# SH-SY5Y neuroblastoma cells treated with phosphatase inhibitor okadaic acid express high molecular weight tau species

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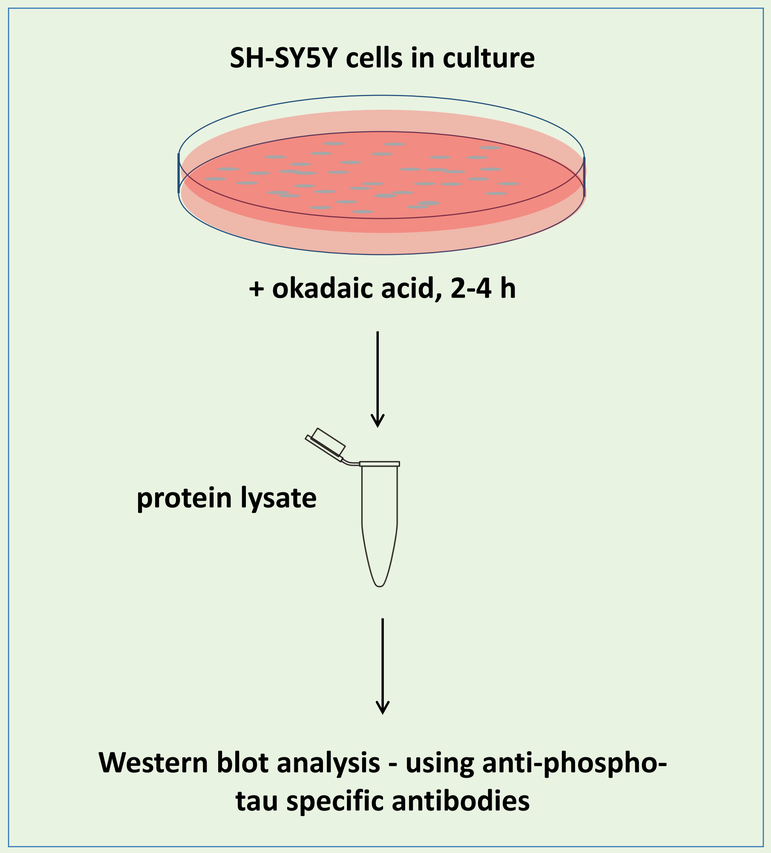
## Summary

**A key feature of Alzheimer's disease early stages is abnormal phosphorylation of tau, a microtubule-associated protein. Tau hyperphosphorylation results from the activity of several protein kinases and downregulation of phosphatase PP2A. To study processes involving phosphorylated tau, cultured cells can be treated with okadaic acid (OA), an inhibitor that primarily targets phosphatase PP2A. Neuroblastoma SH-SY5Y cells express several splicing isoforms of tau and are useful as a cell culture model for investigating molecular pathways important for neuronal tissues due to their neuron-like characteristics.**

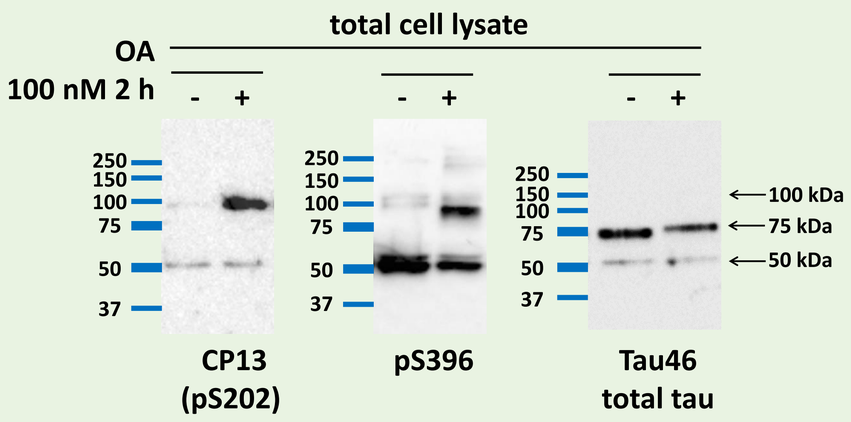
**In order to induce accumulation of hyperphosphorylated tau, we treated SH-SY5Y cells with 20 nM and 100 nM okadaic acid for several hours and analyzed tau phosphorylation at particular amino acid residues using phospho-tau specific antibodies. We observed that okadaic acid-treated SH-SY5Y cells express phospho-tau of the apparent molecular weight around 100 kDa, in addition to the well-described 45-65 kDa tau isoforms. 100 kDa-phospho-tau could be detected by Western blot, using antibodies against tau phosphorylated at Ser202 and Ser396. Alkaline phosphatase treatment of cell lysate removed the signal, confirming phosphorylation-dependent recognition. 100 kDa-phospho-tau is highly soluble and present in a heat stable fraction. Remarkably, this protein was not detected by antibodies Tau46 and Tau5, which recognize amino acid sequences 404-441 and 218-225 respectively, independent of phosphorylation, indicating that these epitopes are absent or inaccessible.**

