Guiding Genomics Use in Cancer Care

Five major cancer organizations are combining forces to propose standards for applying next-generation sequencing to clinical practice.

The Actionable Genome Consortium (AGC) was founded by Dana-Farber Cancer Institute (Boston, MA), Fred Hutchinson Cancer Research Center (Seattle, WA), The University of Texas MD Anderson Cancer Center (Houston), Memorial Sloan Kettering Cancer Center (New York, NY), and the genome-sequencing company Illumina (San Diego, CA). The group plans to define what constitutes an “actionable event” in individual tumors and to recommend best practices for biopsy, sample storage and transport, and DNA extraction; technical performance standards for DNA sequencing; standards for variant identification, annotation, and interpretation used in whole-genome sequencing; and guidelines for the format and content of clinical reports.

“We want to come up with a panel of therapeutic targets that make sense in terms of medical implications and cost,” says Barrett Rollins, MD, PhD, chief scientific officer at Dana-Farber. “We’re focusing on the baseline things that an oncologist would want to know about every patient’s tumor to order tests that have direct clinical applicability or are very likely to.”

For example, the standards might be used to justify testing for specific genetic alterations in non–small cell lung cancers, such as EGFR mutations or ALK rearrangements, that could be targeted with FDA-approved drugs, he says. Insurance companies would use the standards to determine coverage for tests that may improve clinical outcomes.

Knowing the genetic composition of a tumor may also help oncologists match patients to appropriate clinical trials, says Illumina’s Robert Cohen, MBA, director of the AGC.

“In the future, the signature of a tumor will be measured or attempted to be measured in every cancer patient, and that signature should be one way that patients are matched to clinical trials,” he says. “Enrollment of all appropriate patients in clinical trials would become the standard of care.”

A research arm of the consortium will pursue collaborative projects tackling major issues in molecular oncology, says Cohen. One area of focus will be the potential clinical utility of circulating nucleic acids—for example, whether they could be used as a surrogate for tumor biopsies when tissue-based diagnosis is not possible, such as in patients with bone-only metastases.

The AGC plans to publish its recommendations in early 2015, says Cohen. The group will then work with national cancer organizations to develop official guidelines based on the recommendations.

“The time is right to create uniform standards for the molecular analysis of tumors,” says Eric Holland, MD, PhD, director of solid tumor translational research at Fred Hutchinson. “We’re going from a phase of scientific discovery to implementation and what it really means to society.”

CTC Clusters More Likely to Cause Metastasis

Scientists know that circulating tumor cells (CTC) can clump together in groups of two to 50, but how these so-called CTC clusters form and their functional significance has remained unclear.

Now, new research shows that CTC clusters are tumor fragments, held together by the cell junction protein plakoglobin, that break off into the bloodstream. In breast cancers, these clusters are 23 to 50 times more likely to cause metastasis than single CTCs. These findings suggest that targeting pathways involving plakoglobin expression could be beneficial in reducing tumor dissemination (Cell 2014;158:1110–22).

“What kills patients is the metastasis,” says co–senior author Shyamala Maheswaran, PhD, an associate professor at the Center for Cancer Research at Massachusetts General Hospital Cancer Center in Boston. “We now have better insight into a mechanism that increases the efficiency of the metastatic process.”

Mary-Claire King, PhD, professor of genetics and medicine at the University of Washington in Seattle, won the 2014 Lasker-Koshland Special Achievement Award in Medical Science for her contributions to medical research and human rights. Bestowed by the Albert and Mary Lasker Foundation, the annual Lasker Awards “recognize the contributions of scientists, clinicians, and public servants who have made major advances in the understanding, diagnosis, treatment, cure, or prevention of human disease” and carry a $250,000 honorarium.

In 1974, King began a meticulous analysis of more than 1,500 families of women with breast cancer, concluding that a single gene was responsible for breast cancers in some families. After analyzing DNA from hundreds of participating relatives, she reported in 1990 that a section of chromosome 17 was responsible for early-onset breast and/or ovarian cancer in some of the families. She named the gene locus BRCA1. She also developed DNA-based analysis to help families prove genetic relationships and reunite kidnapped and once-missing children with their biologic families.

In September, Louis J. DeGennaro, PhD, was named president and CEO of The Leukemia & Lymphoma Society (LLS), roles he has held on an interim basis since February; he joined the organization in 2005. DeGennaro has more than 25 years of research, drug development, and management experience in academia and the private sector, including positions at the Max Planck Institute in Munich, Germany, and Wyeth Pharmaceuticals in Princeton, NJ. Headquarters in White Plains, NY, LLS funds cancer research, supports educational and public-policy efforts, sponsors scientific conferences, and provides financial assistance to patients.
Nicola Aceto, et al.

Cytokeratin is shown in red and the nuclei are shown in blue.

A CTC cluster from a breast cancer patient, isolated using the CTC-Chip. Wide-spectrum cytokeratin is shown in red and the nuclei are shown in blue.

