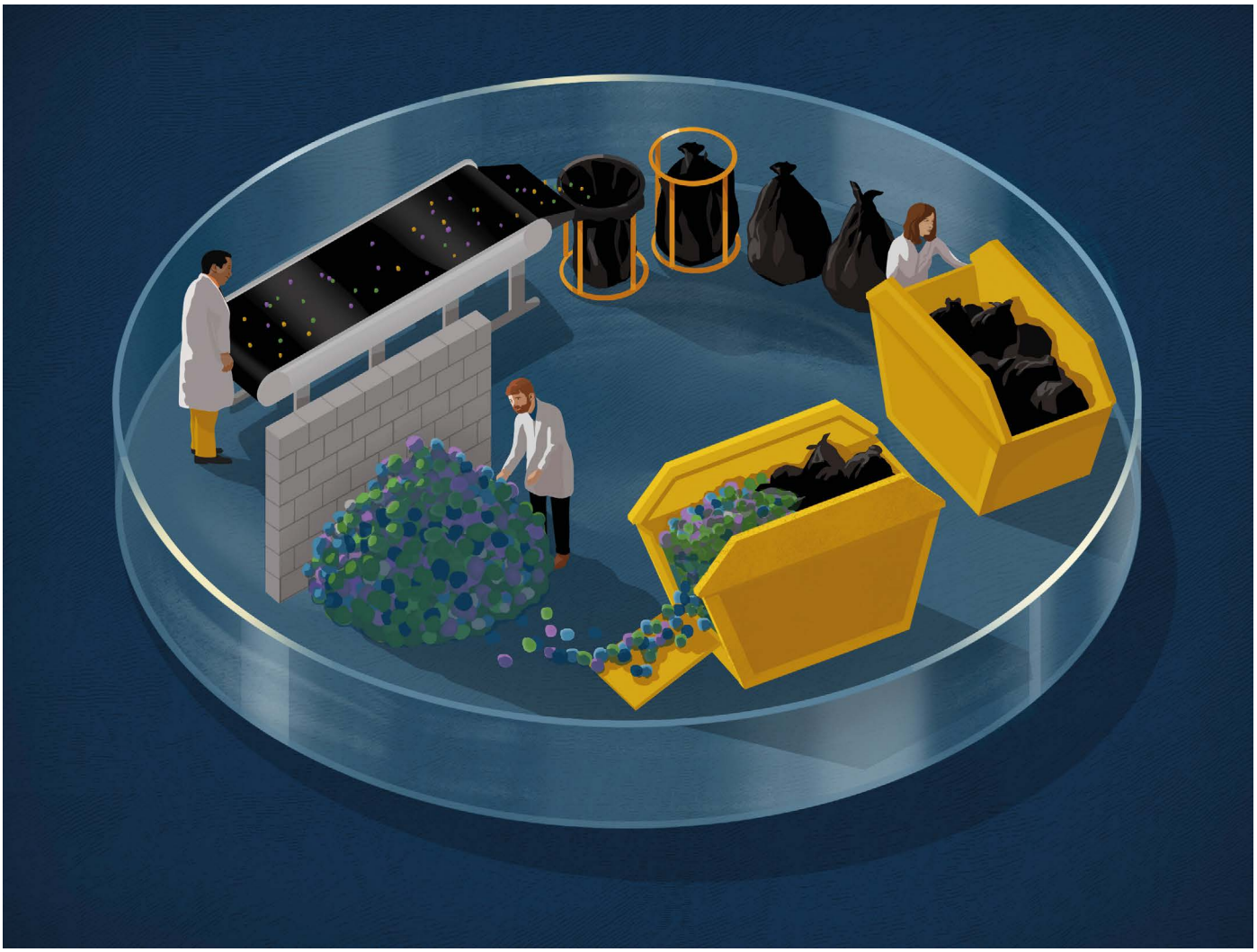


SAM FALCONER

Take out the cellular trash

Biological clean-up mechanisms could hold the key to combating age-related disease. **By Elie Dolgin**

Salwa Sebti was growing impatient. In 2014, she and her colleagues at the University of Texas Southwestern (UTSW) Medical Center in Dallas had begun tracking mice that had a genetically enhanced ability to detoxify their cells. The goal was to test the anti-ageing effects of boosting autophagy, the biological housekeeping process by which cells rid themselves of damaged components. But it was almost two years — a timespan roughly equivalent to 70 years in humans — before the mice showed any clear signs of health improvements.

