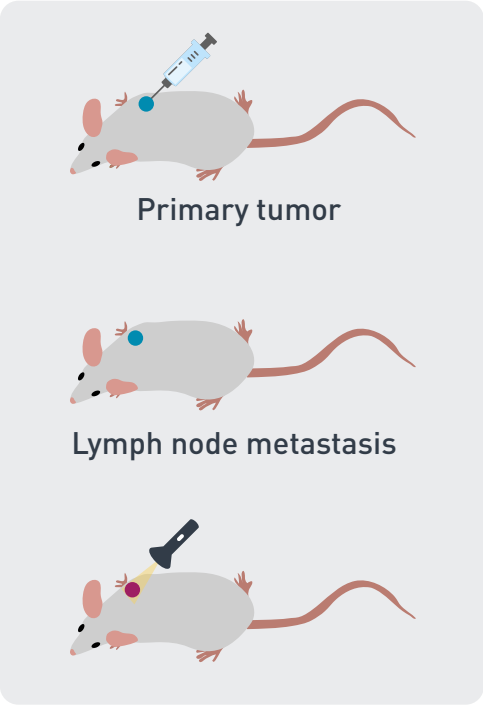
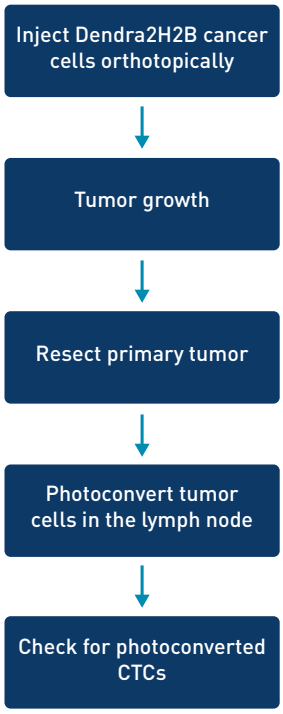
Transit of Tumor Cells Through Lymph Node Vasculature

Why are lymph node metastases often a precursor to more aggressive disease?





Photoconvertible Cancer Cells in Mice

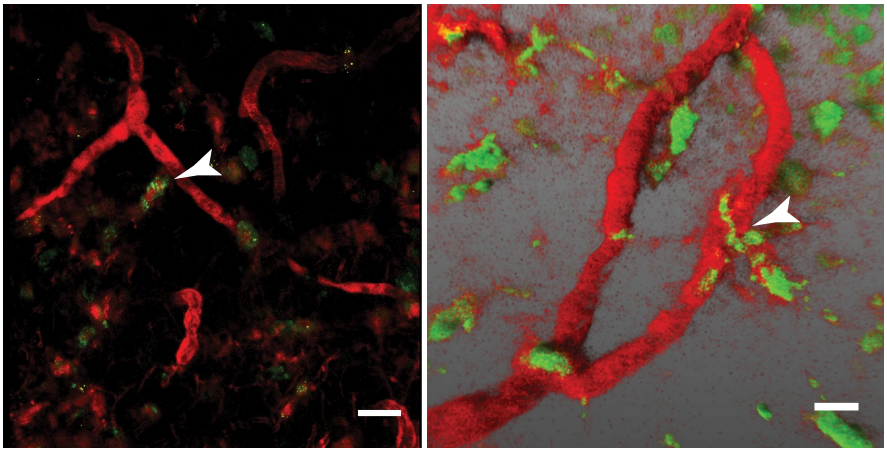
Cancer cells expressing the photoconvertible protein Dendra2H2B were injected into mice. Approximately 20 days later, the primary tumor was surgically removed, and tumor-draining lymph nodes were photoconverted using a diode for five consecutive days. Blood was then analyzed with a flow cytometer for the presence of red fluorescent circulating tumor cells, which are shed into the blood from the lymph nodes.

Lymph node metastases are associated with poorer outcomes for most cancers, but the reason this happens has been a subject of controversy. One camp held that lymph nodes played no unusual role; they theorized that perhaps only very aggressive cancers reached the lymph nodes, or metastases were discovered more often there because lymph nodes tend to get biopsied more routinely. A second group held that lymph nodes are, for some reason, often the first site of metastases and that these tend to accelerate the spread of cancer to other sites.

