Marie: Hello and welcome to *The Parkinson's Research Podcast: New Discoveries in Neuroscience*. I'm your host, Dr. Marie McNeely, and I've partnered with The Michael J. Fox Foundation for Parkinson's Research to bring you to the forefront of the field of neuroscience to discuss the latest advances and discoveries with leading experts.

The Michael J. Fox Foundation created this podcast for researchers, clinicians, and industry professionals with the hope that these conversations and the resources we share will advance your efforts and partnerships to improve brain health. We're welcoming guests with a range of experiences and viewpoints. The views expressed belong to the guests themselves.

Today, we are thrilled to be welcoming our guest, Dr. Zhenyu Yue. Listeners, Zhenyu is the Aidekman Research Professor in the Department of Neurology and Friedman Brain Institute at the Icahn School of Medicine at Mount Sinai. He is also the Director of the Center of Parkinson's Disease Neurobiology and Director of Basic Research of Movement Disorders at Mount Sinai. Today, we will be talking more about Zhenyu and his research related to the autophagy-lysosomal pathway and neurodegeneration. So, Zhenyu, welcome to the show today. How are you?

Zhenyu: I'm good. Thanks for having me.

Marie: Well, we are excited to have you with us, and I'm looking forward to learning more about you and your work. Perhaps we can start by talking about your background. So, Zhenyu, can you tell us a little bit more about yourself and the path that you took to get to your current position?

Zhenyu: Sure. I am the Professor of Neurology in the Department at the Icans School of Medicine at Mount Sinai. I did my undergraduate education in cell biology and vertebrate genetics in China. And then I came to the United States to pursue my PhD study. So, my PhD study is related to molecular biology and biochemistry, and I was working under the mentorship of Aaron Shatkin, a member of the National Academy of Science at Rutgers. And I studied virus biology and mRNA modification, human capping enzymes. And then I went to Rockefeller University as a Howard Hughes Medical Institute Fellow. And I became interested in neuroscience, and then I studied mouse genetics, and then also became interested in autophagy.

So, after my post-doc training, I got a job at Mount Sinai Department of Neurology and became an assistant professor. I was interested in creating an animal model to model human neurodegenerative disease such as Alzheimer's or Parkinson's disease. I think back then our colleagues in the Department of

Neurology were very much focused on movement disorders including Parkinson's disease.

So, I was influenced by my colleagues, and then I decided to generate a Parkinson's disease animal model. Back then there was a gene called the L-R-R-K 2, "LRRK2". The mutation of LRRK2 was shown associated with the most common genetic form of Parkinson's disease. That's why I became interested in that and then I used the BAC transgenic approach to create the LRRK2 transgenic model for modeling Parkinson's disease.

Marie: Well. very cool to hear a little bit more about your background, Zhenyu. And I think, for our listeners out there who might be in various areas of Parkinson's research, they might not be familiar with autophagy. Can you start by maybe setting the stage and talking a little bit more about what autophagy is, the role that it plays, both in neurons and glia, and how it is regulated.

Zhenyu: So, I started my autophagy research when I was a post-doc at Rockefeller University. I was inspired by the work of Dr. Ohsumi, who won the Nobel Prize in 2016 for his discovery of autophagy. Back then, really, autophagy — it's a mystery, really nobody knows about it.

I found a gene called a Beclin 1 that shows an interaction with another protein that is synaptic-related, and we suspected that it could link to the neurodegeneration. So, I became interested in autophagy research. So, the fundamental role of autophagy is the clearance and the degradation through the lysosome pathway. And autophagy is also known as a recycling pathway that wraps up the garbage inside the cells, delivers to the lysosome, which is an organelle to degrade cellular contents. So, this is a very fascinating process, especially in the cell and the neurons. And then it was unknown back then, and my interest was to really uncover, the function of autophagy in neurons and the link to neuron degeneration.

