Health workers dealt with an Ebola outbreak in West Africa in 2014, but can studies of lysosomal disorders provide a potential means of prevention?

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BIOMEDICINE

A rare opportunity

Problems with the lysosome cause more than just lysosomal storage disorders. This crucial cellular component has a surprising role in several common and complex conditions.

BY KELLY RAE CHI

Niemann–Pick type C is a rare lysosomal storage disorder (LSD) that affects 1 in about 150,000 people. Around 95% of cases are caused by a defect in the *NPC1* gene, causing cholesterol to accumulate in the lysosome in cells throughout the body, with devastating consequences. Someone affected by Niemann–Pick is unlikely to survive beyond the age of 20. At first glance, the disease seems to have little connection with Ebola, a virus that is transmitted through bodily fluids to cause severe haemorrhagic fever, and which killed more than 11,000 people in West Africa between 2014 and 2016. But it turns out that Niemann–Pick type C might offer a lead for blocking transmission of the virus: human cells that lack the *NPC1* gene are resistant to Ebola.

It was a huge surprise to discover[1] that Ebola's receptor is not on the surface of the cell but inside it — and moreover is inside the lysosome, says cell biologist Fran Platt, who studies Niemann–Pick disease at the University of Oxford, UK. As recently as a decade ago, the lysosome was viewed as an unremarkable organelle, considered to be little more than a packet of digestive enzymes within each cell. But all that has changed.

"The lysosome has been repositioned now," says neuroscientist Steven Walkley of the Albert Einstein College of Medicine in New York, who studies LSDs and contributed[2] to the Ebola work. The lysosome, he stresses, is "not to be viewed as a simple cul-de-sac and relatively unimportant, but as a major player in the life of the cell". In the past few years, researchers have realized that the lysosome not only degrades unwanted molecules and old organelles, but is also important for cell metabolism and nutrient sensing.

These functions mean that lysosomes may have a role in many more human diseases than just those designated as LSDs, says Platt. Indeed, Niemann–Pick type C is known informally as childhood Alzheimer's because the two conditions share symptoms and pathology. In children affected by this LSD, neurons accumulate long tangles of tau protein that are identical to those found in the brains of adults with Alzheimer's disease.

In the coming years, rare lysosomal diseases are likely to help researchers understand other diseases too, says Platt. There are more than 50 known LSDs, each with its own effects on the cell, such as disrupting calcium balance, increasing oxidative stress and inflammation,

and altering lipid trafficking. The cell biology may be daunting, but many LSDs are caused by mutations in single genes, so they could provide a simple way to untangle lysosome pathology in more common, complex disorders.

THE LYSOSOME REIMAGINED

The lysosome's makeover was heavily influenced by two lines of research. One began in 2008, in the laboratory of David Sabatini at the Whitehead Institute for Biomedical Research in Cambridge, Massachusetts. His team was experimenting with the mammalian target of rapamycin (mTOR), a kinase that is implicated in a long list of human diseases. Along with other proteins, mTOR forms a complex called mTORC1. When mTORC1 senses amino acids — the building blocks of proteins — it sends signals to boost protein production. The researchers discovered[3] that in cells lacking amino acids, mTORC1 complexes are present in the cytoplasm. But when they added amino acids to the cells, mTORC1 relocated to the surface of the lysosome.

The fact that mTORC1's protein partners are sensitive to amino acids suggested that the lysosome was part of a nutrient-detecting system, an idea that was unpalatable to many researchers. "All of a sudden, this compartment that most thought about as a recycling bin, or trash bin, was clearly also talking to the rest of the cell," Sabatini recalls.

Around the same time that the Whitehead team was linking the lysosome with nutrient sensing, molecular geneticist Andrea Ballabio and his colleagues at the Telethon Institute of Genetics and Medicine in Naples, Italy, were disproving the idea of the lysosome as a dull, unchanging organelle. Their vision was of a dynamic lysosome that responded to environmental changes, and this needed a network of genes to govern its behaviour. So Ballabio's team probed gene databases for co-expressed genes and found[4] hundreds of lysosome genes that they dubbed the CLEAR (coordinated lysosomal expression and regulation) network. They discovered that the CLEAR genes were regulated by a master protein called transcription factor EB (TFEB). "I was astonished," says Ballabio. Until that point, he adds, lysosome genes were thought of only as "housekeeping genes that were not finely regulated".

These two pieces of the lysosome puzzle came together in 2012, revealed by two independent studies. One, a collaboration between the groups led by Ballabio and Sabatini, showed[5] that TFEB and mTORC1 interact on the surface of the lysosome. Under normal conditions, mTORC1 tags TFEB on the surface of the lysosome to keep it inactive. When a cell is starved, mTORC1 stops tagging TFEB, which moves into the cell's nucleus to make new lysosome proteins.

