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Morphometric alterations of Golgi apparatus in Alzheimer's disease are related to tau hyperphosphorylation

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ABSTRACT

The Golgi apparatus (GA) is a highly dynamic organelle, which is mainly involved in the post-translational processing and targeting of cellular proteins and which undergoes significant morphological changes in response to different physiological and pathological conditions. In the present study, we have analyzed the possible alterations of GA in neurons from the temporal neocortex and hippocampus of Alzheimer's disease (AD) patients, using double immunofluorescence techniques, confocal microscopy and 3D quantification techniques. We found that in AD patients, the percentage of temporal neocortical and CA1 hippocampal pyramidal neurons with a highly altered GA is much higher (approximately 65%) in neurons with neurofibrillary tangles (NFT) than in NFT-free neurons (approximately 6%). Quantitative analysis of the surface area and volume of GA elements in neurons revealed that, compared with NFT-free neurons, NFT-bearing neurons had a reduction of approximately one half in neocortical neurons and one third in CA1 neurons. In both regions, neurons with a pre-tangle stage of phospho-tau accumulation had surface area and GA volume values that were intermediate, that is, between those of NFT-free and NFT-bearing neurons. These findings support the idea that the progressive accumulation of phospho-tau is associated with structural alterations of the GA including fragmentation and a decrease in the surface area and volume of GA elements. These alterations likely impact the processing and trafficking of proteins, which might contribute to neuronal dysfunction in AD.

1. Introduction

The Golgi apparatus (GA) is an essential organelle in the processing and targeting of cellular proteins, and its disruption is related to neuronal dysfunctions and human diseases (Gonatas et al., 2006; Hu et al., 2007). It has been shown that the GA undergoes morphological plasticity processes that affect the GA shape and protein content under different physiological and pathological conditions (Anton-Fernandez et al., 2015; Fan et al., 2008; Glick, 2002; Levine et al., 1995). In different neurological diseases, including Alzheimer's disease (AD), the GA of certain populations of neurons becomes fragmented (Baloyannis, 2014; Dal Canto, 1996; Fan et al., 2008; Fujita et al., 2006; Fujita and Okamoto, 2005; Fujita et al., 2002; Gonatas et al., 1998b; Gonatas et al., 2006; Gonatas et al., 1992; Hu et al., 2007; Huse et al., 2002; Liazoghli et al., 2005; Mizuno et al., 2001; Rabouille and Haase, 2015; Sakurai et al., 2000; Stieber et al., 1996). Several studies carried out in animal models of AD have related this fragmentation to the accumulation of either amyloid β (Aβ) or hyperphosphorylated microtubule-associated protein tau—the two main pathological hallmarks of AD (see Jiang et al., 2014; Joshi and Wang, 2015; Liazoghli et al., 2005). However, the precise temporal sequence and the possible relationships between GA fragmentation and the accumulation of Aβ plaques or neurofibrillary tangles of hyperphosphorylated tau during the evolution of AD have not been fully characterized.

Proper functioning of the GA is necessary for Aβ production and for trafficking and maturation of amyloid precursor protein APP and its processing enzymes (Burgos et al., 2010; Choy et al., 2012; Greenfield et al., 1999; Huse et al., 2002; Joshi et al., 2015). In addition, studies using the APPswe/PS1ΔE9 animal model of the disease have reported that the accumulation of Aβ peptides leads to Golgi fragmentation, mediated by cdk5-dependent phosphorylation of Grasp 65, which in turn accelerates APP trafficking and Aβ production (Joshi et al., 2015; Joshi and Wang, 2015). Furthermore, the integrity of the microtubule (Burkhardt, 1998; Cole et al., 1996) and actin (Egea et al., 2006) cytoskeleton is necessary for the maintenance of the structural characteristics and the positioning of the GA. Various studies have suggested the existence of

