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Image courtesy of Dr. Thomas Ried, NCI/NIH

Looking to *SKY* Unlocks Secrets of Cancer Chromosomes

What causes the out-of-control growth of tumor cells? A good way to find out is to study the tumor cells themselves, particularly their chromosomes. In most cases of cancer, these chromosomes have tell-tale abnormalities, ranging from the blatant (an entire chromosome missing, for example) to the less obvious (translocations, in which a piece of one chromosome breaks off and binds to the end of another chromosome).

These chromosomal defects are biological “Rosetta stones” that can yield a wealth of information about the genetic causes of cancer. For example, they can reveal the presence of genes known as oncogenes (which transform once-normal cells into cancer cells) or the loss of genes that help to suppress cancer. But all too often, the chromosome rearrangements that lead to cancer are complex, subtle—and difficult to interpret or even to notice.

At the Einstein Cancer Center, the challenging task of molecular cytogenetics—identifying chromosomal and genetic

abnormalities and determining their role in disease—is headed by Dr. Cristina Montagna, assistant professor of pathology and of molecular genetics.

Dr. Montagna joined the Einstein faculty last August, after spending six years at the National Institutes of Health. There she worked with Dr. Thomas Ried, who developed a powerful new technique for analyzing chromosomes. Dr. Montagna uses this technique, known as spectral karyotyping (SKY), in her research on breast cancer at Einstein. SKY is taking center stage in Einstein’s new Genome Imaging Facility, which Dr. Montagna has set up and directs. This facility—and Dr. Montagna’s recruitment to head it—is supported by philanthropy and a grant from the National Cancer Institute.

Dr. Montagna’s SKY technique excels at revealing translocations and other subtle chromosomal abnormalities that standard karyotyping usually misses. It does so by colorizing chromosomes so

SKY is a technique for visualizing all of a person's (or mouse's) chromosomes in color. A different color is assigned to each chromosome, making it easier to identify and analyze chromosomal abnormalities.

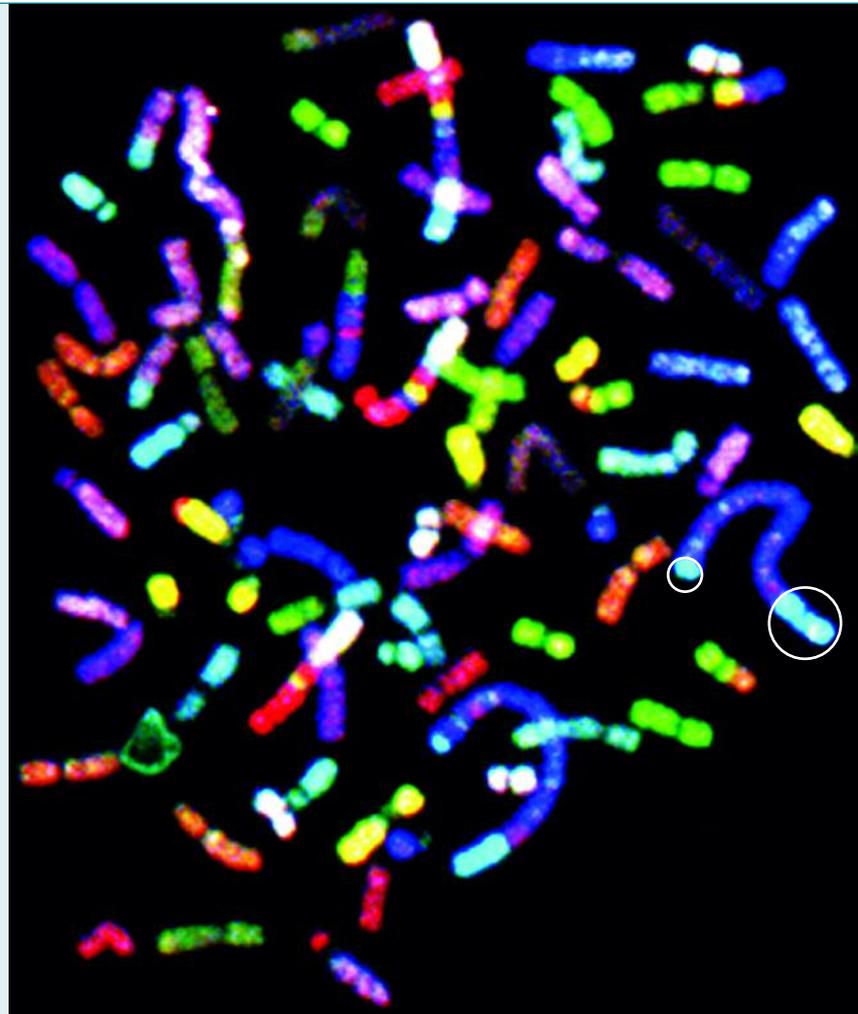
To carry out SKY, researchers use a DNA "library" containing many short sequences of single-stranded DNA called probes. Each DNA probe is complementary to a unique region of each chromosome. The library must contain enough different DNA probes to match up with regions on all the chromosomes of a particular organism.

