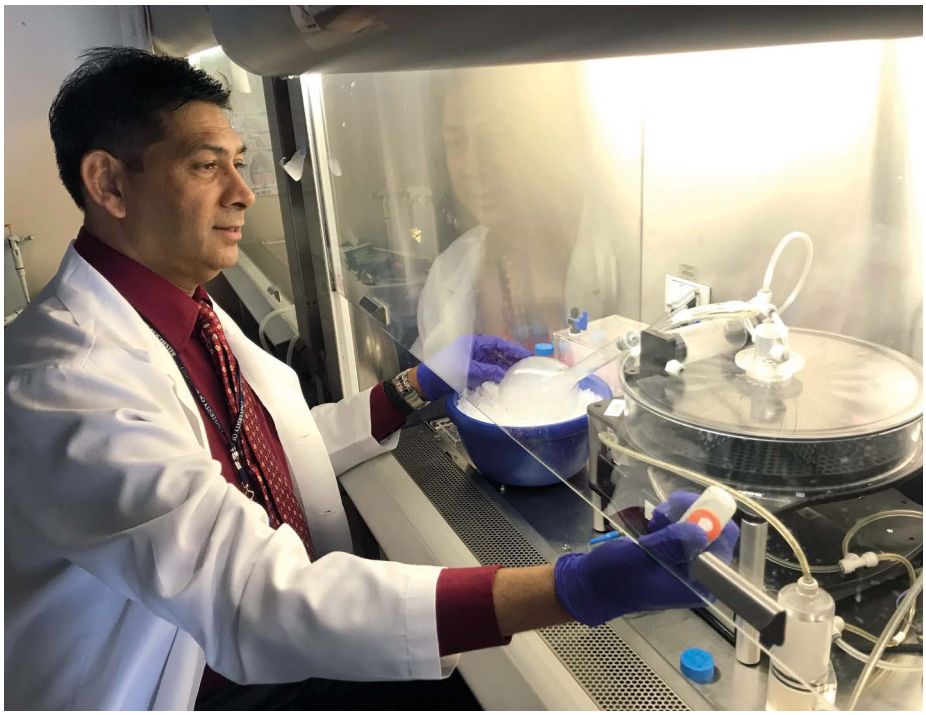


Irfan Rahman exposes cells to smoke as part of research into exosomes and COPD.

IRFAN RAHMAN

# Care packages

## Vesicles released in response to cigarette smoke might trigger COPD, but engineered versions offer possible therapy. **By Jyoti Madhusoodanan**

In the 1980s, researchers found that healthy cells release small, membrane-wrapped packages that are now known as exosomes. They originate deep inside cells, where they are loaded with cargo including specific proteins and RNA before being released to travel beyond the cell.

Initially, researchers thought of exosomes as a means of intercellular communication. "At the time, people thought exosomes were only released to relay neurotransmitters or hormones," says pulmonologist Yang Jin of Boston University, Massachusetts. "Their importance has only been recognized in the last ten years or so."

Now, scientists know that nearly all cells shed exosomes. And Jin and others have found that these vesicles might be key to the symptoms of chronic obstructive pulmonary disease (COPD).

People with COPD — one of the leading causes of death worldwide — experience wheezing, fatigue and chronic coughing. It is especially prevalent in smokers, and research has found that both smokers and people with COPD have an increased number of exosomes circulating in their blood. The contents of these vesicles also differ markedly from those seen in non-smokers without the disease. "We don't know the true triggers of COPD," Jin says. "Looking at the cargo of vesicles in different groups of patients could potentially hold answers about how this disease develops."

In addition to working out the role of exosomes in the development of disease, several researchers are eyeing their therapeutic potential. Early studies suggest that vesicles derived from stem cells can aid tissue repair, and some scientists are considering the possibility of engineering vesicles to carry drugs to diseased tissues. But these efforts have been held back by a dearth of standardized methods to isolate and study vesicles. Advances in techniques over the past few years — and greater scientific consensus in creating standards for research into extracellular vesicles — are pushing the field forward.

