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Towards clinical translation of biomarkers and therapies targeting autolysosomal acidification dysfunction in neuroinflammation and neurodegeneration – an interview with Prof. David Rubinsztein

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Abstract

The article is an interview with Prof. David Rubinsztein, Department of Medical Genetics, and Cambridge Institute for Medical Research, University of Cambridge, Cambridge, UK, conducted by Dr. Chih Hung Lo, Dean's Postdoctoral Fellow of Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore and an incoming Assistant Professor in the Department of Biology and Interdisciplinary Neuroscience Program at Syracuse University, USA, on behalf of *Aging Pathobiology and Therapeutics*.

David Rubinsztein, PhD

David Rubinsztein is Professor of Molecular Neurogenetics and a UK Dementia Research Institute Group Leader at the University of Cambridge. He is Deputy Director of the Cambridge Institute for Medical Research. Dr. Rubinsztein earned his MB ChB, BSc (Med), and PhD degrees from University of Cape Town. He came to Cambridge in 1993 as a Senior Registrar in genetic pathology. His research is focused in the field of autophagy, particularly in the context of neurodegenerative diseases. His laboratory pioneered the strategy of autophagy upregulation as a possible therapeutic approach in various neurodegenerative diseases, and has identified drugs and novel pathways that may be exploited for this objective. He has made contributions that reveal the relevance of autophagy defects as a disease mechanism and to the basic cell biology of this important catabolic process. Rubinsztein was elected Fellow of the Academy of Medical Sciences (2004), EMBO member (2011), Fellow of the Royal Society (2017) and Member of Academia Europaea (2022). He was awarded the Graham Bull Prize (2007), Thudichum Medal (2017), Roger de Spoelberch prize (2017), the Goudie Medal (2020) and 2024 Movement Disorders Research Award from the American Academy of Neurology. He was identified as a Clarivates Analytics Highly Cited Researcher (2018, 2019, 2020, 2021, 2022 and 2023). (https://www.cimr.cam.ac.uk/staff/professor-david-rubinsztein-fmedsci-frs)

Chih Hung Lo: Prof. Rubinsztein, my name is Chih Hung, let me briefly introduce myself. I am currently a Dean's Postdoctoral Fellow at the Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore. I am also an incoming Assistant Professor in the Department of Biology and Interdisciplinary Neuroscience Program at Syracuse University in the United States. My

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research interests focus on studying inflammatory cytokine receptor activation such as TNF receptor 1 (TNFR1) [1] and intrinsically disordered protein aggregation such as tau [2] that drive autophagy and lysosomal dysfunction in neuroinflammation and neurodegeneration, particularly in Alzheimer's disease and related dementia. I have heard your talk at conferences and since then I have been following your research, which is very important in shaping my research program. Therefore, I am honored to have the opportunity to have a conversation with you. My first question may be a very general question. Among the various biological processes, what attracts you the most to delve into the field of autophagic and lysosomal degradation in neurodegenerative diseases?

David Rubinsztein: I have been concerned that many of the proteins had caused the conditions acted by gain-of-function mutations. So, the first disease that I thought about a lot was Huntington's disease. The disease was generally identified about thirty-one years ago. It was clear that it was a gain-of-function mutation, and the protein was poisonous to the cells. It's been so apparent with subsequent discoveries particularly that you know α-synuclein acts in the same way in Parkinson's disease and that tau acts in the same way in dementia. So, we have thought that because they are toxic to the cells, one way of treating such diseases is to lower the levels of the proteins. When I started working on Huntington's disease almost thirty years ago, I wondered whether anti-sense technology would be an appropriate strategy. But we didn't have an expertise and didn't really follow that. But it has of course become a very attractive strategy for some of these conditions in recent years. I always kept the idea that lowering the protein was an attractive strategy. So, in late 1990s to early 2000s, when I became aware of what autophagy was, we started doing some experiments and we first did experiments actually in yeast models. We made yeast models of Huntington's disease and tested yeast genes, but we never published them. Soon after we tested compounds in mammalian cell models that we have made of Huntington's disease and showed that autophagy was likely to be important in that context [3, 4]. So, I said to my student at the time that we are interested in making humans better, so let's focus on humans. For her thesis, she had a lot of yeast data that end up being in the supplementary section or in the discussion. That's the story of how we started, and you know then it became apparent to us that it was more important not only for Huntington's disease, but it was also important for clearing out α-synuclein and tau and many other proteins causing neurodegeneration. That's how we started and that's what's kept us in the field for such a long time.

