Fast, simple-to-use assay reveals the 'family tree' of cancer metastases

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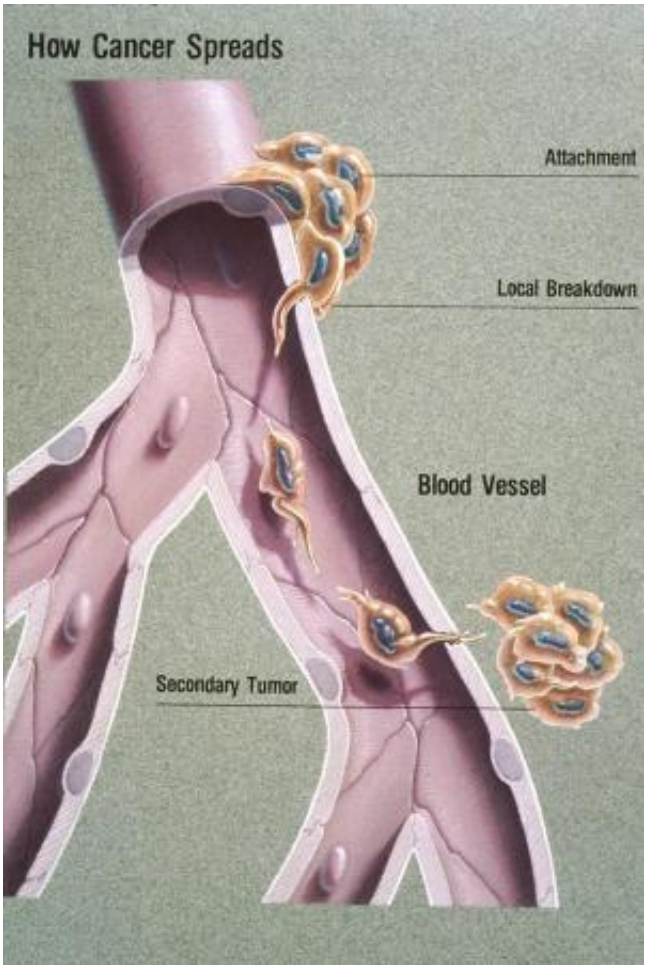

Illustration showing hematogeneous metastasis. Credit: National Cancer Institute

A Massachusetts General Hospital-based research team has developed a simple assay that can reveal the evolutionary relationships between

primary tumors and metastases within a patient, information that may someday help with treatment planning.

The process of metastasis – a tumor's ability to spread to other parts of the body – is still poorly understood. It is not easy to determine whether metastasis began early or late in the development of the primary tumor or whether individual metastatic sites were seeded directly from the original tumor or from an intermediate site. Now a research team has developed a simple assay that can reveal the evolutionary relationships among various tumor sites within a patient, information that may someday help with treatment planning.

"If we could build a 'family tree' of all cancer nodules in a patient, we could determine how different tumors are related to each other and reconstruct how the cancer evolved," says Kamila Naxerova, PhD, of the Steele Laboratory for Tumor Biology at Massachusetts General Hospital (MGH), corresponding author of the report being published in *PNAS Early Edition*. "Usually that would require extensive genetic analysis with complex sequencing methods, but our methodology achieves that goal quickly and with minimal experimental effort."

Cancer researchers are just beginning to investigate the extent and significance of genetic differences among tumor cells – either cells within a discrete tumor or between a primary tumor and metastases in other parts of the body. The authors note that there are two different models of metastasis – one in which an advanced primary tumor disseminates metastatic cells late in its development, which would predict little genetic difference between primary and metastatic cells, and another in which metastasis occurs early in tumor development, which would predict significant genetic differences in metastatic cells that have evolved separately from those in the primary tumor. Some studies have suggested that the two models apply to different types of cancer, but patient data so far has been limited.

Answering important clinical questions – such as whether genetic diversity is a risk factor for aggressive tumor development or how it relates to treatment resistance – requires analyzing samples from many patients with different types of cancer. Using technologies like whole genome or whole exome (the protein-coding portion of the genome) sequencing requires specialized equipment and advanced data analysis and is still relatively expensive. The approach developed by the MGH team focuses on small areas of the human genome – so-called polyguanine (poly-G) repeats that are particularly susceptible to mutation, with genetic 'mistakes' occurring frequently during cell division. While these mutations do not directly relate to the development or progression of a tumor, they can reveal its lineage – how individual tumor cells are related to each other.

In the current paper, the authors adapt Poly-G repeat analysis – initially developed to study lineage relationships between single cells in mice – to the study of human cancer for the first time. Analyzing the poly-G profiles of primary and metastatic colon cancer samples from 22 patients revealed that how the primary and metastatic tumors related to each other was different for each patient. In some individuals there were significant genetic differences between tumor sites, suggesting early metastatic spread; in others, there was little difference between a primary tumor and its metastases. The investigators also identified instances in which the genetic profiles of metastases were similar to those of only some cells in the primary tumor, suggesting that those cells were the source of the metastases, and other cases in which the genetic profiles of metastases from the same primary differed depending on their location.

"We found that there are several paths that can lead to metastatic disease," says Naxerova, a postdoctoral research fellow in the Steele Lab. "We are now applying this methodology to address specific clinically relevant questions about the biology of metastasis in larger

numbers of patients. The method is fast and inexpensive and should be applicable to other types of tumors than colon cancer."

Co-author Elena Brachtel, MD, from MGH Pathology notes that archival tissues from the files of the department were used for this study. "After diagnostic studies on tissue removed during a patient's operation are completed, the formalin-fixed paraffin tissue blocks are stored for several years. Increasingly, new molecular tests can be performed on tissue that was removed from a patient several years earlier, at a time when these tests were not yet available."

Rakesh K. Jain, PhD, director of the Steele Lab and senior author of the paper, adds, "The assay has many potential clinical applications. For example, it could be used to reliably and quickly distinguish a metastasis from a second, independent tumor. Or it could identify the primary tumor in situations where multiple lesions are present and it is ambiguous which one is responsible for seeding metastases." Jain is the Cook Professor of Radiation Oncology (Tumor Biology) at Harvard Medical School.

More information: Hypermutable DNA chronicles the evolution of human colon cancer, *PNAS*, 2014. www.pnas.org/cgi/doi/10.1073/pnas.1400179111

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