



Dying neuron

Synuclein degradation and Parkinson's disease in Athens

Defeating the Shakes

What do Pope John Paul II, Salvador Dali and Muhammad Ali have in common? They all suffered, or suffer, from Parkinson's disease (PD). One protein involved in PD is alpha-synuclein (ASYN). But how does it cause PD-typical neurodegeneration? Some answers from Athens.

In AD 175 the physician Galen described it as "shaking palsy". Much later, in 1817, a detailed study was conducted by English physician James Parkinson, after whom the disease is named. In his study he defined PD as, "Involuntary tremulous motion, with lessened muscular power, in parts not in action and even when supported; with a propensity to bend the trunk forwards, and to pass from a walking to a running pace: the senses and intellects being uninjured." In a significant number of patients, however, cognitive impairment is also observed at advanced stages of the disease.

Greece. The name conjures up deep blue sea, beautiful scenery, good food, happy people, ancient science and philosophy. However, modern Greece is *still* a hotbed of science, as, for example, Leonidas Stefanis proves. He and his colleagues from the Neurobiology section at the Foundation for Biomedical Research of the Academy of Athens (IIBEAA) have just published interesting findings on how aberrant alpha synuclein (ASYN) inhibits chaperone mediated autophagy, thus conferring toxicity to neuronal cells.

ASYN belongs to the synuclein family of proteins which are found in presynaptic terminals. Normal ASYN is involved in maintaining synaptic function and plasticity responses of neurons. On the other hand, it constitutes a major component of the Lewy bodies, cytoplasmic inclusions that characterise PD pathologically.

Indeed the causative agent

More recent data has confirmed that ASYN indeed is the causative agent in PD. In fact, the increased expression and accumulation of wild type ASYN is sufficient to cause PD in humans. No surprise, therefore, that certain missense point mutations in the ASYN gene also lead to ASYN accumulation, causing PD in rare families.

ASYN has the propensity to aggregate and this aggregation property underlies its pathological functions. Therefore, it is of utmost importance to find the reasons for its abnormal accumulation. One possibility

is that the pathways, by which ASYN is degraded, are blocked or inhibited. The degradation pathways, usually acting on such abnormal proteins, include the ubiquitin and autophagic pathways. Dysfunction of either of them might contribute to the pathology of PD as well as various other neurodegenerative conditions.

After completing his MD and PhD at Athens University Medical School, Leonidas moved to Columbia University, New York. Here, his interests in neurodegenerative diseases developed at Prof. Lloyd Greene's lab, where he was involved in research on cellular neurobiology, in particular, mechanisms of neuronal apoptotic death. In parallel, he worked in Robert E. Burke's lab at the Department of Neurology, where he studied behavioural neurology and *in vivo* biology. He fondly remembers, "It was great to do *in vivo* biology, to perform a lesion in one part of the brain and to see the effects of transneuronal degeneration in another region." Within a short space of time he became an assistant professor and started to explore protein degradation in neuronal cells. Twelve years later he moved back from the USA to Athens and established his own lab.

Earlier work from Leonidas' lab shows that ubiquitin and ASYN, two important proteins found in Lewy bodies, co-localised in inclusion bodies in rat PC12 cells following proteasomal inhibition. (*J. Neurochem.* vol. 78(4): 899-908). Several other groups have since shown ASYN accumulation in Lewy body-like inclusions upon proteasome inhibition, and this has given rise to the idea that proteasomal inhibition may be a crucial factor in PD pathogenesis.

Contrastingly, the studies performed by Stefanis's group did not show any increase in ASYN levels following proteasomal inhibition, suggesting that there might be other forms of protein degradation responsi-

ble. The alternative mechanism could be autophagy, a process that degrades cellular components through the lysosomal machinery. Autophagy exists in three types, micro autophagy, macro autophagy and chaperone mediated autophagy (CMA).

Whilst at Lloyd Greene's lab at Columbia University, Leonidas generated stable cell lines expressing wild type and the A53T mutant form of human ASYN, which is responsible for the familial forms of PD. Using these new tools, he observed reduced proteasomal activity in cells expressing the A53T mutant. In addition, electron microscopic experiments showed accumulation of double membrane-bound vesicular structures, suggesting that autophagy was also perturbed (*J. Neurosci.* vol. 21(24): 9549-60).

In a more recent collaborative work, Leonidas' team showed that in *in vitro* purified WT ASYN, but not the mutant forms A53T and A30P, was degraded in isolated liver



From left: Kostas Vekrellis, Maria Xilouri, Tereza Vogiatzi, and group leader Leonidas Stefanis

lysosomes by the CMA process. The mutant ASYN forms appeared to disrupt the process of degradation of other CMA substrates, acting in effect as CMA inhibitors (*Science* vol. 305:1292-5). However, whether these phenomena actually occurred in neuronal cells remains unclear.

