Inhibition of protein phosphatases by okadaic acid induces expression of high molecular weight phospho-tau-immunoreactive protein species in neuroblastoma SH-SY5Y cells

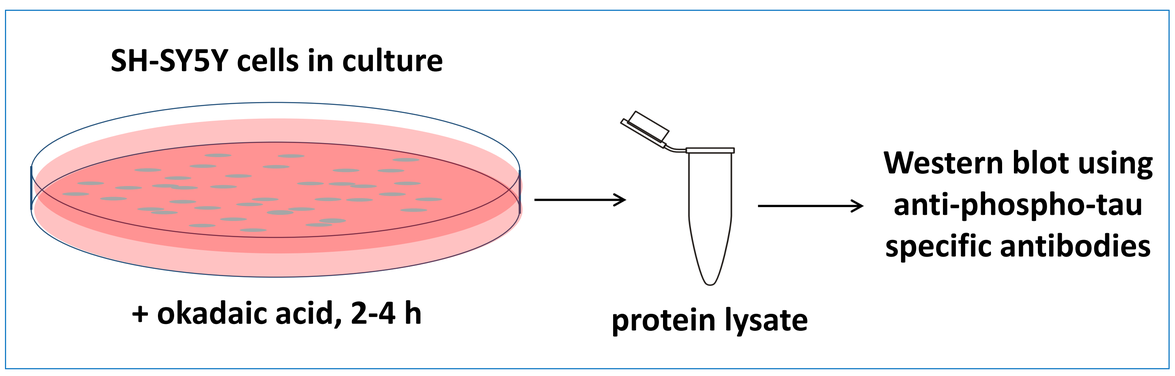
Mirta Boban, Terezija Miškić, Mirjana Babić Leko and Goran Šimić*
Croatian Institute for Brain Research, University of Zagreb School of Medicine
Address: Šalata 12, 10000 Zagreb, Croatia, *E-mail: gsimic@hiim.hr

ABSTRACT

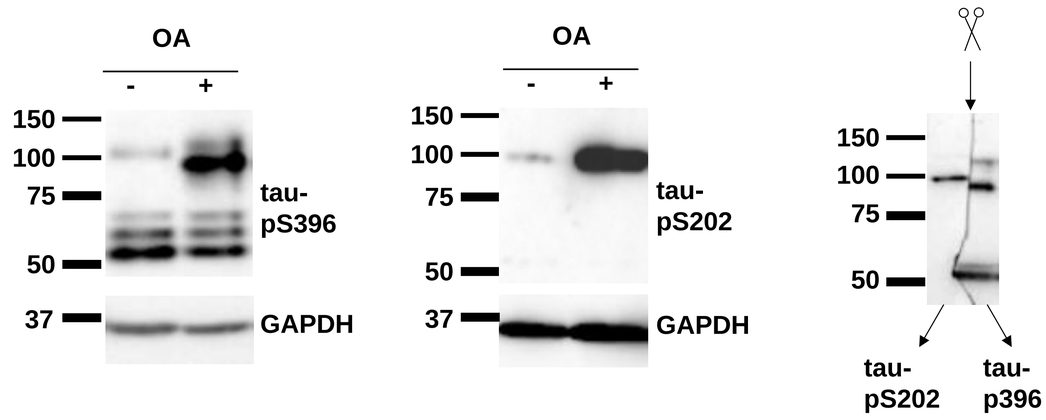
A key feature of Alzheimer's disease (AD) is aggregation of microtubule-associated protein tau in the neurofibrillary tangles (NFT) in the brain. NFT tau is characterized by abnormally high phosphorylation, which may result from the upregulated activity of protein kinases and downregulation of protein phosphatases.

To investigate tau under the condition of protein phosphatase impairment, we treated neuroblastoma SH-SY5Y cells with okadaic acid (OA), an inhibitor of protein phosphatases and analyzed total cell lysates with phospho-tau and total tau antibodies using immunoblot.

