Two stable SHSY-5Y cell lines over-expressing full length and truncated Tau441 as screening tools for tauopathies

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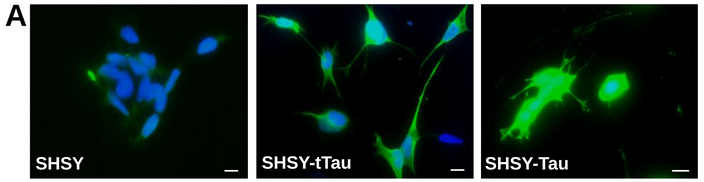
BACKGROUND

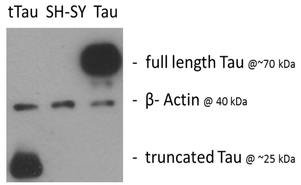
Tau proteins belong to the family of microtubule-associated proteins and play an important role in stabilizing the neuronal microtubules network. They are the major constituents of intraneuronal and glial fibrillar lesions described in Alzheimer's disease and numerous neurodegenerative disorders referred to as 'tauopathies', including progressive supranuclear palsy, corticobasal degeneration, argyrophilic grain disease, as well as the inherited frontotemporal dementia and parkinsonism linked to chromosome 17.

Molecular analysis revealed that hyperphosphorylation might be the important event leading to Tau aggregation resulting in neurodegeneration and dementia. Development of new compounds capable of preventing tau hyperphosphorylation is an increasingly hot topic. Thus, reliable models are needed that reflect tau hyperphosphorylation in human diseases.

METHODS

For this purpose, we generated two stably transfected SHSY-5Y cell lines either overexpressing the longest human Tau441 isoform comprising two disease related mutations (SHSY-Tau) or a truncated version of Tau441 (SHSY-tTau).





SHSY cells overexpressing truncated or full length Tau441 protein.

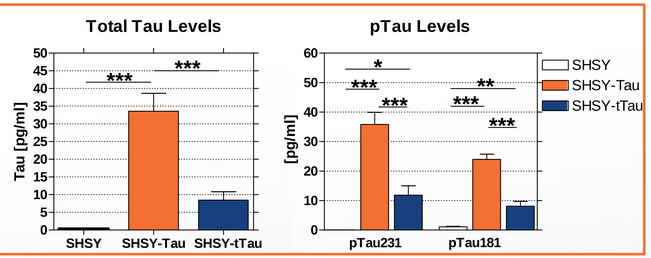
Figure 1: A) SHSY, SHSY-tTau and SHSY-Tau cells were subjected to indirect immunofluorescences using an antibody against human Tau clone AT7 (green) and DAPI (blue) as nucleus counterstain. Bars: 10 µm. B) Protein expression of Tau species in SHSY, SHSY-tTau and SHSY-Tau cells. Shown is one representative immunoblot of human Tau levels in the indicated cell lines. β-Actin was used as loading control. Endogenous Tau levels in SHSY cells were too low to be detected.

RESULTS

Comparison of tau expression and phosphorylation levels in SHSY versus SHSY-Tau and SHSY-tTau overexpressing cells confirmed relevance to human diseases. The phosphorylation pattern of tau can be reliably modulated by distinct kinase inhibitors targeting CDK, JNK or GSK-3β. Effects on tau phosphorylation (residues Thr231 and Thr181) were determined by immunosorbent assays (Mesoscale Discovery).

Hyperphosphorylation at Tau phospho sites Thr231 and Thr181 in SHSY-Tau and SHSY-tTau cells.

Figure: 2: Human total Tau (left graph), pThr231 and pThr181 Tau (right graph) were analyzed by immunosorbent assay in SHSY, SHSY-tTau and SHSY-Tau cells. SHSY-Tau cells showed strongly elevated levels of total Tau compared to SHSY-tTau cells or to SHSY cells. Phosphorylation at sites Thr231 and Thr181 of Tau was highly increased in SHSY-Tau and SHSY-tTau cells compared to untransfected SHSY cells. Statistical significance is indicated by *<0.05, **<0.01, ***<0.001 as determined by One-Way ANOVA (Newman-Keuls Multiple Comparison Test). Data are shown as group mean + SEM (n= 5).



New hypothermia-induced Tau hyperphosphorylation models are available *in vitro* and *in vivo* at QPS Austria

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Kinase inhibitors targeting JNK, CDK or GSK-3β modulate the phosphorylation pattern in SHSY-Tau and SHSY-tTau cells.

