The role of Chaperone-Mediated Autophagy in the nervous system in health and disease

Xilouri[1], M., Papagiannakis[1,2] , N., and Stefanis[1,2*], L.

[1]Center of Clinical Research, Experimental Surgery and Translational Research, Biomedical Research Foundation of the Academy of Athens, Athens, Greece
[2]Second Department of Neurology, University of Athens Medical School, Athens, Greece

Research question and background

Chaperone-mediated autophagy (CMA) is one of the three mammalian autophagic pathways of lysosomal degradation, that involves the recognition of select cytosolic proteins containing the KFERQ motif by the cytosolic chaperone Hsc70. Hsc70 binds the substrates and translocates them to the lysosomal membrane, where, upon binding to the transmembrane receptor Lamp2a, they are unfolded and threaded into the lysosomal lumen, where they are degraded by lysosomal proteases. CMA activity is directly dependent on Lamp2a levels at the lysosomal membrane and on lysosomal Hsc70 levels in the lysosomal lumen. The role and regulation of CMA in humans, and in particular in the nervous system, is unknown. Aging in other tissues is associated with CMA dysfunction, but whether this occurs in the CNS is unclear. We will thus: a) Examine levels of the key CMA molecules Lamp2a and Hsc70 and CMA function in various cell types derived from Parkinson's disease (PD) patients and controls, as well as in PD brains and age-matched controls, and b) Examine brain tissues derived from normal human controls across different age groups to ascertain whether Lamp2a and Hsc70 levels decrease with ageing.

Methods and tissues used

In order to unravel potential alterations in key CMA markers in human brain occurring with aging or under pathological conditions such as PD, we have obtained human post-mortem tissue encompassing the following brain areas: caudate nucleus, substantia nigra, cingulate gyrus and amygdala, derived from control and PD subjects. From controls, we have obtained material across different age groups (age: 30-50, 50-80, 80+, n=3-5 / brain region) and from PD subjects we have acquired material between 60 to 80+ years of age (n=2-8 / brain region). These tissues are homogenized in a lysis buffer that enables the separation of lysosomes from the cytoplasm. The resultant lysosomal and cytoplasmic fractions are then used for the detection of the proteins of interest by western blot.

Results and conclusion

At this stage, we have started analyzing the expression levels of CMA and other lysosomal-related (such as p62, LC3-II, GBA) proteins in cytoplasmic and lysosomal fractions of nigral tissues derived from three PD patients and three age-matched controls (age 70-80). So far, we haven't detected significant alterations in the levels of the CMA markers, Lamp2a and Hsc70, between control and PD patients. These data are rather preliminary and this analysis is still on going. In the next steps, we will complete the assessment of lysosomal markers in the nigral tissues, and start similar analysis in the control samples across different ages. Overall, this study will lead to enhancement of the understanding of the role of CMA in the nervous system, an area of research in its relative infancy.