Understanding the Role of Autophagy in Infectious Disease

Just like people declutter their homes to prevent the accumulation of broken or unwanted items, cells use autophagy to maintain homeostasis. This essential cleaning process enables them to capture and degrade unnecessary or dysfunctional macromolecules, such as damaged organelles, protein aggregates, and microbial pathogens. As a result, autophagy is a fundamental defense mechanism employed by cells to control and clear viral infections.

Welcome to *The Scientist* Speaks, a podcast produced by *The Scientist'*s Creative Services Team. Our podcast is by scientists and for scientists. Once a month, we bring you the stories behind news-worthy molecular biology research.

In this episode, Charlene Lancaster from *The Scientist* spoke with Josephine Thinwa, an assistant professor in the departments of internal medicine and microbiology at the University of Texas Southwestern Medical Center, to learn more about the importance of autophagy in mitigating viral infections and how understanding this process could help physicians treat a rare neurodevelopmental disorder.

<u>Igniting Her Passion for Infectious Diseases</u>

Narrator:

When Josephine Thinwa sat down with her first-year college advisor to talk about her future, she felt conflicted. On one hand, she wanted to continue fostering her love of the scientific method, which was ignited through the many science fair projects she completed in high school. But on the other hand, she loved the idea of treating patients as a physician. Torn between the two options, Thinwa's advisor told her about a former student who earned an MD and a PhD. It was this moment that inspired her to pursue both. Now as a physician scientist specializing in infectious diseases, she remains excited by the idea that patients with difficult-to-treat infections in the hospital can inspire her to ask and answer new questions in the laboratory about how these microbes interact with the body. However, the subject of infectious diseases sparked her interest long before pursuing higher education.

Josephine Thinwa:

I was born in Kenya, East Africa, and immigrated to the United States with my family when I was 11 years old, and I remember a latent culture shock. About a year after being in this country, I woke up one Saturday morning and thought to myself, I have not had diarrhea for the last year.

When I was a kid in Kenya with a lot of family in the rural areas, you are touching everything outside, not washing your hands. I was constantly sick and seeing that change just by moving across the ocean made me want to solve this problem of these infections. It is funny to think that I became an infectious disease doctor as well as somebody who studies infections in a lab, but it really did stem from that moment of thinking that it is not fair that when I was in Kenya, I was sick all the time, and here in the United States, I am not as sick.

Narrator:

Motivated to investigate the pathogens that infected her as a child, Thinwa's PhD project focused on how intestinal epithelial cells initiate inflammation following recognition of invasive bacterial species, such as *Yersinia enterocolitica* and *Salmonella*. Although she anticipated that bacteria would remain the central theme of her medical and research career, that changed once she became a clinician and made a startling realization—physicians do not know how to treat most viral infections. When people feel ill, it is natural for them to seek the help of a doctor for treatment. But physicians are often unable to prescribe any medication to their patients when they are suffering from a viral infection. Compared to antibiotics, scientists have developed far fewer antiviral drugs, which forces people to wait out their illness with medications that only diminish their symptoms rather than targeting the root cause.

Josephine Thinwa:

That was jarring for me to see. And so, I wanted to shift focus to viruses, and decided to join the lab of Beth Levine, who was my postdoctoral mentor. I lovingly call her the mother of autophagy. She has now passed away a few years ago.

Dr. Levine was and continues to be, even posthumously, a force to be reckoned with. She was an incredible physician scientist and actually trained in infectious diseases. And I think what she embodied that was so powerful is how you can take a simple observation and really see its potential. Knowing that a small finding, if you are willing to work at it, be persistent with it, and believe in it, essentially, you can grow it into something that really matters. By the time she entered the autophagy field and some of the key fundamental findings had been described in yeast. But she discovered the first mammalian autophagy gene, beclin.

