Brain Research 1677 (2017) 1–13

Contents lists available at ScienceDirect

Brain Research

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

Research report

Distribution of spleen tyrosine kinase and tau phosphorylated at tyrosine 18 in a mouse model of tauopathy and in the human hippocampus

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ARTICLE INFO

Article history:
Received 8 May 2017
Received in revised form 11 August 2017
Accepted 25 August 2017
Available online 14 September 2017

Keywords:
Alzheimer's disease
AT100
Neurofibrillary tangles
Non-receptor tyrosine kinase

ABSTRACT

Purpose: Spleen tyrosine kinase (Syk) has been shown to phosphorylate tyrosine 18 of tau *in vitro*. It has been proposed that increased immunoreactivity for double-phosphorylated Syk in hippocampal neurons of Alzheimer's disease cases indicates a not yet defined neurodegenerative process. To investigate this possibility we have studied Syk and tau phosphorylated at tyrosine 18 (pTyr18) in transgenic mice and human hippocampi.

Methods: We performed immunohistochemistry, immunofluorescence labeling and Western blotting and compared the distribution of Syk double-phosphorylated at tyrosines 525 and 526 and pTyr18 in human tau transgenic pR5 mice and human hippocampi with low and high Braak stages for neurofibrillary tangle pathology.

Results: pTyr18 appeared early during the course of neurodegeneration in pR5 mice and was widely distributed in the pR5 brain, including neuronal somata and fiber tracts. In contrast, only strongly pTyr18- and AT100-(tau phosphorylated at Thr212 and Ser214) positive neurons with a fibrillary tau pathology in old pR5 mice and microglia displayed immunoreactivity for double-phosphorylated Syk. In human hippocampi, phosphorylated Syk was mainly present in granulovacuolar inclusions in hippocampal pyramidal neurons and did not co-locate with pTyr18 in these neurons. We observed pTyr18-positive neurons and neurons with granular pSyk immunoreactivity already at early Braak stages and their number was markedly increased in Braak stage VI.

Conclusion: Syk appears unlikely to be the major kinase that phosphorylates tyrosine 18 of tau in tauopathy. It possibly phosphorylates tyrosine 18 of tau and regulates other tau kinases in neurons with a fibrillary tau pathology.

1. Introduction

Tauopathies are a group of neurodegenerative disorders that ultimately cause dementia. This group includes Alzheimer's disease (AD), the most prevalent tauopathy, progressive supranuclear palsy, corticobasal degeneration, Pick's disease, argyrophilic grain disease and frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17). Tauopathies are characterized by abundant neurofibrillary lesions in distinct regions of the brain. The lesions consist of intracellular accumulations of abnormal filaments composed of the microtubule-associated protein tau in a hyperphosphorylated state (Tolnay and Probst, 2003; Arendt et al., 2016). Almost all phosphorylation of tau is on serine and threonine residues (Hanger et al., 2009; Martin et al., 2011), but there is also evidence for a pathogenetic role of hyper-phosphorylation of its tyrosine residues. Human tau contains five tyrosine residues (tyrosine 18, 29, 197, 310, and 394 according to the sequence of the longest tau isoform) that are present in all tau isoforms, while mouse tau contains only four tyrosine residues lacking tyrosine 29. Tyrosines 18, 197, and 394 are phosphorylated in paired helical filament-tau (for review see: Lebouvier et al., 2009).

Spleen tyrosine kinase (Syk) and ZAP-70 are members of the Syk family of non-receptor tyrosine kinases. Syk has been shown to phosphorylate tyrosine 18 of tau *in vitro* and in CHO cells (Lebouvier et al., 2008) and is involved in the production of the

Abbreviations: AD, Alzheimer's disease; FTDP-17, frontotemporal dementia with parkinsonism linked to chromosome 17; GVD, granulovacuolar degeneration; GSK3β, glycogen synthase kinase-3β; NFTs, neurofibrillary tangles; pSyk, spleen tyrosine kinase phosphorylated at tyrosines 525 and 526; pTyr18, tau phosphorylated at tyrosine 18; SEM, standard error of the mean; Syk, spleen tyrosine kinase.