The second study[6] was led by cell biologist Shawn Ferguson at Yale University in New Haven, Connecticut, who found TFEB on the surface of the lysosome. "That first observation at the microscope was one of the most exciting," Ferguson recalls. He realized that, by partnering with a transcription factor, the lysosome could help to control gene expression.

Linking mTORC1 with TFEB helped to convince sceptics that the lysosome really is part of an active signalling process, rather than a finishing line, Sabatini says. "That was important for the field. Now you have a situation where the lysosome regulates mTORC1, and mTORC1 regulates the lysosome," he says. "It's really a loop rather than a linear pathway."

These studies help to challenge scientists' labelling of lysosomal diseases as simply problems with storage, because the accumulated materials are also crucial to the cell as a source of building blocks for new molecules. As a result, says Walkley, it might be appropriate "to think of lysosomal disorders as 'deficiency' disorders", although future studies will need to bear out this idea.

"Even infection by bacteria and parasites can be cleared by inducing this pathway."

PATHWAYS TO THERAPY

Understanding the role of the lysosome has huge implications for treating disease. For example, researchers are investigating whether boosting the movement of molecules through the lysosome and related organelles will help to clear unwanted material from the cell. This is promising for Niemann–Pick type C, in which the movement of molecules around the cell is impaired. Several research groups are exploring the use of drugs that induce autophagy — which takes molecules and organelles to the lysosome to be degraded — as a possible treatment for brain disorders, for example.

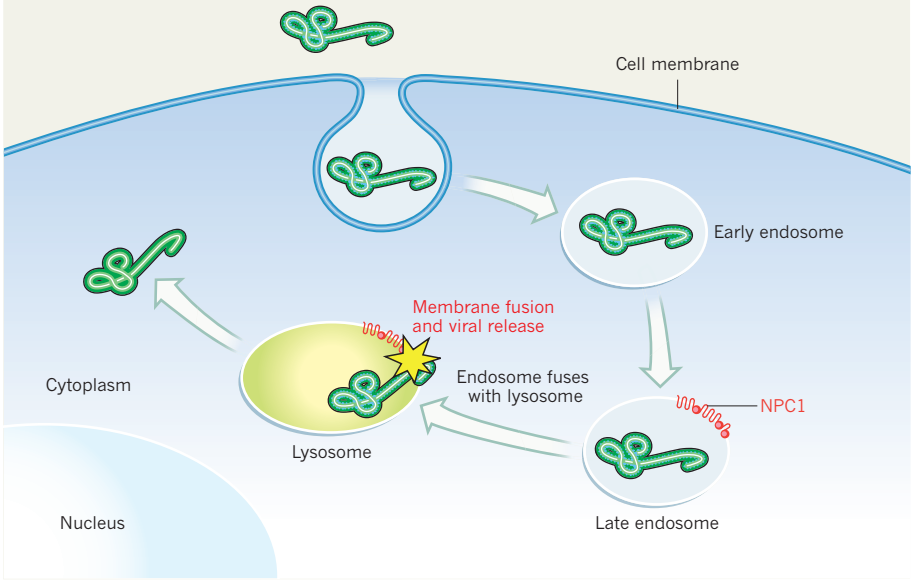
One way to boost the clearance of accumulated molecules in the cell is to increase the amount of TFEB or its downstream targets. Higher TFEB levels generate more lysosomes and stimulate autophagy, which pushes the cell to degrade and eliminate more compounds. And TFEB seems to spur an unconventional but intriguing process called lysosomal exocytosis, in which the lysosome expels its contents outside the cell as a means of removing excess material.

Because TFEB is a transcription factor, it coordinates the activity of hundreds of genes over a sustained period of time. This is much more powerful than many therapies that target a single enzyme involved in post-translational modification, for example, which might work for only a limited period of time, Ballabio says. But this broad and sustained activity could also present safety concerns, because it might feed nascent tumour cells. So Ballabio's group is learning how to activate the pathway safely, and hopes to develop a reversible activator for TFEB. The researchers are also parsing TFEB's downstream targets and their functions, and hope that these studies will generate fresh targets.

Work in animal and cell models by Ballabio and other groups is showing that manipulating TFEB could, in theory, treat a range of conditions, including Parkinson's disease, cancer and obesity. "Even infection by bacteria and parasites can be cleared by inducing this pathway," Ballabio says.

DEADLY DELIVERY

The Ebola virus enters the cell in an endosome and uses the protein NPC1 as its ticket into the cytoplasm, where it can the access the cell machinery and start replicating. However, NPC1 is usually missing in the genetic disorder Niemann-Pick type C, so these cells are resistant to Ebola infection.



COURTESY OF THE BALLABIO LAB

Andrea Ballabio (right) and his team hope to use a pathway in the lysosome to treat a range of conditions.

