Until very recently, scientists thought they had a pretty good basic model for how cells work. DNA was the storehouse of all information, the blueprint for life. Proteins were the building blocks: the bricks, mortar, and switches that actually made a living thing and also made it work. According to the model, while RNA did a good job shuttling the DNA’s instructions from the nucleus to the cytoplasm, it did not serve any larger purpose. And the long stretches of DNA that did not contain information for building proteins were unimportant.

In 1993, scientists started to find the first hints that this long-standing model of how life works might not explain everything. Researchers studying Caenorhabditis elegans worms started finding evidence that short 18–25 nucleotide-long snippets of RNA produced from genes that did not encode protein might be bigger players in the workings of cells than had been realized. These snippets started turning up in a number of different species, showing remarkable conservation and suggesting that some fundamental piece of the cell was starting to make itself known (Figure 1).

Fast forward to the present day. The importance of these short RNA pieces—now dubbed microRNAs—as epigenetic regulators of cell development, survival, and disease is becoming ever clearer. And their popularity as a research topic has exploded. "The basic science of microRNAs is just fascinating," said Curtis Harris, M.D., Chief of CCR’s Laboratory of Human Carcinogenesis, who studies microRNAs as prognostic tools in cancer. "How the microRNA is processed from its gene, its function in normal cell biology, in development, and in disease—since microRNAs are relatively newly discovered, all of these features are still being worked out." A search of the PubMed database turned up 2,611 papers published on microRNAs since 2001, with 1,027 published in 2007 alone.

The links between microRNAs and cancer are also now well appreciated. "The cancer field has now become very excited by microRNAs, in part because they are so new," noted Harris. Cancer researchers also have a clearer view of microRNAs as actors in carcinogenesis. Based on this knowledge, researchers like Harris are investigating how to turn our growing knowledge of microRNAs into clinical tools for cancer prognosis and therapy.

Shooting the Messenger

MicroRNAs are like transcription factors for RNA. Just as transcription factors control a gene’s transcription into messenger RNA, microRNAs control a messenger RNA’s translation into protein. But instead of promoting gene expression, as transcription factors do, microRNAs impede it: MicroRNA-bound messenger RNAs do not get translated, effectively silencing the gene from which they were transcribed. "MicroRNAs bind to the messages of protein-coding genes, either changing the stability of that message or the translation of that message into protein," explained Harris.

The number of microRNA genes tucked away in the genome is unclear, but by some estimates there may be as many as a thousand. Researchers estimate conservatively that about one-third of all
protein-coding genes may be controlled to some extent by microRNAs. They can have such widespread effects because they tend to act globally. “Because they are short and their ‘seed’ sequences [the first six nucleotides in a microRNA, which act as binding sites] are somewhat degenerate, they physically interact with messages that they don’t exactly match,” Harris noted. “So a single microRNA may target 10, 50, maybe even a 100 messages in different genes or pathways.”

Drawing the Lines
The first paper suggesting a link between microRNAs and cancer, published in 2002 by a colleague of Harris, The Ohio State University’s Carlo Croce, M.D., reported that a genomic region deleted in about half of all cases of B-cell chronic lymphocytic leukemia (B-CLL) housed genes for two microRNAs. The development of techniques for microarray and bead-based flow cytometry analyses of microRNA expression soon led to the discoveries of microRNA signatures unique to specific tumors and their cellular origins.

As the research on microRNAs and cancer has gone deeper, particular microRNAs have begun to stand out as potentially causative agents. For instance, microRNAs called miR-155 and the miR-17-92 cluster act like oncogenes, while miR-15a and miR-16-1 appear to function as tumor suppressors. The genetic lesion Croce identified in B-CLL included the genes encoding miR-15a and miR-16-1.

“We are finding that microRNAs can serve a range of purposes in the context of cancer and cancer treatment,” said Harris. “They can tell us a lot about the basic biology of cancer and about what pathways are involved and what might be good targets for therapeutic development. One can, at least in preclinical studies, knock down the expression of a specific microRNA with an antisense strategy and see an anti-tumor effect. They can also be good clinical biomarkers, useful tools for diagnosis and, maybe, for predicting therapeutic outcome.”

Translating Science Together
It was the role of microRNAs as developmental players in cancer, combined with Croce’s B-CLL paper, that gave Harris his entree into the world of microRNAs. “When Carlo made what I think was a seminal observation that microRNAs were associated with cancer, that seemed to be a very exciting finding and one that I thought might have relevance to solid tumors.”

Having known Croce for some time, Harris contacted him and suggested that they work together to look at microRNA profiles in solid tumors. Joining forces, their laboratories produced, in early 2006, an examination of the microRNAomes (the total palette of microRNA expression within a cell) of tissues from a spectrum of solid tumors (e.g., lung, breast, stomach, prostate, and colon). “This paper was one of the first to indicate the extensive involvement of microRNAs in the pathogenesis of solid tumors,” reported Harris. It also suggested that microRNA expression could influence cancer development by controlling protein-coding oncogenes and tumor suppressors (Figure 2).

