Effects of combinatorial tau pseudophosphorylation upon microtubule binding, assembly and the regulation of dynamic instability in vitro

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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, promotes microtubule assembly and regulates microtubule 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. Specifically, we are examining the ability of various pseudo-phosphorylated tau molecules to bind and assemble microtubules as well as to regulate microtubule dynamics. Additionally, we are examining the effects of the prolylisomerase pin1 to influence these effects. Consistent with a variety of cellular studies, pseudo-phosphorylation of tau at serine 404 has little effect on tau's ability to assemble microtubules relative to WT tau in both 4R and 3R isoforms. On the other hand, pseudo-phosphorylation of tau at serine 262 exhibited markedly reduced ability to bind to and assemble microtubules relative to WT tau. Pseudo-phosphorylation at position serine 396 also compromises tau's ability to bind and assemble microtubules although the effect is of lesser magnitude compared to the pseudo-phosphorylation at ser 262. Ongoing studies of the ability of each phosphorylation event, individually and in combination, to affect the ability of tau to influence microtubule behavior should provide valuable insights into mechanisms of normal and pathological tau action.

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