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Glial fibrillary acidic protein isoform expression in plaque related astrogliosis in Alzheimer's disease

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ABSTRACT

In Alzheimer's disease (AD), amyloid plaques are surrounded by reactive astrocytes with an increased expression of intermediate filaments including glial fibrillary acidic protein (GFAP). Different GFAP isoforms have been identified that are differentially expressed by specific subpopulations of astrocytes and that impose different properties to the intermediate filament network. We studied transcript levels and protein expression patterns of all known GFAP isoforms in human hippocampal AD tissue at different stages of the disease. Ten different transcripts for GFAP isoforms were detected at different abundancies. Transcript levels of most isoforms increased with AD progression. GFAPδ-immunopositive astrocytes were observed in subgranular zone, hilus, and stratum–lacunosum–moleculare. GFAPδ-positive cells also stained for GFAPα. In AD donors, astrocytes near plaques displayed increased staining of both GFAPα and GFAPδ. The reading-frame–shifted isoform, $GFAP^{+1}$, staining was confined to a subset of astrocytes with long processes, and their number increased in the course of AD. In conclusion, the various GFAP isoforms show differential transcript levels and are upregulated in a concerted manner in AD. The $GFAP^{+1}$ isoform defines a unique subset of astrocytes, with numbers increasing with AD progression. These data indicate the need for future exploration of underlying mechanisms concerning the functions of GFAPδ and $GFAP^{+1}$ isoforms in astrocytes and their possible role in AD pathology.

1. Introduction

Astrocyte processes envelope neuronal synapses and form, together with the pre- and postsynaptic elements, the tripartite synapse. There is ample evidence that astrocytes are actively involved in modulating synaptic transmission (Halassa and Haydon, 2010; Lee et al., 2012; Seth and Koul, 2008). In response to damage inflicted to the central nervous system, astrocytes can change into a so-called reactive state. This transition termed astrogliosis is characterized by an increase in the expression of their main intermediate filament (IF), glial fibrillary acidic protein (GFAP), by morphological alterations (hypertrophy) and by functional changes (Kato et al., 1998; Pekny and Nilsson, 2005; Simpson et al., 2008; Verkhratsky et al., 2010; Vincent et al., 2010). Intermediate filaments are dynamic structures that are involved in a range of cellular processes during homeostasis and stress; however, the functional consequences of increased GFAP expression in reactive astrocytes are not understood (Middeldorp and Hol, 2011; Sofroniew and Vinters, 2010).

Amyloid plaques are a neuropathological hallmark of Alzheimer's disease (AD), and they are surrounded by reactive astrocytes already at an early stage of the disease (Carter et al., 2012; Kamphuis et al., 2012; Sofroniew and Vinters, 2010). GFAP expression is highly associated with plaque load and, to a lesser extent,

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with the number of neurofibrillary tangles (Hanzel et al., 1999; Muramori et al., 1998; Vehmas et al., 2003). Recently, it has been shown that reactive gliosis limits amyloid deposition (Chakrabarty et al., 2010; Furman et al., 2012), and that the presence of GFAP plays a crucial role in this (Kraft et al., 2012). Furthermore, GFAP/vimentin double knockout mice show a reduced hypertrophy of cell processes and greater sensitivity for spinal cord injury and as well as having a larger infarct volume after ischemia, indicating that GFAP has protective role of GFAP and that a proper IF network is a crucial component of reactive gliosis (Li et al., 2008; Nawashiro et al., 1998, 2000; Wilhelmsson et al., 2004). Several functional consequences of a change in GFAP IF network have been reported. GFAP overexpression, GFAP isoform ratios, and mutations in the tail and rod domain all influence IF network structure and reduce the motility of glioma cells in vitro (Elobeid et al., 2000; Kamphuis et al., 2012; Lepekhin et al., 2001; Roelofs et al., 2005; Yoshida et al., 2007). Astrocytes lacking IFs fail to form a barrier-like structure around amyloid-β (Aβ) deposits (Lepekhin et al., 2001; Lu et al., 2011; Wilhelmsson et al., 2004; Xu et al., 1999). Furthermore, evidence has been presented that the GFAP network is involved in intracellular vesicle motility (Potokar et al., 2007, 2010), and that the IF network serves as a platform for signal transduction (Pallari and Eriksson, 2006) and translation (Kim and Coulombe, 2010).