Chih Hung Lo: Thank you very much. I will continue to follow your research. As my research focuses on understanding autolysosomal acidification dysfunction and related therapeutic targeting, my next question is more relevant to these fields. Alterations in autolysosomal acidification have been detected in different cell types in the brain [5, 6], can you comment on how these cell-type specific pH changes may play different roles in neuroinflammation and neurodegeneration.

David Rubinsztein: I think this is an important question and it's very likely to have serious impact in diseases. In the Alzheimer's context, for instance, Ralph Nixon has provided very nice data for over many years actually, supporting an idea that lysosomal acidification effects might play a primary role in the disease [7]. He has shown that in the situation of APP triplication that you get in Down's syndrome and mutations in the processing enzymes, there is elevated lysosomal pH which is likely an important contributor to the build-up of Aβ [8]. I think your question about cell-type specific changes in lysosomal pH is an interesting question. I don't know much about it, but I suspect that it will end up being an important variable to consider in these diseases. I think what one means by cell-type specific changes is that whether there are changes that occur more in neurons versus microglia or astroglia. I don't think that's one level of the problem and I don't think that much is understood about that. But of course, within different cell populations, there might be differing vulnerabilities and buffering of lysosomal pH which I think is an additional question. I think it's a right area to consider in the future.

Chih Hung Lo: Thank you. I read Ralph Nixon and your recent paper in Nature Reviews Molecular Cell Biology [9], and I have learned a lot from the paper. The following question is still on the similar topic. I'm always wondering is there any possibility to detect this so-called the notion of early autolysosomal acidification because based on what I know so far it's very hard to monitor lysosomal pH in vivo. Is that possible for us to do that in the human brain? It is really a broad question that I hope to get some comments.

David Rubinsztein: It is clearly a very important problem and it's going to be a big challenge. I think at the moment, I don't know how one would do that. But it is possible that with very careful experiments. I have not thought them through, but you know with very careful experiments for instance, when doing mouse experiments where you perturbed lysosomal pH in the mouse brain experimentally and maybe looked for changes either in proteomes or other biomarkers in the CSF, you might be able to get some type of correlations. I think this may probably be your best chance. Clearly doing something where you specifically measure lysosomal pH in the brains of living people, I'm not sure if that is going to be possible. You might be able to develop some type of very clever probe, like PET imaging. It would also have a very fine dynamic range to be used, and you could think of that, but it might be easier to get the CSF. Actually, I used to be very keen on PET probes for various things, especially in potentially measuring autophagic substrate accumulation. My colleagues who are clinicians say that the problem with PET probes is that PET imaging and PET probes are very expensive. In terms of rolling that out with a lot of people that might be hard, but we have got to try to develop whatever methods we can.

Chih Hung Lo: Sure. I think that's very important comment

to alter autolysosomal acidification in the mouse brain and check for proteome changes in the CSF or perhaps in the blood as potential biomarkers [6].

David Rubinsztein: Well, I think particularly you know if one's going to be thinking about therapeutic strategies, if you've got markers of alteration in the pathway that you try to modify, then you can assay that. For instance, if you develop a drug that targets lysosomal pH and it works very nice in vitro or in culture, the question is, will that work in the mouse experiment as you try to join the dots. Ideally when you do the clinical trial in patients, if you can show that the drugs are actually doing what you think it is doing, and then you can relate that to the clinical phenotype. Then you can at least say you have done the experiments to test whether altering lysosomal pH affects the clinical phenotypes in people. That will be much harder to interpret if you don't have those types of biomarkers.

Chih Hung Lo: Actually these have answered my next question which I am going to just briefly talk about. As there are small molecules and lysosome-acidifying nanoparticles under preclinical development stage [6, 10], what are the future directions that can clinically translate them? I believe you have already kind of answered this. Do you have any more comments regarding this point and as this is my last question.

David Rubinsztein: You know this is an important area and I'm glad that you were thinking about it, and others are working on that. We have worked on boosting autophagy biogenesis, but I really think that it's important to explore the other side of the pathways, whether boosting lysosomal activity or decreasing pH is going to be effective methods, especially in diseases where there might be a pH problem or an activity problem. I think that these biomarker issues are important. Another consideration is of course at least about looking at altering pH, one got to be a little bit careful because one might be talking about a therapeutic window there. Because if you decrease pH too much, there are also other issues related to lysosomal defect which I might think that there is an additional consideration. I'm glad people are working in that space, and we have got to consider as many rational therapies as we can. That is clearly a space where there are opportunities to make a difference, and so it's an area that I definitely encourage.

Chih Hung Lo: Sure. Thank you very much. These are all the questions.

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