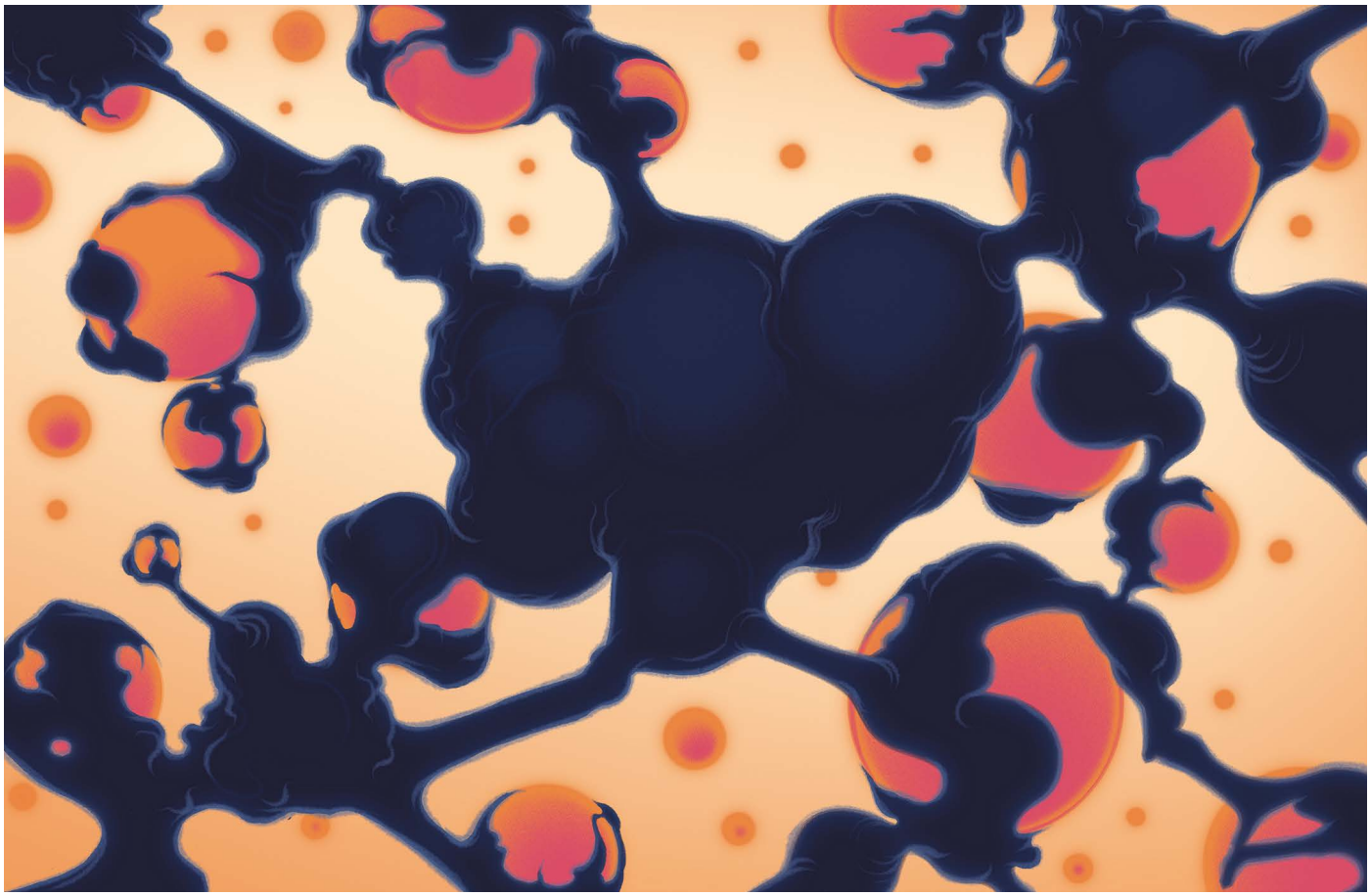


SAM CHIVERS

Zombies in the lungs

The role of senescent cells in chronic obstructive pulmonary disease is beginning to be unpicked. **By Anthony King**

A senescent cell is like a classic movie monster — it exists in an 'undead' state. It no longer divides, but it is resistant to death. It is also supersized, and produces an unusual number of proteins, many of which stoke inflammation. Almost all cells have the capacity to enter this zombie-like state, given the right circumstances. Radiation exposure, too much or not enough oxygen and certain toxins, such as those found in cigarette smoke, can all provide the spark, says James Kirkland at Mayo Clinic in Rochester, Minnesota, who is a leader in the field of cellular senescence.

