Relationship between alpha-synuclein and ER stress in Parkinson's disease

Author: **S. Hasegawa,** Supervisor: D. Hraboš, Collaborator: G. Gonzalez
Department of Clinical and Molecular Pathology, Palacky University Olomouc

Introduction

Endoplasmic reticulum stress followed by **unfolded protein response** is one of the cellular mechanisms contributing to the progression of **alpha-synuclein** pathology in Parkinson's disease and other Lewy body diseases. The process is activated by **chaperon Grp78** that binds to unfolded or misfolded proteins (e.g. modified alpha synuclein), thus activating three signal pathways (PERK, ATF6 and IRE2alpha) which could lead to apoptosis.

We aimed to compare potential activation of endoplasmic reticulum stress in **Parkinson's disease patients' brain** and human cerebral organoid models of parkinsonism.

Methodology

Our study includes brain tissue **from 7 Parkinson's disease patients and 4 healthy controls (Tab. 1).** We analysed expression in situ of **endoplasmic reticulum stress-associated proteins - Grp78, ATF4, ATF6** - in formalin-fixed paraffin-embedded tissue from **substantia nigra** and **hippocampus** using **tissue microarrays.** We analysed the tissue by **proximity ligation assay** to prove **protein-protein interaction of alpha-synuclein and Grp78. Further, we have used human iPSC-derived cerebral organoid models (Fig. 1) of amyotrophic lateral sclerosis / Parkinson – dementia complex (toxin LBMAA)** and **Parkinson's disease (toxin MPP+)** to analyse **Grp78, ATF4 and ATF6.** Cerebral organoids were provided by G. Gonzalez, Dept. of Neurology University Hospital Olomouc.

Results

We analysed the expression of **endoplasmic reticulum stress markers** in histological sections of human brain tissue. We have seen **marked increase of Grp78** in substantia nigra (not shown) and **hippocampus (Fig. 2)** of Parkinson's disease patients. We have seen a **significant increase in proximity ligation assay positive signals** in patients compared to control subjects **(p=0.0002) (Fig. 3).** Eventually, we analysed markers of endoplasmic reticulum stress in **cerebral organoids,** using **two models of neurodegeneration.** We observed a **marked increase in expression of Grp78 in the organoid tissue in both models and in various concentrations (Fig. 4).** Similarly, we have seen substantial **increase in both cytoplasmic and nuclear expression of ATF6 (Fig. 5).** In situ **expression of ATF4 was stable,** independently on endoplasmic reticulum stress inductor.

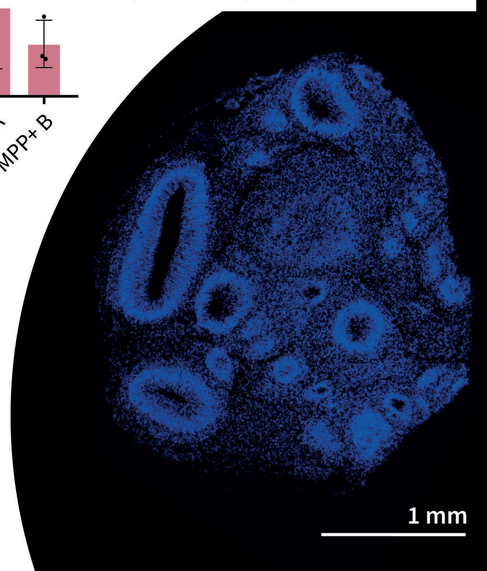
Figure 1. Cerebral organoids characterization

Subject	Age	Sex	Braak stage
CTRL 1	64	M	--
CTRL 2	66	F	--
CTRL 3	71	M	--
CTRL 4	59	M	--
PD1	74	M	5
PD2	67	F	4
PD3	65	M	6
PD4	70	M	5
PD5	83	F	6
PD6	81	F	6
PD7	73	M	6

Table 1. Controls and patients characterization

References

Baek JH, Mamula D, Tingstam B, Pereira M, He Y, Svenningsson P. GRP78 Level Is Altered in the Brain, but Not in Plasma or Cerebrospinal Fluid in Parkinson's Disease Patients. Front Neurosci. 2019;13:697.
Hetz C, Saxena S. ER stress and the unfolded protein response in neurodegeneration. Nat Rev Neurol. 2017;13(8): 477-491.
Villa CE, Cheroni C, Dotter CP, et al. CHD8 haploinsufficiency links autism to transient alterations in excitatory and inhibitory trajectories. Cell Rep. 2022;39(1):110615.