It was worth the wait. The animals' hearts and kidneys had less tissue scarring than usual; spontaneous cancers were kept at bay; and the mice lived approximately 10% longer. As the data finally poured in, Sebti recalls thinking to herself: "Oh wow, we have a strong phenotype."

Other scientists had previously reported similar age-defying benefits of enhanced autophagy in worms and flies. But the UTSW study was breaking new ground. Spearheaded by Beth Levine — a pioneering autophagy researcher who died of cancer in 2020 — and co-led by two of her former postdocs, Sebti and Álvaro Fernández, it was the first definitive demonstration that boosting the autophagy machinery could promote longevity and well-being in a mammal[1].

"This was a proof of concept that it's possible and it's beneficial," Sebti says. By breeding the autophagy-boosted mice together with various mouse models of disease, her team and others have found several more health-promoting benefits. They have shown how autophagy augmentation can preserve stem-cell function in the ageing mouse brain[2], prevent cognitive decline in a mouse model of Alzheimer's disease[3], and overcome ageing-related organ degeneration[4].

Now, a growing number of biotechnology start-ups are trying to replicate those anti-ageing effects by using drug compounds.

Autophagy "is clearly a very fundamental process that is linked to so many different diseases," says Peter Hamley, chief scientific officer of Samsara Therapeutics in Oxford, UK. The company was set up in 2018 to treat neurodegenerative and rare genetic diseases by inducing or restoring the cellular degradation

system. "It's a big opportunity for drug discovery," he says.

It's also a big challenge.

Autophagy is often likened to the trash management system of the cell. And just as municipal waste services involve collection, transportation and ultimately disposal, so too must the cell's autophagy system follow a coordinated, multistep process. It first requires cellular refuse to be bagged up inside sack-like structures known as phagophores. These then mature into cargo containers called autophagosomes, which fuse with degradation hubs called lysosomes. Only then do waste products get broken down.

Any part of that cell-cleaning process could go wrong, and they often do as cells age. But if researchers do not fully understand what aspects of autophagy are defective in any particular disease, drugs that modulate the wrong parts of the pathway could do more harm than good. A therapy could, for instance, help the cell to package more trash. "But if your trash compactor isn't working properly, you're just going to end up with a room full of trash bags," says Tim Sargeant, who studies autophagy at the South Australian Health and Medical Research Institute in Adelaide. "That's one of the dangers here."

As a result, although some anti-ageing researchers and companies have gone all-in on targeting autophagy, others are more circumspect – especially given the lack of solid evidence in people or even mouse models for many of the proposed interventions.

"It's clear that, in general, maintaining autophagy in particular cell types in the body is going to be beneficial for treating many diseases of ageing – so it makes sense to look at autophagy activation," says Stephen Helliwell, vice-president of discovery biology at Rejuveron Life Sciences, a biotechnology company based in Zurich, Switzerland. But, he adds, "it's one thing to do experiments in worms and flies. It's quite another to show that autophagy manipulation is a safe and effective anti-ageing strategy in people."

Choose your target

Some oncologists are striving to inhibit autophagy because it can fuel the growth and spread of established tumours – although it is also known to stop new tumours from forming. Those focused on other diseases of ageing, however, have the opposite goal in mind: they are trying to give autophagy a lift, albeit in different ways.

Some companies, such as Life Biosciences in Boston, Massachusetts, are taking aim at an atypical version of the process, known as chaperone-mediated autophagy (CMA). In this particular type of autophagy, helper proteins called chaperones interact with 'destroy me' signals on target proteins. The chaperones then shuttle their cargo directly to lysosomes, which break down the trash.

MARIAN VEJCIK/GETTY

Parkinson's disease can complicate normally simple activities, such as eating soup.

"CMA activation is exciting because it is a whole new therapeutic target that has not been explored clinically but appears to play a critical role in ageing biology," says Joan Mannick, head of research and development at Life Biosciences.

> **"One of the jokes is that my husband wants to start taking them."**

Cell biologist Ana María Cuervo has demonstrated this in numerous disease contexts, including conditions linked to metabolic dysfunction and neurodegeneration. In 2013, she and chemical biologist Evripidis Gavathiotis, both at the Albert Einstein College of Medicine in New York City, identified a class of molecule that could stimulate CMA by promoting the uptake of chaperone-guided garbage proteins into the lysosome[5].