Marie: Very interesting. So, let's talk about what you have found. What have you discovered so far about the role that it is playing in neurons, as well as in glia?

Zhenyu: So, my lab and then another lab have shown that autophagy is constantly active inside the neurons. It can be induced in a much a higher level when there is a stress, but it's constitutively active to turn over protein aggregates and large protein complexes, as well as injured cellular organelles, such as mitochondria, Golgis, and so on. So, it's a very important homeostatic control process.

It's also very selective. It can recognize the protein cargo or "substrate" — we use the word. For the autophagy degradation — it's mediated through autophagy

receptors. It is a definitely neuroprotective function because without autophagy, the neurons become dysfunctional because of the garbage accumulation.

And then without autophagy, the neuron will eventually die because of too much stress, garbage accumulation, poisoning the neuronal function, and eventually degenerate. It has also a very important role for the glial cells, such as astrocyte and microglia. Although the glia — they share a very similar mechanism, in terms of autophagy machinery and the process, but the glial cell has a very different cargo or autophagy substrate. Without the autophagy the glial cells cease to proliferate and then they become senescent.

Senescence is a state that the cells cannot proliferate and then they become an aged-like stage. And it also regulates the inflammatory response. So, it's just a very important homeostatic control mechanism for both neurons and glial cells.

Marie: Well, that makes sense, and I can imagine how a backup of things in neurons and glia that need to be cleared could cause some problems. So, let's talk about the regulation of this and in what cases do these backups occur?

Zhenyu: So, the regulation of autophagy in neurons and glial cells are extremely complicated. These neurons or glial cells are receiving signal from extracellular space. They will signal to autophagy. There is a whole bunch of genes or proteins that regulate autophagy activity.

One of the best examples is the mTOR that negatively regulates autophagy function. Of course, many of the cargoes, or protein, organelles are degraded through autophagy, through the autophagy receptors, which is very important. The protein molecules that can recognize a specific cargo and then take them to the autophagy machinery, and then eventually degrade it in the lysosome. And that is how the selectivity of autophagy comes from. And then the other very important part of autophagy in neurons in terms of the regulation is they're compartmentalized. So, in other words, a neuron has different compartments.

For example, the cell body, and then there's a long process known as the axon, and then the other end of the process is called the dendrite. The regulation of autophagy in these different compartments are very different. That really shows the complexity of how autophagy is regulated differentially in the different compartments.

One example is the autophagy at the tip of the axon. We call that the pre-synapse. And then we know there's a very important autophagy activity at the synapse. And then autophagy processes cross talk within the synapse trafficking. And then we believe autophagy is very important to control the synaptic function through the degradation of specific sets of autophagy cargoes or synaptic vesicle

proteins. Therefore, controlling the synaptic activity and synaptic transmission of the neurons.

Marie: Very interesting. And you actually have done some research identifying some novel regulators of autophagy. So, can you go into some of the details of the findings that your lab has had in this area?

Zhenyu: Absolutely. We identified autophagy receptors, such as a p62. We also recently identified another very important autophagy receptor, AKAP11. So, let me just start with the p62. The p62 is the best to characterize autophagy receptors. It specifically recognizes our protein aggregates through their ubiquitin or modified form. And then the p62 will show that the p62 can co-localize with many protein aggregates. The aggregates could be the disease-related proteins, such as huntingtin in Huntington's disease and alpha-synuclein in Parkinson's disease.

We have published work recently. We showed that the p62 can interact with the alpha-synuclein. And then that provides a way for selective degradation of alpha-synuclein inside the microglia cells. So, we found that when the alpha-synuclein is secreted by a neuron, it can enter into microglia cells and is specifically recognized by p62.

And the p62 then takes alpha-synuclein to autophagy-lysosome for degradation. In such a way, the microglia assist the neuron to clear the alpha-synuclein inside our brain. And that provides a very important mechanism to maintain the alpha-synuclein at a low level. Because we know that when alpha-synuclein is overexpressed, when there is too much alpha-synuclein, it causes Parkinson's disease. So, it's a very important function for the microglia autophagy to clear alpha-synuclein inside of the brain to maintain the homeostasis to prevent the neuron degeneration.

