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-SYSY 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-SYSY 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-SYSY 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.

CONCLUSIONS
Our results show that treatment of SH-SYSY 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-SYSY cell culture treated with okadaic acid as a model for studying phospho-tau related processes.