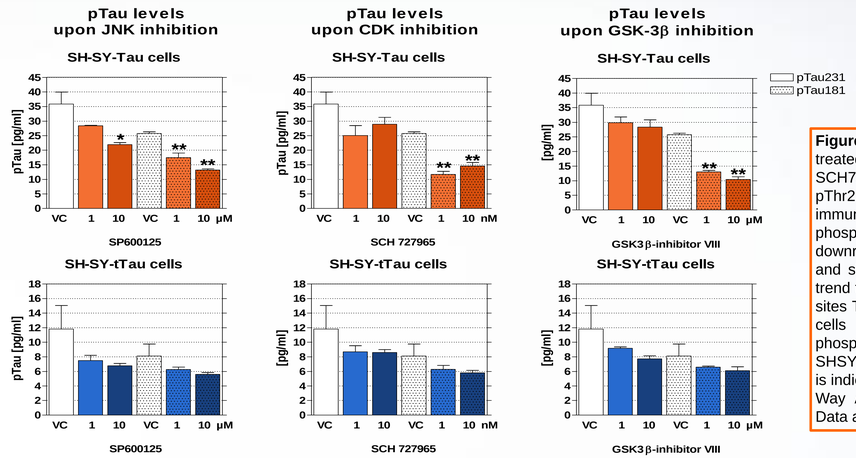

Figure: 3: SHSY-Tau and SHSY-tTau cells were treated with kinase inhibitors SP600125 (JNK), SCH727965 (CDK) and GSK-3β inhibitor VIII for 6h. pThr231 and pThr181 Tau levels were measured by immunosorbent assay. In SHSY-Tau cells, hyper-phosphorylation at site Thr181 was significantly downregulated by JNK, CDK and GSK-3β inhibition and site Thr231 by JNK inhibition. A non-significant trend toward the reduction of hyperphosphorylation at sites Thr231 and Thr181 was observed in SHSY-tTau cells upon kinase inhibition. No effects on Tau phosphorylation was observed in untransfected SHSY cells (data not shown). Statistical significance is indicated by *<0.05, **<0.01 as determined by One-Way ANOVA (Dunnetts Multiple Comparison Test). Data are shown as group mean + SEM (n= 4).

SHSY-tTau cells show elevated APP and Aβ peptide levels and an increased impairment by Aβ1-42 toxicity.

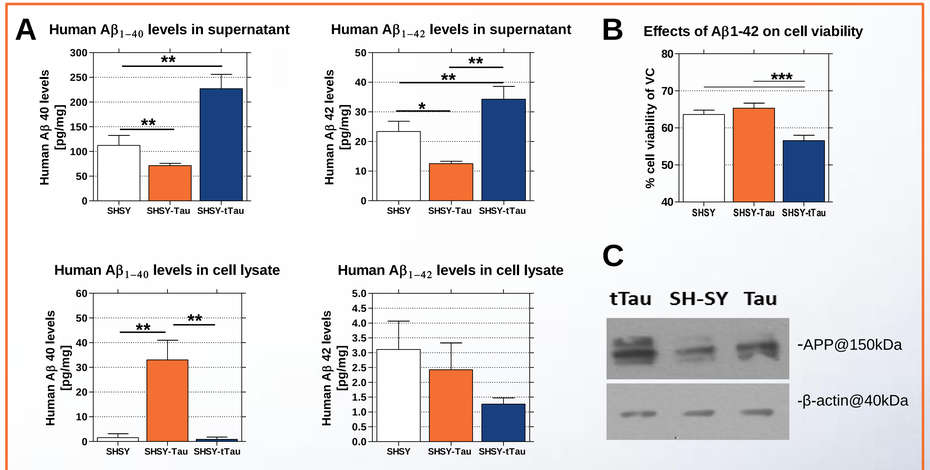

Figure 4: A) Aβ peptides 1-40 and 1-42 were determined by immunosorbent assay in supernatants (top) and cell lysates (bottom) from SHSY, SHSY-Tau and SHSY-tTau cells overexpressing full length or truncated human Tau. SHSY-tTau cells showed increased Aβ 1-40 and 1-42 levels in supernatant whereas SHSY-Tau cells displayed higher cellular Aβ 1-40 levels. **B)** Cells were treated with 1 µM oligomerized Abeta 1-42 for 96h. Cell viability was determined by MTT assay. SHSY-tTau cells were noticed to be more sensitive to Abeta 1-42 toxicity compared to SHSY or SHSY-Tau cells. Statistical significance is indicated by *<0.05, **<0.01, ***<0.001 as determined by One-Way ANOVA (Newman-Keuls Multiple Comparison Test). Data are shown as group mean + SEM (n= 4). **C**) Shown is a representative immunoblot of APP in SHSY-tTau, SHSY and SHSY-Tau cell lysates. β-Actin was used as loading control. SHSY-tTau cells showed elevated APP levels compared to SHSY or SHSY-Tau cells.

CONCLUSION

In summary, we present two cellular systems to analyze the efficiency of Tau modulating compounds. Hyperphosphorylation and its modulation by kinase inhibitors known to be involved in tau phosphorylation are therefore reliable indicators for the suitability of these two cell lines as *in vitro* models for tauopathies.

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