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feedback mechanisms between tau hyperphosphorylation, microtubule organization and GA fragmentation. For instance, it has been proposed that the microtubule-interacting protein tau, which is localized on GA membranes along with many of the tau phosphorylation-related kinases participates in the association between Golgi membranes and microtubules (Farah et al., 2006). It has been suggested that tau pathology precedes GA fragmentation since, in animal models of AD, the overexpression of tau induced GA fragmentation (Liazoghli et al., 2005; Lin et al., 2003). However, other studies showing tau hyperphosphorylation after brefeldin A or nocodazole treatments of HEK293/tau cells led to the suggestion that tau hyperphosphorylation is a downstream event that is a consequence of the disorganization of the GA (Sutterlin et al., 2002; Jiang et al., 2014).

Pioneering studies in AD patients reported that the alterations (including fragmentation and decreases in size) of the GA of hippocampal neurons are unrelated to the content of intracellular NFT in CA1 hippocampal field (Salehi et al., 1995) or preferentially associated with neurons devoid of NTF in the subiculum and entorhinal cortex (Stieber et al., 1996). However, during the course of our research into the possible alterations of the GA in the hippocampus and neocortex of AD patients using different methodological approaches to those used by Salehi et al. (1995) and Stieber et al. (1996), we found that the progressive accumulation of phospho-tau may be associated with the fragmentation of the GA. Thus, in the present study, we have reexamined this issue using double immunofluorescence techniques, high-resolution confocal microscopy and 3D reconstruction and quantification methods to measure the volume and surface area of the GA in neurons from control cases and from AD patients with pre-tangle and tangle stages of hyperphosphorylated tau accumulation. To label the GA, we used antibodies that recognize MG160, an integral membrane Golgi protein commonly used as a GA marker (Anton-Fernandez et al., 2015; Burrus et al., 1992; Gonatas et al., 1998a; Gonatas et al., 1995; Stieber et al., 1995; Stieber et al., 1996; Yamaguchi et al., 2003; Zhou et al., 1997; Zuber et al., 1997).

Importantly, differences in age (Jiang et al., 2014), fixation procedure and postmortem period prior to fixation might affect the structural characteristics of the GA. These factors vary between the control and the AD patient groups used in previous reports (Ellisman et al., 1987; Salehi et al., 1995; Stieber et al., 1996) and might also affect the morphology of the GA, as evaluated in the present study by the expression of MG160. Therefore, in order to evaluate the possible influence of normal aging and the postmortem delay period on the analysis of MG160 expression in the GA and discriminate the interference between these potential changes and those associated with the course of AD, we have analyzed the GA in brain sections from C57BL/6J mice with different ages and from mice with different postmortem times before brain fixation.

The results of the present study indicate that, in AD patients, the GA of numerous cortical and hippocampal neurons undergo specific and profound alterations including fragmentation and a decrease in the volume and surface area of the elements that are immunoreactive for MG160. These changes are likely to affect the protein processing, glycosylation and sorting (Wang et al., 2008; Xiang et al., 2013) and are more pronounced and more frequently found in neurons with a progressive accumulation of hyperphosphorylated tau. Therefore, the perturbation of the microtubule network by tau hyperphosphorylation could alter GA structure and the secretory pathway, impairing the highly regulated processes of protein sorting in neuronal compartments.

