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Research Report

Regulation of tau proteolysis by phosphatases

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ABSTRACT

One pathological hallmark of Alzheimer's disease is the accumulation of highly phosphorylated tau. Since tau phosphorylation inhibits its proteolysis, we examined the impact of endogenous phosphatase activities on tau proteolysis by homogenization of cultured cells and 3xTg-AD mouse brain followed by incubation with or without phosphatase inhibitors. Incubation without phosphatase inhibitors significantly increased tau immunoreactivity against antibody C3 (which reacts with tau truncated at D421), and increased the generation of tau breakdown products. These changes were augmented by lithium treatment and inhibited by constitutively active GSK3β. These findings underscore that tau proteolysis is regulated by a balance of kinase and phosphatase activities.

1. Introduction

One pathological hallmark of tauopathies including Alzheimer's disease is aberrant somatodendritic accumulation of highly phosphorylated tau (Binder et al., 2005; Kanai and Hirokawa 1995; Lee et al., 2001; Tashiro et al., 1997). Accumulation may also induce tau hyperphosphorylation and aggregation (Delobel et al., 2003; Sahara et al., 2002). Cell-free analyses indicate that tau is a substrate for multiple endogenous proteases (Wang et al., 2010) including such cathepsin D (Bednarski and Lynch 1996; Kenessey et al., 1997), calpains (Johnson et al., 1989; Litersky et al., 1993; Park and Ferreira 2005; Yang et al., 1997), caspases (Gamblin et al., 2003; Horowitz et al., 2006; Rissman et al., 2004), proteosomal proteases (David et al., 2002), aminopeptidases (Karsten et al., 2006; Sengupta et al., 2006) and thrombin (Arai et al., 2005).

Phosphorylation of tau inhibits its proteolysis by calpain, caspases and thrombin (Arai et al., 2005; Guillozet-Bongaarts et al., 2006; Johnson et al., 1989; Litersky et al., 1993; Poppek et al., 2006; Yang et al., 1997). By contrast, cathepsin D accelerates the degradation of phosphorylated fetal tau (Kenessey et al., 1997).

Since tau phosphorylation is determined by the balance of kinases and phosphatases activities (Chan et al., 2008; Ekinci et al., 2003; Gong et al., 1994a,b,c; Gong et al., 1993; Shea, 1996; Shea and Didier 1998; Shea and Ekinci 1999; Shea and Fischer 1996), we examined herein the role of phosphatases in tau proteolysis.

Herein, the role of phosphatases in tau truncation and phosphorylation were investigated in lysates derived from NB2a/d1 cells in which GSK3β activity was manipulated and from 3xTg-AD mouse brain. We present evidence that tau truncation at D421 is strongly associated with the activity of protein phosphatase.

2. Results

We first evaluated the impact of lithium treatment or GSKala overexpression on intracellular GSK3β activity in NB2a/d1 cells (Fig. 1). As anticipated, total GSK3β immunoreactiviy in homogenates of cells overexpressing GSKala was significantly

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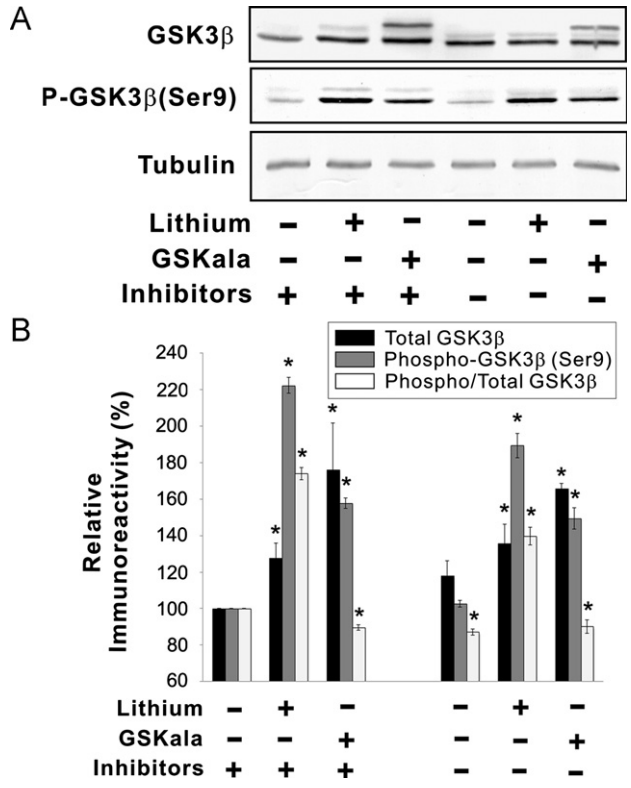

