Comparative analysis of different peptidyl-prolyl isomerases reveals FK506-binding protein 12 as the most potent enhancer of α-synuclein aggregation

Deleersnijder, A., Van Rompuy A.S., Desender, L., Pottel, H., Buée, L., Debyser, Z., Baekelandt V., and Gerard, M.

Laboratory of Neurobiology and Gene Therapy, K. U. Leuven, Kapucijnenvoer 33, B-3000 Leuven, Flanders, Belgium.

Research question and background

FK506 binding proteins (FKBPs) are members of the immunophilins, enzymes that assist protein folding with their peptidyl-prolyl isomerase (PPIase) activity. Some non-immunosuppressive inhibitors of these enzymes have neuroregenerative and neuroprotective properties with an unknown mechanism of action. We have previously shown that FKBPs accelerate the aggregation of alpha-synuclein (α-SYN) in vitro and in a neuronal cell culture model for synucleinopathy. In this study, we investigated whether acceleration of α-SYN aggregation is specific for the FKBP or even the PPIase family. Therefore, we studied the effect of several physiologically relevant PPIases, namely FKBP12, FKBP38, FKBP52, FKBP65, Pin1 and cyclophilin A, on α-SYN aggregation in vitro and in neuronal cell culture. Among all PPIases tested in vitro, FKBP12 accelerated α-SYN aggregation the most. Furthermore, only FKBP12 accelerated α-SYN fibril formation at subnanomolar concentrations, pointing towards an enzymatic effect. Although stable overexpression of various FKBPs enhanced the aggregation of α-SYN and cell death in cell culture, they were less potent than FKBP12. Both in vitro and cell culture data provide strong evidence that FKBP12 is the most important PPIase modulating α-SYN aggregation and validate the protein as an interesting drug target for Parkinson's disease. In order to further corroborate the possible in vivo relevance of our findings, we decided to investigate the presence of the studied PPIases in Lewy bodies (LBs) of Parkinson's disease (PD) patients.

Methods and tissues used

Substantia nigra (SN) tissue sections from a PD patient (Braak stage 6) and a healthy control were obtained from the Netherlands brain bank.

Immunofluorescent double stainings were performed against the following antigens: ubiquitin, FKBP12, FKBP38, FKBP52, FKBP65, CYPA, Pin1 and α-SYN.

Results and conclusion

Confocal analysis revealed that 80-90% of the LBs stained positive for ubiquitin, as expected (Fig. 1A). In addition, in some LBs, we could detect weak staining for FKBP12 or Pin1 in the dense core and/or halo (Fig. 1B and 1G respectively). However, no co-localization between any of the other PPIases and α-SYN was detected. In sections of a healthy control, no LBs could be detected with either antibody. The finding that FKBP12 can be detected in LBs is in agreement with our conclusion that FKBP12 is the most physiological modulator of α-synuclein aggregation.

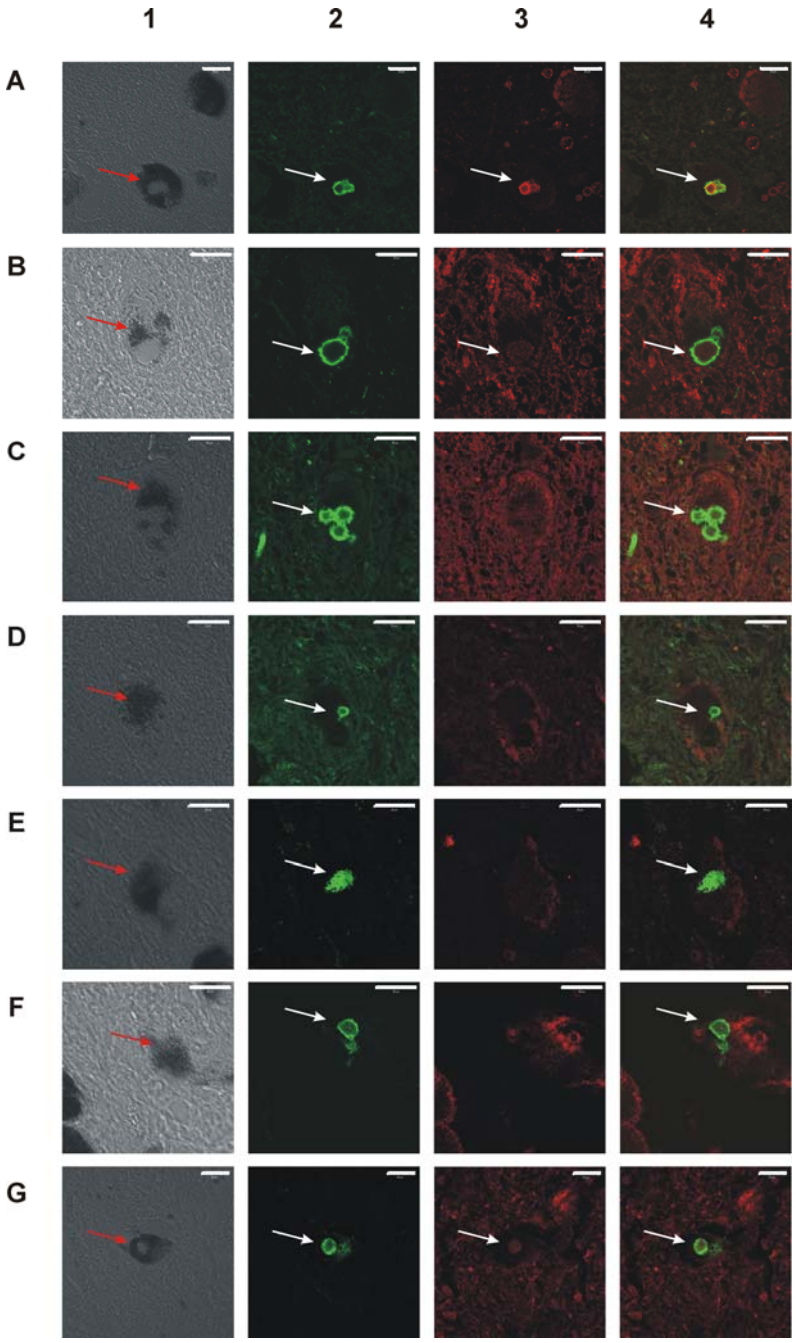

Figure 1: Co-localization of PPIases and Lewy bodies in the brain of a PD patient Confocal microscopy for ubiquitin (A), FKBP12 (B), FKBP38 (C), FKBP52 (D), FKBP65 (E), CYPA (F) or Pin1 (G) and α-SYN positive LBs in the substantia nigra of a PD patient (Braak stage 6). Left panels (1), white light image. Left middle panels (2), immunohistochemical staining for α-SYN is shown in green. Right middle panels (3), immunohistochemical staining for ubiquitin, FKBP12, FKBP38, FKBP52, FKBP65, CYPA or Pin1 is shown in red. Right panels (4), overlap image. Scale bars, 20 μm. Red and white arrows depict the pigment of dopaminergic neurons and Lewy bodies respectively.