Unlike the zombie armies of Hollywood films, senescent cells can be useful. When insults to DNA threaten to push a cell into a cancerous state, senescence can come to the rescue. The potential cancer cell is lulled into a zombie-like state in which it cannot divide, and therefore cannot be cancerous. Senescent cells also draw elements of the immune system, including macrophages and natural killer cells, to their location. This helps to clear up cellular debris and any toxins that might have pushed the cells to become senescent in the first place. Senescence is also involved in wound repair, and even the initiation of childbirth.

But it is not all good news. There is a growing suspicion that senescent cells also have a leading role in triggering age-associated diseases, including chronic obstructive pulmonary disease (COPD). Put simply, researchers are beginning to worry about the zombies in our lungs.

Both of the major risk factors for the disease — ageing and smoking — are known to bring about senescence in lung cells. Evidence also suggests that a high burden of senescent cells in the lungs is involved in the development of some of the features of COPD, such as inflammation and emphysema (damage to air sacs in the lung), although researchers lack the tools to be certain that this is a cause.

Just five years ago, few researchers would associate these cells with COPD — the third leading cause of death worldwide. Now, interest in the role of senescence in chronic lung disease is growing, potentially leading to new treatments.

Biological links

The hallmarks of COPD are emphysema and inflammation — a process involved in the hardening or fibrosis of the airways, causing them to become obstructed. Together,

STOPPING THE SPREAD OF SENESCENCE

Some researchers think that senescence might spread between cells and tissues. Could this explain why people with COPD are also likely to have other conditions?

People with chronic obstructive pulmonary disease (COPD) live with more than just airway obstruction. "One of the features of COPD is that it is almost always associated with other diseases of accelerated ageing, particularly cardiovascular disease," says respiratory scientist Peter Barnes at the National Heart and Lung Institute in London. Cardiovascular disease is both more common and more likely to be a cause of death in people with COPD. Hormonal, metabolic, psychiatric and neurological disorders, as well as gastrointestinal disease, are also more common in people with COPD[6]. And some researchers think that cellular senescence might help to explain the high rate of comorbidities associated with COPD.

Senescent cells have the ability to convert other cells to senescence — even at a distance. "Senescence spreads from cell to cell," says Kirkland. "You can find a predominance of senescent cells at the site of a lung disease, but in those individuals, you find senescence cells elsewhere too."

When Kirkland's group transplanted a relatively small number of senescent cells into young mice, this was enough to cause persistent physical problems for the animals. In older mice, even fewer cells were required to cause a problem. Eliminating the cells alleviated physical dysfunction and helped the mice to live longer[7].

The lungs are well-vascularized organs that are regularly exposed to pollutants in air and can become overwhelmed by repeated toxic insults, which can lead to the development of numerous senescent cells. Some researchers think that this senescence might spread to other tissues.

Lee has begun to look for immune cells in bone marrow and blood in genetically altered mouse models to see whether smoking- and senescence-mediated COPD extends beyond the lung. "Many of our COPD patients have significant skeletal muscle atrophy and mitochondrial abnormalities in muscle cells. So this goes beyond the lung," she says.

these phenomena cause shortness of breath, wheezing, a chronic cough and lack of energy.

COPD is usually diagnosed in people over the age of 40, and around half of all cases can be attributed to smoking. The habit is also known to promote cellular senescence. "Cigarette smoking is an oxidative stress to cells in the lung. That is the mechanism that puts them into senescence," says Peter Barnes, a respiratory scientist at the National Heart and Lung Institute in London. The association has led some to suspect that senescence plays a part in triggering the symptoms of COPD. "We know that senescent cells can produce a low-grade inflammatory response, which is identical to what we see in COPD," says Barnes. And lung biopsies taken from people with mild to moderate COPD also show signs of senescent cells, suggesting that these cells could be a cause, rather than a consequence, of COPD.

Left unchecked, the cells will secrete molecules that promote inflammation and bring about degradation of extracellular matrices – behaviour that is referred to as the senescence-associated secretory phenotype, or SASP. A build-up of senescent cells in the lungs also seems to limit the potential for tissue renewal. "Normally, these senescent cells get cleared, and this allows tissue architecture to be maintained," says Victor Thannickal, a respiratory scientist at the University of Alabama at Birmingham. But in people with COPD, he thinks, clearance cannot keep pace with the cells' creation. "When they don't get cleared, then the accumulation of senescent cells can cause harm," he says.

> **"When they don't get cleared, then the accumulation of senescent cells can cause harm."**

Older people seem to be more susceptible to chronic illness caused by cellular senescence. This could be because the immune system deteriorates with age, which might impede the removal of senescent cells. Constant stimulation by toxins from cigarette smoke and pollution accelerates senescence and might exhaust the immune system, resulting in a slowing down of the body's ability to deal with it. Eventually, a threshold might be crossed "beyond which the lung is incapable of clearing senescence cells", says Thannickal. The higher numbers of cells can then ratchet up pro-inflammatory and pro-senescent secretions, causing tissue disruption.