Now that the lysosome is known to have a crucial role in many diseases, researchers are wondering whether therapeutic strategies for LSDs can inform the treatment of more common disorders. Many LSDs are caused by deficiencies in specific lysosomal enzymes. However, current strategies to replenish those enzymes are of little use for treating neurological diseases, as none of these treatments can cross the blood–brain barrier (see page S154).

Nevertheless, understanding the role of the lysosome in these more common diseases will help to inform treatment. Lysosomal defects may not be the primary cause of the diseases, but treating such defects should help to improve the quality of life for patients, Platt says. "That's the first step we need to achieve."

BRAIN DISEASES

Rare diseases such as Niemann–Pick represent important ways to understand common diseases, especially neurodegenerative disorders, says Platt. The brain is particularly susceptible to defects in the lysosome and other organelles because neurons cannot divide, so small imbalances in the cell's recycling capability will build up in neurons over time. Even healthy older brains get clogged, whereas body cells can dilute any excess storage material by dividing.

Genetic studies have uncovered many such unexpected connections between rare childhood diseases and common adult diseases. For example, Gaucher's disease is caused by a mutation in the gene that encodes the lysosomal enzyme glucocerebrosidase, which is associated with lipid synthesis. The same mutation is now known to be a common risk factor for Parkinson's disease (see page S160).

About ten years ago, scientists found[7] that people with only one working copy of the gene that encodes progranulin — a protein involved in inflammation and wound healing — are likely to develop frontotemporal dementia, a late-onset neurodegenerative disease. Then, about five years ago, researchers described[8] a rare case in which siblings who lack both functional copies of the progranulin gene develop an LSD in early life called neuronal ceroid lipofuscinosis, or Batten disease. It turns out that progranulin is found on the lysosome and is regulated by the master regulator TFEB.

Such stories support the idea that defects involving lysosomal storage may lie on a spectrum, with more severe disruptions taking hold early in life, in the case of LSDs, and milder ones occurring later, says Ferguson. The cases of progranulin and glucocerebrosidase suggest that the severity of disease depends on how much particular lysosome-related genes are expressed in an individual (the gene dosage). "Five years ago, there was barely a whisper on this," Ferguson says, but today these links have brought researchers who study rare and common diseases to the same meetings.

"We've learned a lot from the Niemann–Pick field, and that's helping us understand how to develop therapeutics for Ebola."

Evidence is mounting that defective lysosomes could also be the culprits in common brain diseases. In 2010, neuroscientist Ralph Nixon of New York University's Langone Medical Center found[9] that a gene that is commonly mutated in early-onset Alzheimer's disease is required by lysosomes to function normally. The gene, which encodes a protein called presenilin, allows a large molecular complex called vATPase to assemble on the membrane of a lysosome and acidify its contents, which is essential for breaking down unwanted materials. To Nixon, these results support the idea that presenilin mutations accelerate Alzheimer's disease by causing lysosomal storage problems in the brain.

Although the evidence strongly pointed to a disruption of the lysosome system in Alzheimer's disease, the idea that vATPase had a role "was so out of the box", says Nixon, that many Alzheimer's researchers — particularly those who believed that amyloid is the primary culprit — were sceptical. "It did relate to amyloid, but it implied that there were direct links between the genes and the lysosome that didn't require amyloid to be the initiator of disease." Some researchers challenged Nixon's conclusion, but a study[10] by his group last year affirmed his findings, he says.

Researchers have since found six more brain diseases that involve both vATPase and the lysosome. The idea that presenilin could be a major contributor to the pathogenesis of Alzheimer's disease, by failing to acidify the lysosome, is "no longer a huge stretch", Nixon says. "It's more or less the tip of the iceberg." He predicts that more examples of lysosome dysfunction in common diseases will be found.

The connection between Ebola and Niemann–Pick type C has continued to deepen since the initial studies. Crystal structures published earlier this year demonstrate the relationship beyond any doubt: NPC1 interacts directly with the outer coating of the virus (see 'Deadly delivery'). This work has indicated a potential therapeutic target for Ebola. "We've learned a lot from the Niemann–Pick field, and that's helping us understand how to develop therapeutics for Ebola," says microbiologist Kartik Chandran of the Albert Einstein College of Medicine, whose group collaborated on the first screens that found *NPC1*.

In a bid to prevent Ebola infection, Chandran's group is looking for drugs that can inhibit NPC1. This would be like inducing Niemann–Pick disease in a person for a short time, he says, but it could be particularly effective for health-care workers who have acute exposure to the virus. "This is a new strategy that hasn't been tried before," he adds. And because NPC1 is also the route of infection for other viruses from the same family, it could be useful for treating other filovirus infections, for example by the Marburg virus.

Families affected by Niemann–Pick type C have been instrumental in driving research into treatments to prevent Ebola infection, particularly by providing samples. This new research on Ebola may one day also help these families. In general, research that links rare and common diseases looks like a win–win process. "We must give an answer to these patients affected by rare diseases," Ballabio says. "We can't abandon them." ■

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