Ovarian Cancer Genome Sequencing Unveils Findings

By Vicki Brower

Scientists may now have a better picture of the genomic landscape of ovarian cancer than of any other malignancy. A 15-group team of the Cancer Genome Atlas (TCGA) Research Network sequenced all the protein-coding segments, or exomes, of nearly 500 ovarian tumors.

“Because of the high number of tumors studied, the research fills in a lot of details not attainable with fewer samples,” said Paul Spellman, Ph.D., TCGA computational scientist and an associate professor in the department of molecular and medical genetics at Portland’s Oregon Health and Science University. Results of the sequencing illuminate mutations driving its development, important signaling pathways, and potential weaknesses. These results, published in the June 30 issue of *Nature*, offer a wealth of new information that, in time, could help researchers develop more targeted treatments for women with ovarian cancer.

The study examined genes from the most common and most aggressive ovarian tumor type, serous adenocarcinoma. Researchers confirmed that a mutated TP53 tumor suppressor gene is involved in ovarian cancer: 97% of tumors in the study carried this mutation. Researchers also identified mutations in nine other important genes that occur at much lower but statistically significant rates, including BRCA1 and BRCA2; tumor suppressors RB1 (the retinoblastoma gene) and NF1 (the neurofibromin gene); and a new tumor suppressor gene not previously associated with ovarian cancer, CDK12, a cyclin-dependent kinase.

According to Gad Getz, Ph.D., director of Cancer Genomics Informatics and Computational Biology at the Broad Institute, in Cambridge, Mass., “another important discovery is that, besides these mutations, ovarian cancer is largely driven by copy number changes [of genes] which are amplified or deleted,” he said, adding, “We have drugs for a number of these changes.” For example, the mutations appear to be sensitive to the PARP [poly (ADPribose) polymerase] inhibitors used to treat women with BRCA1- and BRCA2-related breast cancer. Researchers also found a few patients with mutations in the BRAF gene, for which new drugs are being tested in other cancers.

TCGA is a large cooperative project funded by the National Cancer Institute and the National Human Genome Research Institute. TCGA started in 2006 to sequence 20 different tumor types, beginning with glioma. For ovarian cancer, the project has already spun off several important complementary studies. Collectively, these findings could help elucidate a disease that, according to the American Cancer Society, occurs in an estimated 21,990 women per year and kills 15,460 annually, making it the deadliest cancer among women. “Drug research for ovarian cancer has lagged far behind other cancers because of the relatively low numbers of patients, in spite of the fact that it kills more proportionately than many other malignancies,” said Douglas Levine, M.D., associate attending surgeon and head of the gynecological research lab at the Memorial Sloan-Kettering Cancer Center, in New York.

Ovarian Cancer Hallmarks

The *Nature* study confirms previous research by David Bowtell, Ph.D., professor of cancer genomics and genetics at the Peter MacCallum Cancer Centre, in Melbourne, Australia. He found that the TP53 tumor suppressor gene is nearly always mutated in high-grade serous ovarian cancer and that TP53 damage occurs early in the genesis of ovarian cancer, rather than later as in colorectal cancer (see July 2011 Cancer Discovery and May 2010 *Journal of Pathology*). “Given
that TP53 mutation is both an early and ubiquitous event, what triggers it and whether it is the key molecular event from which the tumor evolves are critical questions,” said Bowtell. If a TP53 mutation could be detected before clinical signs of the disease—when it is already advanced and incurable—treating patients early and arresting further development of ovarian cancer might be possible, he added. Currently, however, no test or drug treatment for this mutation exists.

The TCGA findings also confirmed that disruptions in the BRCA1/2 pathways are another hallmark of ovarian cancer. Defects in the BRCA pathway occur in approximately half of women with high-grade serous tumors. The alterations in the BRCA1 and BRCA2 DNA-repair genes may occur not only as germline mutations but also as independent somatic and epigenetic changes, said Spellman. “This suggests that these tumors may only require one defect to become a malignant phenotype,” he said.

Levine agreed. “That P53 damage is an early event, taken together with three possible types of changes in BRCA1 and BRCA2, may be sufficient to establish a tumor. “In addition to the ubiquitous mutation of the TP53, it is very significant that most other genetic changes were not high-frequency mutations but rather widespread copy number changes such as amplifications and deletions,” Levine continued. Mutations are changes in the DNA sequence of a tumor, and copy number alterations mean that there are too many (amplifications) or too few (deletions) copies of the DNA, but the sequence is intact and not mutated. In many solid tumors, a driver mutation has been identified in key genes and is present in 5%–25% of case patients. But in ovarian cancer, besides TP53, BRCA1, and BRCA2, few high-frequency mutations occur, but many copy number alterations do—more so than in most other solid tumors, Levine explained. “Copy number, in addition to TP53 mutations, is a major driver of ovarian cancer, unlike other solid tumors. This means that we must figure out how targeted therapies will work in copy-number–altered tumors in contrast to tumors with activating somatic mutations,” he said.