Marie: That makes sense. And that sort of answers my next question, which is to talk about how disrupting this delicate balance of the autophagy-lysosomal pathway could lead to Parkinson's or other neurodegenerative diseases. Do you have thoughts on what could be causing this initial disruption that could then, potentially downstream, lead up to that failure to clear alpha-synuclein?

Zhenyu: Let me start with the neurons. So, a neuron is a very special cell type. It's post-mitotic and then it's polarized. And it is extremely sensitive to loss of protein degradation pathways such as ubiquitin, proteasome, autophagy, lysosome. And there is an evidence that shows that there are mutations in those proteolysis pathway that would put the stress on the neuron. And the neuron will degenerate. Now in contrast, other cell types are much more resilient to loss of a certain function of autophagy or lysosome.

So, neurons are extremely sensitive. That also partially explains why autophagy function or dysfunction could cause the neuron degeneration that is linked to the major neurodegenerative diseases. Another interesting aspect is the aging process. We know that autophagy-lysosome function declines in the aging process. And then in the aged brain, the autophagy is much less efficient than the younger brain. And then that also explains why there was garbage, there was protein aggregates, there was damage to the organelles that cannot be immediately turned over or taken care of. Therefore, our aged brain with a very much defective autophagy-lysosome are vulnerable.

And I already explained that without autophagy, the garbage accumulates. The protein aggregates also pile up our neuron as well as the glial cells. And then especially many disease-associated proteins such as alpha-synuclein in Parkinson's, and tau in tauopathy, in Alzheimer's disease and also even in APP, the Aβ, there's also certain aspects of the intracellular — those APP — that really, they are are prone to aggregate.

And then autophagy is an important mechanism to remove those protein aggregates and protect our neurons from the damaging injury, and prevent neurodegeneration. So, that's how autophagy functions. And then in terms of providing the mechanism for preventing the protein aggregates and preventing the neurodegeneration.

Marie: Definitely. And I think Zhenyu, this is a really interesting area of research because this represents what might be a common mechanism across different neurodegenerative diseases that could be targeted. So, let's maybe talk about your recent *Nature Communications* paper where you took a step back and systematically investigated autophagy targets of neurons using integrated proteomics as your approach. So, can you talk a little bit about what led you to this study?

Zhenyu: So, that particular study you mentioned that we recently published in *Nature Communications*, it's a way to provide a landscape of the autophagy degradation in neurons. Because of all the important evidence linking autophagy and lysosome defects to neurodegeneration disease, we thought about an unbiased approach to systematically characterize what the autophagy degrades inside the neurons. So, we utilized both human stem cell-derived neurons as well as animal models that we disrupt the autophagy through genetic deletion of essential autophagy genes, and then followed by quantitative proteomics with the help of our collaborators. So through that, we uncovered a lot of interesting autophagy cargoes or substrates of autophagy that have never been shown before.

So, they also allowed us to have insight into what autophagy degrades and then what in a cellular organelle, the proteins specifically, are an autophagy substrate.

So, we found that ER, which is a very important set of organelles — that seems to be a very important cargo for autophagy.

By ranking all the protein or cargoes, we can see that the ER protein is really on top of the charts. We also identified a lot of interesting proteins related to synaptic protein trafficking, and then we also validated these proteins that accumulate in both the human neurons, as well as the animal model neurons. Therefore providing an explanation why autophagy controls the synaptic functions. So, because of some specific set of synaptic proteins that are normally degraded by autophagy, even under normal conditions; therefore, autophagy can control the synaptic transmission. And this just also allows us to identify an important autophagy receptor that mediates the degradation of a specific protein kinase, I mentioned about the AKAP11, that is an adapted protein for a very important kinase called PKA that regulates the synaptic function and has been implicated in psychiatric diseases such as bipolar, schizophrenia.

