Stable Expression of Tau Fluorescent Mutants as a Model to Screen Different Modulators of AD-related Kinases

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Abstract
The microtubule associated protein Tau is the main component of the neurofribillary tangles (NFT), aberrant structures that appear in the brain of Alzheimer´s disease patients and other tauopathies, such as FTDP-17 or corticobasal degeneration. Tau protein binds to and stabilizes microtubules (MTs) but in pathological states, it aggregates and loses its important functions. These Tau aggregates are composed basically by hyperphosphorylated and truncated forms of tau. Multiple Tau gene mutations are pathogenic for hereditary FTDP-17 disease. These mutations have similar effects to hyperphosphorylation in Tau in AD and result in NFT formation.
We generated stably transfected cell lines that expressed different forms and mutants of Tau protein fused to turboGFP in order to study Tau behaviour in these conditions and perform a fluorescence-based cell assay for the screening of kinase modulators that affect the behaviour or location of Tau protein.

Results

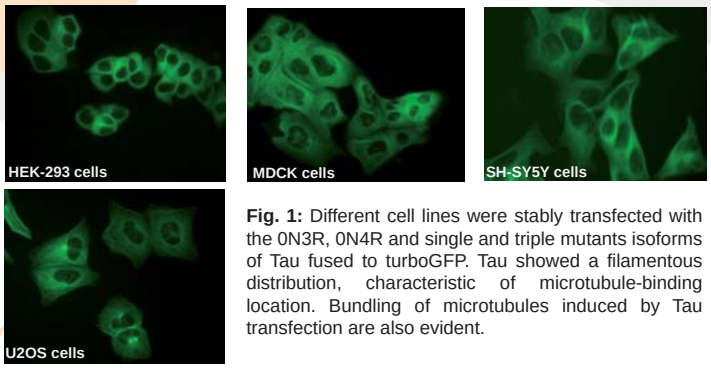

Fig. 1: Different cell lines were stably transfected with the 0N3R, 0N4R and single and triple mutants isoforms of Tau fused to turboGFP. Tau showed a filamentous distribution, characteristic of microtubule-binding location. Bundling of microtubules induced by Tau transfection are also evident.

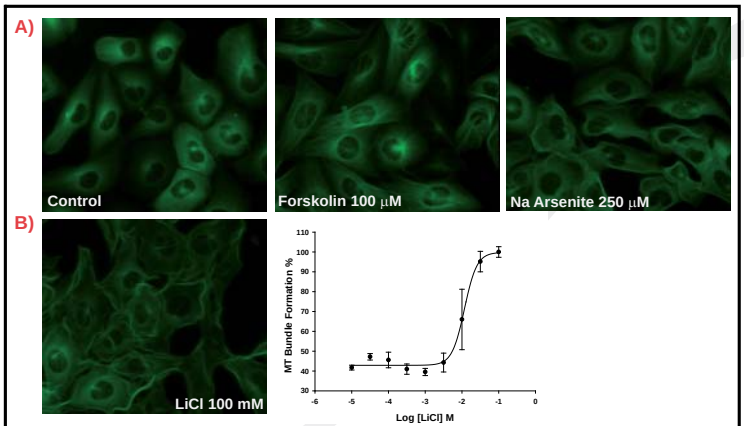

Fig. 3: A) Not only kinase inhibitors like LiCl, but also PP2A modulators (Forskolin) and oxidative stress inducers (Na Arsenite) affect Tau-TM binding to MTs in U2OS cells.
B) Dose-response curve for the GSK3 inhibitor LiCl. IC50 value for LiCl was determined by treating U2OS Tau-TM model cells with concentrations from 100 mM to 10uM during 2h. The intracellular bundle formation is quantified with a BD Pathway 855 High-Content Bioimager and Attovision software. Ic50 for LiCl is 11.75 mM and z' for this experiment was 0,80 +/-0,02.

Materials and methods

HEK-293 and MDCK cells were mantained in DMEM containing 10% fetal bovine serum. SH-SY5Y cells were mantained in RPMI containg also 10% fetal bovine serum. U2OS cells were manteined in F12 medium with 10% fetal bovine serum. Cells were transfected using calcium phosphate or LTX (Sigma). These lines clones stably expressing different forms of turboGFP-tagged Tau were obtained after selection with 500 µg/ml geneticin.
Lithium Chloride, Sodium Arsenite and Forskolin were purchased from SIgma.
Based on the strength of the initial response and lack of cytotoxicity, 43 compounds from a library of 1200 compounds were selected and used at 10 µM in this Tau-tGFP model.
Images and data were carried out with a BD Pathway 855 High-Content Cell Analyzer.