Identifying Dysregulated Genes

Using TCGA data to seek sources of dysregulation other than tumor suppressors, Chad Creighton, Ph.D., assistant professor of medicine at the Baylor College of Medicine, in Houston, searched for microRNAs (miRNAs) that might play an anti-cancer role. These miRNAs are small, noncoding, naturally occurring sections of RNA that govern various aspects of the cell cycle and stem cell differentiation, which are now being studied for their use as drugs to fight cancer. Creighton performed molecular profiles on normal and cancerous tissue samples and identified one miRNA, mir31, which is associated with defects in the p53 pathway in ovarian cancer when its function is lost (see March 2010 Cancer Research).

“This suggests that patients with cancers deficient in the p53 pathway might benefit from therapeutic delivery of this miRNA,” Creighton said. “Our goal is to use miRNAs as drugs, employing nanoparticles for delivery into patients,” he said. Creighton recently submitted to a journal new data that follow up the TCGA study. In that report, his team digs more deeply into the 2010 miRNA study, which combines gene expression and microRNA expression data to identify additional anticancer miRNAs and their associated gene targets.

The TCGA data also revealed defects in the RB1 and PI3K–RAS signaling pathways in 67% and 45% of tumors, respectively. These pathways govern cell growth, proliferation, differentiation, motility, and survival. Drugs for the PI3K pathway already exist and are being tested in ovarian and other cancers. Another commonly dysregulated pathway found was NOTCH, for which drugs are also in development. The study also identified 168 epigenetically dysregulated genes, which indicates the potential use of demethylating drugs in ovarian tumors, Levine said.

Disease Sub-types

The TCGA study also supports earlier work by Bowtell indicating that high-grade serous ovarian cancer can be divided into four distinct molecular subtypes according to expression patterns (see August 2008 Clinical Cancer Research). The new study identifies similar subtypes, called immunoreactive, proliferative, differentiated, and mesenchymal, on the basis of gene clusters and predominant cell types that occur in tumors. “These tumor subtypes have more in common with other types of cancers, such as breast, endometrial, or renal clear cell cancer, than with each other,” said Bowtell.

Bowtell has proposed a model of development of high-grade serous cancers, which the TCGA results support. An initial mutation of TP53 and BRCA or other DNA-repair function occurs, followed by genomic instability and possibly enhanced by overexpression of certain proteins such as cyclin E1. This instability causes widespread copy number changes that in turn drives the tumor’s evolution into one of four subtypes (see November 2010 Nature Reviews Cancer).

Cancer’s Achilles Heel

A study in the July 1, 2011, preprint of the Proceedings of the National Academy of Sciences complements the TCGA results. It uses TCGA sequencing information to functionally test what the mutations and alterations it identified actually do: Do they drive the growth of cancer, or are they merely “passengers” and unnecessary for its development? “Many genetic changes, whether mutations or alterations, occur when a tumor is developing as the genome is unstable,” said principal investigator Bill Hahn, M.D., Ph.D., associate professor of medicine at Boston’s Dana–Farber Cancer Institute. Hahn and colleagues established Project Achilles to find cancer’s weak spots.
The *PNAS* study helps sort out which gene defects identified by TCGA are necessary for ovarian cancer to develop and spread. Focusing on the ubiquitous and often-clustered 1,200 gene amplifications, researchers integrated TCGA's structural studies with functional experiments to winnow out drivers of ovarian cancer by comparing gene amplifications to normal tissue. Using RNA interference to turn genes on and off, they first silenced more than 10,000 genes to find driver genes in 25 ovarian cancer cell lines and then compared them to the amplified chromosomal regions (see December 23, 2008, *PNAS*). Narrowing the list to 65 amplified genes, Hahn found which were essential for tumor growth and ultimately identified the PAX8 gene, responsible the development of the female reproductive tract. PAX8 was present in almost one-fifth of ovarian cancer samples studied and when suppressed, led to cell death (see early edition of July 11, 2011, *PNAS*).

Although PAX8 encodes a transcription factor, making it difficult to shut down, Hahn's team is still pursuing the gene as a drug target. By the end of summer, Project Achilles will have screened 250 cell lines. “Our hope is that with these new studies, the scientific community will have a new way of looking at cancer genomes,” said Hahn.

*Dr. Hahn is a consultant for Novartis.*

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