FEBS Letters 584 (2010) 3227–3232

journal homepage: www.FEBSLetters.org

Microtubule destruction induces tau liberation and its subsequent phosphorylation

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ARTICLE INFO

Article history:
Received 13 May 2010
Revised 9 June 2010
Accepted 10 June 2010
Available online 17 June 2010

Edited by Jesus Avila

Keywords:
Tau
Microtubule
Phosphorylation
Stathmin
Neurofibrillary tangle
Alzheimer's disease

ABSTRACT

Neurofibrillary tangle-bearing neurons, a pathological hallmark of Alzheimer's disease, are mostly devoid of normal microtubule (MT) structure and instead have paired helical filaments that are composed of abnormal hyperphosphorylated tau. However, a causal relationship between tau phosphorylation and MT disruption has not been clarified. To examine whether MT disruption induces tau phosphorylation, stathmin, an MT-disrupting protein, was co-expressed with tau in COS-7 cells. Stathmin expression induced apparent MT catastrophe and tau hyperphosphorylation at Thr-181, Ser-202, Thr-205, and Thr-231 sites. In contrast, c-Jun N-terminal kinase activation, or phosphatase inhibition, led to significant tau phosphorylation without affecting MT structure. These findings suggest that MT disruption induces subsequent tau phosphorylation.

1. Introduction

Tubulin heterodimers, α- and β-tubulin, assemble into microtubules (MTs), which are in a dynamic equilibrium with the non-polymerized form. Filamentous structures provide intracellular cytoskeleton in various cells and are especially enriched in neurons [1–4]. The extent of MT assembly is regulated by many factors such as temperature, protein-modification of tubulin, small molecules like taxol, and some MT-interacting proteins [5–7]. Because MTs have significant roles in a broad range of biological functions, including shaping the neuronal structure and transporting intracellular cargoes, it is reasonable to speculate that MT disruption, if any, profoundly affects neuronal architecture and function [8–11].

Tau proteins were identified as a factor promoting MT assembly and stabilization. MT assemblies are thought to be negatively regulated by tau phosphorylation [12,13]. To date, more than 40 serine (Ser) and threonine (Thr) residues have been identified as possible phosphorylation sites on tau [14]. Although the biological significance of individual phosphorylation sites is not known, it has been known that phosphorylation of Ser-262 (numbered according to the 441-residue tau) has a profound effect on its interaction with MTs [15].

Neurofibrillary tangles (NFTs) are a major neuropathological hallmark in brains affected by Alzheimer's disease (AD) and related diseases. The central nervous system diseases in which NFT formation is predominant are categorized as tauopathies. Because the areas in the brain that form NFTs often exhibit neuronal loss, the formation of these filamentous structures is assumed to be the crucial event in neuronal degeneration [16]. Abnormally phosphorylated tau is a major component of NFTs [17–19], which raises a possible link between phospho-tau accumulation and neurodegeneration and has led us to investigate their causal relationship. NFT-bearing neurons often accompany loss of MTs and tubulin [20–22]. This inverse relation can be observed across various animal models for tauopathy [23,24]. It remains unclear why MTs or tubulin are lost in the affected neurons. MT loss has also been found even in non-NFT-bearing neurons in the AD brain [22], suggesting that MT loss may precede tau phosphorylation and accumulation in brains affected by tauopathy. Therefore, we aimed to elucidate the possible link between MT loss and phospho-tau accumulation. The effects of drug-inducing MT depolymerization on expressed tau have been studied [25], which showed that the tau was dephosphorylated at specific sites. However, the impacts of cell-intrinsic MT-disrupter on tau phosphorylation have never been

Abbreviations: NFT, neurofibrillary tangle; AD, Alzheimer's disease; MT, microtubule; GSK3β, glycogen synthase kinase 3 beta; JNK, c-Jun N-terminal kinase; MEKK, mitogen-activated protein kinase/extracellular signal-regulated kinase kinase kinase; DMEM, Dulbecco's modified eagle's medium; FBS, fetal bovine serum; MOI, multiplicities of infection

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0014-5793/$36.00
doi:10.1016/j.febslet.2010.06.014

addressed. Stathmin is a factor acting on MT disruption and/or a tubulin sequestration and is ubiquitously expressed in many cell types, including neurons [26,27]. Furthermore, SCG10, a neuron-specific homolog of stathmin, is known to increase in the brains of AD patients [28]. To study whether physiological MT disruption affects tau phosphorylation, tau and stathmin were co-expressed in COS-7 cells using adenovirus-mediated transfection.

2. Materials and methods

2.1. Materials

Anti-stathmin antisera against N- and C-terminal positions, STN1 (ASSDIQVKELEKRA) and STC1 (RKNKESKDPADETEAD) were raised against synthetic peptides conjugated with KLH. The anti-tau antibodies used were tau-c, which reacts with pan-tau [29]; AT270, AT8, and AT180, which react with phospho-Thr181, phospho-Ser202 and -Thr205, and phospho-Thr231 and -Ser235, respectively (Innogenetics, Gent, Belgium); PHF-1, which reacts with phospho-Ser396 and -Ser404 (a generous gift from P. Davies) [30]; and PS422 (BioSource International, Camarillo, CA). Anti-α-tubulin (DM1A) and anti-acetylated-α-tubulin (6-11B1) were purchased from Sigma (St. Louis, MO). Okadaic acid was purchased from Roche Diagnostics (Basel, Switzerland).