Some of the clearest evidence linking exosomes to the symptoms of COPD emerged in 2019. While trying to understand how a particular protein exited immune cells, Edwin Blalock, a pulmonologist at the University of Alabama at Birmingham, found it inside exosomes, along with an unexpected travelling companion: the enzyme neutrophil elastase[1].

Elastase is a prominent player in COPD. The enzyme wears down the stretchy fibres of elastin and collagen that keep the lungs flexible. In healthy individuals, cells counter elastase's effects with an anti-protease called α1-antitrypsin (α1AT), and COPD was long considered the result of an imbalance between these two proteins. This view is bolstered by the fact that people with a genetic deficiency in α1AT are at much greater risk of developing COPD — even if they have never smoked — than are non-smokers without the mutation. The idea that higher levels of neutrophil elastase are linked to COPD "has been a cornerstone of the study of COPD for over six decades", says Blalock. "But the levels of elastase typically seen were never high enough to counter α1AT activity. That was the conundrum."

Blalock and his colleagues found that when elastase was packed on the surface of exosomes, it was protected from neutralization by α1AT. These exosomes also bore a marker called Mac-1 that helped them to bind to the extracellular matrix, where elastase then digests matrix fibres. The loss of elastin and collagen from the extracellular matrix causes lung tissue to become less flexible and alveolar spaces to widen, which in turn reduces the efficiency with which the lungs transfer oxygen and carbon dioxide into and out of the body.

When exosomes from people with COPD were injected into mice, the animals developed signs of COPD, including emphysema[1]. "This is the first instance of being able to have exosomes transfer a disease phenotype from a human to a mouse," Blalock says. "It's surprising, especially the rapidity with which the mice developed COPD after they first encountered these exosomes, and I think it points to their potency as effectors of damage."

### Spurring symptoms

Neutrophils are not the only source of exosomes implicated in COPD. In healthy people, lung epithelial cells usually release exosomes containing a protein called CCN1. But Jin's team found that when mice were exposed to cigarette smoke — about the equivalent of around 70 cigarettes a day for 3 months — lung epithelial cells instead released a fragmented form of the protein directly into bronchial fluids[2]. The intact

form of the protein inside exosomes modulated inflammatory proteins in the lung and helped to maintain homeostasis after exposure to cigarette smoke. But the CCN1 fragments not encapsulated in vesicles caused a spike in the production of two proteins that digest the extracellular matrix, causing cells and tissues to die. The reason, Jin suggests, is that smoking and other stressors alter how proteins such as CCN1 are tagged for processing, resulting in the production of abnormal fragments that are not wrapped in an exosome.

Jin and others are also looking at microRNAs in exosomes; these are more stable and easier to detect than proteins. Several microRNAs are enriched in extracellular vesicles from lung epithelial cells exposed to cigarette smoke, according to one study[3]. Researchers found that one of these, miR-210, reduced autophagy, a process that is essential to clearing away damaged cells. The microRNA also increased the formation of collagen and cells associated with fibrosis, which stiffens lungs. All these functions could contribute to the development of COPD, says Takahiro Ochiya who studies exosomes at Tokyo Medical University, lead author of the study.

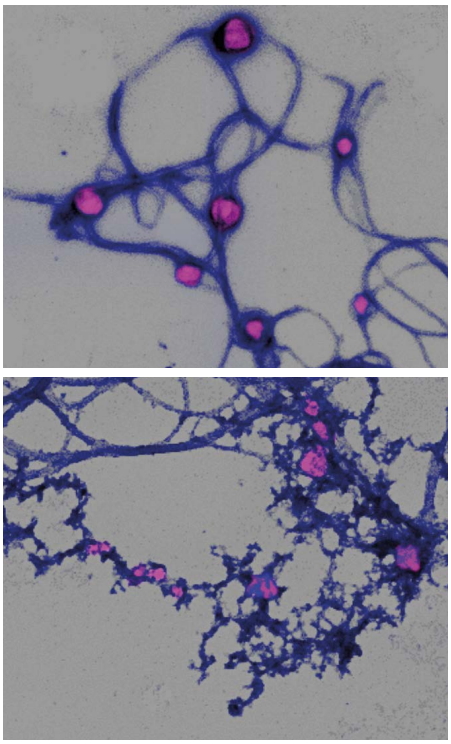
Because exosomes carry multiple molecules, it has long been hoped that their contents could be used as diagnostic or prognostic biomarkers. Not all those who smoke develop COPD, and not all those who have COPD are smokers. The contents of extracellular vesicles might help to "figure out whether a person has the potential to develop emphysema or not", Jin says.

