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Autophagy in neurodegeneration: recent advances, autophagosome formation, and therapeutic innovations—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 (CIMR), University of Cambridge, Cambridge, UK, conducted by Aracely Garcia-Garcia of Histology Department, Faculty of Medicine, Universidad Autonoma de Nuevo Leon (UANL), Monterrey 64460, Mexico, on behalf of *Aging Pathobiology and Therapeutics*.

David Rubinsztein, PhD

David Rubinsztein is a 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 the University of Cape Town. He came to Cambridge in 1993 as a Senior Registrar in genetic pathology. His research is focused on 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 essential 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), the Thudichum Medal (2017), the Roger de Spoelberch Prize (2017), the Goudie Medal (2020), and the 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).

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Aracely Garcia-Garcia: Professor Rubinsztein, it is an honor to interview you. First, I would like to introduce myself. My name is Aracely Garcia Garcia. I am a professor at the School of Medicine at Universidad Autonoma de Nuevo Leon in Mexico. My research focuses on the effect of antioxidant molecules and autophagy inducers on Parkinson´s disease and aging models. I have prepared some questions, and I would be very grateful if you could share your thoughts and experiences with us. As your research is focused on the field of autophagy and in the context of neurodegenerative diseases, could you explain why autophagy is essential for brain function and what evidence in humans underscores its relevance?

David Rubinsztein: I think the important papers showing that basal autophagy is very important for brain function come from Noboru Mizushima and Masaki Komatsu [1, 2], who made the first neuron-specific conditional knockout autophagy genes in mice. When they did that, they got increased protein aggregation and cell death, and eventually,

of course, mice died. They got the same results from mice not having two different genes, which was also quite nice. Over the years, it has become clear that neural autophagy is very important in a number of contexts. It's important for neuronutrient recycling, for degradation of misfolded aggregated-prone proteins, degradation of dysfunctional organelles like mitochondria, where I suspect you know much more than I do, and other features like regulation of senescence and maybe even regulation of neural transmission. I think people are still learning more about the roles of autophagy in neuronal function. I think in terms of what evidence supports its relevance in humans, there are mutations described in key genes involved in autophagy. Interestingly, in humans, the predominant phenotype is a neurodevelopmental phenotype, while the mice knockouts clearly get neurodegeneration. I'm not sure that humans don't also get neurodegeneration, it might be difficult to assess that on the background of severe neurodevelopmental phenotypes. There are human diseases which speak to that, and I think they hint that they get neurodegeneration as well. I think that the combination of the human natural mutations, the mice knockouts, and what has been done in culture systems really argues that this is a very important process modulating risk and disease. The one other thing I would say is that when we think about autophagy and neurodegeneration, it might be helpful to divide our thoughts, if possible, into two different categories. Many of the neurodegenerative diseases we are interested in, like Huntington's disease, are situations where the disease protein is not only an autophagy substrate but it also negatively regulates autophagy. From the studies in mice and sometimes in cultured cells, we can see if you have an autophagy defect in those types of diseases, you are increasing the levels of the poisonous protein, which is doing other bad things as well, in addition to affecting functions that autophagy regulates in a normal situation. Do you see what I'm getting at? So, it's a two-arms attack on the brain, which is different to what happens if we just knock out autophagy in a normal brain. I have started to think that maybe we need to consider these as slightly different questions, but I hope that gives a flavor of what my current views of the situation are.

Aracely Garcia-Garcia: So, autophagy tends to decline with age, which may contribute to neurodegeneration. What do we know about mutations in autophagy-related genes and their role in human neurodegeneration?

David Rubinsztein: Mutations in ATG7 have been described in a number of cases. There's also an ATG5 mutation that's been described, and there have been two mutations described. They all seem to get, as I said, this neurodevelopmental phenotype. I think that we shouldn't exclude the possibility of neurodegenerative phenotypes, and there are hints that they might occur. It would be interesting to follow those types of individuals over time. Ideally, it would be nice to be able to get their brains eventually to do a pathological examination to see if there are features of neurodegeneration. What's interesting about the ATG7 mutations is that it looks like there are very low levels of ATG7. There might be hypomorphs to some extent, meaning they might have a little bit of residual function. It's not entirely clear to me, but some of the individuals really live a very long time, I think, you know, into their 70s. It is quite intriguing when you compare that to the mice situation. I think it's important to realize that with some of these autophagy genes, at least the cell culture data that has been reported by Mizushima's lab [2], and I think the experience of others, you really need to lower the levels of these autophagy genes to very, very, very low levels in order to have a clear effect on autophagy. So, you could have a situation where if you have got two percent function remaining, that is still enough to keep things going a little bit and cause maybe a slightly different phenotype to a complete null. The one other thing I would say to back up one of my earlier answers is that our focus on autophagy and neurodegeneration has been very neuron-centric. I think that for moving forward, we need to match more work on the roles of autophagy in non-neuronal cells. We started doing a little bit, and others have done very nice work, as Junying Yuan has done very nice work on autophagy in microglia poisonous in the Alzheimer's context [3]. I think that we need to now start understanding what autophagy does in non-neuronal cells as well as how they might impact neurodegenerative diseases.