Autophagy disrupted?

To see if autophagy is really involved, Maria Xilouri and Tereza Vogiatzi in Leonidas' lab created inducible rat and human neuronal cell lines, expressing WT and

A53T ASYN, and two corresponding mutant forms. These mutants lack the CMA targeting motif; therefore, they cannot be degraded by CMA and, at the same time, cannot influence degradation of other CMA substrates. (*PLoS ONE* vol. 4(5): e5515).

While lysosomal degradation was significantly reduced in A53T ASYN-expressing rat PC12 cells and human SH-SY5Y cells, there was no change in the double mutant that lacked the CMA recognition motif. This is the first demonstration in a cellular context that perturbations of lysosomal function mediated by aberrant ASYN are due to its effects on CMA. Interestingly, aberrant ASYN did not affect survival in these proliferating cells.

And finally there's cell death

In a parallel study, Kostas Vekrelis showed susceptibility of these cells to WT ASYN-mediated toxicity upon neuronal differentiation (*J. Neurochem.* vol. 109(5):1348-62). Interestingly, CMA dysfunction and an increase in the expression levels of the macroautophagy marker LC3 II were observed in these neuronally differentiated cells expressing WT ASYN, indicating accumulation of autophagosomes. In the neuronally differentiated cells, A53T seemed to cause lysosomal damage that was more generalised and not confined to CMA. In this case as well, there was a significant increase in the levels of LC3-II.

The main question that Leonidas' group wanted to answer was whether the lysosomal effects, mediated by CMA dysfunction, could be responsible for the toxicity mediated by aberrant ASYN. They found that neuronal toxicity induced by both WT and A53T ASYN was significantly reduced in the case of the mutants that were not targeted to CMA. Another issue that they tackled was whether the observed compensatory induction of macroautophagy could have detrimental effects. And indeed, in macroautophagy inhibition experiments they showed, for the first time, that aberrant induction of macroautophagy was, in part, responsible for abnormal ASYN-mediated toxicity.

Leonidas and co. next went on to examine the same in primary cortical neurons. In this setting, only A53T ASYN caused CMA impairment and autophagosome accumulation, again dependent on CMA targeting. Over-expression of all forms of ASYN resulted in significant reduction in cell viability of cortical neurons, with the A53T mutant exhibiting the most toxic effect. Interestingly, the double mutant was significantly

less toxic than the single mutant, suggesting that CMA targeting may mediate some of the aberrant effects of A53T ASYN. Improving CMA function may not only serve to facilitate ASYN degradation but also to mitigate deleterious consequences of aberrant ASYN on this system. Like in the neuronal cell lines, the autophagosome accumulation was detrimental in that its molecular or pharmacological inhibition improved survival of A53T ASYN-expressing neurons.

This study very explicitly illustrates the phenomenon of CMA dysfunction induced by aberrant ASYN in various neuronal cell lines and primary neurons. To sum up the findings, aberrant ASYN inhibits CMA function in all types of cells and all conditions, as indicated by reduction in CMA-dependent protein degradation. This leads to compensatory induction of macroautophagy, as shown by autophagosome accumulation, which, in turn, leads to macroautophagy-mediated cell death.

Apart from shedding light on the manner in which aberrant ASYN leads to dysfunction and death in neurons, this study highlights a new avenue for PD therapy, based on manipulation of the CMA pathway. Accordingly, stimulation of CMA could lead to enhanced ASYN degradation; at the same time it could overcome the inhibitory action of aberrant ASYN and, therefore, could prevent neuronal loss in PD patients.

A key to preventing neuronal loss?

Maria remembers, "It was not an easy job to do all the transfections. Especially in primary cells, double transfections with viral vectors was not easy." Tereza agrees. "Nevertheless, Maria and I were a good team." Though they had neatly shown the above phenomenon, it was not easy to publicise. They first submitted it to *PLoS Biology* but received the manuscript back with a depressing note, "Not very novel". But they subsequently had it published in *PLoS ONE*.

And the future? With lots of energy and ideas in the bag, Leonidas' lab is presently concentrating on several projects, including the relation between Gaucher's disease, ASYN and PD; the role of UCH-L1 (an enzyme involved in ubiquitination) in PD; and understanding the complicated relationship between ASYN and lysosomes.

"After all that much research, the protein degradation theme has survived as a mechanism relevant to neurodegenerative diseases; in fact, the field is getting more complex and varied. There is always something new to do", says Leonidas.

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