But not everyone is on board with linking senescence to both the symptoms and the causes of COPD. Cellular senescence has not yet been proved to be the prime driver of COPD. "We know that smoking triggers COPD and senescence, but perhaps senescence is a by-product of ageing or a by-product of COPD," says Irfan Rahman, a biochemist at the University of Rochester Medical Centre in New York. In 2018, he reported that although ageing and cigarette smoking caused senescence in the lungs of mouse models of COPD, this did not worsen the severity of their symptoms[1]. "We were unable to find a link between senescence and COPD in response to tobacco smoke, at least in mice," he says.

Other researchers, however, think that cellular senescence will turn out to be a crucial driver of COPD. When Patty Lee, a pulmonologist at Duke University School of Medicine in Durham, North Carolina, interrupted early expression of senescence genes in mouse models of COPD, she found that these rodents did not go on to develop emphysema[2].

Search for signs

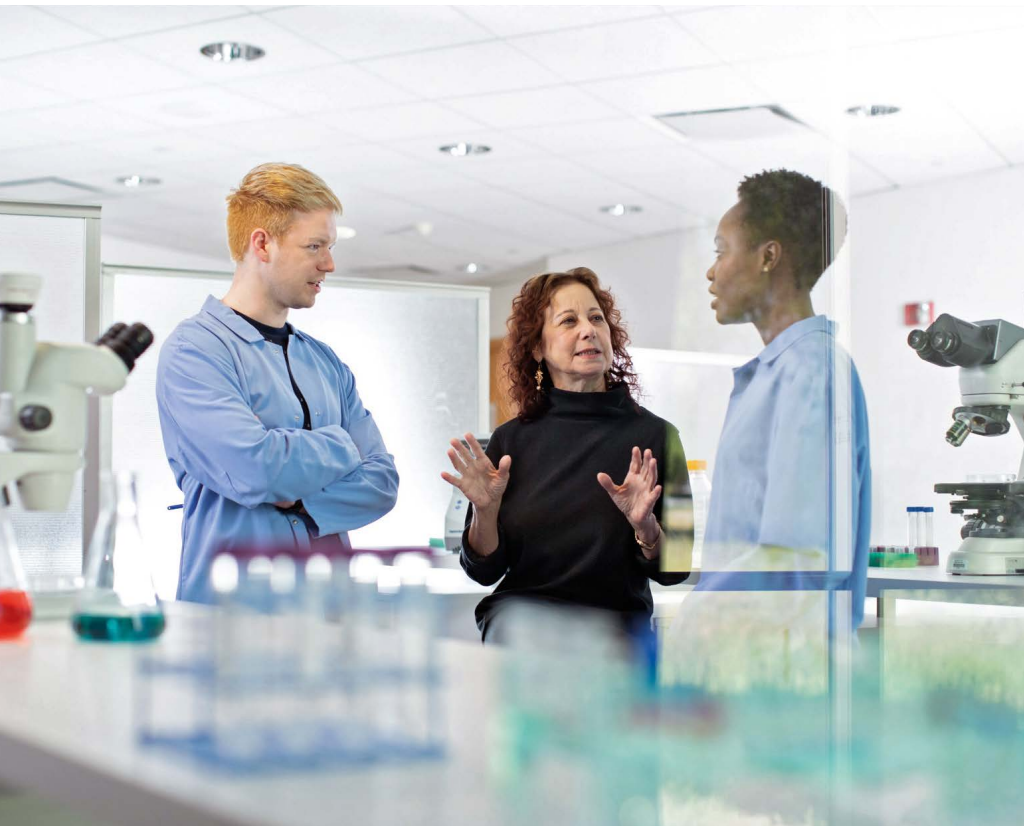
One reason for researchers' uncertainty is that senescent cells in the body can be difficult to distinguish from healthy cells. "It's a mess," says Judith Campisi, a cell biologist at the Buck Institute for Research on Aging in Novato, California. "We now have maybe a dozen different biomarkers of senescent cells, but the problem is that no single biomarker is exclusive to senescent cells." Reliable methods of detection and tracking are needed to fully understand the role of senescent cells in COPD, including the possibility that they are involved in the development of other conditions that commonly occur alongside COPD (see 'Stopping the spread of senescence').

Kirkland's group is working on blood, urine and epigenetic tests so that researchers can get a handle on the burden of senescent cells in each patient. As well as certain secretory factors, the researchers want to monitor exosomes – small vesicles that senescent cells secrete in large quantities (see page S10). "With exosomes, you can tell which cell type shed them, and then you can look at their cargo for markers of senescence," says Kirkland. Others, such as Lee, think advances in imaging will make it possible to see inside cells in the lung to detect and quantify their senescence.