So by this really unbiased proteomics approach, our study provided a landscape of autophagy degradation in neurons and a very important resource for many researchers who are interested in autophagy and lysosome in neurodegenerative diseases as well as psychiatric diseases.

Marie: Definitely, and I know one of your areas of expertise is in model development for these kinds of studies, and I'm curious to maybe get a little bit of insight into your model selection. You mentioned that you've been looking at human stem cell-derived neurons and also different animal models. Can you go into a little bit more detail about these models you selected and why those were the most appropriate choice for these kinds of studies?

Zhenyu: That really relates to our question about modeling the disease using all kinds of organisms. So, traditional organisms or species we use are rodents, flies, and C. elegans. And these have provided tremendous knowledge for us to understand how the disease progressed and to test the idea of therapeutics. Recently, the IPS neuron, or the stem cell-derived neuron, has become so important for us to understand the disease because we realized there was a huge difference between the human cells and also the animals in terms of their disease mechanism study, and as well as the progression, and understanding the disease mechanism.

So, the human stem cells we used in this case for deriving into the dopaminergic neuron, which is the most vulnerable cell type in Parkinson's disease. And then this has provided a very important tool for us to really answer the question about the human disease and how the genetic mutation and how the defective autophagy can contribute to Parkinson's disease.

Marie: Very interesting. And going back to this *Nature Communications* paper, the results specifically, can you talk about what are the implications or the potential impacts of your findings identifying these autophagy receptors and the cargo as potential targets, and what this means for the field?

Zhenyu: To answer that, there was a really interesting paper published in *New England Journal of Medicine*. They actually found the autophagy gene ATG7 — they found six families, independent families. They all have this mutation in ATG7, and then it caused neurodevelopmental disorders. So, our study used the same gene mutation in ATG7 in both the human stem cell-derived neurons, as well as the animal model, show that the many defects in the neurons, including the clearance of proteins in ER, Golgi, mitochondria, as well as synaptic vesicle trafficking.

So, our study really provided insight into the disease mechanism for how these patients could have in their neurons, in their brains. So, our results really help explain exactly what went wrong in their neurons without the autophagy gene ATG7. Of course, there also has an implication in development of biomarkers, as well as the treatment of autophagy defects in neuron-related diseases, such as the lysosome storage, as well as Parkinson's disease, which has a lot of flavor in lysosome defects. So, our study provided the idea where we should look at or how we identify the autophagy defects, and how we develop the therapeutics targeting autophagy-lysosome to restore the lysosome-autophagy defects in those affected neurons.

Marie: Absolutely. And I think it would be really ideal to be able to identify or detect these autophagy deficits early on in the course of someone's disease. So let's talk about biomarkers. That was a perfect transition. I know The Michael J. Fox Foundation is providing support for a project that you're working on now that's actually focused on identifying some sensitive, quantitative biomarkers to both monitor, as well as predict, autophagy-lysosome activity. So, Zhenyu, can you walk us through the aims of this study?

Zhenyu: Well, yeah, we are grateful to The Michael J. Fox Foundation to support our research. So, that work is really related to the study that I just mentioned, the unbiased approach to identify autophagy cargo, the substrate in neurons. So, by leveraging our findings, we propose to develop autophagy-lysosome biomarkers in Parkinson's disease.

The rationale being that there has been really important evidence showing through the human genetics and pathological study, it's showing that there's a function of autophagy-lysosome in the development of the Parkinson's disease. Therefore, our goal is to identify the autophagy and lysosome biomarkers or molecules in the patients. So, we propose to identify and then study a panel of

autophagy cargo proteins. So, the rationale is that these autophagy defects will cause the accumulation of autophagy cargoes in the Parkinson's disease patients' samples, including their brain and also the biofluid. So, the goal is to see whether or not there is any change of these autophagy biomarkers in the Parkinson's disease patients, the post-mortem tissues as well as the biofluid.

