The Little Transcript that Could

Discovering microRNA's big role in gene expression and cancer

By Michelle Siao

"miRNA may become essential not only in elucidating myriad genetic processes, but also in finding the Achilles' heel of cancer."

Big finds don't always enter with a big splash. When microRNA (miRNA) was first identified in the flatworm *C. elegans* in 1993, it went largely unnoticed. The Human Genome Project was well underway, captivating both the scientific and public communities with the prospect of decoding the 3 billion nucleotides of DNA that encode our genetic blueprint. Yet amidst this thrill, why has the past half-decade spawned excitement about noncoding RNAs that make up only about 1% of our total number of genes (1)?

It turns out that miRNAs, tiny strands just 21-25 nucleotides long, play a key role in regulating gene expression. As a result, they influence a spectrum of biological processes, including the timing of development and cell division (2,3). Recent research also shows that miRNA expression may be involved in the self-renewal and differentiation pathways of stem cells, which themselves are at the frontier of therapeutic research (4). It is these properties that are leading scientists to relate aberrant miRNA production with tumorigenesis (5). Furthermore, researchers at the Broad Institute of MIT and Harvard have demonstrated the great potential of utilizing miRNA to improve the speed, accuracy, and cost of cancer screens as well as our general understanding of cancer (6). At a time when cancer is the second leading cause of death in the United States (7), it is possible that miRNA may become essential not only in elucidating myriad genetic processes, but also in finding the Achilles' heel of cancer.

Small Molecules with Great Responsibilities

The central dogma of molecular biology states that the genetic code, in the form of DNA, is transcribed into messenger RNA (mRNA), whose information is then translated into proteins critical to cellular structure and function. Findings on miRNA, however, have revealed a new dimension of complexity in this process. The trick to controlling the expression of genetic information encoded in DNA lies in the code of miRNA itself. After undergoing an intricate biosynthetic process (Figure 1), miRNA is trimmed so that its code is complementary to that of its target mRNA. It can therefore bind to the mRNA, which it then either cleaves or hinders from being translated into proteins (2,3). Since proteins are essential for cellular structure and function, changing the level of their production can have enormous consequences and may be detrimental to the cell.

In retrospect, it is easy to see how even our first encounters with miRNA hinted

at the molecule's involvement in cancer. In 1993, Victor Ambros and his colleagues at Harvard University showed that the *lin-4* gene in *C. elegans* encodes not the expected protein, but a 22 nucleotide long RNA, the first miRNA identified (8). Because of its partial complementarity to *lin-14* mRNA, this miRNA is able to regulate the levels of the LIN-14 protein, which determines temporal organization of early larval development (9). Mutations in *lin-4* resulted in, among other defects, an abnormal differentiation of cells similar to that found with tumor cells.

Yet the field was so new that the term miRNA had not yet been coined, so novel that no one had reason to believe that this RNA interaction was relevant to species other than flatworms, much less humans. A crucial turning point occurred seven years later when the developmental timing miRNA, *let-7*, was identified (10). Though the discovery was initially made in *C. elegans*, this molecule was found to be conserved across a wide spectrum of species, from flies and zebrafish to sea urchins and frogs to mice, chickens and humans (11).

Since then, more than 3000 miRNAs with a variety of functions have been identified in animals, plants, and viruses (5). A sampling of miRNAs' responsibilities includes control of cell death (12), neuronal development (13), differentiation of blood cells (14), leaf development in plants (15,16,17), and brain development (18). Time is increasingly showing us the scope of miRNAs' influence on our bodies. These small molecules regulate approximately 30% of genes in humans (19). Over 200 human miRNAs have been identified, and recent estimates place the total number as high as 1000 (20). Of all the miRNA genes, more than half are located within genomic regions associated with cancer.

When Cell Death Leads to Life

The paradox of cancer is that the robust survival and proliferation of cells kills the organism that harbors it. Normal cells carefully monitor themselves, limiting their numbers and, if necessary, committing cellular "suicide" for the benefit of the organism. That is, if a cell senses the incurrence of an irreparable and dangerous mutation, it undergoes apoptosis, or programmed cell death, to prevent propagation of the error. Cancer cells have one or more mutations that render them insensitive to signals that prevent cell division or induce apoptosis.

