

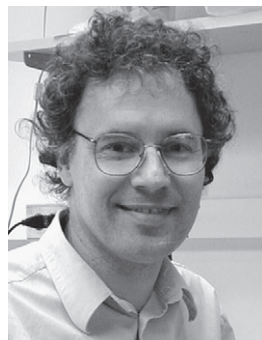
Studies Define Role of microRNA in Cancer

Once ignored completely or overlooked as cellular detritus, short snippets of RNA discovered only a little over a decade ago are turning out to be crucial regulators of cell growth, differentiation, and death.

These small 20- to 23-nucleotide non-coding sequences, dubbed microRNA (miRNA), help regulate protein production in the cell by binding to a complementary site in protein-coding messenger RNA (mRNA), effectively preventing it from being translated, and, in some cases, targeting it for destruction.

It now appears that at least some of the more than 200 miRNA sequences discovered in the human genome contribute to the development of cancer. Three papers published simultaneously in the June 9 issue of *Nature* demonstrate that miRNAs are not only part of the process that leads to cancer but in some cases may act as oncogenes or tumor suppressors in their own right. Furthermore, miRNA levels in tumors may possibly be used to classify cancers, even those of ambiguous origin. One of the articles goes so far as to suggest designating some miRNAs as “oncomiRs.”

It should not be surprising that miRNA has now been associated with cancer, according to the researchers



Victor Ambros

studying the role of miRNAs in the cell.

“Nothing surprises me anymore, particularly when it comes to miRNAs,” said Victor Ambros, Ph.D., of

Dartmouth

University in Hanover, N.H., the first scientist to isolate an miRNA, lin-4 from the nematode *Caenorhabditis elegans*. “Many of us have started profiling miRNAs in tumors and tumor cell lines with the hope that some of the miRNAs would be good markers for important tumor behavior, particularly behavior that might be clinically relevant. I think the basis for that hope is that these are important regulatory molecules. Now, it appears from these papers that our hopes may be well founded. This is very promising indeed.”

In the most comprehensive look to date at the distribution of 217 miRNAs in normal cells of a diverse array of cancers, including solid tumors, leukemias, and lymphomas, Todd Golub, M.D., and his colleagues at the Broad Institute of the Massachusetts Institute of Technology and Harvard University in Cambridge, Mass., found they could classify tumors in a hierarchical clustering that reflected the developmental origin of the tissues. Moreover, even within a single developmental lineage, they found they could subdivide acute lymphoblastic leukemia samples into three categories based on their mechanism of genetic transformation. Finally, in a test of the robustness of their technique, the researchers predicted the cellular origin of poorly differentiated tumors and correctly classified 12 of the 17 samples. When the researchers tested the same samples using an mRNA-based classification system, they got one out of 17 correct.

“This is sort of an extension of Golub’s work showing that you can classify cancers based on mRNA distribution,” said Harvey Lodish, Ph.D., professor of biology at Massachusetts Institute of Technology. “It looks,

rather interestingly, like miRNAs might work better. It's not obvious why, but they do."

Some of the answers as to why miRNA seem specific to particular tumor type may come from mechanistic studies of their role in particular cancers. The first hints of miRNA's role in cancer came from Frank Slack, Ph.D., and his colleagues at Yale University, who showed that an miRNA called lethal-7 (let-7) can act as a tumor suppressor that directly regulates the RAS oncogene. (See News, Vol. 97, No. 9, p. 626, "Blocking Cancer With RNA Interference Moves Toward the Clinic.")

Gregory Hannon, Ph.D., of Cold Spring Harbor Laboratory and Scott Hammond, Ph.D., of the University of North Carolina, Chapel Hill, led an investigation into the mechanism of action of a genomic sequence on chromosome 13 known to be amplified in several forms of lymphoma, including B-cell lymphomas, and other tumor types. Previous studies had shown two potential genes in the region, but only one, annotated as c13orf25, was overexpressed. This region does not harbor a protein coding sequence, but instead a cluster of seven miRNAs. Further investigation showed five of these miRNAs are overexpressed in B-cell lymphoma derived cell lines compared to normal B cells.

To understand the role of the miRNAs in the tumorigenic process, the researchers used a mouse model of B-cell lymphoma. The animals carry a transgene overexpressing the C-myc oncogene, which is overexpressed in many human cancers.

The researchers infected hematopoietic stem cells with a retrovirus carrying the miRNA gene cluster. Animals overexpressing the miRNA developed lymphoma and died more quickly than mice not infected with the miRNA gene cluster. Genetic analysis showed the tumors were derived from immature pre-B cells.

"These miRNAs are expressed very early in development, and their level goes down as cells mature," said Hammond. "They appear to be a cell lineage regulator, a small group of

miRNAs that regulate cell fate. They are associated with an immature cell type. To some extent that's what cancer



Scott Hammond

is, so it's not surprising that we should get these results." In a complementary paper, Joshua Mendell, M.D., Ph.D., and colleagues at Johns Hopkins University, Baltimore, demonstrate some of the same miRNAs studied by the Hannon group are themselves regulated by C-myc. They in turn attenuate translation of the transcription factor E2F1, itself a protooncogene.

The authors suggest the miRNA intermediaries act in a kind of feedback loop to keep tight control over proliferation signals. In this respect, the very same miRNA species that appeared to be oncogenes in the Hannon paper seem to be acting as tumor suppressors in the Mendell group's experiments.

"What's interesting about those two papers is that they seem to come to somewhat different conclusions about some of the same miRNAs, which illustrates the complexity of the situation," said Ambros.

Mendell explained the apparent paradox of an miRNA acting as both an oncogene and tumor suppressor gene by suggesting their action changes depending on the cell and tissue type in which it is expressed.

"If you think about it, different tumors are known to have different genetic events that drive their proliferation, so depending on what the dominant targets are in a given tumor, that could actually dictate the way the miRNA cluster functions," he said.

In an editorial accompanying the articles, Paul Meltzer, Ph.D., of the National Human Genome Research Institute, succinctly summarizes the largest issue facing the miRNA field: "a great deal of experimental work remains to be done in validation."

The fact is that very few miRNA targets have been identified and validated using experimental approaches.

"The main point to keep in mind is that miRNAs downregulate," said Lodish. "In that sense they are opposite to transcription factors, which turn on genes. One thinks of them as switches, but more likely [they are] modulators."

Lodish and his MIT colleague David Bartell, Ph.D., are studying the role of miRNAs from various hematopoietic cell lineages. They established in a *Science* paper published in 2004 that miRNAs are a key component of hematopoiesis.

"The problem is that it is hard for us to figure out the rules that determine the consequences when an miRNA interacts with a target mRNA, or even whether there will be a consequence at all," said Ambros. "When we stare at an miRNA sequence, and even have a computer stare at it, we



Johns Hopkins Gazette

Joshua Mendell

are still at the moment faced with more complexity than we can easily get a grip on. But it's fair to say that there will be rapid progress in this area, especially as genetic approaches are increasingly applied to test the predicted miRNA-target interactions."

Lodish pointed out that doing experiments with miRNA is much more difficult than with messenger RNA profiling. "You need more material and the steps are more involved," he said. "It will make it trickier to move into the clinic. ... We know there are miRNAs that affect cell fate. But that's a long way from saying we are going to convert this into something therapeutically useful. Our experience with cancer is a) it's going to take a long time, but b) it will happen."

—Karyn Hede