

JULY 28, 2005

Researchers Pinpoint Genes that Drive Spread of Breast Cancer to Lungs

Howard Hughes Medical Institute researchers have identified a telltale set of genes that causes breast cancer to spread and grow in the lungs, where cancer cells often flourish with lethal consequences.

The researchers said that the genes are more than markers that identify the presence of metastatic cancer. These genes are mediators that enable fragments of breast cancer tumors to take root in the lungs.

The scientists are hopeful that their research will give clinicians a new set of molecular tools to test tumor biopsies for the activity of these specific genes. This, in turn, should help guide treatment by permitting the early diagnosis of breast cancers that will ultimately metastasize to the lung.

"It's all about selection, but it's a combination of Darwin and Murphy. These cells are mutating in a largely random fashion, so a mutation that gives a cell an advantage will be selected for in a Darwinian manner. And like Murphy's Law, if a cell can get nasty, it will get nasty."

- Joan Massagué

The genes that have been identified produce proteins that may move to the top of the "most wanted list" of prime targets for therapies to thwart metastasis, said the study's senior author Joan Massagué, a Howard Hughes Medical Institute investigator at Memorial Sloan-Kettering Cancer Center. Massagué and his colleagues are optimistic that their technique can be extended to other types of metastatic cancer, where it should aid in identifying genetic characteristics of metastatic tumors to aid diagnosis and treatment.

Massagué, postdoctoral fellow Andy J. Minn, and graduate student Gaorav P. Gupta, led the research team that published its findings in the July 28, 2005, issue of the journal *Nature*.

Until now, researchers had taken a largely ad hoc approach to identifying genes that might influence how breast cancer spreads. Such studies usually involved overactivating individual candidate genes and observing whether that change in activity affected the cancer's ability to spread. Such piecemeal studies did not yield a comprehensive, unbiased picture of the broad genetic changes that drive metastasis, according to Massagué.

More recent advances, including large-scale surveys of gene activity, have revealed that a tumor's metastatic potential can be identified and defined as a characteristic genetic signature that reflects gene activity in primary tumors. But those techniques only go so far, said Massagué. Those signatures are useful markers of metastasis, but they do not necessarily reveal the identity of specific genes that drive the metastatic process, he said.

In earlier studies in mice, Massagué and his colleagues pioneered a novel approach that led to the identification of a “toolbox” of genes active in metastatic breast cancer cells that help those tumors spread to bone. The new studies reported in *Nature* build on that earlier work, but with a twist: This time the researchers set out to identify genes that spur metastasis of cancer cells to lung, and to demonstrate that those genes play a role in the establishment of human tumors.

“Reasoning as biologists, we felt that when tumor cells escape into circulation and reach the various organs, these organs are adapted by millions of years of evolution to maintain their integrity and to eliminate those cells,” said Massagué. “So, the few metastatic cells that overcome those barriers must have a very special genetic endowment. And since the lung and the bone each impose very strong but different selective pressures, they must be selecting for different genetic abilities. And if this is true, there have to be different sets of active genes for the metastatic cells to survive in each of these organs.”

Thus, in their experiments, Massagué and his colleagues injected mice with cultured cells originally taken from the lung of a breast cancer patient with metastatic cancer. They selectively isolated the specific cancer cells from those mice that were distinguished by aggressive lung metastasis. “In essence, we used a mouse as a cell sorter to pinpoint the cancer cells,” said Massagué.

Once they had isolated the aggressive metastatic cells, the researchers then analyzed the activity of the cells' genes using DNA microarrays—which are capable of measuring the activity of a vast number of genes at once. This analysis revealed a “metastasis signature” of genes whose activity uniquely distinguished the cells that aggressively spread to the lung. Generally, these

genes code for proteins that are receptors on the cell surface or external secretory proteins that influence the external “microenvironment” of the cells.

“While our resulting genetic signature correlated very well with the ability of these cells to form metastases in the lungs, the next question was whether these genes were actually mediating that metastasis, or were they simply markers,” said Massagué.

The researchers next tested the effects of either overactivating or suppressing the activity of those genes in cancer cells in various combinations. They found that different combinations of those genes did, indeed, profoundly affect that metastatic capacity of those cancer cells in the lungs.

“Up to this point, we had carried the research as far as we had with our previous studies of bone metastasis,” said Massagué. “Now, however, we proceeded to explore the effects of these genes in primary human tumors. And that is where we found the most amazing surprise.”

In analyzing the genetic signatures produced by tumors that had been excised from 82 patients, the researchers found that those patients whose cancers had metastasized to the lung exhibited the patterns of gene activity characteristic of lung metastasis that they had identified in their cell studies. The researcher also analyzed data from another group of patients in the Netherlands, finding that those patients' genetic metastasis signature also correlated with lung metastasis in those patients.

According to Massagué, further study of the roles of the metastatic genes indicated that some genes might enhance survival of particular cancer cells in the primary tumor and spread to the lung, while others might afford those cells growth advantages once they take hold in the lung.

“It's all about selection,” said Massagué. “It's a combination of Darwin and Murphy. These cells are mutating in a largely random fashion, so mutation that gives a cell an advantage will be selected for in a Darwinian manner. And like Murphy's Law, if a cell can get nasty, it will get nasty.”

Studies are now underway to understand the predictive value and function of each of the metastatic genes identified, said Massagué. Once progress has been made on that front, Massagué believes the new knowledge could have profound implications for both diagnosis and treatment of breast cancers.

“Clinicians tell me that the management of a patient who is likely to relapse—or likely to relapse to the bone versus the lung—is very different than managing non-relapsing patients,” he said. “So at minimum, such knowledge of the likelihood of relapse will help the patient and the physician to be prepared. Also, knowing the organ in which the metastases may recur makes it feasible to follow up more effectively with tests on that patient after

removal of the primary tumor, to look for any sign that the tumor is coming back.”

In terms of potential for treatments to block metastasis, said Massagué, “Since many of these genes encode receptors or products that the cells secrete, they are pharmacologically more accessible to be blocked than genes whose products act inside the cell. What’s more, compounds already exist to block some of the genes we have identified or the cell functions they regulate. The next step is to begin testing these compounds in animal models to see if they can block metastasis.”

More broadly, said Massagué, the technique of isolating metastatic cells and identifying their characteristic genetic signatures can be readily extended to metastasis of breast cancers to other organs, as well as metastasis in other cancers. He and his colleagues are now collaborating with other laboratories to compare the genetic signatures of other cancers that metastasize to the lung.