ALL IN THE GENES

Cell biologist at SRI develops method that could chart a new course for treating cancer and other diseases at their source

Leaning forward in his office chair, hands clasped, Dr. Burton Yang is smiling.

A senior scientist in molecular and cellular biology at Sunnybrook Research Institute (SRI), Yang has reason to smile. In the last 10 years he has published more than 40 peer-reviewed papers, many in high-impact journals. Grant money is rolling in, and his lab group numbers six, including two postdoctoral fellows and a visiting scientist. Researchers in other countries invite him to speak about his work—on this day he has just returned from Iowa City—and in 2009 *Nature Cell Biology* published a discovery he calls "the most important" of his career.

That discovery is a method for expressing single microRNAs, and it has the potential to advance significantly microRNA research throughout the field of molecular biology, particularly in gene therapy to treat cancer and other diseases.

MicroRNAs are a type of ribonucleic acid (RNA), and they are very small—relative to a cell, they are like dust in a house. The sole job of RNA, scientists believed until recently, was as "messenger" in cell development: copying the genetic instructions contained in deoxyribonucleic acid (DNA) in an intricate series of interactions called transcription. Some RNAs, in turn, are translated into proteins that, dependent upon their DNA and the genetic environment in which they were transcribed, become the hundreds of cell types—and trillions of cells—that comprise the human body.

In the early 1990s, the notion of RNA as mere messenger in this orchestral process was shattered when scientists discovered small-interfering RNA and microRNA, and showed they could regulate gene expression—the move from DNA to RNA to protein—which had dominated molecular biology for five decades. Researchers quickly discovered other classes of RNA, and the number of RNA categories soon exceeded 20.

The multiple roles of these RNAs have attracted keen attention from scientists, but interest in microRNA has been particularly intense. As Yang noted in his Iowa City presentation, the number of articles with the word "microRNA" grew from two in 2002 to more than 2,600 in 2010 in a sample of the world's top journals. This interest has been driven by a growing understanding of the ability of microRNA to regulate the expression of other genes and, in some cases, to encourage gene expression.

Dr. Michael Julius, vice-president of research at Sunnybrook Health Sciences Centre and a professor of immunology and medical biophysics at the University of Toronto, says the impact of microRNA on molecular biology has been revolutionary. "The description of microRNA and its capacity to alter gene expression has been fundamental to our recent understanding of cell development," says Julius. "It's actually been nicknamed the 'micromanager' of the genome."

Before 1993, says Julius, scientists thought the next 50 years of molecular biology would be about understanding protein-DNA interaction, through proteins like transcription factors, for example, that tell DNA to turn on or off. "That notion has now been turned on its head, and we're looking at RNA-DNA interactions," he says.

A further level of complexity, says Julius, lies in the ability of microRNAs to change their chemical sequence. This feature enables them to interact with new varieties of genes and play a direct role in disease development and the delicate genomic balance that is good health. The importance of understanding microRNA has thus become paramount.

Enter Yang's single-microRNA expression method.

A big problem in the study of microRNAs is that they frequently "express" as clusters. Researchers have been able to test and measure the function of particular clusters, but not the specific microRNAs, and thus their specific roles, within those clusters. One of the most-studied clusters, made up of seven microRNAs, is called