A variety of GFAP isoforms have been described. To date, at least 9 splice variants of GFAP transcripts have been identified in different species (human, mouse, rat) (Kamphuis et al., 2012; Middeldorp and Hol, 2011). The canonical form, GFAPα, has 9 exons and is specifically expressed in astrocytes. GFAPβ has an alternative upstream transcriptional start site in the 5'UTR (Condorelli et al., 1999b; Feinstein et al., 1992; Kamphuis et al., 2012). GFAPγ lacks exon 1 and includes the last 126 bp of intron 1-2 (Zelenika et al., 1995). Four splice variants, GFAPΔEx6, GFAPΔ164, GFAPΔ135, and GFAPΔEx7, skip sequences in exon 6/7, and were identified in AD tissue, focal lesions assocated with chronic epilepsy, and a specific human astrocyte subtype (Boer et al., 2010; Hol et al., 2003; Kamphuis et al., 2012; Middeldorp et al., 2009b). These splice variants encode for a frameshifted GFAP protein, termed GFAP^{+1}, with a frameshifted carboxy-terminus against which we have raised a specific antibody named GFAP^{+1} (Hol et al., 2003; Middeldorp et al., 2009b). In an article by Zelenika et al., a transcript including the last 284 bp of intron 8–9 was reported, which we found to be highly expressed in the mouse brain and which we termed GFAP-zeta (GFAPζ) (Kamphuis et al., 2012; Zelenika et al., 1995). In GFAPδ and GFAPκ isoforms, exons 8 and 9 are replaced by intron 7–8 sequences (Blechingberg et al., 2007; Kamphuis et al., 2012; Nielsen et al., 2002; Roelofs et al., 2005). GFAPδ is highly expressed in proliferating cells in the subventricular zone and in the subpial layer of the human brain (Roelofs et al., 2005; van den Berge et al., 2010). The different splice variants result in different GFAP isoforms with variable C-terminal regions. Because the tail domains are important for the assembly of GFAP filaments and for the binding of interacting proteins (Nielsen et al., 2002; Perng et al., 2008), a differential expression may result in a different GFAP cellular IF network, leading to a different morphology and even an altered function of astrocytes. Recently we published an extensive survey on the gene expression of GFAP isoforms and GFAP protein distribution in the mouse brain and in response to plaque-related gliosis in an AD mouse model (Kamphuis et al., 2012). The gene expression profile of the GFAP-isoforms was shown to remain invariable with age and did not alter as a consequence of plaque-related gliosis. At the protein level, only GFAPα and GFAPδ could be localized, with GFAPδ present at relatively high levels not only in neurogenic cells in the subventricular and subgranular zones but also in astrocytes located in the subpial region (Kamphuis et al., 2012).

In previous articles, we reported observations on the differential expression of GFAPδ (Roelofs et al., 2005; van den Berge et al., 2010) and GFAP^{+1} (Boer et al., 2010; Hol et al., 2003; Middeldorp et al., 2009b) in distinct types of astrocytes in the human brain; however, a systematic description of all human GFAP isoforms in relation to AD pathology has not yet been published. To this end, we investigated the changes in GFAP isoform transcript levels and protein distribution in AD tissue and at different stages of the disease. We focused on the hippocampus, which is one of the brain areas earliest and most severely affected by AD (Braak and Braak, 1991; Gomez-Isla et al., 1997). We collected tissue from a large number of donors that were classified for amyloid stage (Thal et al., 2000a) and Braak stage of neurofibrillar degeneration (Braak and Braak, 1991), as indicators for the progression of AD.

2. Methods

2.1. Human post-mortem brain material

Human post-mortem brain material was obtained from the Netherlands Brain Bank (NBB). Snap-frozen (non-fixed and without freeze protection) tissue of the hippocampal area was obtained. For all 75 donors, the medical history was retrieved by the NBB, and an extensive neuropathological assessment was performed by the NBB on multiple brain areas. Next to the staging for amyloid load (Thal et al., 2000a), all donors were also Braak staged for tau (Braak and Braak, 1991). The hippocampal area included the dentate gyrus (DG), area CA1-4, and variable parts of subiculum and, occasionally, entorhinal cortex.

Details of the donors, ranked according to their amyloid score (O, A, B, C), are presented in Supplementary Table 1. Donors were sex and age matched as much as possible, but the amyloid O donors of the frozen tissue were significantly younger compared to the amyloid B ($p < 0.047$) and amyloid C ($p < 0.023$) donors. The age of the amyloid O group (n = 13) ranged from 51–96 years (mean, 70 years); amyloid A (n = 8) ranged from 64–90 years (mean, 76 years); amyloid B (n = 10) ranged from 64–93 years (mean, 83 years); and amyloid C (n = 34) ranged from 57–96 years (mean, 80 years). In addition, 10 donors were included that did not have an NBB-documented amyloid score, but were scored for tau pathology. The post-mortem delay ranged from 3–11 hours (mean 6 hours) and did not differ between groups. In addition, the pH of the CSF (no significant differences among groups), brain weight (reduced by 15% in amyloid C vs. amyloid O; $p < 0.001$), and ApoE genotype were provided for most donors.

2.2. Cloning GFAP splice variants into pcDNA3

Expression plasmids containing full length isoforms were constructed by modifying the pcDNA3-GFAPα full-length plasmid (Roelofs et al., 2005). (For details, see Supplementary Material and Methods.)

2.3. RNA isolation and real-time quantitative polymerase reaction assays

Cryosections of 10-μm thickness were cut, and 20 sections were collected in a tube kept cold at −20 °C. Tissue sections were subsequently thawed and dissolved by adding 1 mL of TRIzol (Life Technologies) at room temperature, followed by thorough vortexing. RNA was isolated from the 400 μL aqueous phase by precipitation at −20 °C overnight in 400 μL isopropanol, with the addition of 1 μL of 20 μg/μL glycogen (Roche). The quality of the RNA was determined on a Bioanalyzer (Agilent Technologies) and expressed