Increased mucins pinned to worsening cystic fibrosis symptoms

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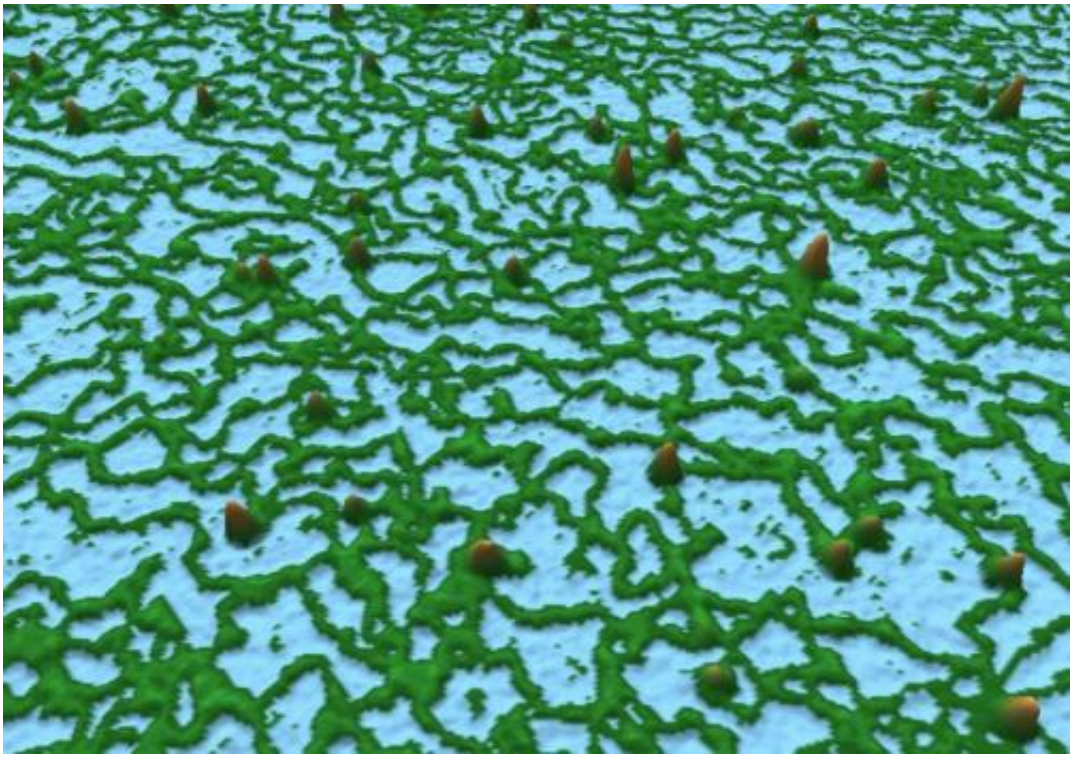

This image shows mucins, large proteins that form a network through bonds to give mucus its thickness and elasticity. Credit: Lab of Mehmet Kesimer, PhD

UNC School of Medicine researchers have provided the first quantitative evidence that mucins – the protein framework of mucus – are significantly increased in cystic fibrosis patients and play a major role in failing lung function.

The research, published today in the *Journal of Clinical Investigation*, shows that a three-fold increase of mucins dramatically increases the water-draining power of the mucus layer. This hinders mucus clearance in the CF lung, resulting in infection, inflammation, and ultimately lung failure.

"Our finding suggests that diluting the concentration of mucins in CF mucus is a key to better treatments," said Mehmet Kesimer, PhD, associate professor of pathology and laboratory medicine and co-senior author of the JCI paper.

Ashley Henderson, MD, assistant professor of medicine and co-first author of the JCI paper, added, "We think this study shows why nebulized hypertonic saline [sterile salty water] improves the hydration of the CF airway, improves the patient's mucus clearance and, in so doing, increases lung function."

The UNC study also casts further doubt on a controversial 2004 study that disputed the theory that mucins play a major role in CF.

This work, a collaboration of 13 UNC scientists, is part of an extensive UNC lung research program based in the new Marsico Lung Institute, which is led by Richard Boucher, MD, co-senior author of the JCI study.

"This paper points to a therapeutic strategy to rectify this problem of mucus clearance and provides signposts, or biomarkers, to guide development of novel therapies," said Boucher, the James C. Moeser Eminent Distinguished Professor of Medicine. Also, by measuring mucin concentration in patient mucus, doctors could learn whether therapies are working and to what degree.

Scientists and doctors have known for a long time that failing to clear mucus is the major reason why CF patients face chronic lung infection

and inflammation. But the mechanisms of this failure have not been well understood.

Normally, when we breathe, the mucosal layer of our lungs trap the contaminants – dust, pollutants, bacteria – naturally found in air. Then, epithelial cells with hair-like cilia brush the mucus up and out of our lungs. In people with cystic fibrosis, though, this process doesn't work as well because they lack a properly functioning CFTR gene. They continually battle infections and must work hard to clear mucus from their lungs.

This is where mucins come into play. Mucins give mucus its gel-like thickness and elasticity. "Without mucins, mucus would have the viscosity of blood," Kesimer said. "The vast majority of mucus is water, but 30 to 35 percent of the remaining solid material is made up of mucins. They form a network of bonds that serves as a framework."

This is why Kesimer and his UNC mentor, the late John Sheehan, PhD, Distinguished Professor of Biochemistry and Biophysics, suspected that something must happen to mucins in the CF lung. They and others knew that CF mucus is typically drier than normal mucus.

Back in 2004, however, other researchers used a standard immunologic analysis to show that mucins were decreased in CF secretions. They suspected DNA was the main culprit that caused problems in CF mucus. Sheehan and Kesimer were skeptical, as was Henderson, a clinician who saw CF patients and had been a research fellow in Sheehan's lab. They set out to conduct various novel experiments to physically measure the amount of mucins in CF secretions and normal mucus.

In one experiment, they used a technique called size exclusion chromatography: in a column, they added custom-made beads that had small pores. Smaller proteins could enter the pores while mucins could

not. Through this separation, Kesimer and Henderson's team isolated the mucins and simultaneously measured their concentration using a refractometer.

By using sputum samples from CF patients, the researchers found that CF mucus contained three times as many mucins than did normal samples. They also conducted experiments to show that mucin overabundance led to a six-fold increase of the pressure between the mucus layer and the ciliated layer.

This finding affirms the CF disease model that UNC researchers published in the journal Science in 2012. In essence, in a CF patient, the increased osmotic pressure of the concentrated mucus layer crushes the ciliated cells so that mucus is not cleared. The lung becomes a breeding ground for bacteria. This leads to more mucins, more mucus, inflammation, and subsequently lung failure.

Moreover, Kesimer's team showed precisely why the 2004 research was flawed. Those researchers used a classic antibody based immunologic technique called a western blot, which measures the expression of a given protein – in this case mucins – based on an antibody response to that protein.

But, as Kesimer pointed out, antibodies must latch onto proteins at specific sites on the proteins' surfaces. When Kesimer conducted the western blot, he got the same result as the 2004 researchers. But then he used a technique called mass spectrometry to find that CF secretions are full of proteases – enzymes that break down molecules. The mass spectrometry showed that the proteases degraded the mucins, essentially "erasing" many of the sites where antibodies could bind without disrupting the structural integrity of mucins.

"For that reason, we saw less antibody response using the western blot,"

Kesimer said. And so it looked as if there were fewer mucins. "But by using more accurate methods, we clearly saw the increase of mucins. In fact, we've analyzed many samples of sputum from patients with other chronic pulmonary diseases and we saw the increase in mucins in them, as well."

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