Okadaic acid treated SH-SY5Y neuroblastoma cell line as a model to study Tau protein phosphorylation and oligomerization

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INTRODUCTION
A key feature in the early stages of Alzheimer's disease 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, a phosphatase inhibitor that primarily targets PP2A. SH-SY5Y cells express several splicing isoforms of tau and are useful as a cell culture model for investigating pathways important for neuronal tissues due to their neuron-like characteristics.

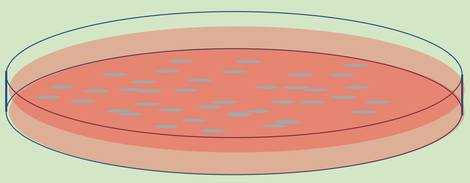
METHODS
To induce accumulation of hyperphosphorylated tau, *in vitro* cultured SH-SY5Y neuroblastoma cells were treated with 100 nM phosphatase inhibitor okadaic acid for indicated periods of time. Cells were harvested and protein lysates prepared. Tau protein levels and phosphorylation status at specific amino acid residues were analyzed by immunoblot (western blot) using antibodies against N-terminal part of tau (CP27) or middle part of tau (Tau5) and phospho-tau specific antibodies (CP13 and anti-tau-pSer396). Levels of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were used as a loading control. In the experiment that required inhibition of protein synthesis, we treated cells with 100 mM cycloheximide for 15 minutes prior to adding okadaic acid.

RESULTS
We observed that okadaic acid treated SH-SY5Y cells express tau protein of the apparent molecular weight around 100 kDa, in addition to the well-described 45-65 kDa tau isoforms. High molecular weight tau was detected by western blot using antibodies against tau phosphorylated at amino acid residues Ser202 and Ser396, as well as by the CP27 antibody that recognizes N-terminal part of tau.

Cells that have been pre-treated with cycloheximide, a general inhibitor of protein synthesis, express 100 kDa tau protein upon treatment with okadaic acid, showing that high molecular weight tau is not newly synthesized in response to okadaic acid treatment, and may thus represent tau oligomer.

Biochemical characterization showed that 100 kDa tau is stable in the presence of strong denaturing and chaotropic agents urea and guanidine. 100 kDa tau did not dissociate in the solution containing β-mercaptoethanol, indicating that the potential oligomer does not require disulfide bonds.

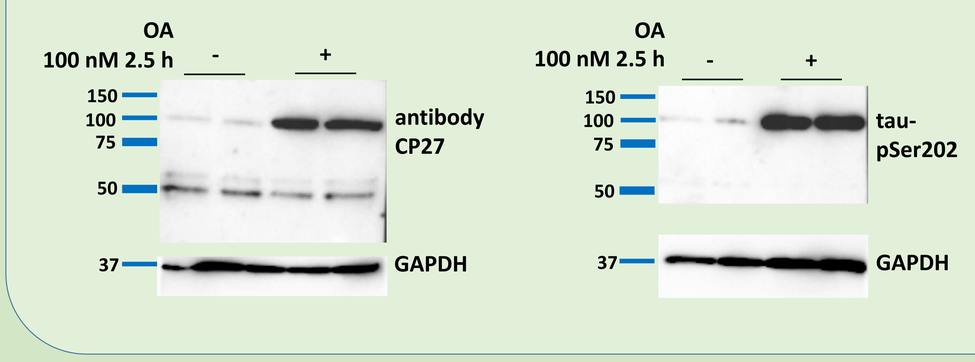
SH-SY5Y cells in culture



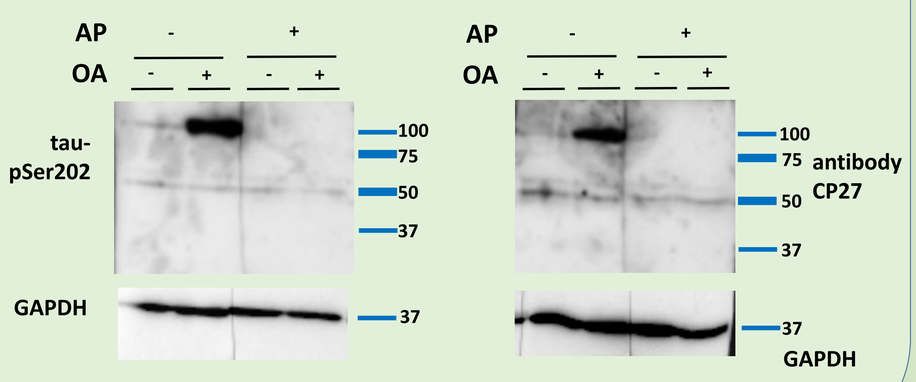
+ okadaic acid, 2-4 h

Western blot analysis - using anti-phospho-tau specific antibodies

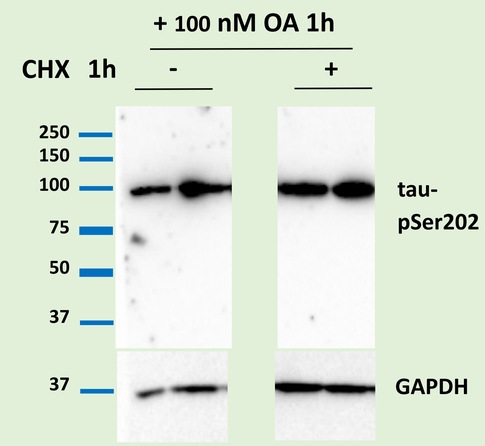
Cultured neuroblastoma cells treated with phosphatase inhibitor okadaic acid (OA) express high molecular weight (100 kDa) tau protein phosphorylated at amino acid residues Ser202 and Ser396.



Antibody CP27 against N-terminal part of tau does not recognize 100 kDa tau on membrane pre-treated with alkaline phosphatase (AP), in a preliminary experiment.



Induction of high molecular weight phospho tau by okadaic acid does not require new protein synthesis.



single membrane photographed under the same conditions

CONCLUSIONS
Our results show that treatment of SH-SY5Y cells with phosphatase inhibitor okadaic acid induces formation of high molecular weight tau protein that may represent a large splicing isoform of tau or tau oligomer. In support of the latter possibility, a previous study using fluorescently labeled tau transfected into HEK293 cells indicated that tau oligomerizes upon cell treatment with okadaic acid (Tak et al., PLOS One 2013, 8:12). We biochemically characterized 100 kDa tau and excluded a possibility that this protein is newly synthesized as a response to okadaic acid treatment. Our findings contribute to characterization of SH-SY5Y cell culture treated with okadaic acid as a model for studying phospho-tau related processes.

High molecular weight phospho-tau is stable in the presence of reducing agent β-mercaptoethanol and in buffers containing strong denaturing and chaotropic agents urea and guanidine-hydrochloride