Aracely Garcia-Garcia: Autophagosome formation is often described as originating from double-membrane structures called phagophores. Recently, your research group has highlighted differences between mammalian and yeast autophagosome formation. Could you elaborate on these findings and their significance?

David Rubinsztein: Until very recently, we thought, like everybody else, that autophagosomes came from cup-shaped precursors. So, you got this cup-shaped structure with a single opening, where the opening was then closed, and you have a mature autophagosome. I think the work over the years has shown that the autophagosomes are essentially an outgrowth of the recycling endosome system, specifically involving the RAB11A recycling endosome. These are not just a subset of autophagosomes; they represent all the autophagosomes, as far as we can tell. Our experience with these cultures suggests that autophagosome formation has this very strong dependency on RAB11A [4]. The autophagosomes are not cup-shaped structures. We were always wondering, and when we initially described the LC3 conjugation on the RAB11A compartment, what things would look like. They are not cup-shaped structures; they are finger-like outgrowths of the RAB11A compartment, and then these fingers actually occupy a large volume. The fingers then grasp autophagic substrates and then close down, and the gaps between the fingers are sealed by the ESCRT complex. That is a prerequisite or consequence of the completed autophagosome containing its content from the RAB11A compartment. So, if you stop the closure, you don't have its dissociation, so you get the structure still stuck on the RAB11A compartment [5]. That was a real surprise for us, and it was enabled by looking with super-resolution structured illumination microscopy at cells, where we made various perturbations in

the autophagy pathway. Then, we could go back and look at normal cells and know what we were seeing. I think the reason why people probably missed it before is that these finger-like structures actually occupy quite a large area, and it's very difficult to identify those fingers by EM. Using markers and immunogold makes the structural studies rather difficult, and so there are those challenges. I think in this particular problem, we got lucky with the super-resolution microscopy, having the right type of resolution and also capturing the sort of the larger volumes that are occupied by the finger-like structures compared to the small autophagosomes after the closure. Of course, there will be other challenges.

Aracely Garcia-Garcia: Thank you. I also noticed that in your work, you have some involvement in clinical trials. It's very interesting that several drugs currently used for other conditions, such as felodipine and rilmenidine, which are used to treat hypertension, are now known as autophagy inducers. Could you discuss the progress of autophagy inducers in clinical applications?

David Rubinsztein: It's a good question. Ideally, we'd like to find drugs that we can use in patients with these awful diseases and, ideally, find drugs that delay the onset of the disease. If you got Huntington's disease, most individuals will have a family history, so if you can stop the onset or delay it by many years, then you have effectively cured the disease. It's much better to stop the onset of the disease and slow the progression once you're already sick; it's probably easier biologically to do as well. If you're going to do that, you need to have drugs that are going to be safe and very well tolerated. We have done quite a lot of repurposing screening, and felodipine is one of the most advanced of the most recent batch. What we learned with felodipine is that when you do these repurposing studies in mice, you've got to really pay a lot of attention to pharmacokinetics. You've got to try to do your mice experiments so that you mimic the plasma concentrations and, ideally the brain concentrations of the drug that a human would be seeing if they were taking the drug for hypertension. So that you would see in a human with the use, taking the drug for another purpose. We did that very carefully with felodipine, and we devised the experiments so that we could test that hypothesis. At least the experiments within the constraints we have, suggested that felodipine at concentrations that are human-like can induce autophagy in the mice brain. At the moment, we are conducting a safety study, which I should say is greatly facilitated by having my clinical collaborator, Prof. Roger Barker at Cambridge University, who is really directing the clinical trial [6]. We are looking at it in early Huntington's patients with different doses, and we are looking at safety. So far, it seems to be safe, as one would expect, because this has been used in many hypertensive patients. We are also going to be looking at some preliminary biomarkers to see if we get any hints that it might be active and useful in Huntington's patients, you know, in follow-up studies, so that is where we are. Another compound that I'm excited about comes from a study published last year, where we showed that the anti-HIV drug maraviroc could ameliorate toxicity in Huntington's mice models. It does so by preventing the microglial chemokines, CCL3/4/5, from activating the neuronal chemokine receptor CCR5, which would impair autophagy [7]. Essentially, maraviroc is a CCR5 blocker, so we're trying to pursue that work; it is like the earliest stage. We're doing some further mice studies to try to make a stronger case before we try to motivate human studies with that compound.

