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MicroRNAs Intimately Involved in Cancer

In discoveries that may open a new chapter in understanding and diagnosing cancer, Howard Hughes Medical Institute investigators and their colleagues have established that tiny microRNAs provide a novel genetic route to the initiation of some forms of cancer.

The researchers published their discoveries in independent papers in the June 9, 2005, issue of the journal *Nature*. Their findings show that distinctive patterns of activity of microRNAs (miRNAs) in cancer cells can be used to diagnose cancers, reported HHMI investigator Todd R. Golub, who is at the Dana-Farber Cancer Institute and the Broad Institute of MIT and Harvard.

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— **Todd R. Golub**

Golub and co-authors H. Robert Horvitz and Tyler Jacks, both HHMI investigators at MIT, showed that miRNA expression profiles can be used to both classify human cancers and to distinguish normal cells from those that are cancerous. The new study shows that microRNA expression profiles can even distinguish cancerous cells that cannot otherwise be identified on the basis of their outward appearance.

In a second paper published in *Nature*, Gregory Hannon, Scott Lowe and their colleagues showed that a specific cluster of microRNAs can cause lymphomas in mice. Hannon and Lowe, who were recently named as HHMI investigators at Cold Spring Harbor Laboratory, say that given the new findings, they agree with a proposal calling for cancer-causing microRNAs to be dubbed "oncogenic micro RNAs," or "oncomiRs," just as cancer-causing genes are called oncogenes. Senior author Scott Hammond of the University of North Carolina at Chapel Hill and Carlos Cordon-Cardo at the Memorial Sloan-Kettering Cancer Center also contributed to the studies.

A third paper in *Nature* from Joshua Mendell and colleagues at The Johns Hopkins University School of Medicine reported that some microRNAs cooperate with a gene already known to cause human cancers.

"[T]hese three studies change the landscape of cancer genetics by establishing the specific miRNAs expressed in most common cancers and investigating the effects of miRNAs on cancer development and cancer genes," wrote Paul Meltzer, of the National Human Genome Research Institute, in a *News and Views* commentary in the same issue of *Nature*.

MicroRNAs, which are no more than a couple of dozen nucleotides in length, appear to regulate a broad array of physiological and developmental processes. However, their regulatory roles remain largely mysterious, as the functions of only a few of more than 200 known microRNAs have been established. Unlike the large messenger RNA (mRNA) molecules that code for cellular proteins, the tiny microRNAs regulate gene activity by interfering with mRNAs.

According to Horvitz, the idea for their study has its origins in developmental biology. "Since the discovery that microRNAs control specific cell divisions in the nematode *C. elegans*, I have speculated that there might be a relationship between microRNAs and human cancer," he said. "Our collaborative study establishes a striking correlation between patterns of microRNA expression and cancer and offers the prospect of using microRNA expression patterns to help in the diagnosis and treatment of cancer."

Golub said that while there had been hints that individual miRNAs were switched on or off in cancers, "there hadn't really been a broad view of miRNA profiles in cancer until this work. The first question was, is there anything interesting to find at all? We suspected microRNAs might be involved in cancer because they play important roles in embryonic development."

Golub noted that the small size of microRNAs posed a challenge for using conventional microarray techniques to analyze gene activity. In microarray analysis, vast numbers of DNA genes are attached to a small slide. That slide is then treated with a mixture of fluorescently tagged messenger RNA isolated from cells whose activity is to be analyzed. The RNA molecules attach to their complementary DNA counterparts on the slide. The amount of mRNA, as measured by fluorescence, reflects a gene's activity. However, such mass gene expression profiling is all but impossible with miRNAs because their short sequences are not as distinctive and thus "cross-hybridize" with one another, said Golub.

So, Golub and his colleagues invented a quick, inexpensive, accurate bead-based technique for analyzing miRNA activity. Their technique involves attaching to color-coded plastic beads DNA sequences complementary to the individual miRNAs whose levels they wish to probe. The beads can be made in 100 different colors, enabling analysis of the levels of many different miRNAs.

