# Stanford scientists find new marker to identify severe breast cancer cases

April 14 2010

Women with breast cancer whose tumors express high levels of a particular genetic marker are significantly more likely to die from their disease than are those with more normal levels, according to researchers at Stanford University School of Medicine. The finding implies that blocking the action of the marker — a newly recognized type of RNA — could one day be an effective way to prevent metastasis and improve survival for these women, who make up about one-third of all breast cancer patients.

"We've found that this RNA, called HOTAIR, is a really important player in human health," said Howard Chang, MD, PhD, an associate professor of dermatology and a member of the Stanford Cancer Center. "When it becomes dysregulated in breast cancer, it drives the tumor cells to metastasize and worsens a woman's prognosis."

It does this, the researchers believe, by tinkering with the cells' genome, laying bare regions that are normally kept bundled up and silent in adult cells and silencing others that are normally expressed. "As a result, the cells' gene expression profiles begin to look much more like those of embryonic fibroblasts, and they acquire attributes that allow them to thrive in other parts of the body," said Chang.

Chang is the senior author of the work, which will be published on April 15 in the journal *Nature*. He was named a Howard Hughes Medical Institute Early Career Scientist in March 2009. He collaborated with researchers at Johns Hopkins University School of Medicine, Academic

Medical Center in the Netherlands, the Broad Institute of Harvard, the Massachusetts Institute of Technology and Applied Biosystems Inc. to conduct the research.

Until recently, RNA was thought to exist mostly to carry protein-building instructions from the DNA in the nucleus to protein-making factories called ribosomes in the cell's cytoplasm. However, it's become more clear in the past few years that RNA is much more versatile. Some types can affect how genes are expressed by binding to and even modifying the structure of DNA.

Chang and his colleagues have been studying HOTAIR — named for "HOX antisense intergenic RNA" — for several years now, trying to understand why RNA molecules that aren't translated into proteins are so prevalent and what other things they might be doing. "There's something like 10,000 of these large intervening non-coding RNAs, or lincRNAs, in the human genome," said Chang. "They represent a new class of genes that don't make protein, but that affect gene expression in some mysterious way."

In 2007, Chang and his colleagues reported that HOTAIR plays an important role in helping cells know their location in the body and what they are supposed to become. It works by activating a group of enzymes called the Polycomb Repressive Complex 2, or PRC2, involved in DNA packaging. The finding was the first to show that lincRNAs can affect the expression of distant genes on other chromosomes and gave an inkling of their global importance in the body.

In the current study, Chang and his colleagues compared levels of HOTAIR expression in normal human breast tissue (obtained from breast reduction surgeries conducted at Johns Hopkins Hospital) with that of primary breast cancer tumors and tumors that had metastasized to other parts of the body. They found that about one of every three

primary tumors expressed levels of HOTAIR that were over 100-times higher than that of normal breast tissue. Metastatic tumors expressed levels of HOTAIR that were hundreds or thousands of times higher.

"In many studies, changes in gene expression in cancer cells are pretty subtle," said Chang, "and require analyzing dozens of genes to make an accurate call. Here we saw differences of 100- to 1,000-fold."

They then repeated the analysis with frozen samples from 132 breast cancer patients from the Netherlands Cancer Institute whose subsequent medical histories were well documented. They found that those women whose primary tumors expressed high levels of HOTAIR were approximately three times more likely than their peers to have their tumors metastasize and to die in the subsequent 15 years. The association held regardless of the woman's tumor size, stage or hormone-receptor status.

Although the correlation between HOTAIR expression levels and a cancer's aggressiveness could be a useful prognostic tool, the researchers still didn't know what, if anything, HOTAIR was doing in the tumor cells. So Chang looked at breast cancer cell lines that can be grown in the laboratory. Most of these cell lines express relatively low levels of HOTAIR and grow tamely in laboratory dishes.

However, when the researchers engineered the cells to express HOTAIR levels similar to those seen in some patients' primary tumors, the cells were able to clump together in colonies and gained the ability to migrate through an artificial barrier mimicking the biological "walls" that separate compartments within tissues — a key first step in metastasis. Conversely, blocking HOTAIR expression in a rare cell line that sported higher levels hampered its ability to accomplish the same feats.

The researchers further showed that cells lacking PRC2 expression did

not respond in the same way to HOTAIR expression. Cells over-expressing HOTAIR showed altered patterns of PRC2 binding and differences in expression levels of more than 800 genes — many implicated in breast cancer progression.

To watch the cells in action, the scientists injected human breast cancer cell lines into the tail veins of laboratory mice. HOTAIR-expressing cells were uncommonly able to metastasize: About eight- to ten-times more new tumors were found in the animals' lungs than in animals injected with cells expressing lower levels of HOTAIR.

"It's really amazing," said Chang. "When this RNA is expressed inappropriately, it causes metastasis and the expression of all these genes to change. In normal development, these genes are only bound by PRC2 in areas of the body where HOTAIR is expressed. So it's as if these cells are, in a way, changing their identity."

If so, the findings have implications that extend beyond cancer. HOTAIR comes from a region of the genome well-known for acting as a kind of positioning system during development — helping cells know where they are in the body and what they should do there. So it makes sense that changes in HOTAIR expression tweak the cells' sense of purpose. In fact, the patterns of PRC2 binding in the HOTAIR-expressing cells resemble not those of mild-mannered breast epithelial cells but of more capricious embryonic skin cells. These cells are more developmentally flexible and may move about the body more readily.

"The effects that these lincRNAs can have in cancer progression are much more complex than we had anticipated," said Chang. "It's possible they could be used as biomarkers, but they may also be important in therapy." If possible, blocking HOTAIR's expression in tumor cells may be a valuable way to inhibit metastasis and improve a patient's prognosis, the researchers believe.

Provided by Stanford University Medical Center