The Importance of Autophagy for Fighting Viral Pathogens

Narrator:

Levine uncovered beclin and many other essential autophagy genes by examining interactions between the host and viral pathogens. Mammalian cells rely on autophagy to recycle basic building blocks, such as amino acids, lipids, or monosaccharides, from cellular components or foreign particles. Researchers have classified this process into two major categories: bulk and selective autophagy. Cells employ bulk autophagy to non-selectively capture cytosolic contents. Alternatively, selective autophagy uses receptors to identify specific cargo, such as intracellular viral capsids, and target them for degradation. Consequently, selective autophagy is critical for antiviral immunity, and this process could serve as a potential target for new drugs once scientists understand it better. To identify novel regulators of autophagy during viral infection, Xiaonan Dong, a postdoctoral fellow on Levine's team, had just completed an extensive genome-wide siRNA knockdown screen prior to Thinwa joining the group.

Josephine Thinwa:

The way the screen was done, cells were infected with Sindbis virus, which is an RNA virus, and herpes simplex virus, which is a DNA virus. The goal of using distinct types of viruses was to try to find common regulators that are necessary for activating autophagy in response to these viral infections that could be generalizable to maybe many different types of viruses.

That generated over 200 potential regulators of autophagy. When I joined the lab, I was actually going to work on something a little bit more GI, GI microbiome and how that may influence viral infections and autophagy. But Dr Levine in her sort of abounding wisdom said why do not you as a secondary project look at this list that Xiaonan has just completed and see if you can find a gene that you want to characterize and see if it really pans out to be a regulator of autophagy. So, it took me six weeks to comb through that list and decide which one to pursue, and in the end, landed on CDKL5.

The Significance of Focusing on CDKL5

Narrator:

CDKL5 or cyclin-dependent kinase-like 5 is a protein that is ubiquitously expressed in most cells and tissues within the human body and is essential for neuronal function and survival. Approximately 20 years ago, scientists determined that loss-of-function mutations in the gene encoding CDKL5 resulted in a devastating neurodevelopmental condition called CDKL5 deficiency disorder. Children with this rare disease suffer from intractable seizures that even modern epileptic drugs cannot address. The idea that a critical kinase could play a role in autophagy during viral infections thrilled Levine.

Josephine Thinwa:

She always had a very great nose for when something matters. And I remember having a conversation with her when I decided to pursue my project on CDKL5 and its regulation of autophagy during viral infection. I remember that she was excited that it mattered. This CDKL5 matters to humans. It matters to human health, so we should pursue it. I love that about her, that she cared about the science, but also the humans who this science could impact.

This idea that by understanding its role in autophagy through the lens of viruses could potentially bring us a little bit closer to understanding how it functions in neurodevelopment was part of why I wanted to study CDKL5 in the context of autophagy.

CDKL5's Role in Autophagy During Viral Infections

Narrator:

Through her investigation, Thinwa and her team determined that CDKL5 is an important regulator of autophagy during viral infection. They showed that mice lacking CDKL5 were more susceptible to

infection with Sindbis virus, herpes simplex virus, and chikungunya virus than wildtype mice. These boosted infection rates resulted in higher mortality rates and increased neuronal cell death in the brain, which suggests that CDKL5-mediated autophagy is vital to neuronal and host survival.

Josephine Thinwa:

Autophagy is particularly important for neurons because they do not replicate like other cells. When you have a long-lived cell that is essential for the survival of the host, having a process that essentially keeps the cell healthy, keeps the cell clean, eliminates, for example, damaged mitochondria that produce an excess of reactive oxygen species is important. These are also highly metabolic cells, right? Because they are constantly firing and communicating with our cells, so they have lots of turnover of organelles. And so, those organelles when they do become damaged have to be degraded and eliminated. When you have a viral infection that impacts a neuron, autophagy becomes essential for the survival of that neuron. The brain is an immune-privileged site. We know that high levels of inflammation will kill the patient. So, these cell-intrinsic mechanisms of viral resistance, like autophagy, are really important, because you cannot have a flooding of inflammation and inflammatory cells come to this immune privilege site, which in itself would actually harm the host.

Narrator:

When Thinwa and her team knocked out CDKL5 in HeLa cells, they observed a robust accumulation of viral capsids, where these aggregates were not contained within autophagosomes. They wondered if the loss of CDKL5 prevented the autophagy machinery from recruiting the viral components. In a paper from 2010, Levine and her team established that the core autophagy receptor p62 was important for capturing Sindbis virus capsids. Since then, numerous publications have shown that p62 interacts with capsid proteins from several different types of viruses. Based on these papers, Thinwa decided to examine if CDKL5 interacts with p62. Her team demonstrated that CDKL5 phosphorylates p62 and this posttranslational modification allows p62 to better capture viral capsid proteins and target them to the autophagosome for degradation.

