Investigating potential players in α-Synuclein aggregation in Parkinson's disease

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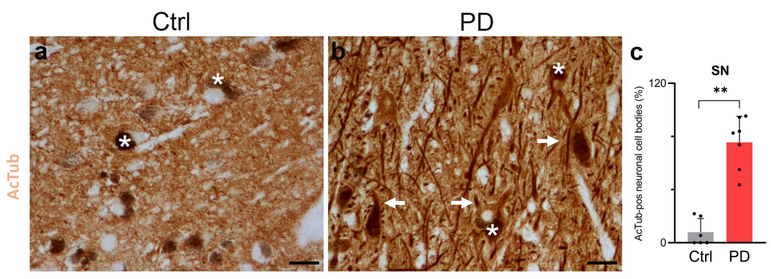
Introduction

The aggregation and spreading of α-Synuclein (α-Syn) are critical events in the pathogenesis of Parkinson's disease (PD). If the involvement of many mechanisms has been suggested for α-Syn aggregation process, including acetylation of microtubules[(1,2)], others have been involved in the cellular attempts to counteract α-Syn aggregation, spreading and toxicity. Among these, the modulation of molecular chaperones to alleviate the burden of aggregated α-Syn is one promising approach that is currently under investigation. A well-known chaperone highly expressed in the brain is clusterin (CLU). CLU is known to have a role in various biological functions, such as response to stresses, apoptosis, and inflammation. Moreover, it is involved in the clearance of misfolded proteins. Indeed, CLU functions as an ATP-independent chaperone, showing characteristics akin to a "holdase". This suggests its relevance in the pathological process, since it could prevent the aggregation of misfolded proteins[(3)]. Despite some studies in cellular models indicate the capability of CLU to limit the aggregation of α-Syn[(4)] and to mitigate the toxicity linked to α-Syn oligomers[(5)], studies investigating the role of CLU in *post-mortem* human brain of PD patients are currently lacking.

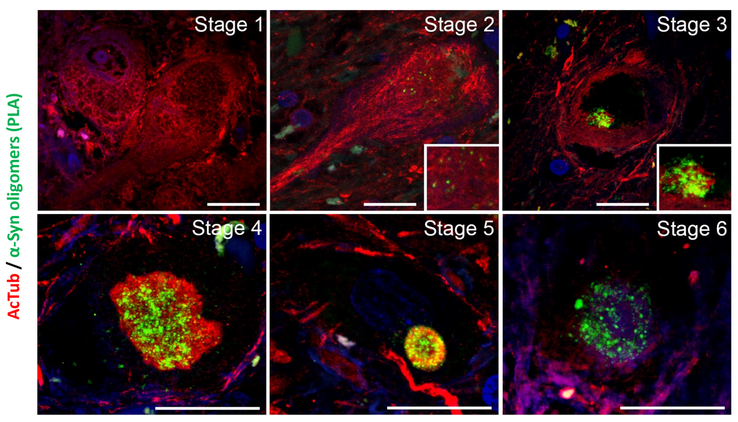
Aim

The aim of this work is to investigate potential pathways involved in α-Syn aggregation process.
In particular, we focused on *Substantia nigra* of *post-mortem* human brain affected by PD at Braak stage 6 and evaluated the distribution of both **acetylated α-tubulin** and **CLU** and their interplay with α-Syn, to verify their involvement in the aggregation process leading to Lewy body formation.

1. *Acetylated α-tubulin redistribution is linked to the early steps of α-Synuclein aggregation[6]*

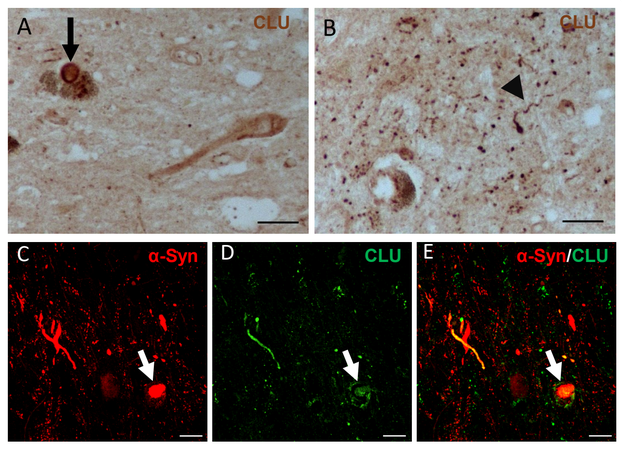


Acetylated α-tubulin accumulates in neuronal cell body in PD patients. (a,b) Acetylated α-tubulin (AcTub) staining in *Substantia nigra* of *post-mortem* human brain obtained from control (Ctrl) or PD affected (PD) subjects reveals that AcTub is accumulated in neuronal cell body of PD samples (arrows). Scale bar, 20 µm. **(c)** Graph represents the quantification of AcTub positive neuronal cell body, expressed as percentage of the total neurons. Mann-Withney test, p<0.01. SN: *Substantia nigra*, asterisks = neuromelanin.