2.2. DNA constructs and expression in COS cells

Generation of recombinant adenovirus was performed as previously described [29]. Briefly, stathmin cDNA encoding amino acids 1–145 was amplified and inserted between the HindIII and XbaI sites of the pFLAG-CMV2 vector. The FLAG tagged-stathmin open reading frame was inserted into the pAxCAwt vector (Takara) at the SwaI site (designated pAxCA-F-stathmin) and the resulting cosmid was used to generate recombinant adenoviruses (designated AxCA-F-stathmin) by the COS-TPC method following the manufacturer's instructions. Adenoviruses containing cDNA of the longest isoform of human brain tau (AxCA-Tau4RWT), LacZ (AxCAi-LacZ), c-Jun N-terminal kinase (JNK) 3 (AxCA-JNK3), and Δ mitogen-activated protein kinase/extracellular signal-regulated kinase kinase kinase (MEKK) (AxCA-ΔMEKK) were also used [29]. COS-7 cells were maintained in Dulbecco's modified eagle's medium (DMEM) containing 10% fetal bovine serum (FBS) in a 5% CO_2/air chamber. For adenoviral expression, COS-7 cells were cultured in a six-well dish and exposed to recombinant viruses at various multiplicities of infection (MOI) in DMEM containing 5% FBS.

2.3. Cell fraction and Western blotting

COS-7 cells were gently washed with phosphate buffered saline 3 days after infection and lysed in Laemmli's sample buffer. Each of the fractions having equal start-cell counts were separated by 8% SDS–PAGE and electroblotted onto nylon membranes for immunoblot analyses as previously described [29]. MT-bound and -unbound tau were prepared as described previously [25]. Okadaic acid was added 1 day after infection of AxCA-Tau4RWT, and cells were lysed 24 h after treatment.

2.4. Immunocytochemistry

Cells were fixed with 4% paraformaldehyde for 10 min and permeabilized by 0.5% Triton X-100, followed by incubation with primary antibodies. Bound antibodies were visualized with Alexa dye-conjugated secondary antibodies (Molecular Probes) and observed with a confocal laser scanning microscope (Radiance 2000 KR3; Bio-Rad).

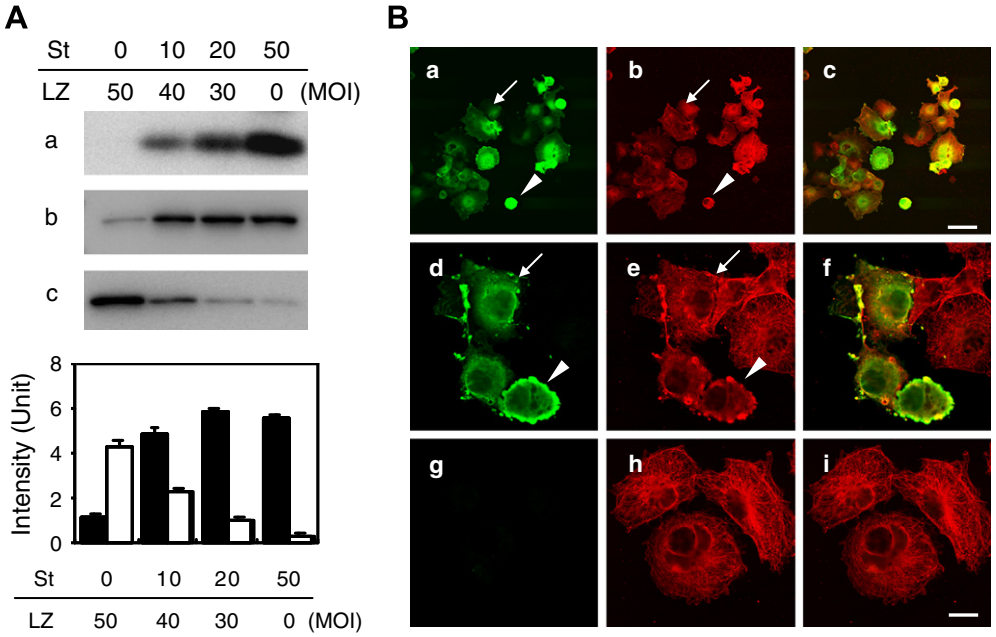

Fig. 1. Adenoviral induction of stathmin induces MT catastrophe in COS-7 cells. MT stability of COS-7 cells infected with AxCA-F-stathmin was analyzed by biochemical and immunocytochemical procedures. (A) Three days after infection with AxCA-F-stathmin (St) or AxCAi-LacZ (LZ) cDNA at the indicated MOI, the total amount of stathmin was evaluated by STC1 (a). Free (b) and polymerized tubulin (c) were fractioned as described in Section 2 and quantified by Western blotting using the anti-α-tubulin antibody, DM1A. The lower panel shows quantitative analyses of free (closed) or polymerized (open) tubulin in COS-7 cells. Each data point represents the means and standard error (S.E.) for three independent experiments. (B) 50 MOI of AxCA-F-stathmin-infected COS-7 cells (a–f) were co-labeled with STC1 (green) and DM1A (red), and observed by confocal laser scanning microscopy. It should be noted that the low level of stathmin expression induced mild MT disruption (arrow); however, high levels of stathmin expression caused cell to shrink (arrowhead). These abnormal cells were not observed in AxCAi-LacZ-infected cells (g–i). Merged images are shown in c, f, and i. Scale bars: a–c, 100 μm; d–i, 25 μm.