So, we are right now, thanks to The Michael J. Fox Foundation support, we are going through the number of the biomarkers from the autophagy-lysosome, trying to identify their changes in the spinal fluid and also through the IPS neurons derived from Parkinson's disease patients. And by culturing these neurons, we try to analyze the secretion of some of the autophagy-lysosome markers. So, the notion is that when the autophagy-lysosome is impaired, the secretion of the certain autophagy cargoes will increase, therefore allowing us to understand the change of the autophagy and also thinking about the defect in our Parkinson's disease samples. This provides important tools for us for identifying the biomarker in Parkinson's disease through our autophagy marker study.

Marie: Very interesting. And can you give us a status update, Zhenyu, on how the project is going so far?

Zhenyu: We just started this project about a few months ago. And so far, we are trying to identify using the stem cell-derived neuron in culture medium and are working with our collaborator by unbiased proteomics approach to identify any change of autophagy markers inside and outside of the neurons. So, this is just the beginning of the work. Hopefully I will be able to tell more progress and our finding, especially using the biofluid of human samples in our Parkinson's disease patients.

Marie: Definitely. And Zhenyu, if you have had to think about kind of the barriers. What are some of the biggest challenges involved in developing this set of autophagy biomarkers?

Zhenyu: Well, I would say the biggest challenge is the biofluid collection from the biobank. I think to get high quality with the samples with a great clinical association or study, you know, they are biofluid samples. It's just really difficult to get them.

I know there's also a huge effort in the field to set up the banks to collect the samples from humans, from our patients. But it just so far is not good enough. We need more. We need the high-quality samples to help our research. It's thanks to The Michael J. Fox Foundation as well as NIH — the NINDS — and they are putting lots of effort. And then I think that this is a really wonderful resource, but we need more.

We need all sorts, from the clinician and our patients, very importantly, to work together to get the samples to allow us to really take a good look of the change of the different molecules in these biofluids and the post-mortem tissues to identify the reliable, reproducible biomarkers for the purpose of the diagnosis and also the discovery of the drugs.

Marie: Absolutely. And I'd love to think about the future. When we talked about this project, you mentioned it's just recently begun. What are the potential impacts of this research? Or perhaps what are the next steps along that pathway?

Zhenyu: So, our study expects to provide a panel of autophagy-lysosome markers for better predicting the autophagy-lysosome impairments in the Parkinson's disease patients. And also offer opportunity for determining an autophagy-lysosome pathway biomarker capable of predicting Parkinson's disease status, enabling to stratify patient population and improve clinical trial designing — how that's done.

Marie: I like this application for clinical trials. I think that's really important and something that the field definitely needs. Do you see this potentially moving into the clinic as well? If you're able to identify these biomarkers, is it something where someone would just sort of get this annual screening as part of their everyday health care?

Zhenyu: Absolutely. There was always a hope that our finding will lead to the biomarker for autophagy-lysosome pathway. That's my hope to get a decent number of high quality biofluid from PDBD banks, allowing us to really identify the autophagy biomarkers. And then it's our hope to really provide this opportunity to develop the diagnostic for predicting even the process, especially the prodromal or the early stage of Parkinson's disease progression, allowing the early intervention. So, the whole idea of the biomarker is really to show a certain population in a human that even before they have the motor symptoms, the panel of our autophagy marker that would think that predicts the lysosome-autophagy defects in human patients before the onset of the motor symptoms. So this allows us to really provide an early diagnosis before even the disease onset.

Marie: Definitely. I think the potential impacts are very exciting. And I'm looking forward to seeing the results from this study as you continue to make progress on it. But perhaps we can talk about things that are moving the field forward. You know, in terms of tools, resources, collaborations, you mentioned some things that could help you specifically. What do you see as resources that are really helping to accelerate Parkinson's research?

