7.5 mL of a 36% sucrose solution and 2.7 mL of a 15% sucrose solution in buffer A, in 10 mL ultracentrifuge tubes (Ultraclear, Beckman, Izasa, Tenerife, Spain). Sucrose gradients were centrifuged at 150,000*g* for 18 hours at 4 °C in a Beckman SW41Ti rotor. Fractions (F_1–F_6) of 2 mL were collected from the top to the bottom and the final pellet, corresponding to the precipitated detergent soluble fractions, that is, non-rafts fractions, were collected and resuspended in 200 μL of buffer A and frozen at −80 °C until analyses. Protein contents in each fraction were determined by the Bradford method.

2.3. Lipid raft protein characterization

For the characterization of proteins associated with lipid rafts, samples from the 6 fractions obtained were diluted in SDS loading buffer (625 mM Tris-HCl pH 6.8; 1% SDS, 10% glycerol, 5% β-mercaptoethanol; 0.01% bromophenol blue), boiled at 95 °C for 5 minutes, and loaded on 12.5% SDS-PAGE followed by Western blotting. Equal volume amounts of the different raft fractions were loaded. Gels transferred to Hybond P were processed for immunoblotting, using the following antibodies: rabbit polyclonal anti-caveolin-1 (diluted 1:200; Santa Cruz Biotechnology, Dallas, TX, USA) and anti-flotillin-1 antibody (diluted 1:1000; Becton Dickinson Biosciences, Madrid, Spain), as markers of raft scaffolding proteins, to characterize raft-enriched fractions. Other antibodies were used against non-raft membrane protein markers as controls of lipid raft purity: mouse monoclonal antibodies against the non-raft membrane proteins α1 subunit of the Na^+/K^+ ATPase (Upstate-Merck Millipore, Barcelona, Spain), and clathrin (Sigma Aldrich, Madrid, Spain), both diluted at 1:1000. Additional antibodies were mouse anti-HSP90 (diluted 1:1000; Stressgen Biotechnologies, Madrid, Spain), mouse monoclonal OXPHOS (diluted 1:10,000; Mitosciences), rabbit polyclonal SOD 2 (diluted 1:2000; Stressgen Bioreagents, Zurich, Switzerland), and rabbit polyclonal GRP97 (diluted 1:200; Santa Cruz Biotechnology). All these antibodies were used to re-blot onto the same membrane. Visualization of antibody labeling