Treatment dreams

Although much about the role of senescent cells in COPD is not understood, some researchers are already contemplating targeting senescence to treat COPD and other age-associated conditions.

Treatments that aim to stymie inflammation in the lungs, such as cytokine blockers, have



JIM HUGHES PHOTOGRAPHY

Judith Campisi (centre) and her colleagues are testing drugs that destroy senescent cells.

not yet proved effective. "The thinking was that inflammation drives the pathological changes in COPD, and a way of stopping the disease is to reduce this inflammation," says Barnes. But disappointing results led to speculation that it might be better to deal with the source of inflammation — senescent cells.

The first approach to tackling senescence is a class of drugs called senostatics, which block the molecular pathways that lead to senescence. The best-studied example is the mTOR pathway, a molecular system involved in cell growth and survival, and in protein manufacturing. In 2018, researchers showed that activation of the mTOR pathway in lung vascular cells or alveolar epithelial cells of mice prompted senescence in the lungs and caused COPD-like problems[3]. Barnes suggests that inhibiting the mTOR pathway could be a valuable therapeutic approach.

Rapamycin, a natural compound that inhibits mTOR, has been shown to increase lifespan in mice, possibly by putting a dampener on senescent secretions. Similarly, the widely prescribed diabetes drug metformin, which increases production of the mTOR inhibitor AMPK, reduces emphysema and inflammation in mice[4]. "We don't have evidence in humans, but we think these studies are feasible," says Barnes. A trial is under way to see whether metformin can reduce age-related disease in people in the United States.

A second potential approach is to destroy senescent cells. But this is no mean feat. In the lab, senescent cells survive conditions that easily kill normal cells. They also secrete compounds that act as a shield against their own killer secretions. This "allows them to survive, while killing everything around them", says Kirkland. To defeat them, researchers are looking to drugs that can knock out these cells' shields. "Senolytics work by transiently disabling those pathways, for just a few minutes, and allow the senescent cells to commit suicide," Kirkland explains. Unity Biotechnology, a start-up company in Brisbane, California, has had promising results from an initial phase I trial to treat osteoarthritis of the knee with its senolytic, UBX0101. Calico Life Sciences in San Francisco, California, a biotech company backed by Google, is also eyeing senolytics.

"We were unable to find a link between senescence and COPD in response to tobacco smoke."

In a 2019 study, Kirkland tested a combination of two senolytic agents in 14 people with idiopathic pulmonary fibrosis, a respiratory condition involving irreversible scarring of the lungs[5]. The compounds — a cancer drug called dasatinib, and a compound found in many fruits and vegetables called quercetin — triggered the death of senescent cells and improved the participants' physical function. A phase II study is under way. "The big question is, will it work for COPD," says Campisi, who co-founded Unity Biotechnology. "We are working on that now."

Hope and hype

Some researchers are wary, however, about getting too carried away. Removing every senescent cell in the lungs might have damaging side effects. "Which cell types senolytics target is going to be important," warns Lee. Safety concerns partly explain the initial focus on idiopathic pulmonary fibrosis — on average, people die within four years of diagnosis. "Senolytics should start off with very serious life-threatening conditions for which there is no good treatment," says Kirkland. "We don't know the side effects of these drugs yet."

Some fear that research on the potential clinical relevance of senescent cells could be overwhelmed by hype. One COPD researcher, who wanted to remain anonymous, said they had never heard of senescence five years ago, but it now seems almost obligatory to mention the phenomenon in grants or papers on COPD. Thannickal similarly notes that what was a trickle of reports on the topic five to ten years ago has turned into a waterfall. But Barnes argues that there is good reason to pay attention to senescence. After all, COPD affects one in ten people over the age 40, and there is an urgent need for treatments that do more than just manage symptoms. "It is such a common disease," he says. "It is really good to test out some of these ideas."

The zombie hordes of films and books are usually defeated. And although it is too early to tell whether COPD and other age-associated diseases will follow the same script, zombie cells are at least now firmly in researchers' cross hairs. "There's recognition now of the importance of senescence and lung ageing in COPD," says Lee. "It is certainly on our target list," agrees Kirkland. "We and other labs here are working around the clock."

Anthony King is a science writer in Dublin.

1. Rashid, K. *et al. Sci. Rep.* **8**, 9023 (2018).
2. Kim, S.-J. *et al. Aging Cell* **18**, e12914 (2019).
3. Houssaini, A. *et al. JCI Insight* **3**, e93203 (2018).
4. Cheng, X.-Y. *et al. Oncotarget* **8**, 22513–22523 (2017).
5. Justice, J. N. *et al. EBioMedicine* **40**, 554–563 (2019).
6. Yin, H.-L. *et al. Medicine* **96**, e6836 (2017).
7. Xu, M. *et al. Nature Med.* **24**, 1246–1256 (2018).