Zhenyu: So, all the work that we have done — so, we have created a large set of proteomics data and mass spec data. These are tremendous resources. And we also created genetic animal models, stem cell-derived animal cell models for modeling Parkinson's disease, as well as the autophagy defects. And also we

integrated lots of the resources from the human to the mouse model. And these are the resources that provide important tools as well as the idea or understanding of the research. We also, through the work, we established the collaboration with multiple laboratories and also multiple institutes. This is a wonderful team.

And we are also fortunate to establish our Parkinson's Disease Center at Mount Sinai just two years ago. This is also part of our research to integrate the laboratories that work on very different aspects of the research through the computation analysis, human stem cells, animal models, pathology, human post-mortem tissues, and so on. So, this is a really wonderful team. And I appreciate the opportunity that we have this Center to have a synergy to have a large collaboration to really tackle a very important and difficult disease such as Parkinson's disease.

Marie: Definitely. And I think you're specifically tackling some important areas of Parkinson's disease research, but there are still many unanswered questions. So Zhenyu, when you look towards the future, what do you see as maybe the most important or the most promising future directions or areas of opportunity in the field?

Zhenyu: So, there are many unanswered questions. And I think the most exciting part is to really translate our research into clinic application. But this is also the biggest challenge. How do we do this transformative work?

So far, I have mentioned about The Michael J. Fox Foundation supported the research in terms of autophagy biomarkers using our platform and also a study in biofluid using Parkinson's disease patient samples. And I think this is the most exciting part. And we hope to see those really translational studies coming out from our lab, establishing the diagnostic tools as well as therapeutic ideas into the cure for Parkinson's disease. I also want to mention that we have developed nanobodies, a specific antibody targeting LRRK2 genes, which is one of the most important genetic connections to Parkinson's disease. So, the nanobody currently we're developing has a very high specificity and then it allows us also to provide additional resources for targeting this very important kinase, which is implicated in Parkinson's disease.

And then more importantly, we mentioned about autophagy defects in Parkinson's disease as well as other neurodegenerative diseases. We're also in the process of developing autophagy compounds or drugs in terms of restoring autophagy-lysosome function and providing a therapeutic development for Parkinson's as well as other neurodegenerative diseases.

Marie: Well, Zhenyu, it sounds like you have a lot of fascinating projects in the works that are quite promising. And we appreciate you joining us on the show today. And I'd love to end our conversation by just talking about how the work that you're doing is bringing us closer to the big goals of finding a cure for Parkinson's or contributing to improved therapies for people who have Parkinson's now.

Zhenyu: Yeah, I want to acknowledge our students, postdocs, and our trainees for their hard work in the laboratory towards understanding the disease. And we are excited about projects and future opportunities to translate our basic mechanism finding into clinical applications, in particular, the development of autophagy-lysosome biomarkers and autophagy-related drugs. I believe our work provides an opportunity to identify the biomarkers in autophagy-lysosome pathways, better predict potential disease subtypes and status, and importantly help stratify the patient population and improve the clinical trial designing. Additionally, we are developing autophagy drugs that target specific stage of autophagy impairment we believe implicated in affected neurons in Parkinson's disease. And we hope to develop better diagnostic tools and treatments targeting autophagy in the future.

Marie: Well, thank you so much for sharing your insights and your research with us on the show today. It's been such a pleasure to chat with you.

Zhenyu: Well, again, thanks for having me, and thanks to the listeners.

Marie: It's been wonderful to chat with you. And listeners, it's been great to have you here with us as well. If you want to know how The Michael J. Fox Foundation can help your research, please visit MichaelJFox.org/researchresources. And you can find new episodes of this show each month on the MJFF website or on your favorite podcast platform. When you have a moment, please subscribe to our show to make sure you don't miss our outstanding lineup of upcoming episodes. We look forward to connecting with you again in our next episode of *The Parkinson's Research Podcast*.