Peeking into a Tumor’s Future
Apart from the questions of development and pathogenesis are those of progression. The ability to predict an individual’s clinical outcome or risk for recurrence has been an

Figure 1: The roles of microRNAs—simple short pieces of RNA that do not encode protein—in the control of nearly all critical cellular processes have gained widespread and rapid appreciation.
MicroRNAs, in this context, are a new class of biomarkers that will be useful not only with clinical stage, but also with other biomarkers such as genomic or proteomic changes.

With this reasoning in mind, Harris, Croce, and their colleagues started narrowing their view of microRNA expression profiles to focus on prognosis. In 2006, they released a paper, authored by now-Harris lab alumnus Nozomu Yanaihara, M.D., Ph.D., revealing that the expression pattern of microRNAs in lung tumors correlated not only with tumor type, but also with prognosis. It was one of the first studies to tie microRNAs to a patient’s prognosis following surgery, independent of tumor stage.

Broad Effects

“The same things we know about stage 1 lung cancer and the limitations of clinical staging can be said for stage 2 colon cancer,” Harris noted. “There is a similar need to identify those individuals who have a good or poor prognosis independent of stage. You can get a good idea on a population level who is going to face recurrence and who is not, but on an individual basis, you have to be cautious when deciding who needs what therapy and how aggressively a patient needs to be treated.”

Knowing that they could predict lung cancer outcomes, Harris and Croce teamed up again to see if they could achieve the same results in colon cancer. The time and place for this study were ideal—Harris has studied colon cancer for a number of years and has a long-standing cohort of patients from whom he has collected tissue and detailed clinical and family histories. “In addition,” Harris said, “I knew of a cohort in Hong Kong that would provide us with a validation population that would allow us to confirm any results we found in our locally-based cohort. I wanted to make a diverse comparison by looking at two very different cohorts. If a typical U.S. population and, in this case, a typical Chinese population show the same result, it is more likely that your data will be generalizable to a broad range of people with colon cancer.”

And once again, Harris’ and Croce’s collaboration has proven fruitful. A new paper, released in January of this year and co-authored with Yanaihara by Harris laboratory Cancer Prevention Fellow Aaron Schetter, Ph.D., M.P.H., and Postdoctoral Fellow Jane Sohn, Ph.D., showed that microRNA profiles could be used to predict both prognosis and clinical outcome, another first in the microRNA world. In addition, they found that the levels of...
The question of predicting survival and response is not limited to colon and lung cancers. One cancer for which there is a significant need for new prognostic tools is liver cancer. Currently, surgical resection or transplantation are the best options available for liver cancer patients; however, based on assessments of liver function, tumor size, and stage, only 10 to 20 percent of patients are eligible for these surgical options. Even those who are able to undergo surgery face an uncertain future; the frequency of metastasis and/or recurrence is very high.

A New View of Liver Cancer

Knowing that microRNAs were starting to show promise for prognosis in other tumors, Xin Wei Wang, Ph.D., Head of the Liver Carcinogenesis Section in CCR’s Laboratory of Human Carcinogenesis, began to look at whether such patterns could be applied to liver cancer as well. By comparing cancerous and noncancerous liver tissues, Wang and his collaborators identified 20 microRNAs whose expression correlated with risk of metastasis and did so with greater accuracy than classical pathology staging. They also found that this pattern itself could be used as an independent measure for predicting a patient’s clinical outcome.

Wang looks at these results in terms of clinical benefit. Methods for microRNA isolation and analysis are advancing rapidly, which creates conditions for turning microRNA profiling into a standard procedure for liver cancer patients. Having a profile in hand that can distinguish high- and low-risk patients would allow clinicians to decide early after a diagnosis how aggressive a treatment approach to take and give insights into how to personalize treatment for an individual patient.

Looking to a MicroRNA-Based Future

“We are only five or so years separated in time from the first suggestions that microRNAs could be involved in cancer,” said Harris. “In that short time, we have come very far, and in my opinion, microRNAs are going to be very significant biomarkers for diagnosis and prognosis in a number of cancers. I also anticipate that microRNAs may be useful clinical targets, which is a longterm goal for us to determine.”

But he is the first one to declare that there is a great deal more work to be done. “I’d like to see our prognosis results replicated in a number of different populations, so that we can see how broad they are,” mused Harris. “Ours was the first report on the use of microRNAs to predict therapeutic outcome, and as such these data need to be confirmed. But it is an exciting time and an exciting opportunity, being engaged at the early stages of translating a fundamental discovery.”
Curtis C. Harris, M.D.
Chief, Laboratory of Human Carcinogenesis

“It all started in a small farming community in Kansas and at state science fairs,” Curt Harris quipped when asked about his background. But he came to CCR with an inborn instinct for translational medicine. “One of my medical school mentors, with whom I had done some collaborative research, suggested that I should continue my work at NCI. I started out with a small laboratory while finishing my clinical training and haven’t ever thought about leaving.”