**To clarify the identity of 100 kDa-phospho-tau, we investigated the possibility that it represents a tau oligomer. In support of this possibility, a previous study using fluorescently labeled tau transfected into HEK293 cells indicated that tau oligomerizes upon cell treatment with okadaic acid. We noticed that 100 kDa-phospho-tau is stable in the presence of strong denaturing and chaotropic agents urea and guanidine. It did not dissociate in the solution containing β-mercaptoethanol, indicating that the potential oligomer is independent of disulfide bonds.**

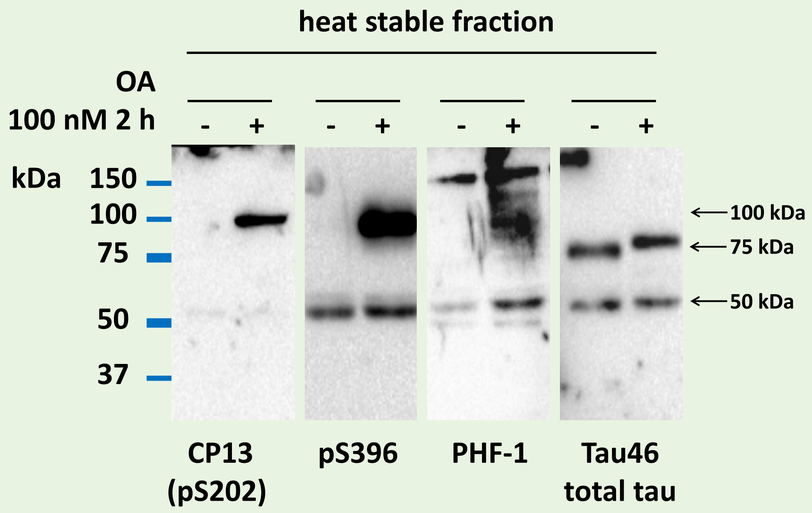
**Taken together, we found that treatment of SH-SY5Y cells with okadaic acid leads to expression of a 100 kDa protein reactive to anti-phospho-tau antibodies CP13 (ptau-Ser202) and pSer396 (ptau-Ser396). While not fully characterized, this protein may represent a phosphorylation-induced tau oligomer.**