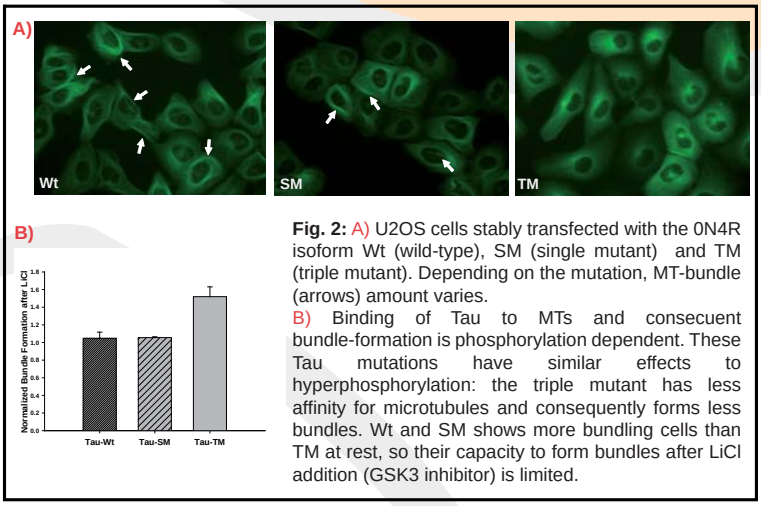

Fig. 2: A) U2OS cells stably transfected with the 0N4R isoform Wt (wild-type), SM (single mutant) and TM (triple mutant). Depending on the mutation, MT-bundle (arrows) amount varies.
B) Binding of Tau to MTs and consecuent bundle-formation is phosphorylation dependent. These Tau mutations have similar effects to hyperphosphorylation: the triple mutant has less affinity for microtubules and consequently forms less bundles. Wt and SM shows more bundling cells than TM at rest, so their capacity to form bundles after LiCl addition (GSK3 inhibitor) is limited.

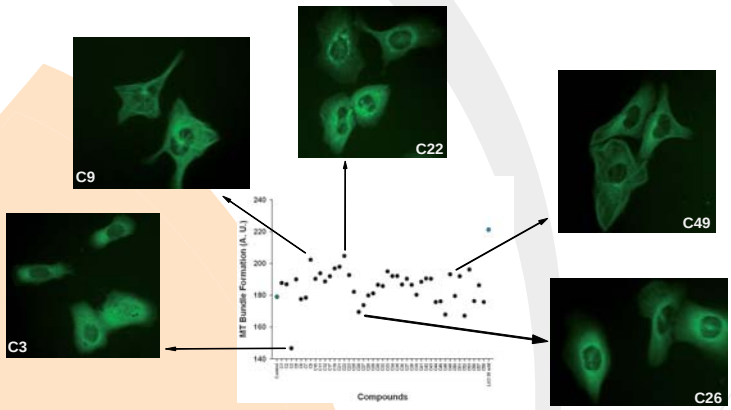

Fig. 4: Screening in Tau-TM U2OS cells of representative and less toxic compounds from a library of 1200. Negative and positive (LiCl 30 mM) controls are shown in green and cyan, respectively. Some of the compounds have effect on Tau distribution: C3 and C26 shows no binding to MTs and in some cells Tau appears in nucleus; C9 and C49 shows more MT bundles; C22 shows an odd punctuate pattern around the nucleus.

Conclusions

1. **Stably Tau transfected cells are viable.**
2. **Tau-turboGFP shows a filamentous distribution, characteristic of microtubule-binding location.**
3. **Fusion of turboGFP does not affect the functional properties of Tau.**
4. **Transfected Tau construct induces bundles of microtubules.**
5. **Mutations in Tau have different effects on bundle formation.**
6. **Distinct compounds over Tau-tGFP cells affect bundle formation.**
7. **BD Pathway 855 High-Content Bioimager is able to quantify number and intensity of bundles and therefore, study Tau behaviour and location.**
8. **According to the previos point, our assay could be valid for the screening of new modulators of AD-related kinases.**

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