In mouse models, these compounds have helped to ameliorate signs of Alzheimer's disease[6] and have restored the functionality of blood-forming stem cells in the bone marrow[7]. The putative drugs worked so well, in fact, that "one of the jokes is that my husband wants to start taking them," Cuervo says.

She and Gavathiotis licensed the technology to Life Biosciences and now serve as scientific advisers to the company. With support from the non-profit Alzheimer's Drug Discovery Foundation in New York City, Life Biosciences is optimizing the compounds for use by humans. Clinical trials involving people with age-related neurodegenerative conditions should follow.

Because CMA relies on this highly specific signalling mechanism, drugs that stimulate the process should "only degrade things that have been primed by the chaperone to be degraded", says Cuervo. This offers potential safety advantages over strategies designed to activate the less-discriminating cellular demolition process implicated in the typical autophagy pathway, she argues.

The strategy is limited to protein targets that engage with CMA, however. It is not an option for other proteins, or for promoting the clearance of other types of cellular debris, such as entire organelles. For that, companies are exploring ways of inducing general autophagy, either in a non-selective manner or with an eye to eliminating structures such as mitochondria, the energy powerhouses of the cell.

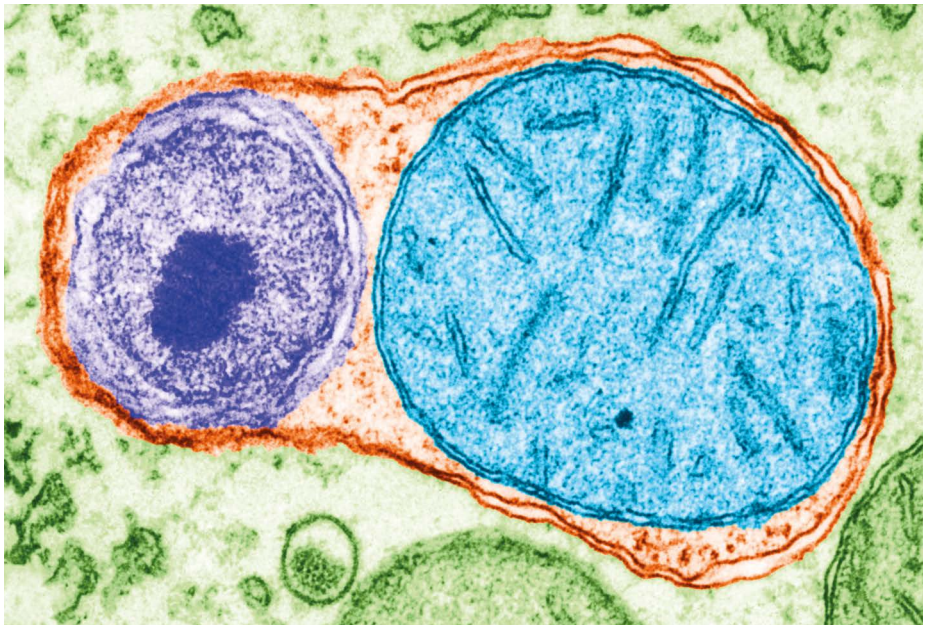
Scientists at MindRank AI in Hangzhou, China, for instance, used predictive algorithms informed by machine learning to identify molecules capable of promoting the degradation of damaged mitochondria, a process known as mitophagy. Their virtual screen yielded a list of 18 compounds. Working with gerontologist Evandro Fang at the University of Oslo and pharmacologist Jia-Hong Lu at the University of Macau, the company then used human cells, worms and mice to winnow this list down to two drug candidates, both of which target a protein called PINK1 – an important mediator of mitochondrial quality control. Late last year, the researchers showed how the drugs helped to improve memory in worm and mouse models of Alzheimer's disease[8].

This kind of approach has begun to attract interest from large pharmaceutical companies. Last year, for example, drug giant AbbVie, based in North Chicago, Illinois, agreed to purchase Mitokinin, a small biotech company in San Francisco, California, that is developing PINK1-targeted agents for the treatment of Parkinson's disease. "By speeding up mitophagy, you cause an increase in the clearance of defective mitochondria," explains Nicholas Hertz, Mitokinin's co-founder and chief scientific officer – and that seems to improve neuronal function and brain health.

A new purpose

When it comes to drugs with broad autophagy-inducing potential, several medicines already being widely used seem to fit the bill. For example, metformin, a common diabetes treatment, and the immunosuppressant rapamycin, which is used to prevent transplant rejection, both fire up autophagy signalling through their effects on mTOR, a master regulator of several steps on the autophagy pathway.