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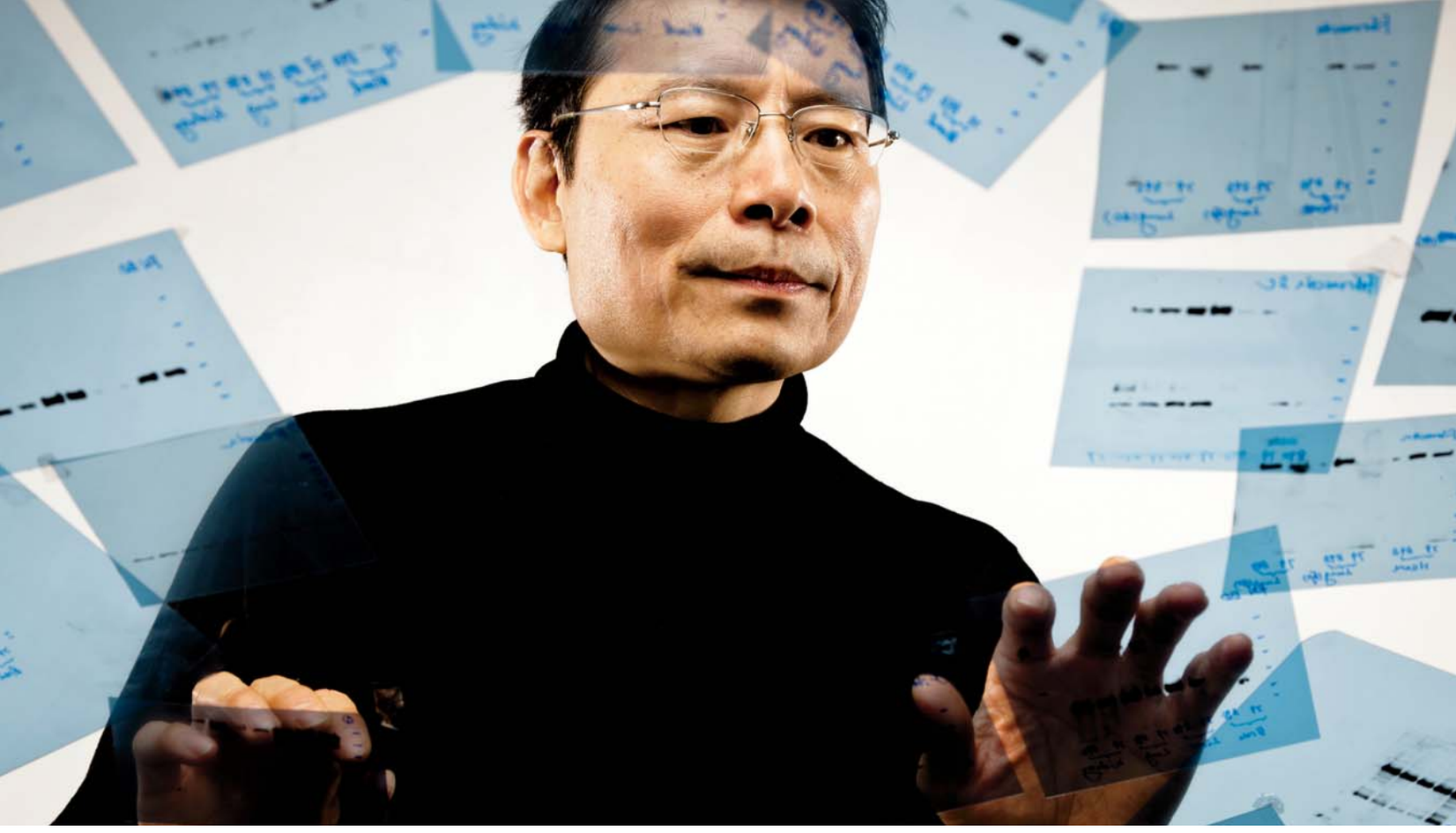

DR. BURTON YANG

miR-17~92. Researchers have strongly linked overexpression of this cluster with numerous cancers, showing it both encourages cancerous cell proliferation and regulates genes that would otherwise keep malignant cell growth in check.

In the early 2000s, Yang and his lab were studying a type of protein called versican that plays a role in blood vessel and tumour growth. Looking at this protein and small-interfering RNAs, they had an idea for how they might express a single, mature microRNA to study its particular role, but their experiments testing this idea failed.

From that failure, however, they had an idea about why their new construct wasn't working. "Mature microRNAs are short, only about 20 nucleotides [the molecules that comprise RNA and DNA], so the enzymes that process them into mature microRNAs were unable to recognize them," says Yang, who is also a professor in laboratory medicine and pathobiology at U of T.

The solution, they discovered, was to use something called precursor microRNAs. Precursor microRNAs are 70 to 100 nucleotides long, and are therefore more recognizable to the enzymes that bind, slice and process them into a mature state.

Moreover, the lab expressed a single microRNA—miR-17, of the miR-17~92 cluster—in a Petri dish and in mice—a first in the field. "Using cells only, we don't have a true physiological condition," says Yang. "But in the body of the animal, we're able to do it the right way. The body will not, for example, produce an unrealistic amount of microRNAs as might happen in a Petri dish, so you get quality control."

With the resulting transgenic mice, Yang and his lab precisely mapped several functions of miR-17. They found that when miR-17 was overexpressed, the mice developed much smaller bodies and organs, and fewer types of blood cells. They also found miR-17 produced those effects by acting on specific proteins called fibronectin and FNDC3A—a finding that matched results from other researchers showing a link between those proteins and miR-17. Yang and his group are now tweaking miR-17 expression to gauge its effect on blood and cancer cells. They hope this work will lead to new therapies to regenerate damaged blood vessels in patients with heart disease, and to shrink or halt tumours in those with cancer.

Building on Yang's breakthrough, other labs have begun using the single-microRNA expression method for their own studies in several clinical areas. Those labs have yet to publish results, but Dr. Wei-Yang Lu, a former colleague of Yang's at SRI, now an associate professor in physiology and pharmacology at the University of Western Ontario, is using the method to study the role of miR-17 in the regulation of brain functions. Lu's preliminary data has shown that neurons in the brain of miR-17 transgenic mice are fragile when blood flow is restricted, suggesting that greater expression of miR-17 may increase the severity of stroke-induced brain damage. These results, he says, are "very exciting."

Yang, however, is quick to qualify the impact of his findings: "MicroRNAs are very complicated. One can regulate expression of many things, and that expression can be regulated by other microRNAs, so there may be something going on [that] we did not see."

That said, scientists have discovered over 1,000 microRNAs that influence interactions in more than 60% of the human genome, with profound effects on health. Given these findings, a method that promises better understanding of individual microRNA function should keep cell biologists busy—and Yang smiling—for some time to come.

—Jim Oldfield

Yang's work was supported by the Canada Foundation for Innovation; the Canadian Institutes of Health Research, including a China-Canada Joint Health Research Grant with the National Natural Science Foundation of China; the Heart and Stroke Foundation of Ontario; and the Ministry of Research and Innovation.