Researchers discover how breast cancer spreads to lung

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The spread of breast cancer is responsible for more than 90 percent of breast cancer deaths. Now, the process by which it spreads -- or metastasizes -- has been unraveled by researchers at Johns Hopkins.

Reporting in two papers, the researchers have discovered the switch that enables breast cancer cells to travel to and be received in the lungs.

The results appear in two separate papers, one in the September 12 issue of the Proceedings of the National Academy of Science Early Edition and the other in the August 22 issue of Oncogene.

"Metastasis transforms breast cancer from a local, curable disease, to one that is systemic and lethal," says Gregg L. Semenza, M.D., Ph.D., the C. Michael Armstrong Professor of Medicine, director of the Vascular Program in the Institute for Cell Engineering and a member of the McKusick-Nathans Institute of Genetic Medicine at Johns Hopkins. "Metastasis was long thought a late event in cancer progression, but we have now shown metastasis to be an early event that is dependent on HIF-1"

Discovered by Semenza's team nearly 20 years ago, the HIF-1 protein controls genes that enable cells to survive in low oxygen, like cells in solid tumors. More recently, others have found that in patients with breast cancer, an increase in HIF-1 activity correlates with increase in metastasis and decreased survival.

To uncover the role of HIF-1 in breast cancer metastasis to the lungs, the research team first looked at the lung, which is prepared for the arrival of metastatic cells by enzymes that are produced by the breast cancer cells. Using human breast cancer cells, the research team examined the genes that encode these enzymes and found regions where HIF-1 could bind to the DNA. Since HIF-1 is active in low oxygen, the team genetically engineered and reduced the amount of

HIF-1 the cells could make, then examined how active the enzyme-producing genes were in cells grown in normal or low oxygen levels. They found that the cells were unable to produce these enzymes without HIF-1.

The team next implanted some of these same human breast cancer cells -- some that made normal amounts of HIF-1 and some that made reduced amounts -- into mice and examined the lungs after 45 days. Compared with breast cancer cells that made normal amounts of HIF-1, those making less HIF-1 resulted in smaller tumors and fewer changes in the lung, leading them to conclude that HIF-1 is critical for lung metastasis.

In order for breast cancer cells to spread to lungs, they must leave the breast, enter blood vessels that lead to the lungs, and exit those same vessels. "Blood vessels are pretty tight, a cell has to work pretty hard to get through the vessel wall," says Semenza.

"Since HIF-1 triggers the lung to prepare for arriving breast cancer cells, we wondered if HIF-1 also is involved in getting cells into and out of blood vessels."

Semenza's team used breast cancer cells grown in low oxygen to examine the activity of 88 genes known to play a role in metastasis.

Looking for genes that are turned on in response to low oxygen, they found one called angiopoietin-like 4 and one called L1 cell adhesion molecule, known as ANGPTL4 and L1CAM for short. Further examination of the DNA around these genes revealed regions where HIF-1 could bind, and removing HIF-1 from cells rendered them unable to turn on the two genes.

When breast cancer cells turn on ANGPTL4, it helps them travel through blood vessel walls, the team found by injecting these cells either with normal or "knocked-down" levels of ANGPTL4 into mice and examining their lungs. Cells lacking HIF-1 and containing extra ANGPTL4 were better able to invade the lungs than cells without extra ANGPTL4; the researchers concluded that ANGPTL4 promotes cell exit from blood vessels. And they found the same to be true for L1CAM.

Lastly, a few years ago, Semenza's team found that digitalis/digoxin, commonly used to treat irregular heartbeats, can block HIF-1 production and can stop liver and prostate cancer cells from growing.

To see if digitalis could do the same with metastatic breast cancer, the researchers transplanted human breast cancer cells into mice.

After two weeks, they gave the mice daily injections of digitalis or saline. They found both fewer and smaller lung metastases in mice treated with digitalis.

"This is really exciting," says Semenza. "The therapeutic range for digoxin is well established, and our findings warrant clinical trials to determine if these doses are enough to sufficiently block HIF-1 and slow breast cancer growth and metastasis."

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