In worms and mice, these drugs increase



DON W. FAWCETT/SCIENCE PHOTO LIBRARY

A colour-enhanced transmission electron micrograph of an autophagic vacuole.

lifespan and overall well-being. But because they have many molecular effects, "you can never be sure what is due to autophagy," says Beat Nyfeler, a chemical biologist at the Novartis Institutes for BioMedical Research in Basel, Switzerland.

Unfortunately, the molecular promiscuity of mTOR signalling, which also controls protein synthesis, and the nonspecific nature of the drugs can also result in unwanted side effects. "I think to really address the power of activating autophagy one would need to find something mTOR-independent," Nyfeler says.

By repurposing an existing drug, David Rubinsztein, a cellular neurobiologist at the University of Cambridge, UK, has identified a blood-pressure medication that induces autophagy through other means. Called felodipine, it works by blocking calcium channels, both to relax blood vessels — hence its use to treat hypertension — and to increase autophagic activity.

In mouse models of Parkinson's and Huntington's disease, Rubinsztein and his colleagues have shown that the drug promotes the clearance of aggregate-prone proteins from the brain, resulting in behavioural and functional improvements[9]. A clinical trial is now planned to evaluate whether felodipine treatment can reduce levels of mutant huntingtin protein in the spinal fluid of people with early-stage Huntington's disease.

Other companies, meanwhile, are looking to develop selective autophagy-activating agents directed at different therapeutic targets. At Samsara Therapeutics, scientists have approached the problem by exposing cultured cells to tens of thousands of drug compounds and then running assays designed to detect any changes in autophagy function. This work has yielded three promising drug leads, Hamley says, which the team is now trying to validate.

Some companies are taking a more target-focused approach. At Caraway Therapeutics in Cambridge, Massachusetts, president and chief scientific officer Magdalene Moran uses human genetics to guide her team's drug discovery. Mutations in several lysosome-associated proteins are known to cause devastating neurodegenerative disease, so Moran and her colleagues reasoned that promoting the function of those same proteins could have therapeutic benefit. "What we're really looking to do is enhance the capacity of these existing pathways to address disease," she says.

"We're essentially educating the autophagy pathway to go after a disease target."

Casma Therapeutics, also in Cambridge, Massachusetts, has its sights set on a few specific targets that would rev up autophagy in cells for which the decomposition system is impaired. But the company — which Levine helped to found with Andrea Ballabio, scientific director of the Telethon Institute of Genetics and Medicine in Naples, Italy, and others — is advancing a platform technology that would target any troublesome cellular component and send the unwanted material flowing through the autophagy pathway. As Casma's chief scientific officer, Leon Murphy, explains: "We're essentially educating the autophagy pathway to go after a disease target and to eliminate that from the diseased cell."

Research teams from Tohoku University in Sendai, Japan, and Fudan University in Shanghai, China, first described this approach in 2019, each detailing a different way to design compounds that escort target molecules to phagophores for demolition. The Tohoku team made a mitochondria-targeting degrader[10], whereas the Fudan group made a selective degrader of mutant huntingtin proteins[11] — a strategy now being pursued by PAQ Therapeutics, a Fudan University spin-off company in Cambridge, Massachusetts.

Researchers who have long studied autophagy but found little industry interest welcome the attention that drug companies are finally paying to the field. Some think the power of autophagy could be harnessed in any number of different ways — including with creative dosing strategies to maximize the therapeutic effect. "Maybe you want to use it periodically instead of chronically to avoid the negative effects of too high autophagy," says Congcong He, a cell biologist at Northwestern University's Feinberg School of Medicine in Chicago, Illinois. The mice used in Sebti's landmark 2018 study were created by He, who has unpublished data validating this 'less is more' approach to autophagy induction.

There could also be synergies between different parts of the process. "It may be valuable to both turn on the degradative process itself, and then also to enhance the delivery of a certain substrate into the autophagosome," says Skip Virgin, a scientific co-founder of Casma who now leads research efforts at Vir Biotechnology, an immunotherapies company in San Francisco, California, that is also studying autophagy.

This would be like putting more garbage trucks on the road and increasing the size of trash incinerators to ensure clean and safe streets. Along the road to ageing, cells might benefit from similar investment in waste management.

Elie Dolgin is a science journalist in Somerville, Massachusetts.

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