In one experiment, researchers injected immunodeficient mice with a 1:1 mixture of human breast cancer cells engineered to express either a green marker or a red marker. Primary breast tumors developed and retained an equal distribution of green- and red-tagged cells. When scientists analyzed the CTC clusters, they found 91% were positive for both markers, suggesting the clusters do not result from the proliferation of a single tumor cell in the bloodstream.

To confirm that clusters are not the result of single CTCs coming together in the bloodstream, researchers injected green-tagged breast cancer cells into the right mammary fat pad and red-tagged breast cancer cells into the left, causing mice to develop two separate tumors. Investigators found 96% of CTC clusters were of a single color, indicating that these CTC clusters were not aggregating in the vasculature but originated from primary tumor fragments.

Although researchers found that CTC clusters make up only 2% to 5% of all CTCs, the clusters contributed to about half of lung metastases in breast cancer models.

Using CTC-Chip, a microfluidic device that captures CTCs from blood samples, the researchers found that patients with metastatic breast cancer and CTC clusters had reduced survival compared to those without clusters. Mean progression-free survival was 160.5 days for patients with only single CTCs compared with 32.6 days for patients with CTC clusters in more than three blood samples obtained at different times.

When researchers conducted RNA sequencing of single and clustered CTCs from breast cancer patients, they found that CTC clusters overexpressed plakoglobin, a component involved in cell-to-cell adhesion. Suppressing plakoglobin expression in breast cancer cells caused cell clusters to fall apart, disrupting cell-to-cell contact between breast cancer cells, but not normal breast cells, and reducing their metastatic potential.

Although researchers did not conduct RNA analysis of CTC clusters in other epithelial cancers, Maheswaran says plakoglobin might keep their CTC clusters together, too.

What remain unclear are the cues that drive clusters to be shed into the blood and the biological properties that enable them to be highly potent in initiating metastasis, researchers note.

“Prevention of metastasis is the holy grail in cancer,” says Maheswaran. “Our work provides a pathway that might potentially be targetable if we understood the mechanism in more detail.”

**KNSTRN Deemed an Oncogene**

Scientists have discovered a new oncogene for cutaneous squamous cell carcinoma (SCC), the second most common skin cancer. The oncogene, known as KNSTRN, appears to be mutated by exposure to UV light (Nat Genet 2014;46:1060–2).

“Finding a new oncogene was very exciting,” says Carolyn Lee, MD, PhD, a clinical instructor in dermatology at the Stanford University School of Medicine in Palo Alto, CA, and the study’s lead author. “It’s important for our understanding of how SCC tumors develop, and it may eventually provide insight into molecular mechanisms with therapeutic implications.”

Lee and her colleagues hit on KNSTRN while investigating genetic causes of cutaneous SCC. They performed whole-exome sequencing on a series of SCCs and patient-matched normal skin samples, yielding a set of 336 candidate cancer genes. They then sequenced these 336 genes in another set of 100 cutaneous SCCs and patient-matched normal skin cells in a targeted search for SCC-associated mutations.

The three most frequently mutated genes included the well-known tumor suppressor genes TP53 and CDKN2A, as well as KNSTRN, a “gene that we were unfamiliar with,” Lee says.

The mutational patterns the scientists found were “characteristic of exposure to UV light,” which is consistent with well-established data linking SCC to sun exposure. In addition, the mutations clustered in an N-terminal region, including a “hotspot” substitution of phenylalanine for serine at codon 24.

That the mutations clustered in one place provides evidence that KNSTRN is an oncogene, according to Lee. Mutations in tumor suppressor genes, such as the BRCA genes implicated in breast and ovarian cancers, usually scatter evenly throughout the gene, she explains, whereas in oncogenes, they more often accumulate in hotspots.

What little data are available on kinastrin function suggest it normally modulates the segregation of chromosomes during mitosis. Lee’s new findings suggest that mutant kinastrin disrupts sister chromatid cohesion and chromosome segregation, and may result in aneuploidy.

To investigate the gene’s oncogenic potential, Lee and her colleagues introduced normal and mutated KNSTRN into normal human skin cells. They found that the mutated gene disrupts chromosome segregation during cell division. More direct evidence that mutant KNSTRN is tumorigenic came when they found that it accelerates tumor growth in a mouse model of cutaneous SCC. Lee’s search of publicly available TCGA data suggests KNSTRN might also play a role in melanoma, but she says its potential role in other cancers isn’t known.

Kenneth Tsai, MD, PhD, a dermatologist and researcher at The University of Texas MD Anderson Cancer Center in Houston who is not affiliated with the study, says the discovery that a single UV-mediated point mutation can turn KNSTRN into an oncogene that accelerates cutaneous SCC tumor growth is important, particularly because other well-known oncogenes such as mutant RAS are not found with high frequency in the disease in humans.

“What we need now is a deep characterization of its function in the cell,” he says, “and then we need to figure out how to disable it.”
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