Effects of tau pseudo-phosphorylation at residues thr231, ser262, ser396, and ser404 upon regulation of microtubule dynamic instability in living cells

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Tau is a neural specific microtubule-associated protein that is crucial for the proper development and maintenance of the nervous system. Mechanistically, tau binds directly to microtubules (MT), promotes MT assembly and regulates MT dynamics. Given that microtubules are key to many neuronal cell functions, it is not surprising that abnormal tau behavior has long been associated with numerous neurodegenerative diseases, including Alzheimer's disease and related dementias. Biochemically, a large body of data implicates abnormal tau phosphorylation as a key to pathological tau action. Historically, most work on tau phosphorylation has assessed the effects of single phosphorylation events; however, in vivo, tau molecules are multiply phosphorylated. In this work, we sought to compare the effects of single and combinatorial tau phosphorylation events. One technical strategy widely used to overcome a variety of technical difficulties in studying protein phosphorylation is "pseudophosphorylation", i.e., the substitution of aspartic or glutamic acid residues to mimic phosphorylated amino acids. Here, we have used pseudophosphorylation to study the mechanistic effects of individual and combinatorial phosphorylation at four key phosphorylation sites in tau, i.e., thr231, ser262, ser396 and ser404. To examine the ability of various pseudo-phosphorylated tau molecules to regulate MT dynamic instability in cells, we microinjected identical amounts of either wild-type or singly or combinatorially pseudo-phosphorylated tau molecules into EGFP-tubulin-expressing MCF7cells and measured the dynamic instability behavior of individual microtubules by time-lapse microscopy. Consistent with variety of in vitro and in vivo studies, 4R wild-type significantly decreased catastrophe frequency, shortening rate as well as overall MT dynamicity compared to buffer injected control cells. On the other hand, pseudo-phosphorylation of tau at serine 262 resulted in significantly compromised MT dynamicity relative to 4R wild-type molecule. Ongoing studies of the ability of each phosphorylation event, individually and in combination, to affect the ability of tau to influence MT behavior should provide valuable insights into mechanisms of normal and pathological tau action.

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