Clinical trial data was available to support both sets of hypotheses. But now, researchers from Massachusetts General Hospital Cancer Center have used intravital microscopy—putting a live animal under a microscope to image disease processes over time—and photoconvertible proteins to label cancer cells, whose migration through the body can then be tracked, to try to settle this question. By tracing the path of tumor cells into and out of the lymph nodes of mice, they were able to determine that lymph node metastases can spread cancer cells to distant sites via lymph node blood vessels. That paper was published in *Science*[1] in March, alongside a paper from the Clinical Institute of Pathology at the Medical University of Vienna that addressed the same question using different tools.

CAPITALIZING ON A TECHNOLOGICAL ADVANCE

The Mass General Cancer Center team had been following technical developments in many fields and determined that the tools had advanced enough to detect the movement of cancer cells through the body of the mouse.



Cancer Cell Migration Toward Blood Vessels

Cancer cells (green) that invaded the lymph node cortex are shown here wrapped around blood vessels at the arrowheads. The blood vessels were stained red by intravenous injection of rhodamine-dextran. Images were obtained by multiphoton microscopy, in which pulsed high-intensity lasers are used to excite individual molecules.

"The technology had caught up to the question, so we were able to weigh in on whether cancer cells can be spread from lymph node metastasis," says Timothy P. Padera, PhD, associate professor of radiation oncology at Mass General Cancer Center and a co-author of the paper.

To track the spread of cancer, Dr. Padera and his colleagues first implanted the mice with murine cancer cell lines that expressed the fluorescent fusion protein Dendra2H2B, which can change colors under the application of light. They tried three murine cancer cell lines: breast cancer, melanoma and squamous cell carcinoma. After implanting the cells in a primary tumor site, they surgically removed the primary tumors and used a laser diode in the draining lymph node to transform the cells that metastasized there from green to red fluorescence. The assumption was that any red cells they found elsewhere in the body would have migrated from the lymph node and not directly from the primary tumor.

When the blood and lung tissue of the mice were examined, the researchers found red cancer cells circulating in the blood and growing in the lungs of the mice carrying breast cancer and melanoma, evidence that the metastases had come directly from the lymph nodes. The same results did not hold for the mice carrying squamous cell carcinoma.

The team then set out to test whether metastases could also originate from the primary tumor without transiting the lymph node. They photoconverted a fraction of the primary tumor to red fluorescence and not the lymph node. They then detected these red cells in the blood of the mice. Next, they surgically removed the lymph nodes from mice before injecting breast cancer cells into the mammary fat pad. Two weeks after removing the primary mammary fat pad tumor from these mice, they found metastases in the lung, offering further evidence that the lymph node was not needed to spread the cancer cells. In other words, what they found is that metastases can spread in at least two ways: from the lymph node and from the primary tumor.

LEAVING THE LYMPH NODE

Next, they wanted to understand what route cancer cells take to leave the lymph node and invade other organs. Prior research had shown that lymph node metastases often develop along a chain of lymph nodes in human patients, suggesting that cancer cells travel via the lymph vessels from one node to another. But the researchers were somewhat surprised to find that the cancer cells exit lymph nodes via blood vessels rather than the lymph vessels. They discovered this by using image analysis, which showed a close association between the metastatic

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cancer cells and the lymph node blood vessels—an association not found with lymphatic vessels.

To confirm that metastatic cancer cells have an affinity for these blood vessels of the lymph node, they used time-lapse intravital microscopy to measure their movements. Sure enough, cancer cells migrated toward the blood vessels or directly in the blood of the mice. Knowing this predilection for blood vessels may help researchers design cancer therapies in the future.

"What we are going to do next is understand the biology of these cancer cells," says Ethel R. Pereira, PhD, lead author of the paper and research fellow in radiation oncology at Mass General Cancer Center. They will look for chemokines—signaling molecules that regulate cell homing and migration—or growth factors in the microenvironment of the lymph nodes that may play a role in directing these cancer cells toward blood vessels. Such chemokines might serve as therapeutic targets that could inhibit vascular invasion and spread.

Understanding this biology could also help physicians risk-stratify patients according to the likelihood that their lymph node metastasis may advance. And that should help with treatment decisions: who should have a higher dose of radiation or chemotherapy, and who can be spared toxic treatment.

"Right now nearly everyone gets chemo or systemic therapy," says Dr. Padera. "It would be nice to have a better sense of whether lymph node metastases are driving progression in individual patients in order to determine who really needs chemotherapy and who can be spared. We want to develop drugs that will attack cancer cells where they need to be treated."

1 Pereira E, Kedrin D, Seano G, et al. Lymph node metastases can invade local blood vessels, exit the node, and colonize distant organs in mice. ***Science***. 2018; 359(6382):1403-1407.

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