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Cancers Use "Cellular Bookmarks" to Target Favorite Sites of Metastasis

Howard Hughes Medical Institute researchers and their colleagues have discovered that non-malignant bone marrow cells establish “cellular bookmarks” in target organs that guide the spread of cancer cells to their predetermined destination.

The researchers said their findings could have a major impact on how oncologists assess the likeliness of metastasis to specific organs. Their discovery may also help identify subsets of high risk cancer patients who are prone to distant metastases. Those patients would likely benefit from a more aggressive adjuvant therapy to prevent cancer relapse.

Ultimately, understanding how cellular bookmarking works at the molecular level could lead to new information that may help thwart metastasis, a major cause of death among cancer patients, said one of the study's senior authors, Shahin Rafii, a Howard Hughes Medical Institute investigator at Weill Medical College of Cornell University.

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- **Shahin Rafii**

The researchers, led by David Lyden and Rafii, published their findings in the December 8, 2005, issue of the journal *Nature*. Lyden and his colleagues are at Memorial Sloan-Kettering Cancer Center and the Weill Medical College.

Rafii and Lyden's group had established that a specific subset of bone marrow derived cells (BMDCs)—which are comprised of hematopoietic progenitor cells capable of dividing and forming colonies—are recruited by tumors to aid in the growth of new blood vessels. The generation of new

blood vessels occurs through a process called angiogenesis. In previous studies, the researchers had shown that co-recruitment of hematopoietic BMDCs expressing the angiogenic factor receptor, VEGFR1, along with the vascular cells accelerated the assembly of newly formed blood vessels and tumor growth.

“In the current paper, we set forth another novel concept by demonstrating that a non-malignant cluster of VEGFR1-positive hematopoietic BMDCs were recruited to a pre-metastatic niche, thereby establishing a permissive docking site prior to the arrival of the circulating tumor cells,” said Rafii. Biologists use the term “niche” to describe a specialized cellular microenvironment that provides support to specific types of cells. A “pre-metastatic niche” is a cellular microenvironment that is specialized for the development of metastatic tumor cells.

In experiments with mice that had been implanted with highly metastatic lung cancers or melanoma cells, the scientists discovered that BMDCs did, indeed, arrive at the pre-metastatic sites before the arrival of cancer cells. The researchers also found that such clusters appeared prior to the development of metastases in mice genetically predisposed to developing tumors—a system that closely mimics how cancers develop.

The researchers showed that interference with the mobilization of VEGFR1-positive cells from the bone marrow and incorporation into the pre-metastatic niche resulted in a significant decrease in subsequent tumor metastasis. Moreover, depleting VEGFR1-positive cells or inhibiting the function of VEGFR1 itself was also sufficient to retard tumors from spreading to their common predestined metastatic sites.

Remarkably, tumor types determined the pattern of organ localization of BMDCs. By releasing soluble factors, tumor cells were directing BMDCs to spread to the sites they were supposed to go, Rafii said. For example, melanoma cells that have the capacity to metastasize to virtually every organ, released factors that directed incorporation of VEGFR1-positive BMDCs to all of the organs that are known to be the common sites for melanoma metastasis.

Furthermore, the scientists identified a number of regulatory molecules, including the adhesion molecule VLA4, and the protease MMP-9, which were necessary for BMDCs to establish the pre-metastatic niches in target organs and for tumor cells to find and attach to those niches. VLA4 enables attachment of BMDCs to components of tumor stroma, such as fibronectin. “In particular, we discovered that soluble factors released by the tumor cells selectively stimulated the production and deposition of a matrix molecule, fibronectin, which provided a docking site for the attachment of BMDCs before arrival of the tumor cells,” said Lyden.

Rafii and Lyden's group made another clinically relevant observation when they found numerous VEGFR1-positive clusters in various tissues obtained from patients with breast, lung and esophageal cancers. Conventional diagnostic techniques, such as light microscopy, may fail to detect very small micrometastatic tumors in the lymph nodes positioned in the immediate vicinity of the primary tumor, said Rafii. But the presence of VEGFR1-positive clusters might indicate undetected micrometastases, or impending metastasis. This would suggest that these particular patients may be at higher risk for tumor metastasis and thus should be treated more aggressively with adjuvant chemotherapy to prevent future recurrence of the tumor.

Rafii said that the findings in this paper also raise the intriguing proposition that tumor metastatic potential may not only be dependent on the oncogenicity of the cancer cells, but also on the existence of developmentally pre-metastatic niches or “hot spots” in the body that are receptive to metastatic cells.

“It is conceivable that the number and capacity of these hot spots to permit attachment of tumor cells may be determined by the genetic makeup of any given patient,” said Rafii. “For example, the propensity and magnitude of incorporation of VEGFR1-positive cells into various organs may differ from one patient to another, and might explain, for example, why subsets of patients with early-stage colon cancer are more prone to liver metastasis, while others with an identical stage of cancer and oncogenic repertoire are cured of their disease with timely surgery and adjuvant chemotherapy.”

When oncologists diagnose a tumor at an early stage—such as colon, lung or breast cancers—they face a dilemma about what to do after the surgeon has removed the tumor, particularly when pathological examination does not show any evidence or microscopic metastasis. For example, on average, only thirty percent of patients with fully resected primary tumors may relapse, while others are most likely cured of their disease.

“Therefore, it is unnecessary to expose patients at low risk for relapse with adjuvant therapy by delivering high doses of toxic chemotherapy which is usually associated with significant morbidity. However, there is a possibility that the presence of VEGFR1-positive hematopoietic BMDC clusters in the resected ‘tumor free’ tissues portends poor prognosis. As such, these patients may benefit from treatment with aggressive chemotherapy to eradicate any small metastatic foci, decreasing the chances of tumor relapse,” Rafii said.