Blalock suggests that future studies of exosomes from activated neutrophils should examine whether these vesicles occur in all smokers, or just a sub-population who might be at greater risk of COPD as a result. "If it's only a sub-population of otherwise healthy smokers, are they the ones to go on to develop COPD?" he says. "If that's the case, we would have a biomarker to identify the people who smoke who are likely to get the disease."

**"If we keep an open mind, there may be multiple cell types or vesicle types we could use for therapeutics."**

## Microscopic mules

Knowing the molecular triggers that exosomes carry is also a step towards finding drug targets and designing better therapies. Elastase is one possibility. Because the enzyme is shielded from its natural inhibitor while attached to exosomes from activated neutrophils, it is possible that an intervention that dissociates elastase from the exosome could make the enzyme susceptible to a person's α1AT once again, Blalock suggests.



ED BLALOCK/KRIS GENSCHMER/CAMILLA MARGAROLI

**Elastase on neutrophil-derived exosomes (pink) breaks down collagen (blue).**

Engineered vesicles could also be used to carry drugs to specific sites of tissue damage. "Current therapies for COPD are just analgesic or palliative," says Irfan Rahman, who studies environmental medicine at the University of Rochester in New York. "We give steroids, β-agonists or bronchodilators just to open up the lungs, but the destruction continues."

Last year, Rahman and his colleagues reported that, in mice, vesicles derived from mesenchymal stem cells protected lung tissues from the damage caused by exposure to cigarette smoke[4]. And in ongoing studies, Ochiya and his colleagues are evaluating whether a spray delivered directly into the trachea, containing vesicles harvested from healthy lung cells, can reverse the damage caused by COPD.

Jin's team is taking a different approach. Instead of using vesicles derived from healthy cells, it is aiming to manipulate the contents of vesicles to deliver drugs, proteins or microRNAs to treat the symptoms of lung disease. Because vesicles share the surface markers of the cells they are derived from, they can be directed specifically to diseased tissues. "This decreases a lot of side effects that are caused by medications affecting non-target tissues," Jin says.

These and other exosome-based therapies for a variety of conditions, including cancer and Alzheimer's disease, are still in preclinical development – numerous experimental and regulatory hurdles remain.

## Better standards

Most vesicle-based therapeutic strategies in COPD currently rely on vesicles released from cultured cells. But these vesicles vary widely in their contents and how they're formed, making it tough to isolate a pure sample of exosomes and to standardize therapeutic effectiveness. "We still have no gold-standard method to harvest vesicles," Ochiya says.

Exosomes are the only vesicles known to be produced by a cell's internal membranes. One approach to purifying samples of vesicles down to exosomes has been to look for signs of a vesicle having passed through this production pipeline. However, focusing on how the vesicle is formed, rather than its function, might stymie efforts to develop therapeutics, says molecular biologist Kenneth Witwer of Johns Hopkins University in Baltimore, Maryland. Focusing on function could expand the range of potential therapies beyond stem-cell derived exosomes. "If we keep an open mind, there may be multiple cell types or vesicle types we could use for therapeutics," he says. Witwer is one of a number of researchers to propose methods for characterizing vesicles that shift the focus from how they are made to their size, cargo or function.

Demonstrating that a particular batch of vesicles have uniform physical features, and then showing the vesicles' potency in a functional assay, "would help regulators assess whether a vesicle-based product is essentially the same from one batch to the next", Witwer says.

This standardization is crucial if exosome-based therapies are to become a reality. At present, people with COPD are treated with bronchodilators or other drugs that stave off symptoms, but do little to halt the underlying tissue damage. Therapies that rely on exosomes derived from stem cells could perform better than stem cells themselves, particularly because "exosomes may be able to go places where a cell can't", Blalock says. "There may be therapeutic niches that can only be accessed via exosomes."

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