Josephine Thinwa:

I was surprised that it was a direct substrate. We did not know that it would necessarily be phosphorylated by CDKL5. We identified the phosphorylation site in an area of p62 that seems to be important for how p62 is shuttled around. We found the phosphorylation site is close to where the nuclear localization signals are, but also close to the LC3-interacting domain. And LC3 is a protein that p62 anchors onto to attach to the developing autophagosome. We are still trying to fully understand the mechanism of how this phosphorylation alters the activity of p62 to allow it to better recruit capsid.

Looking Beyond Viral Infections

Narrator:

Viral infections stress host cells in several ways including the proteotoxic stress generated by the accumulation of viral antigen aggregates, the oxidative stress caused by the increased production of reactive oxygen species, and the transcriptional and translational shutoffs induced by viruses to minimize host antiviral responses. But autophagy interfaces many of the pathways activated by infection or induced by other stressors. As a result, CDKL5 could be important for mechanisms beyond viral infection.

Josephine Thinwa:

We are actively pursuing this question of is it possible that CDKL5 is important for helping our cells deal with different types of stress. So, not just stress caused by viral infection, but other types of stress that occur in the cell and autophagy would also be involved. So, let us say proteotoxic stress. So, not just viral antigens accumulating, but we know that neurons can accumulate proteotoxic proteins. That is the basis of many neurodegenerative disorders where we see this accumulation of aberrant proteins, like tau and amyloid. Could it be that CDKL5 actually aids the cells in eliminating those types of aggregate proteins, as well? So, we are actively pursuing some of these questions and we think that CDKL5 plays an even greater role than what we appreciate with just studying infections.

It is taking me out of my comfort zone a little bit though, because I am a virologist, immunologist, cell biologist. Now I am becoming more of a neuroscientist. My goodness, how many hats am I going to wear? But you know in this day and age of research, science is now a community pursuit. It is not just me becoming a jack of all trades. It is also really forming a community of people who are interested in some of these questions and we can get to answer them in a very robust way.

The Importance of Studying Autophagy in the Context of Viral Infections

Narrator:

Over the years, scientists have developed drugs effective against specific viruses, such as HIV or hepatitis C. While these treatments have helped millions of people, every time a new viral pathogen causes an outbreak, physicians do not have any tools in their toolbelt to help their patients fight this virus. Thinwa is hopeful that this work will emphasize the importance of understanding the intrinsic mechanisms that cells employ against viruses and these findings lay the groundwork to start producing broad-spectrum antivirals.

Josephine Thinwa:

Viruses are masters of subverting and even manipulating the autophagic process for their own advantage.

Viruses sometimes have multiple virulence factors that are dedicated towards disrupting autophagy. In some ways, it is reassuring because it basically tells us that autophagy is important as an antiviral mechanism. But it is also frustrating because it also means that we have to be creative if we really have to think of a way to mobilize autophagy towards fighting viruses. If we understand how autophagy is being regulated and activated, and how the virus is essentially subverting the mechanism of autophagy, we can find ways to maybe activate autophagy more downstream of where a lot of the viral virulence factors are targeting.

And so, this work is important to figure out how each cell can resist a viral infection and how we can mobilize the mechanisms that are already intrinsically there.

The beauty of studying viruses is that they become part of the cell and they understand cell biology better than we will ever understand. So, by trying to uncover what they are doing to the cell, you end up learning all of this really beautiful biology of how our cells work. That is the wonderful unintended consequence of doing this research.

Outro

Thank you for listening to *The Scientist* Speaks. This episode was produced by the Creative Services Team for *The Scientist* and narrated by Charlene Lancaster. Please join us again in October, as we discover the potential of volatile organic compounds to serve as early Parkinson's disease biomarkers. To keep up to date with this podcast, follow *The Scientist* on social media and subscribe to *The Scientist* Speaks wherever you get your podcasts.