A growing amount of evidence indicates that miRNA is intimately involved in the orchestration of proliferative and apoptotic pathways. Sometimes the overexpression of miRNA exacerbates cancer, as was demonstrated in an experiment conducted by He *et al* (21). This group used a transgenic mouse model of B-cell lymphoma, Eμ-Myc, which usually develops the fatal disease within 4-6 months. Some of the mice were infected with a murine stem cell virus to introduce copies of *miR-17-19b* into the animals' cells. The increased level of this miRNA in the mice accelerated both disease onset and death, which occurred at an average of 51 and 65 days after treatment, respectively, marking *miR-17-19b* as a potential cancer-causing oncogene.

On the other hand, lowered expression of certain miRNAs can also be undesirable. One study identified two miRNAs as repressors of a proliferative transcription factor, E2F1, hinting at the critical tumor suppressing function of these sequences (22). Also, reduced levels of *let-7*, one of the first miRNAs discovered, are not only observed in lung cancer, but also cor-

miRNA biogenesis in animals

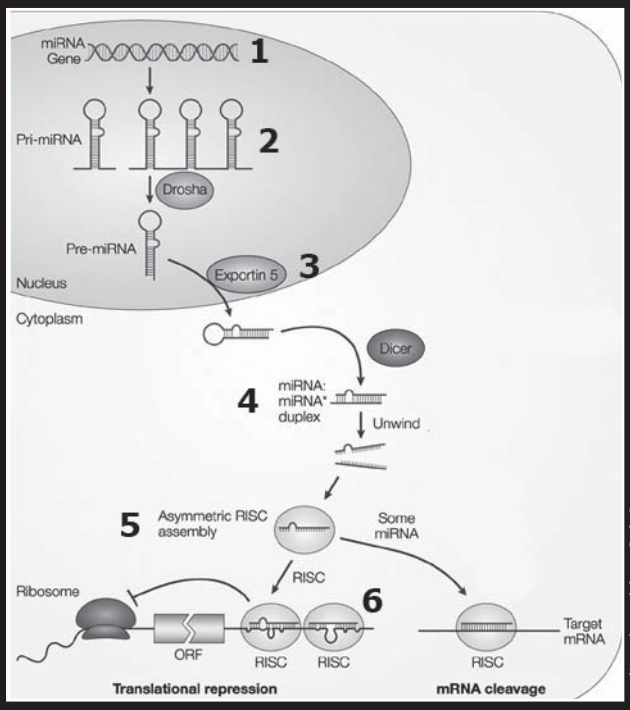

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Figure 1.
1. Inside the nucleus of a cell, primary miRNA (pri-miRNA) is made according to genetic instructions.
2. An enzyme, Drosha, cleaves pri-miRNA into a 60-70 nt stem-loop precursor (pre-miRNA).
3. A transporter called Exportin 5 guides the pre-miRNA out of the nucleus into the cytoplasm of the cell.
4. The RNA-cleaving enzyme Dicer cuts the pre-miRNA's hairpin structure into a duplex of mature miRNA strand and a strand complementary to it (miRNA*).
5. After an enzyme separates the strands of the duplex, only the mature miRNA transcript attaches to an RNA-induced silencing complex (RISC).
6. The miRNA has partial complementarity to an untranslated region (3'-UTR) at the end of its target mRNA. Therefore, RISC to which miRNA is bound can locate mRNAs intended for translational repression or cleavage.

relate with shorter postoperative lung cancer survival and hinder the growth of lung cancer cells *in vitro* (23). Another experiment illustrated the importance of normal functioning miRNA levels by studying *let-7* mutants. Cells with the mutant transcript developed stem-cell-like properties, such that they and their daughter cells divided continuously into identical cells instead of differentiating into specific tissues as they normally should (10).

Stem Cells Versus Cancer Cells: A Subtle Difference

The proliferative properties of cancer cells are reminiscent of the self-renewing character of stem cells. Indeed, these two cell types are so similar that a new hypothesis bridges them, suggesting the existence of "cancer stem cells" that cause leukemia and tumors. According to this model, a limited number of cancer stem cells can proliferate and build up tumors. This idea is supported by observations that only a small percentage of cancer cells can form colonies *in vitro*. If true, working out the subtle differences between regular tumor cells and tumor stem cells would improve clinical methods. Instead of targeting rapidly dividing tumor stem cells and allowing slowly dividing tumor stem cells to regenerate, as current treatments do, new treatments could specifically target cancer stem cells to permanently extinguish the cancer (24).

