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Calcyclin binding protein and Siah-1 interacting protein in Alzheimer's disease pathology: neuronal localization and possible function

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ABSTRACT

The calcyclin binding protein and Siah-1 interacting protein (CacyBP/SIP) protein was shown to play a role in the organization of microtubules. In this work we have examined the neuronal distribution and possible function of CacyBP/SIP in cytoskeletal pathophysiology. We have used brain tissue from Alzheimer's disease (AD) patients and from transgenic mice modeling 2 different pathologies characteristic for AD: amyloid and tau. In the brain from AD patients, CacyBP/SIP was found to be almost exclusively present in neuronal somata, and in control patients it was seen in the somata and neuronal processes. In mice doubly transgenic for amyloid precursor protein and presenilin 1 there was no difference in CacyBP/SIP neuronal localization in comparison with the nontransgenic animals. By contrast in tau transgenic mice, localization of CacyBP/SIP was similar to that observed for AD patients. To find the relation between CacyBP/SIP and tau we examined dephosphorylation of tau by CacyBP/SIP. We found that indeed it exhibited phosphatase activity toward tau. Altogether, our results suggest that CacyBP/SIP might play a role in AD pathology.

1. Introduction

Alzheimer's disease (AD) is the most common neurodegenerative disorder. It is characterized by a progressive loss of neurons and cognitive functions. Pathologic hallmarks of AD are extracellular amyloid plaques, composed of the amyloid-β (Aβ) peptide and intracellular neurofibrillary tangles (NFTs) that predominantly comprise tau protein (Braithwaite et al., 2012). The pathogenic mechanism of AD is still poorly understood and thus further studies on factors or proteins that might be important in the development of AD are necessary. One such factor might be calcyclin binding protein and Siah-1 interacting protein (CacyBP/SIP), a protein expressed at high levels in the brain (Jastrzebska et al., 2000) and having an age-dependent subcellular localization in neurons (Filipek et al., 2008).

CacyBP/SIP was discovered as a S100A6 (calcyclin) target (Filipek and Kuznicki, 1998; Schneider and Filipek, 2011) but later shown to interact with some other S100 proteins (Filipek et al., 2002a) and with Siah-1 (Matsuzawa and Reed, 2001), Skp1 (Bhattacharya et al., 2005), tubulin (Schneider et al., 2007), actin (Schneider et al., 2010), and extracellular signal-related kinase (ERK)1/2 (Kilańczyk et al., 2009). CacyBP/SIP is present in various tissues with the highest level in brain (Jastrzebska et al., 2000) and in some cell lines such as mouse neuroblastoma NB2a (Filipek et al., 2002b). The high level of CacyBP/SIP in neurons (Jastrzebska et al., 2000) and its interaction with tubulin (Schneider et al., 2007) suggested its involvement in developmental changes of the neuronal cytoskeleton. This supposition has been confirmed by the finding that subcellular compartmentalization of CacyBP/SIP in rat brain neurons depends on age. In young rats CacyBP/SIP was present in the cytoplasm and in neuronal processes, and in aged animals it was seen only in the cytoplasm and not in neuronal processes (Filipek et al., 2008). Such changes in localization of CacyBP/SIP were similar to those of tau, a major neuronal microtubule-associated protein, and suggested a possible role of CacyBP/SIP in cytoskeletal physiology and pathophysiology (Filipek et al., 2008). Interaction of CacyBP/SIP with actin, another cytoskeletal protein (Schneider et al., 2010), might

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also implicate a role for CacyBP/SIP in cytoskeletal organization. Furthermore, a substantial sequence similarity between CacyBP/SIP and phosphatases from the mitogen activated protein (MAP) kinase phosphatase family and its ability to dephosphorylate ERK1/2 kinase (Kilanczyk et al., 2011) suggest that the involvement of CacyBP/SIP in cytoskeletal dynamics might rely on its dephosphorylating activity toward key cytoskeletal components.

Microtubules, composed predominantly of α- and β-tubulin subunits, are the major structural components of the neuronal cytoskeleton that plays an important role in neurodevelopment, aging, and neurodegenerative disorders (Baas and Qiang, 2005; Brandt et al., 2005). Tau protein is important in the assembly and disassembly of dynamic microtubules and is associated normally with axonal rather than somatodendritic microtubules (Binder et al., 1985). AD is characterized by a substantial redistribution of tau into the somatodendritic compartment as neurofibrillary pathology, which is in the form of intra- and extracellular tangles, and as dystrophic neurites either throughout the neuropil or within neuritic plaques (Harrington, 2012). Neurofibrillary tau pathology involves the formation of paired helical filaments (PHFs) that comprised tau protein. Tau is an ideal substrate for phosphorylation by multiple kinases in the somatodendritic compartment in AD (Gong et al., 2005).

In the present work we have examined immunohistochemically the neuronal localization of CacyBP/SIP in association with phosphorylated tau (p-Tau) and β-tubulin, in the brains from AD patients and from 2 lines of mice separately transgenic for amyloid and tau. Moreover, we checked the level of CacyBP/SIP and the ratio of the major CacyBP/SIP forms in control and pathologic mouse brain material by applying sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and 2-dimensional (2D) electrophoresis, respectively. Because CacyBP/SIP has recently been shown to exhibit phosphatase activity toward ERK1/2, we also analyzed whether it could dephosphorylate tau, a protein that becomes hyperphosphorylated in AD pathology.