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https://doi.org/10.1016/j.brainres.2017.08.029
0006-8993/

pathological Aβ-peptide and its clearance across the blood brain barrier (Paris et al., 2014). Syk is broadly expressed in hematopoietic cells and has important roles in signaling in both lymphoid and myeloid cells (Cheng et al., 1995; Turner et al., 2000); it is also expressed in various non-hematopoietic cells (for review see: Yanagi et al., 2001).

Compared to spleen, much lower levels of Syk are expressed in the brain. In the developing rat brain, subpopulations of neurons display Syk/ZAP-70 immunoreactivity mainly in their axonal processes (Hatterer et al., 2011). When compared with controls, activated Syk (pSyk), as indicated by phosphorylated tyrosines 525/526 in the activation loop, was significantly increased in brains with Nasu-Hakola disease, a rare autosomal recessive disorder that is characterized by presenile dementia and multifocal bone cysts. Hippocampal pyramidal neurons in the brains of AD cases were also found to display intense pSyk immunoreactivity. The authors hypothesized that an increased level of pSyk in neurons may indicate a not yet defined neurodegenerative process (Satoh et al., 2012). Further evidence suggests a role of Syk in AD. Syk inhibition lowered Aβ levels and tau hyperphosphorylation at several epitopes possibly due to inhibition of glycogen synthase kinase-3β (GSK3β), a major tau kinase (Paris et al., 2014).

To explore a possible role of Syk in tauopathy, we studied the distribution of Syk phosphorylated at tyrosines 525/526 and of tau phosphorylated at tyrosine 18 (pTyr18) in human tau transgenic pR5 mice, an established model for tauopathy (Götz et al., 2001; Pennanen et al., 2004, 2006; Deters et al., 2008; Köhler et al., 2013; Klingebiel et al., 2017) and in the hippocampi of AD cases and controls.

2. Results

2.1. Early and widespread phosphorylation of tyrosine 18 of tau in the brain of pR5 mice

In brain sections from human tau transgenic pR5 mice five weeks of age, the earliest time point examined, many neuronal somata displayed a weak to moderate pTyr18-immunoreactivity. The pTyr18 antibody also stained the neuropil and fiber tracts such as the fimbria, mossy fibers, alveus, stria terminalis and the anterior commissure (Figs. 1a; 2a–d). Omission of the primary antibody precluded staining (Fig. 1b, and d). pTyr18 immunoreactivity was increased in the brains of older pR5 mice (Fig. 1a and c; Fig. 3a–k).

As reported previously, hyper-phosphorylated tau can be detected in pR5 neurons with antibody AT8 that recognizes tau phosphorylated at Ser202 and Thr205 (Götz et al., 2001; Deters et al., 2008; Köhler et al., 2013). pTyr18- and AT8-positive somata occurred in the same regions in consecutive sections stained for light microscopy, but AT8-immunoreactivity was mainly seen in the soma and apical dendrite of neurons, whereas the neuropil and fiber tracts displayed strong pTyr18 immunoreactivity (Fig. 4a and b). There was no pTyr18 and AT8 immunoreactivity in sections from non-transgenic littermates (Fig. 4c, and d; Fig. 5l).