Therefore, research on the role of miRNA in normal stem cells may clarify the mechanisms by which cancer stem cells proliferate, allowing us to make progress in reining in cancer. In a recent study, Hatfield *et al.* created stem cells with a mutation in *dcr-1*, which disrupts the production of functional miRNA (4). This modification delayed the critical transition between two cell cycle stages that determine the initiation of cell division (Figure 2). These stages, G1 and S, represent phases of growth and DNA replication, respectively, that precede a second phase of growth and finally mitosis, the division of the cell into two identical daughter cells. It can therefore be concluded that miRNA is required for stem cells to pass the G1/S checkpoint consistently; miRNA, then, may also make cancer cells unresponsive to signals that arrest the cell cycle at this checkpoint. As we refine our knowledge of miRNA, we may be able to modulate levels of transcripts or their targets to keep proliferation in check.

Screen Out, Map Out, Stamp Out

In addition to helping researchers hunt for possible cancer-causing genes, miRNAs may become the preferred tool in screening for cancer. Todd Golub of the Harvard Medical School and the Broad Institute of MIT and Harvard and his colleagues have developed a bead-based profiling method for identifying cancerous tissue (6). The beads employed in this system are made of polystyrene and are linked to capture probes with short nucleotide sequences complementary to particular miRNAs. When the beads bind to their miRNA targets, they change color according to which miRNA is attached so that further analysis can yield the identity and concentration of a microRNA in a given sample.

This method provides a highly attractive alternative to the prevailing technique, which is called mRNA profiling. One reason is that mRNA is more easily degraded in samples, whereas miRNAs are more readily preserved. Moreover, a bead-based screen comes at a lower cost than one based on mRNA and generates results more quickly. It also leaves more room for additional tests, since new beads with new complementary sequences can easily be added to screen for novel miRNAs. In addition, it features improved accuracy in distinguishing cancerous and normal tissue samples. Even when employed to make difficult diagnoses, the miRNA proved to be more accurate. Finally, the bead-based screen revealed more than the current state of tissues – it divulged the history of the cancer samples. The patterns of miRNA expression could, for example, distinguish gastrointestinal cancers from lung and breast cancers, whereas mRNA profiles did not yield this information (Figure 3).

Other research groups are following the miRNA trail to cancer. A study published last year in the *New England Journal of Medicine* discovered an miRNA signature associated with chronic lymphoctyic leukemia (CLL), a cancer of the blood (25). These researchers followed a method similar to bead-based profiling, using a microarray with probes for specific miRNAs to compare and contrast normal and cancerous tissues. They deduced that a set of 13 miRNAs with differential levels of expression served as a signature for cancer and could distinguish the aggressive form of cancers from the slower-developing form. Some of these miRNAs could also distinguish between samples from cancer patients who had a long interval between diagnosis and therapy and samples from patients who had promptly begun therapy. Additionally, they found that mutations in miR-

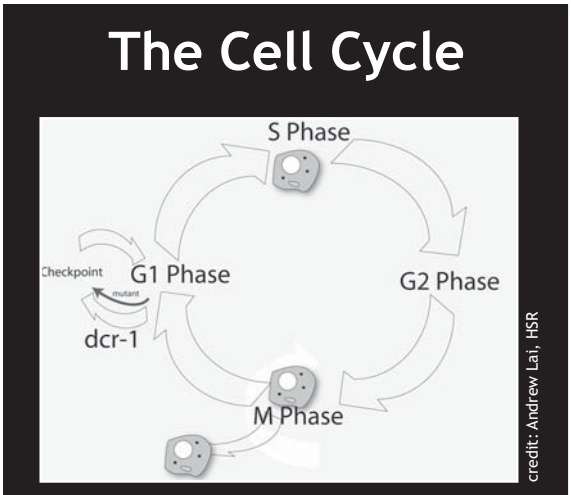

Figure 2. The cell cycle has a series of phases that lead to cell division. The transition from G1 to S phase requires miRNA (open arrow). A dcr1 mutation that prevents miRNA processsing arrests the cell cycle at this checkpoint (shaded arrow).