Using a technique developed by Horvitz and his colleagues, they then amplified the miRNA from target cells, applied it to the beads and stained it. Using a cell-sorting technique, they determined the identity of each miRNA by its bead color and the abundance of each by the intensity of the stain on

each bead.

Using the new techniques, the researchers discovered that they could distinguish a diverse range of human cancer cells from one another on the basis of their miRNA expression patterns alone. They could also use miRNA activity patterns to distinguish subtypes of acute lymphoblastic leukemia, which differ in their genetic origin.

"These findings were both surprising and interesting, because with standard microarray analysis of messenger RNA, for every different question that you want to ask, you use totally different mRNAs that are informative for that particular question. So, it would be simply out of the question to find two hundred messenger RNAs that would be universal indicators of characteristics of cancers," Golub said.

The researchers also tested their technique on cancers that present particular diagnostic challenges, because they appear very similar under the microscope. In those studies, they found that they could classify such cancers with good accuracy. They also found that the technique could distinguish tumors from normal cells, with most miRNAs suppressed in tumors.

Finally, working with Jacks, the researchers tested how effective the technique was in distinguishing tumors from normal cells taken from mutant mice with lung cancer. "Our concern was that our previous findings might have been artifacts, because some of the tumors came from one source and the normal cells from another," said Golub. However, he said, the animal studies confirmed that the technique could distinguish cancerous and normal cells. Golub said that the mouse studies demonstrated that the same miRNA detection system could be used in both mice and humans, to enable comparative studies.

According to Golub, a key biological question is whether the distinctive miRNA profiles they have found in cancers represent causation of cancer by miRNAs, or merely an association.

However, in their *Nature* paper, Hannon, Lowe, Hammond and their colleagues, established a clear causative role for a specific cluster of miRNAs in the blood cancer B-cell lymphoma. In their study, they compared normal human cells and tissues to those with B-cell lymphomas in their expression of the genes for a cluster of miRNAs called mir-17-92. Studies by other researchers had shown that the gene region, or locus, containing this cluster is amplified in several types of lymphoma.

Using a custom microarray technique developed recently by Hammond, the researchers found that the tumors showed substantial increase in activity of the cluster of miRNA genes.

Next, working with co-author Scott Powers of Cold Spring Harbor, Hannon and his colleagues tested the mir-17-92 miRNA gene activity in human tumor samples of lymphoma and colorectal cancers. They found overexpression of the miRNA genes in the former but not the latter. "That finding gave us

confidence that we might be looking at something that would be clinically relevant," said Hannon.

Finally, the researchers tested the effects of overexpression of the miRNA cluster in mice carrying the oncogene *c-myc* — employing a mouse model often used by Lowe and his colleagues.

"Although the *c-myc* mice develop tumors, we found that overexpressing the mir-17-92 locus in those mice caused the tumors to arise much more quickly, be much more aggressive and kill the animals faster," said Hannon. Specifically, he said, the addition of the miRNA genes appeared to reduce the natural cell death, called apoptosis, which tends to hold tumors in check.

While the major focus of cancer research has been on genes that code for proteins, said Hammond, "now we have to at the very least also consider non-coding genes for miRNAs, when we think about the kinds of genetic alterations that can contribute to tumors." Such considerations, he said, will likely influence methods of classifying and diagnosing cancers, as well as treating them.

Major questions remain about the mechanisms by which miRNAs affect cancers and their prevalence in cancer, said Hannon. "This is by no means a final answer about the role of miRNAs in cancer," he said. "But it's the first really definitive link where we can show with biological experiments that microRNAs can act as an oncogene."

Hannon said that — given the apparently central role miRNAs play in causing cancers — he supports the proposal by Frank Slack of Yale that such miRNAs be called "oncogenic micro RNAs," or "oncomiRs," and that mir-17-92 be designated oncomiR-1.