Decoding tumor genomes reveals clues to spread of deadly breast cancer

April 14 2010

Using powerful DNA sequencing technology to decode the genomes of cancer patients, scientists at Washington University School of Medicine in St. Louis are getting an unprecedented look at the genetic basis of a highly lethal breast cancer that disproportionately affects younger women and those who are African-American.

They have decoded the genome of a 44-year-old African-American woman with so-called "triple negative" breast cancer and the genomes of her breast tumor and a metastatic tumor that quickly developed in her brain. By comparing the DNA sequences of the three genomes, the scientists identified 20 genetic changes in a subset of cells in the woman's breast tumor that likely played a role in the metastasis that killed her within months, they report April 15 in *Nature*.

The research suggests that sequencing entire genomes of cancer patients as well as their primary and metastatic tumors can reveal clues to the way the tumors spread and can set the stage for developing more effective drugs that target key genetic errors.

"We are getting an intimate look at the lethal spread of a breast cancer, which is now possible because we can sequence entire genomes quickly at a reasonable cost," says senior author Elaine Mardis, PhD, co-director of Washington University's Genome Center. "This work lays the foundation for understanding the genetic basis of tumor progression and metastasis, and for identifying new drug targets that can improve the outlook for women with this disease."

The patient, the first African-American to have her genome sequenced, had an aggressive, "basal-like" subtype of breast cancer. These genetically unstable tumors account for about 10 percent of all breast cancers and are often called triple-negative because they lack any receptors to which treatment can be targeted. They grow rapidly and typically do not respond well to chemotherapy.

That was the experience of the woman in this study. She initially received chemotherapy to shrink a large breast tumor and then had a mastectomy. When surgeons found evidence of treatment-resistant tumor cells in her lymph nodes, she received radiation therapy, but it did not halt the cancer. The tumor spread to the woman's brain, and she died within a year of her diagnosis.

In the current study, the researchers also sequenced the genome of the patient's breast tumor after growing it in a mouse, to determine whether the animal model can reproduce the genetic profile of the human cancer. The tumor was inserted into the mouse before the patient received cancer treatment.

"The tumor grown in the mouse was remarkably similar to the patient's own breast tumor," Mardis says. "The human tumor and the mouse counterpart shared all of the same mutations and the same fateful course: both spread to other sites. This similarity suggests that mouse models can be valid preclinical surrogates of metastatic disease to evaluate new cancer drugs."

After decoding the genomes of the patient and her tumors, the researchers, led by Li Ding, PhD, research associate professor of genetics, painstakingly sifted through many billions of bases of DNA, identifying 48 single base-pair changes, or mutations, that were shared among the breast tumor, the metastatic tumor in the brain and the tumor grown in the mouse. Then, they looked to see how many cells in the

breast tumor and brain metastasis carried each of these mutations.

Surprisingly, they found 20 mutations that occurred at relatively low levels in the patient's breast tumor that also were present in a much higher percentage of the metastatic tumor cells, suggesting that genetic alterations in the primary tumor drove the spread of the disease.

"This indicates that a small subset of cells with a lethal mutation repertoire break free from the primary tumor, circulate in the body, set up residence in other organs and grow aggressively," say co-lead author Matthew Ellis, MD, PhD, the Anheuser-Busch Endowed Professor in Medical Oncology. "Mutation enrichment is likely to be important for tumor spread since the mutation enrichment pattern in the brain metastasis and the tumor grown in the mouse strongly overlapped, indicating that the process was not random."

For years, scientists studying cancer have asked whether metastasis is driven by mutations that occur after tumor cells arrive at a distant site or whether the primary tumor carries the genetic instructions that unleash the spread of the disease. The Washington University researchers found that the metastatic tumor contained only two additional mutations that were not present in the patient's breast tumor, as well as a missing chunk of DNA. But none of these genetic alterations appeared to be important to the cancer's spread, they said.

They are now decoding the genomes of additional women with basal-like breast cancer to determine whether mutations in their primary tumors also influence the spread of the disease.

Whole genome sequencing involves spelling out all 3 billion letters of an individual's DNA. In this study, the researchers sequenced each of the patient's four DNA samples about 30 times to ensure accuracy.

Unlike sequencing studies that focus only on genes, which make up just 1 percent of the entire genome, whole genome sequencing captures the full breadth of genetic alterations, including large insertions, deletions and other structural changes. Indeed, the researchers found numerous structural changes in the tumor genomes they analyzed, including 28 large deletions, six inversions and seven translocations, an indication of the "genomic chaos" underlying the patient's cancer.

Researchers sequencing only genes miss many of these alterations because they either occur beyond the reach of genes in long stretches of non-coding DNA or in large chunks of missing DNA. For example, the investigators found two large, overlapping deletions in all three tumor samples that rendered both copies of a gene called CTNNA1 non-functional. Because both copies were knocked out, looking only at protein-coding genes would not have revealed the change. Interestingly, loss of this gene has been linked in previous studies to a loss of cell adhesion in human breast cancers, a precursor to metastasis.

The researchers also found a single mutation in the CSMD1 gene, which has been associated with poor survival in invasive ductal breast cancer and is frequently deleted in some colorectal and head and neck cancers. Alterations in the patient's JAK2 and NRK genes also have been observed in other breast cancers.

The new research builds on earlier work by the same team researchers, who less than two years ago became the first to sequence the entire genome of a cancer patient, a woman with leukemia. That research also was published in Nature. Last year, they reported sequencing the genome of a second leukemia patient in the New England Journal of Medicine.

Since then, the Washington University scientists have decoded the normal and tumor genomes of about 150 cancer patients, including those with breast, lung and ovarian cancer as well as glioblastoma, a brain

tumor. They have recently begun sequencing the genomes of 600 pediatric cancer patients, as part of a joint project with St. Jude Children's Research Hospital.

"We've learned some significant lessons about cancer from sequencing the genomes of individual patients and their tumors, but it's clearly just the tip of the iceberg," says Richard K. Wilson, Ph.D., director of The Genome Center and a co-author of the new research. "Moving forward, we'll be comparing tumor genomes from many patients with the same type of cancer to find common genetic alterations. This comprehensive understanding of cancer can aid in the development of new approaches to cancer diagnosis and treatment."

More information: Ding L, Ellis MJ, Weinstock GM, Aft R, Watson M, Ley TJ, Wilson RK, Mardis ER et al. Cancer remodeling in a basal-like breast cancer metastasis and xenograft. Nature. April 15, 2010.

Provided by Washington University School of Medicine