His research interests, at first glance, cover a range of topics. “My lab’s research is diverse, which reflects being a physician-scientist. We have a strong motivation for understanding basic research and translating that knowledge into the clinic.” What truly excites him, though, are moments of unexpected convergence. “I love it when there are two parallel lines of research in the laboratory, and there is a connection that we never would have predicted, leading us into something much more interesting.”

This eye for convergence has fueled his work on biomarkers and prediction. “We have done a lot of work on molecular pathogenesis of cancer and how normal cells become cancer cells,” Harris said. “We try to look at cancer from a scientific standpoint, a clinical standpoint, and a public health standpoint.”

Harris finds working with his growing web of laboratory alumni a fruitful and enjoyable aspect of his job. “We maintain what we like to call the ‘LHC Family.’ I find it very satisfying to collaborate with former fellows who have cultivated their own independent careers and with whom I can have a collegial relationship.”

Harris earned his medical degree from the University of Kansas School of Medicine and did his clinical training at the University of California at Los Angeles and at NIH.

Nozomu Yanaihara, M.D., Ph.D.
Harris Laboratory Alumnus

Nozomu Yanaihara worked with the Harris lab from 2004 to 2007 as a Research Resident and a Visiting Fellow from the Jikei University School of Medicine in Tokyo, Japan. “I am a gynecologist and an obstetrician by training, with a focus on gynecological oncology. When I decided to come to the National Cancer Institute for additional experience, one of my mentors, a former Fellow in Curt’s lab himself, suggested I contact him.”

Now an Assistant Professor of Obstetrics and Gynecology back at Jikei University, Yanaihara maintains an active relationship with his colleagues in the Harris laboratory. “One of the biggest things I learned at CCR,” Yanaihara noted, “was that while researchers from different countries may struggle with language barriers, there are no barriers in research as long as we have shared goals.”

His interests in microRNA and cancer, fueled by his work at CCR, have now crossed into his work in Japan. “I took part in several microRNA-related projects while at NCI, including the lung cancer prognosis prediction project. I am now applying the techniques and results that I brought back to prognostic research in gynecological cancers. My hope is to carry out this work collaboratively with Curt and others back in the United States.”

Yanaihara received both his M.D. and his Ph.D. from Jikei University.
Aaron Schetter, Ph.D., M.P.H.  
Cancer Prevention Postdoctoral Fellow

Aaron Schetter’s path to the Harris lab has followed a route that places him squarely at the intersection of public health and basic science. “I started off doing basic research studying cell biology in *C. elegans,*” said Schetter. “After I finished my Ph.D., I wanted to switch to a field that was more relevant to human disease. After deciding to study cancer, I joined the NCI’s Cancer Prevention Fellowship Program.”

Launched in 1987, this program trains multidisciplinary experts in cancer prevention. Scientists, clinicians, and other health professionals are encouraged to earn an M.P.H., followed by mentored research with NCI investigators. “It’s given me a completely different set of skills than what I developed as a Ph.D. student,” Schetter noted.

After completing the academic portion of the program, Schetter looked for a laboratory where he could put those skills to work. “It’s difficult to find labs where you can do both basic science and epidemiology well. Curt’s lab is a large and diverse group, with people doing basic science and ones who exclusively do this kind of epidemiologic work, so to me it seemed a good fit.”

The translational aspect of Harris’ microRNA research also drew him in. “I saw how the microRNA work could rapidly turn into something that could affect human disease itself. MicroRNAs are pretty easy to work with in this regard. You can see which ones are altered in colon cancer and then move quickly into functional studies testing your hypotheses.”

Schetter’s experiences give him some pretty broad options for the future. “I haven’t decided yet whether to go the academic route or the industry route, but regardless I hope to continue in the field of discovering and testing biomarkers and therapeutic targets in cancer.”

Schetter earned his Ph.D. at Cornell University and his M.P.H. at the University of California at Berkeley.

Jane Sohn, Ph.D.  
Postdoctoral Fellow

With a research background in microbiology, Jane Sohn brings a different perspective to research on cancer. “I came to Curt’s lab to work on colon cancer and a related disease called inflammatory bowel disease (IBD). It’s thought that bacteria contribute to cancer risk by inducing inflammation. Though the etiology of IBD is not known, it is a disease of inflammation, and IBD patients have an increased risk for colon cancer.”

Her current work stems from a realization she had while working on her Ph.D. “I was working in a laboratory that focused on bacterial pathogenesis and realized that I was interested in looking at the interface between inflammation and cancer. MicroRNAs may help us better understand this interface.”

Sohn finds the environment within the Harris lab to be truly unique. “Curt’s lab does three different things: basic research, translational research, and molecular epidemiology. NIH encourages basic scientists to collaborate with others doing translational science or epidemiology, and here it is already happening just within this lab.

“I came from a basic science setting,” Sohn noted, “and now have an appreciation for human disease that I didn’t have before.”

Sohn did her doctoral work at the Massachusetts Institute of Technology.

Aaron Schetter, Ph.D., M.P.H. (left), and Jane Sohn, Ph.D. (right)