New therapeutic target identified for acute lung injury

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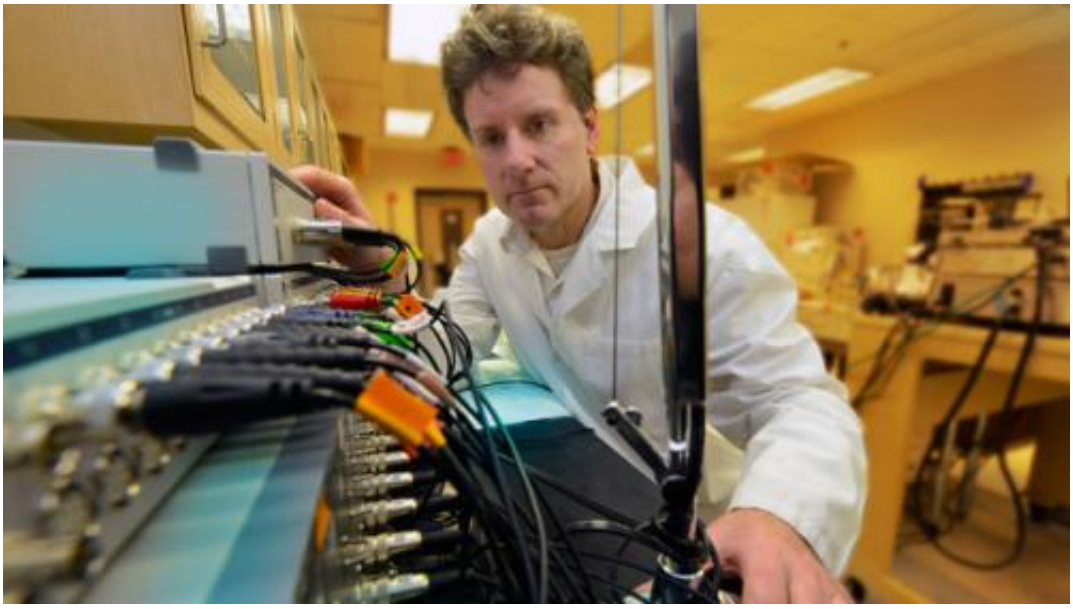

This is Dr. Stephen Black, cell and molecular physiologist at the Medical College of Georgia at Georgia Regents University. Credit: Phil Jones

A bacterial infection can throw off the equilibrium between two key proteins in the lungs and put patients at risk for a highly lethal acute lung injury, researchers report.

Bacteria can alter a single amino acid in the protein RhoA, pushing its activity level well above that of Rac1 and prompting blood vessels to leak and flood thousands of tiny air sacs in the lungs, said Dr. Stephen

Black, cell and molecular physiologist at the Medical College of Georgia at Georgia Regents University.

The study in *The Journal of Biological Chemistry* also proposes a biological shield that appears to protect RhoA from the potentially lethal alteration.

"Activation of RhoA appears to be an early, early event and it's a pathologic activation," Black said. "The cell can't regulate it anymore. It just stays on," he said, comparing the aberrant activity level to a rapid-fire gun with no pause for reload.

Causes of acute lung injury include common bacterial infections that cause maladies such as pneumonia; severe trauma that induces shock; multiple transfusions; burns; and meconium aspiration, when newborns inhale waste products. Mechanical ventilation used to support ailing lungs can also cause or compound the problem that occurs in about 150,000 Americans annually, killing about one-third, and leaving many with damaged lungs.

While overactive RhoA was believed to be a culprit, just how it contributed was unknown. Researchers also knew that nitration – adding a nitro group to a protein to change its function – occurs at high rates in acute lung injury and normalizes when the stimulus, such as a bacterium, is removed.

Using human lung cells and mass spectrometry, they found the amino acid Y34 was altered in this condition. Then, using 3-D computer modeling, they mapped out exactly how that alteration affects RhoA function. They found it turns RhoA into a steady-firing protein.

While antibiotics can wipe out the bacterial stimulus, patients must survive long enough for the body to resume making normal RhoA. "This

is one of the insidious things. You can clear the bacterial infection and still die," Black said. The good news is that the proteins turn over in just a few days.

Nitration occurs, in this case, when peroxynitrite is added to a protein, a move previously believed lethal to the protein. However, MCG researchers found nitration can also activate RhoA.

Once they found the specific modification, they designed a peptide that mimics the normal protein sequence and placed it on top of the area of nitration. "Think about it as a shield," Black said. For these early experiments, researchers gave the peptide shield before the stimulus to see if they were even on the right track. They've since redesigned the peptide to reduce undesirable effects on normal RhoA signaling and are now giving it the way patients may one day get it: after changes to Y34 already have occurred. "We have high hopes for what we call the gen-2 peptide," said Black, citing early evidence that it can normalize RhoA activity.

Y34 is one of about 200 amino acids in RhoA. "We are looking at one amino acid in one protein in an extraordinarily complex disease and it makes it better," said Black.

To ensure tight regulation of the consistency of blood vessel walls, Rac1 and RhoA normally have the opposing functions of pulling together and pushing apart the endothelial cells that line them. "The balance of those determines whether you are going to be tight or leaky," Black said. "If you can clear the stimulus, you can theoretically make the cells knit back together again."

Still unclear is how long RhoA can sustain its bacteria-modified super-pace and whether non-bacterial causes of acute lung injury prompt the exact RhoA alteration.

The researchers performed simultaneous studies in an animal model. The research was funded by a National Institutes of Health Program Project grant.

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