Aracely Garcia-Garcia: Okay, thank you very much. I am sure that you have heard about many supplements that claim to induce autophagy. What are your thoughts on these products?

David Rubinsztein: If one thinks about those products as a generic question, the key question is not whether they induce autophagy at a certain concentration in tissue culture cells or even in mice. The question is, do those products induce autophagy at those concentrations that you would see in a human taking them at the conventional dosage? I don't know what the answer to that is. I think if they do, that's great, but the lesson we've learned, and we've become much more sensitized to over the last two years, is that pharmacokinetics are very important. I think that if one's making a claim about a certain compound, one really needs to do those studies. Now, the question is, to go back to Dr. Lo's question, how are you ever going to show that in a human brain? Then you have got to do studies where you do the pharmacokinetics in humans; then you work out what the concentrations are, and the half-lives, *etc*., in the human plasma, because you could do it in the CSF if you got the right patients and if you can persuade people to have lumber punches. But you do those experiments, and then you try to do those experiments in a model organism like mice, where you mimic the concentrations, and then you see, well, when you mimic the concentrations, does it work like that in the brain? For instance, if you got CSF data of a compound, you can see whether the CSF concentration of your supplement induced autophagy in neurons in culture. That's a start, but I'm not sure that there's that much literature where people really try to join the dots in that way. But I encourage it because it would help you understand how those supplements might be working or not.

Aracely Garcia-Garcia: Thank you very much. Lastly, I would like to hear how you go about selecting PhD students and postdocs for your research team and what qualities you value most in your team members.

David Rubinsztein: That's a hard question. I have been lucky, and I'm lucky at the moment. Over the years, I have had very good people in my lab, and at the moment, I have got very good people in my lab. I enjoy working with people that often amaze me. They are very talented. It is clearly an important process to try to select people. I don't have the easy answers. I mean, I think you know, ideally we want people in our lab who are passionate about science, who are eager to learn, who work well in the team, who have the capacity to develop well, and the question

is, how do we select them? I wish I had the magic answer. I think what is quite helpful is when I interview people, firstly, I try to spend time talking to them about science and as well as informally, just to get a feel of how they take as people. For science, almost always, I will ask them to do a brief presentation about some work that they have done, and the purpose of that is really to see how much they have been involved in thinking about their project. Sometimes, you know, if it's a PhD student, they've done very little science, they have done a rotation project somewhere, but you can get a feel from that, and how curious they have been, how involved they have been about thinking about what the project means, and what the results mean. One other factor is that people, as far as I am concerned my lab would say the same, actually, that people applying for jobs in our labs, they need to read some of the papers that we have done. They need to at least show an interest in our domain of work, that's also important. But it's quite difficult. Sometimes, somebody comes along, and it's clear that they are a star. They are extremely bright. They ask very perceptive and probing questions. They have an excellent understanding of the field or surprisingly good understanding of the field before they've come. My experiences being that, you know, sometimes somebody comes from a different background, and they are just fantastic. As an example, in the last few years, I have had a number of people come from yeast genetics backgrounds that haven't even been yeast cell biologists sometimes. They have been really yeast geneticist. But they all had the right attributes, and they have ended up being real stars in the lab. I think if you can identify people who've got the desire to learn to develop as scientists, and if you can spot that, it's great. Because for me, one of the great pleasures is to see how people develop and mature as scientists in the lab, and so if you have got the right people for that, it makes your life as PI fantastic.

Aracely Garcia-Garcia: Thank you very much Professor Rubinsztein. Your work is deeply inspiring, and it holds great promise for the development of therapies to combat neurodegenerative diseases.

David Rubinsztein: Thank you for your interesting questions.

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