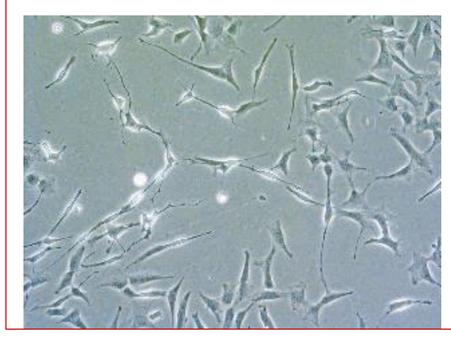
In addition to the well-described 50-65 kDa tau isoforms, we observed that both undifferentiated and retinoic acid- and brain derived neurotropic factor-differentiated SH-SY5Y cells treated with OA express high molecular weight protein species immunoreactive with anti-tau-pS202 and -pS396 antibodies. The apparent molecular weight of 100 kDa indicated a possibility of tau dimer. In support, high molecular weight tau immunoreactive proteins (HMW-TIP) were detected in a heat-stable fraction. However, we were unable to detect HMW-TIP using anti-total tau antibodies. This could be due to protein truncation or epitope masking within the oligomer, or a possibility that HMW-TIP represents a tau-unrelated protein. Our biochemical characterization showed that HMW-TIP were stable under reducing conditions and in the presence of strong denaturing agents, such as urea and guanidine, as well as upon alkaline phosphatase treatment.

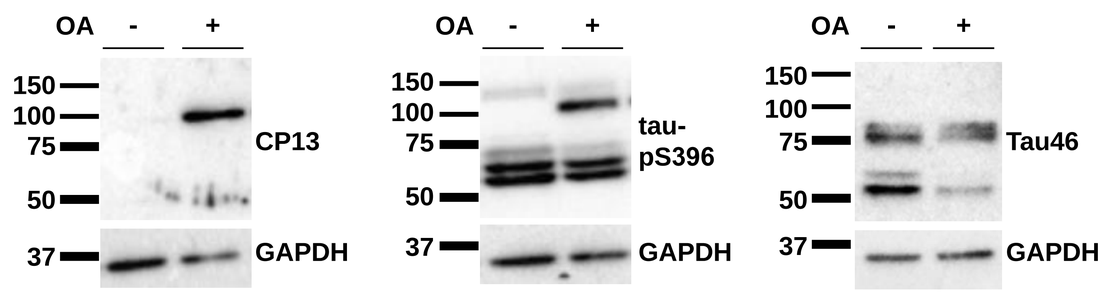


1. OA treatment of SH-SY5Y cells induces expression of a high molecular weight protein species immunoreactive to phospho-tau.