Conclusion

To our knowledge, **this is the first study that shows in situ protein-protein interaction of alpha-synuclein and Grp78 in Parkinson's disease-affected human brain.** Our data prove the long-proposed role of unfolded protein response in the pathogenesis of human synucleinopathies. Moreover, **we have established an important experimental models of Parkinson's disease using iPSC-derived cerebral organoids** and proved their **potential in studying underlying mechanisms of endoplasmic reticulum stress.**

The project was supported by FNOl, 00098892 and IGA-LF-2024-021.

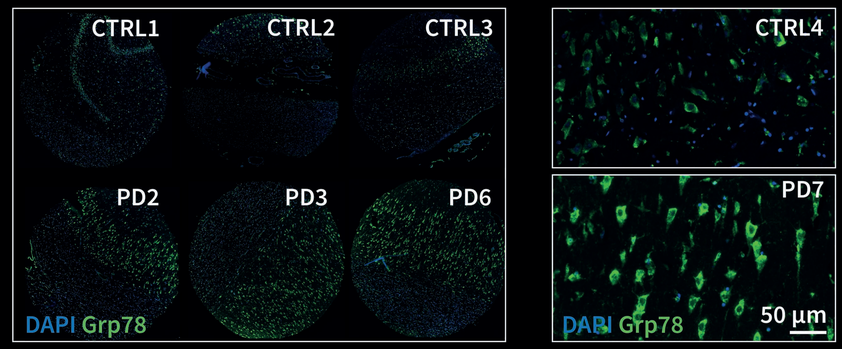


Figure 2. Grp78 expression in hippocampus

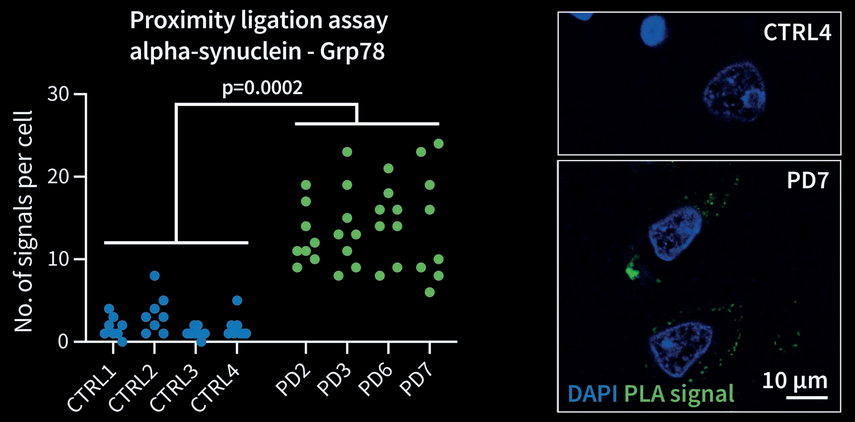


Figure 3. Proximity ligation assay analysis in hippocampus

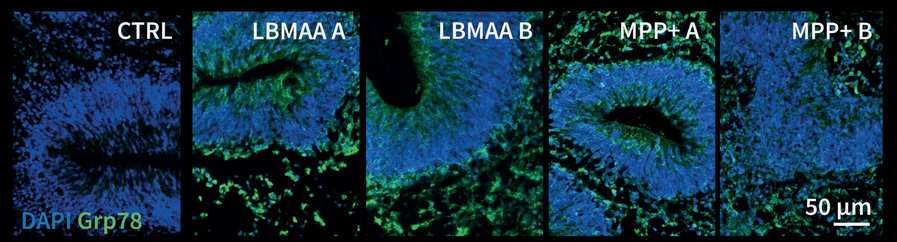


Figure 4. Grp78 expression in cerebral organoids

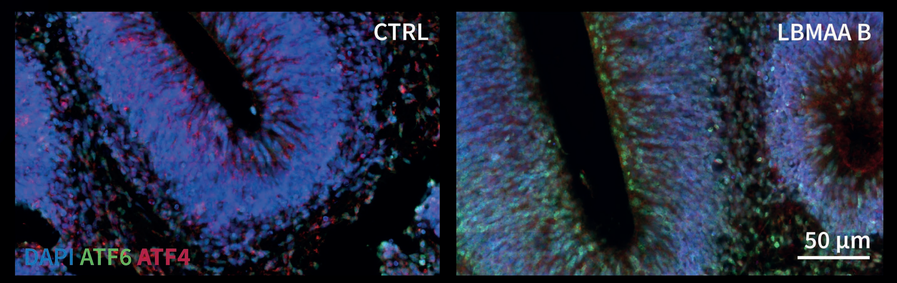


Figure 5. ATF6 and ATF4 expression in cerebral organoids