2. Methods

2.1. Human material

Human brain tissues from 10 patients (age 72–94 years) with neuropathologically diagnosed AD and from 7 control patients (age 43–85 years) were analyzed. Following the Austrian legal procedures, every patient who dies in a hospital can undergo autopsy for diagnostic and/or scientific purposes. Based on the request from the Ethical Committee of the State of Upper Austria upon hospital admission, patients sign a written consent for scientific evaluation of their brain after death. The neuropathologic diagnosis of AD was rendered using the Consortium to Establish a Registry for Alzheimer's Disease (Mirra et al., 1993) and National Institute on Aging-Reagan diagnostic criteria (1997). The Braak staging system for tangle formation was also used (Braak and Braak, 1991). Briefly, after removal from the skull, the brains were fixed for 1 week in 4% formaldehyde. Brain regions were then cut and embedded in paraffin. For immunohistochemical analysis, slices of hippocampal and adjacent cortex of 5 μm thickness were used.

2.2. Animal models

All mice (control and transgenic) were housed in an environmentally controlled room (temperature 23 °C ± 1 °C, 12 hours light/12 hours dark cycle) in groups of 4 per cage. The study was conducted with the approval of the local ethics commission (Polish Law on the Protection of Animals) and carried out in accordance with the Principles of Laboratory Animals Care (NIH publication No. 86-23). All efforts were made to minimize the number of animals used for experiments and their suffering.

Mice B6C3-TgN(Prn-APP695)$_3$Dbo Tg(PSEN1)$_5$Dbo/J, designated as APP+PS1, with overexpression of mutant amyloid precursor protein (K595N/M596L) and mutant presenilin 1 (A246E) were used as a model of Aβ pathology and B6C$_3$F$_1$/J mice were used as controls (The Jackson Laboratory, Jax Mice and Service). Characterization of these mice is given by Borchelt et al., (1997). The expression of both mutated proteins together accelerates the deposition of Aβ in mice, with deposits appearing by the age of 9 months.

A transgenic model of tau aggregation in vivo (line 1 tau mice) was created in which the mouse expresses truncated tau (amino acids 296–390 of the longest human 441-residue tau form) that is fused with a membrane localization signal sequence to nucleate aggregation (Zabke et al., 2008). Expression in neurons is under the control of the murine Thy1-promoter. The tau transgenic model (referred to as line 1 tau mice throughout the text) was established in mice of the outbred NMRI strain (Harlan–Winkelmann, Paderborn) that served as a wild type control. The truncated tau species represent the proteolytically stable core of the PHF left after exogenous proteolysis (Wischik et al., 1988) and a fragment that is capable of generating proteolytically stable tau aggregates in vitro (Wischik et al., 1996). The brain of line 1 tau mice shows an age-dependent increase in tau immunoreactivity. The tau aggregates are neither argyrophilic nor thioflavin-S positive, but similar to the amorphous aggregates described in the early stages of AD. The anatomic pattern and progression of the tau-immunoreactivity in these mice is similar to that described by Braak staging in AD (Braak and Braak, 1997), initiating in the hippocampus and entorhinal cortex before progressing to the neocortex. Mice are not impaired in motor function but display cognitive impairment in spatial learning in the T-maze (Zabke et al., 2008).

2.3. Perfusion and processing of mouse brain

Female mice were deeply anesthetized with Vetbutal and the perfusion procedure was begun when all reflex responses to cutaneous stimulation were absent. Generally, tissues were fixed by intra-aortic perfusion with cold (4 °C) phosphate buffered saline (PBS), containing heparin (5 IU of heparin per 1 mL of buffer), followed by 4% paraformaldehyde in PBS (fixative solution), and then followed by 5% glycerol and 2% dimethyl sulphoxide (DMSO) in PBS. The brains were removed, placed for 1 hour in the fixative solution and then immersed for cryoprotection in 10% glycerol and 2% DMSO and subsequently in 20% glycerol and 2% DMSO. All perfusion solutions contained 1 mM Na_3VO_4 (sodium orthovanadate), a phosphatase inhibitor, to minimize postmortem dephosphorylation of proteins.

For immunohistochemical analysis, sections were cut coronally through the forebrain at a 40 μm thickness with a freezing stage microtome. The consecutive sections were collected throughout the hippocampal region (from −0.94 mm to −4.04 mm posterior to Bregma; Franklin and Paxinos, 2008) of each mouse. From 4 series of the sections collected, 1 set was processed for CacyBP/SIP immunoreactivity, the second for p-Tau(Ser-404) immunoreactivity, the third for β-tubulin immunoreactivity, and the fourth corresponding series of sections was stored at −20 °C in a cryoprotective solution as a reserve.

2.4. Immunohistochemistry and immunofluorescence

For immunohistochemical and immunofluorescence analyses the following antibodies were used: polyclonal anti-CacyBP/SIP (developed and described by Filipek et al. [2002b]), rabbit polyclonal antibodies to neuron specific β III tubulin (clone name ab 18207, Abcam), polyclonal anti-p-Tau(Ser-404) (Santa Cruz),