2.2. Strong pTyr18 immunoreactivity in neurons with fibrillary tau pathology in old pR5 mice

Neuronal somata strongly labeled for pTyr18 occurred in the pyramidal cell layer of the hippocampus, subiculum, amygdala,

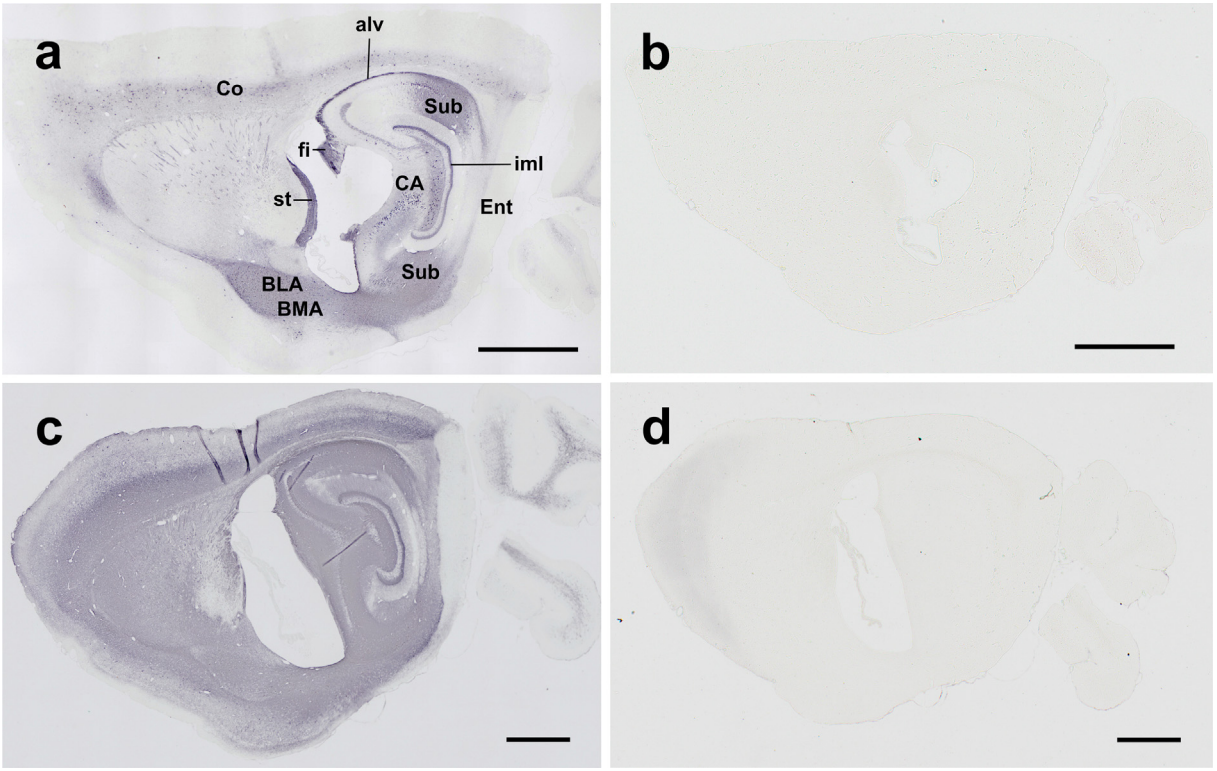

Fig. 1. Overview of pTyr18 immunoreactivity in the brains of pR5 mice. 3.3′-Diaminobenzidine-stained sagittal sections are shown. (a) In a five-weeks-old brain, immunoreactive neuronal somata are mainly located in layer six of the dorsal cortex (Co), hippocampus (CA), subiculum (Sub), basolateral (BLA), and basomedial amygdaloid nucleus (BMA). There is also prominent staining of the inner molecular layer of the dentate gyrus (iml), alveus (alv), fimbria (fi) and stria terminalis (st). (b) Compared with a five-weeks-old brain pTyr18 immunostaining is increased in the neuropil in an 18.5-months-old brain. The outer layers of the entorhinal cortex (Ent) remain unstained. Please note pTyr-18 immunoreactivity in the cerebellum. (c, d) There is no staining when the primary antibody is omitted. Results shown are representative for all three five-weeks-old and all three 18–19 months-old pR5 mice that were examined. Scale bar (a–d), 1 mm.