Fig. 1 – Manipulation of GSK3β activity: panel A presents representative immunoblots of homogenates, incubated with or without phosphatase inhibitors, probed with antibodies against total GSK3β, GSK3β phosphorylated at ser 9, and tubulin (as a loading control) as indicated. Note the overall increase in GSK3β immunoreactivity following GSKala overexpression. Note also the increase in Ser-9 immunoreactivity, indicative of inactive GSK3β, following lithium treatment. Panel B depicts the mean ± standard error of immunoreactive species from 3 such immunoblots. The densitometric value for homogenates derived from nontransfected cells that had not been treated with lithium incubated phosphatase inhibitors was defined as 100%,; values for other conditions are presented relative to those respective values. Ratios of GSK3β phosphorylated at Ser-9 vs. total GSK3β indicates that lithium treatment inhibited GSK3β activity and GSKala overexpression increased GSK3β activity. The absence of phosphatase inhibitors during homogenization did not alter these activity ratios.

enhanced compared to that of untreated cells ($p<0.05$) (Fig. 1). Immunoreactivity of GSK3β phosphorylated at Ser9 from the homogenate of lithium-treated cells was prominently increased ($p<0.001$), indicative of inhibition of activity. Immunoreactivity ratios of phosphorylated at Ser9 and total GSK3β confirmed an increase in overall intracellular GSK3β activity. Relative levels of total GSK3β and GSK3β phosphorylated at Ser9 were essentially unchanged in the presence or absence of phosphatase inhibitors.

We examined the level of tau truncation by caspase. C3 recognizes a neo epitope generated by caspase-mediated tau cleavage at D421 (Gamblin et al., 2003). C3 immunoreactivity within NB2a/d1 cell homogenates was increased by 22% within 4 h of incubation in the presence of phosphatase inhibitors. This increase was doubled by lithium treatment, but was reduced following expression of GSKala (Fig. 2). When cellular homogenates were incubated in the absence of phosphatase inhibitors, C3 immunoreactivity was increased by >100% after 4 h of incubation. Lithium treatment of cells did not affect this increase, but this increase was curtailed to 57% by prior expression of GSKala within cells.

Tau levels were also monitored via nonphospho-dependent and phospho-dependent antibodies. Tau1 recognizes tau that is not phosphorylated on Ser195/198/199/202 (Szendrei et al., 1993). No significant change was observed in Tau1-reactive full-length tau in homogenates of untreated cells following incubation in the presence or absence of phosphatase inhibitors. However, homogenates of lithium-treated cells displayed a 34% increase in Tau1 immunoreactivity after 4 h incubation in the absence of phosphatase inhibitors ($p<0.05$), suggesting that phosphatases within cellular homogenates were capable of net dephosphorylation at Ser195/198/199/202 (Szendrei et al., 1993). No change was observed for homogenates from cells expressing GSKala (Figs. 2 and 3).

Tau1-reactive breakdown products were observed in homogenates from untreated and lithium-treated cells, but not in homogenates from cells expressing GSKala (Fig. 2). Both the overall level of these breakdown products, as well as the appearance of additional lower molecular weight breakdown products, were increased following incubation of homogenates from untreated and lithium-treated cells in the absence of phosphatase inhibitors; expression of GSKala curtailed this increase (Figs. 2 and 3).

No significant change was observed in PHF1-reactive full-length tau following incubation of homogenates in the presence or absence of phosphatase inhibitors regardless of prior treatment with lithium or GSKala expression (Figs. 2 and 3). PHF1 recognizes tau phosphorylated at Ser396/Ser404 (Otvos et al., 1994). Similar levels of PHF1-reactive breakdown products were observed in homogenates from untreated and lithium-treated cells following incubation for 4h in the presence or absence of phosphatase inhibitors. These breakdown products were not observed following incubation homogenates from cells expressing GSKala either with or without phosphatase inhibitors (Fig. 2).

The use of NB2a/d1 cells allowed us to manipulate intracellular kinase activities. To probe the physiological relevance of these findings in NB2a/d1 homogenates, we also incubated homogenates 3xTg-AD mouse brain at 26 months of age (by which time tau pathology is robust)(Oddo et al., 2003) in the presence or absence of phosphatase inhibitors. C3 and Tau1 immunoreactivity within 3xTg-AD mouse homogenates was increased following 4 h of incubation in the absence of phosphatase inhibitors (Fig. 4). There was a prominent decrease in intact Tau1 in the presence of phosphatase inhibitors. In the absence of phosphatase inhibitors, Tau1 immunoreactivity underwent cleavage within 30 min, with generation of a prominent ladder of breakdown products. There was a gradual decrease of PHF1 immunoreactivity in the presence of phosphatase inhibitors, while no significant change was observed after incubation in the absence of phosphatase inhibitors. It is noteworthy that PHF1 immunoreactivity at $T=0$, i.e., even prior to incubation, in the absence of phosphatase inhibitors is much