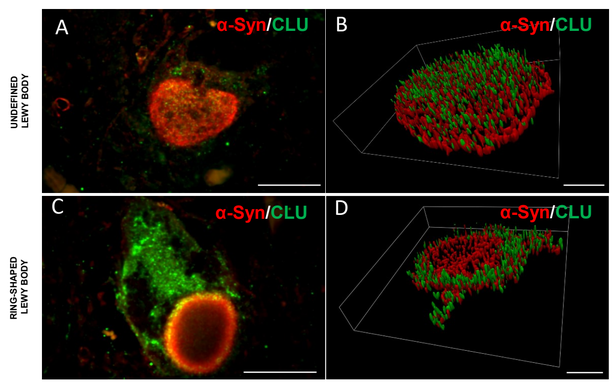


Acetylated α-tubulin redistribution is linked to the early steps of Lewy body formation. The double staining to detect AcTub (by classical immunofluorence assay, in red) and α-synuclein (α-Syn) oligomers (by Proximity Ligation Assay, PLA, in green) reveal the presence of 6 different stages. *Stage 1*: AcTub is strongly present in the soma of neurons. *Stage 2*: AcTub is still accumulated inside the cell body, some small spared α-Syn oligomers appear. *Stage 3*: AcTub and α-Syn oligomers start to accumulate in a small aggregate. *Stage 4*: AcTub is completely accumulated in an aggregate, containing α-Syn oligomers. *Stage 5*: AcTub is restricted to the external border of a ring shaped aggregate positive for α-Syn oligomers. *Stage 6*: the aggregate is negative for AcTub, only few α-Syn oligomers are still present. Scale bar, 20 µm. Nuclei are counterstained with Hoechst.

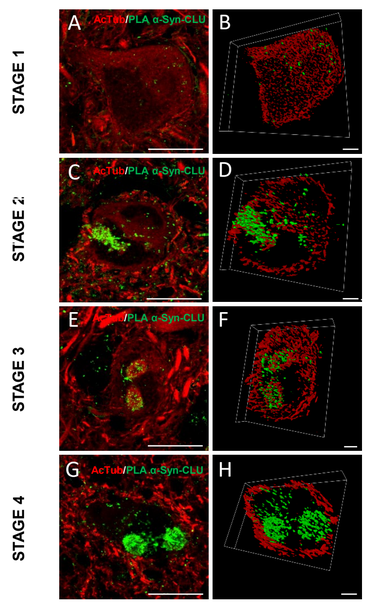
2. *CLU is involved in Lewy body maturation process*



CLU is present in PD pathological inclusions. (A,B) IHC performed on *Substantia nigra* indicate that CLU (brown) staining is present in PD pathological inclusions, as Lewy bodies (arrow) and Lewy neurites (arrowhead). Scale bar, 25 µm. **(C-E)** Double IF assay revealed that CLU (green) is present in the 100% of Lewy bodies (arrow) recognized by α-Syn staining (red) (*n* = 51 Lewy bodies in *n* = 7 PD patients). Scale bar, 25 µm.



CLU distributes differently in Lewy bodies with undefined and ring-shaped structures. (A) In undefined Lewy bodies, recognized by α-Syn staining (red), CLU (green) seems to be widespread distributed inside the inclusion; 3D reconstruction in **(B). (C)** In ring-shaped Lewy bodies recognized by α-Syn staining, CLU appears only in the external ring of the aggregate; 3D reconstruction in **(D). (A,C)** Scale bar, 15 µm. **(B,D)** Scale bar, 5 µm.



CLU strongly associates with α-Syn in the early phases of Lewy body maturation process. The interplay between CLU with α-Syn (detected by PLA α-Syn/CLU, in green) during Lewy body formation changes in different stages, detected by AcTub (in red). **(A)** In *stage 1*, AcTub is strongly accumulated in the soma of neurons and low levels of α-Syn/CLU is scattered in the cytoplasm; 3D reconstruction in **(B)**. **(C)** In *stage 2,* AcTub starts to accumulate in small aggregates concomitantly with a remarkable increase in α-Syn/CLU signal; 3D reconstruction in **(D)**. **(E)** In *stage 3,* AcTub forms an external ring, while α-Syn/CLU signal is homogeneously distributed inside the aggregate; 3D reconstruction in **(F)**. **(G)** In *stage 4*, AcTub staining is weakly present, while α-Syn-signal is present all over the inclusion; 3D reconstruction in **(H)**. **(A,C,E,G)** Scale bar, 25 µm. **(B,D,F,H);** Scale bar, 10 µm.

Conclusion

- **Changes in AcTub** redistribution inside neuronal cell body indicate that alteration of microtubule activity but an early step in neurodegeneration, since it seems to **anticipate the appearance of α-Syn oligomers**.
- **CLU is associated with α-Syn inside Lewy bodies** and, notably in the **early phases of Lewy body formation**, eventually reaching a plateau where it remains stable in the later stages. We hypothesise that, during the first phases of Lewy body formation, **CLU** could convey **α-Syn** from the **cytoplasm** to the **aggregate** due to its **chaperone activity** but remaining engulfed and associated with α-Syn into the dense structure of the Lewy body also during the latest stages.

In conclusion, the study *of post-mortem* human brain gives us crucial insights into ~~on~~ potential players in the neurodegenerative processes that can lead to Lewy body formation.

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