27-28 APRIL 2017

1ST EUROTAUMEETING

Or how to know everything about Tau protein and associated diseases ?

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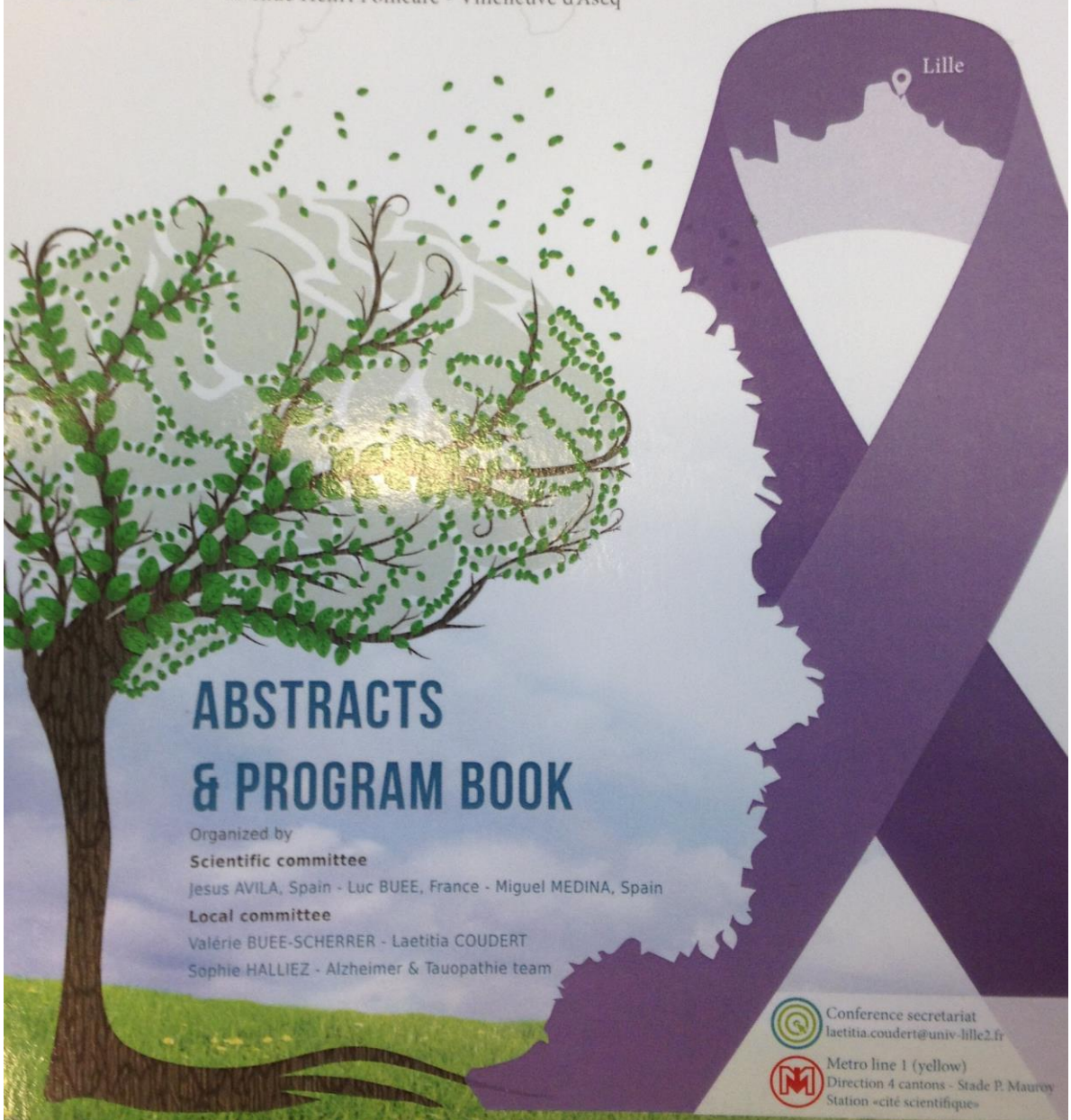

ABSTRACTS & PROGRAM BOOK

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P06: SH-SY5Y neuroblastoma cells treated with phosphatase inhibitor okadaic acid express high molecular weight tau species

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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, 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 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 okadaic acid in 20 nM and 100 nM concentration for several hours and analyzed tau phosphorylation at specific amino acid residues using phospho-tau specific antibodies. We observed that okadaic acid treated SH-SY5Y cells express a high molecular weight anti-phospho-tau reacting protein (highMW-ptau-RP) of the apparent molecular weight around 100 kDa, in addition to the well-described 45-65 kDa tau isoforms. HighMW-ptau-RP 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. HighMW-ptau-RP is highly soluble and present in a heat stable fraction, a characteristic of tau. Remarkably, this protein was not detected by antibody Tau5, which recognizes amino acid sequence 218-225 independent of phosphorylation, indicating that this epitope is absent or inaccessible. To clarify the identity of highMW-ptau-RP, 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 highMW-ptau-RP 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. We are currently testing whether highMW-ptau-RP is stable in formic acid, which is known to dissociate aggregates of misfolded proteins related to neurodegeneration. We are also considering an alternative possibility that highMW-ptau-RP is Big tau, a tau isoform containing the sequence from exon 4a, which is primarily expressed in the peripheral nervous system. 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.