NAs might be associated with cancer. Errors in five specific miRNA codes were found in 15% of cancer patients tested whereas none of the normal samples possessed these errors. And when mutated versions of the *miR-16-1* and *miR-15a* genes were introduced into cells, the levels of the corresponding miRNA products plummeted. This is significant because these miRNAs have been shown to facilitate apoptosis; lowered levels thus seem to permit cells to become cancerous (26).

So are we close to discovering a cure for cancer? Not yet. There is still much that requires clarification from the standpoint of both oncology and miRNA research. Among the challenges ahead are determining whether miRNAs might be cell-specific, causing tumors in some tissues and promoting protective apoptosis in others (5). The subtleties of miRNA regulatory mechanisms also remain to be clarified. And, as is expected, new discoveries lead to debates. Last February, Carlo Croce of Ohio State University and his colleagues published a study that found an miRNA signature for tumors. Like in the Golub study, microarray analysis showed that miRNA could identify cancerous tissues and classify them according to organ of origin. The two studies disagree, however, as to whether certain miRNAs are overexpressed or downregulated, though the discrepancy may be due to differences in sample population size and methods of analysis (27).

Still, such issues can be resolved with time, and we are already on the way to matching cancers with signatures of deregulated miRNAs. Since their discovery in worms just over a decade ago, these tiny transcripts have brought science a long way. With them as a guide, our understanding of cancer and genetic expression is certain to power discoveries in the years to come. H

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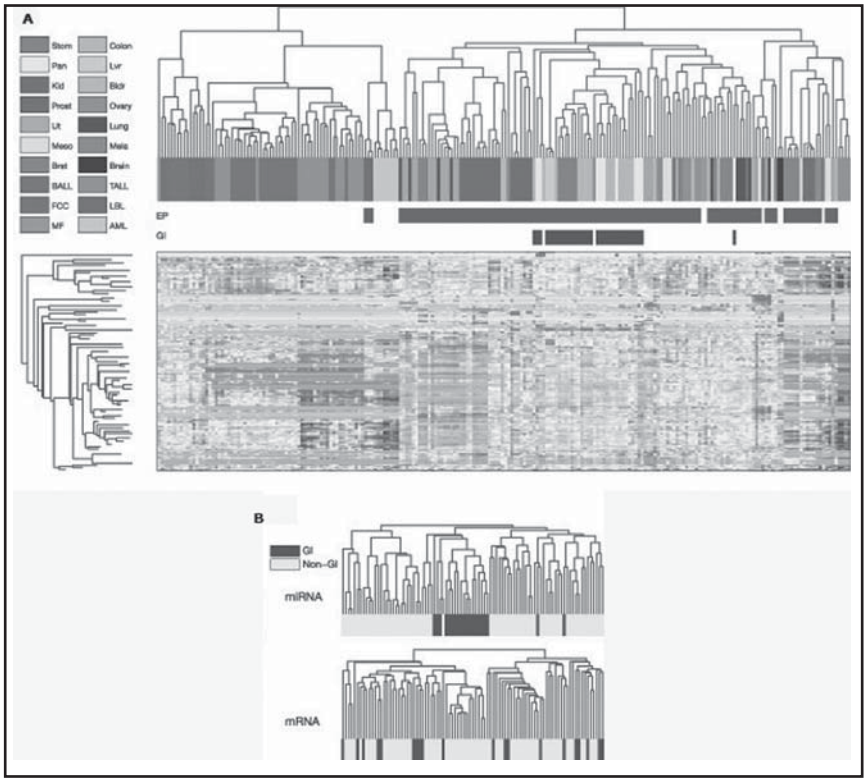

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Figure 3. Clustering of miRNA profiles. A) miRNA expression profiles can group similar tissue sample together based on similarity. Clustering can also help distinguish the origin of a cancer sample, as demonstrated by the grouping of epithelial (EP) and gastrointestinal (GI) tissues. Here, different miRNAs are represented in rows while samples are presented in columns. B) miRNA profiles can cluster GI (yellow) and non-GI (blue) tissues, while mRNA profiles do not distinguish groups efficiently.

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