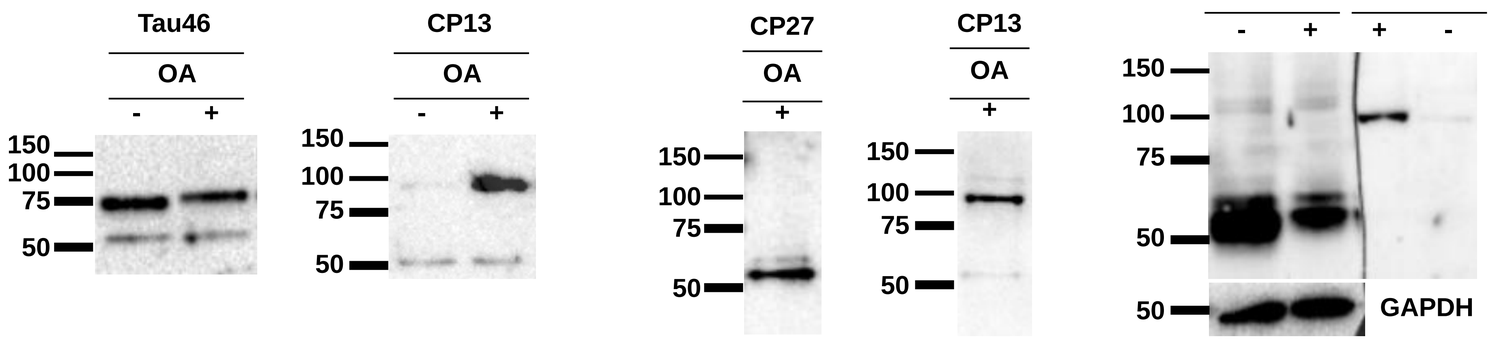


2. HMW-TIP is detected in OA-treated SH-SY5Y cells differentiated into neuron-like cells.

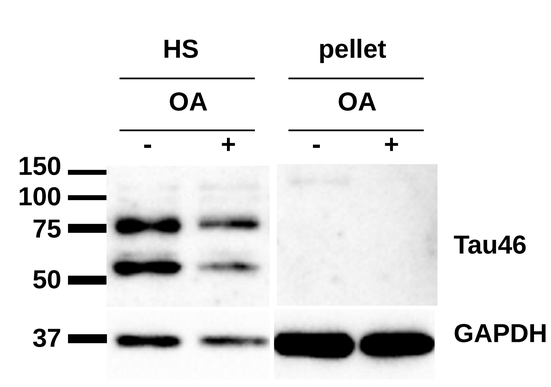




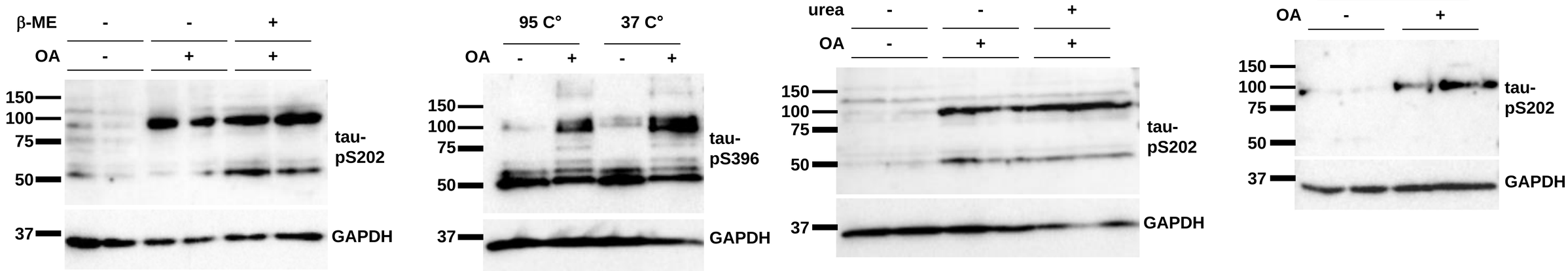
3. HMW-TIPs is not detected by anti-total tau antibodies.



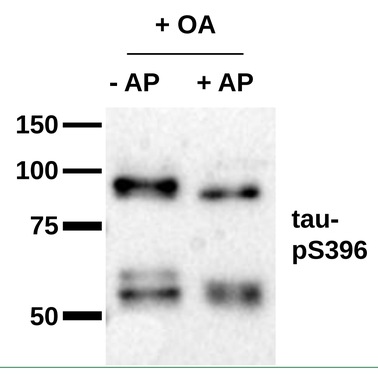
4. HMW-TIP is present in heat-stable fraction.



5. HMW-TIP stability under reducing and denaturing conditions.



6. HMW-TIP stability upon protein de-phosphorylation.



CONCLUSION

In conclusion, we show that protein phosphatase inhibition by okadaic acid induces the appearance of HMW-TIP, which may represent tau oligomer or tau cross-reactive phospho-proteins.

ACKNOWLEDGEMENTS

Funded by the Croatian Science Foundation HRZZ (IP-2014-09-9730).