Each probe is then labeled with a colored fluorescent molecule that corresponds to a particular chromosome. So all probes complementary to human chromosome 1, for example, might be labeled with yellow molecules, those that match up with

chromosome 2 labeled with red molecules, and so on.

When the fluorescent probes are added to chromosomes, they hybridize (bind) to the chromosomal DNA and paint the entire set of chromosomes a rainbow of colors. Using computers and microscopes, scientists can now analyze the painted chromosomes to see if any of them exhibit translocations or other abnormalities.

At right is a SKY image of chromosomes from cells of a human breast tumor. The image reveals numerous translocations, each of which occurs when a piece of a chromosome breaks off and binds to the end of another chromosome. In the circled areas, two pieces from chromosome 13 (colored blue) have translocated to chromosome 8 (colored purple). ■



that each is "painted" a different fluorescent color. That's especially helpful for spotting chromosomal translocations—among the most common chromosomal abnormalities in breast cancer and other solid tumors.

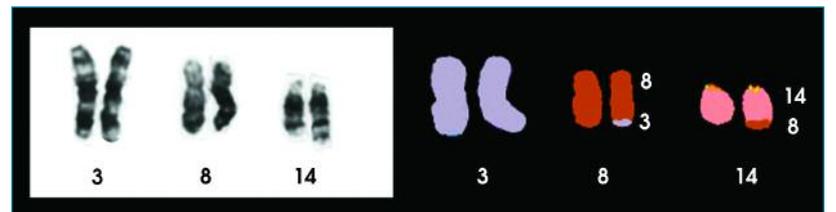
Recently, while using SKY to study the chromosomes in mouse models of breast cancer, Dr. Montagna and her NIH colleagues noticed that a small region of mouse chromosome 11 had essentially gone wild: Not only had it duplicated itself within chromosome 11, but copies had also translocated to several other chromosomes. Additionally, this region appeared in the SKY image as numerous tiny paired specks—so-called "double-minute chromosomes" that often signal the presence of cancer-causing genes known as oncogenes.

"With SKY, these chromosome amplifications were quite obvious in this mouse breast cancer," says Dr. Montagna. "Yet they weren't at all striking in the corresponding chromosomal region of human

breast-cancer cells, and no oncogenes had yet been identified there."

Spurred on by what SKY revealed in the mouse, Dr. Montagna and her colleagues focused on this chromosomal region in both mice and the corresponding human chromosome, known as chromosome 17. Sure enough, detailed analysis revealed that this region of both mouse chromosome 11 and human chromosome 17 contained a single gene, *Sept9*, that had never before been associated with breast cancer and was clearly acting as an oncogene.

Dr. Montagna calls this "a very good example" of how SKY can help reveal chromosomal abnormalities that are important for human cancers. "SKY is a powerful technique that provides us with beautiful images—beautiful but also deadly, and we're always aware of that," she says. ■



On the left, in black and white, is a standard karyotype of three pairs of chromosomes (3, 8 and 14) from a human non-Hodgkins lymphoma tumor. Chromosome 3 looks abnormal, apparently because a section of chromosome 14 has translocated to it. A much different picture is revealed when the same tumor cells are analyzed using SKY (colored chromosomes on the right). Chromosome 3 actually is normal, but chromosome 8 (which seemed normal in the standard karyotype) is shown to contain a portion of chromosome 3. As for chromosome 14, standard karyotyping shows it has acquired a translocation, but its origin is uncertain. SKY analysis clearly shows that chromosome 14's translocation has come from chromosome 8.