A genetic 'gang of 4' drives spread of breast cancer

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Studies of human tumor cells implanted in mice have shown that the abnormal activation of four genes drives the spread of breast cancer to the lungs. The new studies by Howard Hughes Medical Institute researchers reveal that the aberrant genes work together to promote the growth of primary breast tumors. Cooperation among the four genes also enables cancerous cells to escape into the bloodstream and penetrate through blood vessels into lung tissues.

Although shutting off these genes individually can slow cancer growth and metastasis, the researchers found that turning off all four together had a far more dramatic effect on halting cancer growth and metastasis. Metastasis occurs when cells from a primary tumor break off and invade another organ. It is the deadliest transformation that a cancer can undergo, and therefore researchers have been looking for specific genes that propel metastasis.

In the newly published experiments, the researchers also found that they could reduce the growth and spread of human breast tumors in mice by simultaneously targeting two of the proteins produced by these genes, using drugs already on the market. The researchers are exploring clinical testing of combination therapy with the drugs—cetuximab (trade name Erbitux) and celecoxib (Celebrex)—to treat breast cancer metastasis.

The research team, led by Howard Hughes Medical Institute investigator Joan Massagué at the Memorial Sloan-Kettering Cancer Center, published its findings in articles in the April 12, 2007, issue of the

journal Nature and in the online early edition of the Proceedings of the National Academy of Sciences on April 9, 2007.

In an earlier study, Massagué and his colleagues had identified 18 genes whose abnormal activity is associated with breast cancer's ability to spread to the lungs. In the new study published in Nature, Massagué and his colleagues at Sloan-Kettering, along with researchers from Hospital Clinic de Barcelona and the Institute for Research in Biomedecine in Spain, focused on four of these genes. These genes, which code for proteins called epiregulin, COX2, and matrix metalloproteinases 1 and 2, were already known to help regulate growth and remodeling of blood vessels, said Massagué.

"Our understanding of the genes for these four proteins and their behavior in metastasis led us to hypothesize that they might be cooperating with each other in a way that would give an advantage to cells in the primary tumor," said Massagué. "These same genes, we believed, might also be used for some related purpose in the target organ, the lung."

To test this idea, the researchers silenced various combinations of the four genes in human breast cancer cells that had metastasized to the lung, and then tested these cells in mice. To silence the four genes, the scientists used a technique called RNA interference, in which RNA molecules are tailored to suppress expression of target genes.

"We found that depriving aggressive metastatic tumor cells of these genes decreased both their ability to grow large aggressive tumors in the mouse mammary gland and also the ability to release cells from these tumors into the circulation," said Massagué. "The remarkable thing was that while silencing these genes individually was effective, silencing the quartet nearly completely eliminated tumor growth and spread."

Microscopic analysis of blood vessel structure in the tumors revealed that knocking down all four genes greatly reduced growth of the tangle of blood vessels typically seen in tumors. Further experiments revealed that the tumor blood vessels that did form allowed fewer cancer cells to escape into circulation.

The researchers next explored how loss of the four abnormal genes affected the metastatic capability of the cells in the lung. They injected cells deficient in the four genes directly into the circulatory system of mice. "When these cells reached the lung capillaries, they just got stuck there," said Massagué. "We concluded that metastatic cells use these same genes to loosen up cells in capillaries, so that the cells can penetrate the lung tissue to grow there.

"These findings provide a beautiful explanation for how the genes that we identified in breast cancer patients as being associated with lung metastasis manipulate blood vessels to give them an advantage both in the primary tumors and in the lung," he said.

Two drugs already on the market act directly on proteins produced by the genes Massagué's group had been studying. Cetuximab is an antibody that blocks the action of epiregulin and is used to treat advanced colorectal cancer. Celecoxib is an inhibitor of COX2 that is used as an anti-inflammatory, and is being tested in clinical trials against many types of cancer. The researchers also tested whether cetuximab and celecoxib would work effectively in concert to reduce metastasis in mice.

"We found that the combination of these two inhibitory drugs was effective, even though the drugs individually were not very effective," said Massagué. "This really nailed the case that if we can inactivate these genes in concert, it will affect metastasis," he said.

Massagué said that while clinical trials of the drug combination are being discussed, "there are already treatments to diminish the chance of metastasis in breast cancer, so such trials would have to be designed very carefully to understand how and whether the new drug combination would be of additional benefit." In the article published in the Proceedings of the National Academy of Sciences, Massagué and his colleagues explored how the entire group of 18 genes, called the 'lung metastasis gene-expression signature' (LMS) influenced both breast tumor growth and spread to the lungs. Co-authors on the paper were from the University of Chicago, The Netherlands Cancer Institute, Veridex L.L.C., The Cleveland Clinic and the Erasmus Medical Center in The Netherlands.

"There has been an undeniable link between tumor size and growth and metastatic risk, but the molecules and mechanisms underlying this link have remained unresolved," said Massagué. "The hypothesis we wanted to test was that these signature genes play a role in both primary tumor growth and metastasis to the lung."

After analyzing 738 human breast cancer tumors, the researchers concluded that those in which the LMS genes were abnormally active were, indeed, more likely to develop lung metastases. They also found that the activity of these LMS genes gave cancer cells a growth advantage by allowing tumors to develop a rich network of blood vessels to deliver oxygen and nutrients, said Massagué.

Although large tumors are more likely to metastasize, Massagué said his group's findings indicated that the activity of the LMS genes was also critical to the metastasis process. "As the tumors grow and become enriched with LMS-positive cells, because the genes give them an advantage, they reach a point where the tumor becomes richly vascularized," said Massagué. "Then, they can massively execute the advantage the LMS genes provide them to metastasize to the lung."

Massagué said he and his colleagues will explore in more detail the function of other LMS genes, in addition to the four reported in the Nature paper. They plan to investigate whether shutting down other LMS genes will affect metastasis of breast cancer to the lung, and whether the LMS genes influence breast cancer metastasis to other sites, such as the bone and brain. Finally, they will explore whether the LMS genes play a corresponding role in metastasis of other cancers -- such as sarcoma, melanoma and colon cancer -- to the lung, said Massagué.

Source: Howard Hughes Medical Institute