



A CTC cluster from a breast cancer patient, isolated using the CTC-Chip. Wide-spectrum cyokeratin 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.” ■