2. Materials and methods

Human brain tissue was obtained at autopsy from two sources: from seven patients with AD (aged 64–89) and from control human brain tissue from five individuals (aged 40–66) who died due to an accident or other cause and were free of any known neurological or psychiatric illness (Table 1). The AD brain and some control brain tissues were obtained from the *Instituto de Neuropatología* (Dr. I. Ferrer, *Servicio de Anatomía Patológica*, IDIBELL-*Hospital Universitario de Bellvitge*, Barcelona, Spain; IF4, IF10, IF12, IF13 and M16 cases), from the *Banco de Tejidos Fundación* CIEN (Dr. A. Rábano, *Área de Neuropatología*, *Centro Alzheimer*, *Fundación Reina Sofia*, *Madrid*, Spain; Vk11 case) and from the Neurological Tissue Bank (*Biobanc-Hospital Clínic-IDIBAPS*, Universidad de Barcelona, Spain; Bcn 5, Bcn7, Bcn8, Bcn13 cases). Control human brains were also obtained from Dr. Ricardo Insausti, *Facultad de Medicina*, *Universidad UCLM* (Albacete, Spain; AB1, AB2). Following neuropathological examination, the AD stages were defined according to the CERAD (Consortium to Establish a Registry for Alzheimer's Disease; (Mirra et al., 1991) and the Braak and Braak criteria (Braak and Braak, 1995); Table 1). Information regarding TDP43 inclusions was available for four of the seven patients used (Bcn5, Bcn7, Bcn8 and Bcn13). None of them showed TDP43 inclusions in CA1 or temporal neocortex, and only Bcn7 had TDP43 inclusions in neurons of the amigdala.

The postmortem delay between death and tissue processing ranged between 1.5 and 5.5 h (Table 1), and the brain samples were obtained following the guidelines of the Institutional Ethical Committees, which also granted approval. Tissue from some of these human brains has been used in previous studies (Blazquez-Llorca et al., 2010; Blazquez-Llorca et al., 2011).

Upon removal, the brains were immediately fixed in cold 4% paraformaldehyde in phosphate buffer (PB: 0.1 M, pH 7.4), and after 2 h, the tissue was cut into small blocks and post-fixed in the same fixative for 24–48 h at 4 °C. However, one human patient (AB1) was intraarterially perfused through the internal carotid arthery <1 h after death with a saline solution followed by 4% paraformaldehyde in PB. The brain was then removed and post-fixed as mentioned above. After fixation, all the specimens were immersed in graded sucrose solutions and stored in a cryoprotectant solution at −20 °C. Serial sections (50 μm) of the cortical tissue were obtained using a vibratome (St. Louis, MO, USA), and the sections from each region and case were batch-processed for immunohistochemical staining. The sections immediately adjacent to those stained immunohistochemically were Nissl-stained in order to identify the cortical areas and the laminar boundaries.

2.1. Immunofluorescence

For immunofluorescence experiments, free floating serial sections (50-μm thick) were first rinsed in PB and then pre-treated in 1.66% H_2O_2 for 30 min to inactivate the endogenous peroxidase activity and were then preincubated for 1 h in PB with 0.25% Triton-X100 and 3% normal serum of the species in which the secondary antibodies were raised (Vector Laboratories, Burlingame, CA, USA). The sections were then incubated for 48 h at 4 °C in the same stock solution containing the following primary antibodies in the combinations indicated: rabbit anti-MG160 (Abcam, 1:100), mouse anti-NeuN (Chemicon, 1:2000), mouse phospho-PHF-tau pSer202 + Thr205 antibody (AT8, 1:2000, Pierce Endogen).

After rinsing in PB, the sections were incubated for 2 h at room temperature in the appropriate combinations of Alexa 488- or Alexa 594-conjugated goat anti-mouse or goat anti-rabbit antibodies (1:2000; Molecular Probes, Eugene, OR, USA). Sections were also stained with a nuclear stain DAPI (4,6-diamidino-2-phenylindole; Sigma, St. Louis, MO, U.S.A.). After rinsing in PB, the sections were treated with Autofluorescence Eliminator Reagent (Chemicon) to reduce autofluorescence, mounted in antifade mounting medium (ProlongGold, Invitrogen) and studied by confocal microscopy (Zeiss, 710).

From the temporal neocortex and the CA1 region of the hippocampus, we obtained image stacks recorded at 0.35 μm intervals through separate channels with a 63x oil-immersion lens (NA, 1.40, refraction index, 1.45). The number of optical planes in the confocal stacks ranged from 40 to 142 (mean = 84.5) in the neocortex and 69 to 183 (mean = 108.3) in the hippocampus. ZEN 2012 software (Zeiss) was used to construct composite images from each optical series by combining the