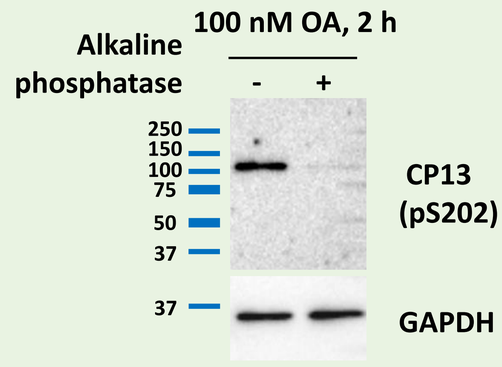

## SH-SY5Y cells treated with 100 nM okadaic acid express 100 kDa tau phosphorylated at Ser202 and Ser396



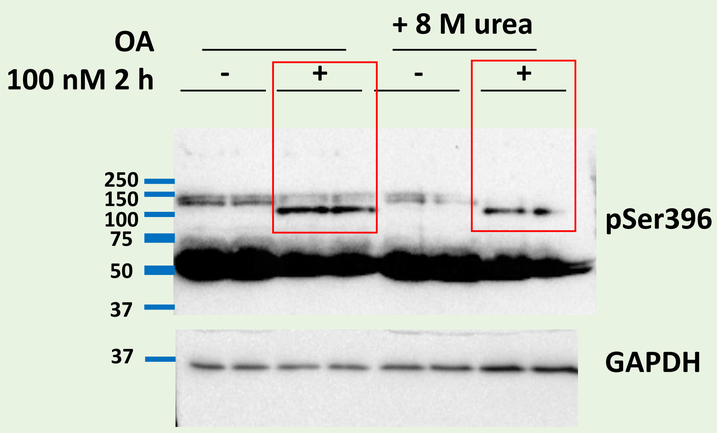

## 100 kDa-phospho-tau is present in the heat stable fraction of protein lysate

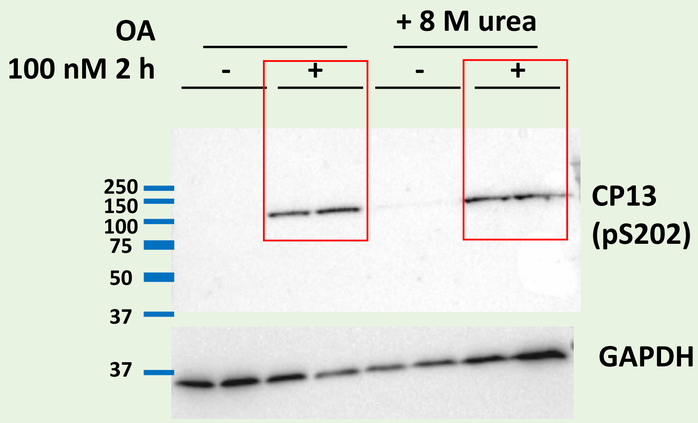


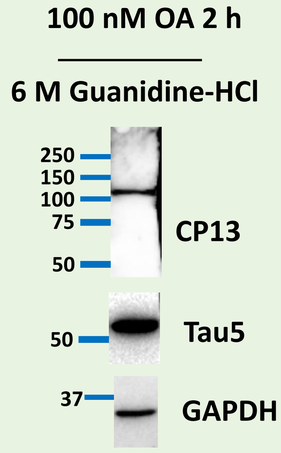

## Alkaline phosphatase treatment abolishes detection of 100 kDa-phospho-tau by phospho-specific antibody



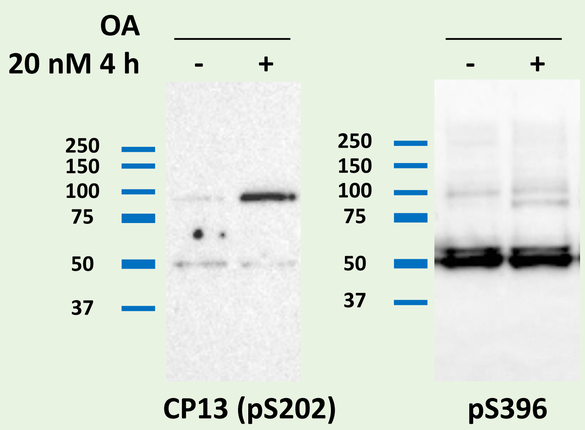

## 100 kDa-phospho-tau is stable in buffers containing β-ME, 8 M urea or 6 M guanidine-hydrochloride



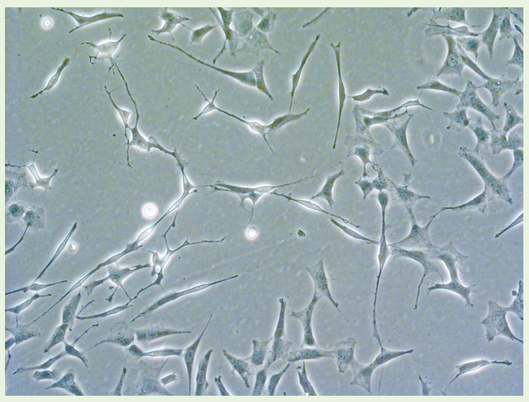




## SH-SY5Y cells treated with 20 nM okadaic acid express 100 kDa tau that is phosphorylated on Ser202, but very little on Ser396




## 100 kDa-phospho-tau is also expressed in SH-SY5Y cells differentiated with retinoic acid



SH-SY5Y cells (P17)
→ 5 days in DMEM/10 % FBS/10 µM retinoic acid
→ 2 days in DMEM/ 1 % FBS / BDNF