Verification of αB-crystallin and Hapln2 expression in brain of patients with Parkinson's disease.

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Research question and background

A large body of evidence now suggests that chronic neuroinflammation is a common feature of the ageing brain and some neurodegenerative disorders. However, the molecular and cellular mechanisms underlying the regulation of innate immunity in the CNS remain elusive. In our recent studies, we found that astrocytic dopamine D2 receptor (Drd2) activation normally suppresses neuroinflammation in the CNS through an αB-crystallin-dependent mechanism. Astrocytes null for Drd2 became hyper-responsive to immune stimuli with a marked reduction in the level of alpha B-crystallin, leading to enhanced vulnerability of dopamine neuron to neurotoxicity. Based on these findings from animal study, we would like to address question whether αB-crystallin is associated with Parkinson's disease. This was also prompted by our previous proteomic data in which expression levels of αB-crystallin were up-regulated in PD brain compared with control.

Methods and tissues used

Immunohistochemical staining was carried out in the brain sections of four PD and three control subjects. We also looked at the specimens of other brain disorders, such as Park. dis. with dementia, Amyotrophic lateral sclerosis, Progressive supranuclear palsy, Alzheimer's disease, Multi-system atrophy, Huntington's disease, Lewy bodies variant, Fronto-temporal dementia ubiquitine + MND.

Results and conclusion

We found that the overall levels of αB-crystallin immunosignals were elevated in PD compared with control. Numerous αB-crystallin-immunopositive Lewy body-like aggreates were observed in PD. A substantial fraction of glial cells in the ventral mesencephalon of PD patients displayed increased Cryab immunoreactivity, compared with controls. The results had been presented in the manuscript submitted to *Nature*. However, in the final revision, this part of data was removed as per request by the reviewers and editor. This may be attributable to the small sample size (n=3-4) and lack of mechanistic interpretation on the formation of αB-crystallin-immunopositive aggregates in PD brain in this study. Although these data were not formally published, it does indicate the association between αB-crystallin and PD pathogenesis. The rest of data is recently published in *Nature* entitled 'Suppression of neuroinflammation by astrocytic dopamine D2 receptors via αB-crystallin' (**2013; 494; 90-94**).

In our αB-crystallin immunohistochemical analysis of other brain disorders, we observed immunosignals in some of these cases, including Progressive supranuclear palsy, Multi-system atrophy, Huntington's disease, Lewy bodies variant, Fronto-temporal dementia ubiquitine + MND, but not in Park. dis. with dementia, ALS and Alzheimer's disease. Because of the small sample sizes of each brain disease, the association between αB-crystallin and those brain disorders needs to be further verified in future investigations.

We also performed Hapln2 immunohistochemical staning on PD tissue sections. Unexpectedly, we found that the hybridoma clones used in the staining showed strong cross-reactivities with α-synuclein. Thus, it is no longer meaningful to perform staining on PD brain sections again.