Micromanaging Cancer

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The discovery of RNA interference triggered a "silent" revolution that overturned our understanding of how gene expression is regulated.[1] The previous model was that gene expression was activated during transcription, the process of copying the DNA sequence into RNA, when transcription factor proteins bind to promoter sequences in DNA upstream of the gene's coding sequence. Now it is clear that an additional important mechanism of gene regulation occurs at the next stage of translating the messenger RNA (mRNA) into protein. Small, noncoding RNAs that have a stem loop structure, called microRNAs, regulate translation by binding to partially complementary sequences of about 22 bases in length in the untranslated region of the mRNA downstream from the coding sequence and interfere with or silence its translation into protein.

In humans, some 1000 distinct microRNAs have been identified. The pattern of expression of microRNAs varies between cell lineages and within a lineage as the cell differentiates or responds to environmental cues. Each microRNA can regulate the expression of hundreds of genes. Some microRNAs act as fine-tuning rheostats to adjust a cell's functional state, and others are master regulators of fundamental pathways that govern whether a cell proliferates, differentiates, survives, or dies, by regulating the expression of multiple genes participating in a common process.

MicroRNAs not only regulate normal cell development but also play an increasingly recognized important role when things go awry in cancer.[2] Shortly after the discovery that RNA interference occurs in mammals (only 8 years ago), Calin and colleagues[3] discovered that the deletion of a microRNA cluster (miR-15a and miR-16) was the link between a common chromosomal translocation and chronic lymphocytic leukemia. MicroRNAs are frequently located at sites of chromosomal instability or amplification and can act as oncogenes or as tumor-suppressor genes. Some microRNAs promote or inhibit cancer in most cell types (e.g., the miR-17-92[4] and let-7[5] families), whereas others appear to have more restricted roles in particular tumor subtypes or in processes that are important in tumor progression, such as in angiogenesis and metastasis. Because of the critical role of microRNAs as regulators of the fate of cells, analyzing and manipulating the world of small RNAs within cancer cells may provide powerful new avenues for diagnosis, estimating prognosis and response to therapies, drug discovery, and therapeutics. MicroRNA profiling may well be the most accurate way to identify a cell type and its functional state and consequently to identify cancers of unknown primary origin and to subtype tumors.[6,7]

The potential uses of microRNA profiling in subtyping human cancers to provide more accurate prognosis and prediction of response to therapy are illustrated in the article by Ji et al. in this issue of the *Journal*.[8] The investigators profiled microRNA expression in three separate cohorts of patients with hepatocellular carcinoma. They found that expression of a single microRNA family, miR-26, which is found in normal hepatocytes and is more highly expressed in the livers of women (who are less susceptible to hepatocellular carcinoma than men), was reduced in a subgroup of samples from patients with this disease. Patients with reduced miR-26 expression (in the lower 50th percentile) had significantly reduced survival during a 6-year period but were more likely to have a response to adjuvant therapy with interferon alfa. Moreover, the expression profile of protein-coding genes in tumors that had lost miR-26 expression was dramatically different: tumors with low miR-26 expression had up-regulated not only interleukin-6 and nuclear factor κB (NF-κB) but also many genes regulated by these proinflammatory molecules. Consistent with the findings here, it was already known that high levels of interleukin-6 (even in serum) and NF-κB are linked to a poor prognosis in patients with hepatocellular carcinoma and that estrogen inhibits the production of interleukin-6.[9]

Tumor microRNA and mRNA profiling reflects gene expression of the whole tumor, which includes contributions from the tumor cells as well as tumor stroma and any infiltrating immune cells. This should be kept in mind in interpreting the thousands of microRNA profiling stud-

ies in cancer that are just now being published. In this case, it may well be that interleukin-6 is being stimulated in Kupffer cells, rather than in tumor cells. Therefore, the proinflammatory signature of tumors with low miR-26 expression may be an indirect effect of the lack of the microRNA in the tumor cells on neighboring cells. In the study by Ji et al., about 90% of the patients also were long-term carriers of hepatitis B virus (HBV). If the salient distinction of poor-prognosis hepatocellular carcinoma is inflammation, which might also modulate the expression of the cancer-promoting HBV, then it might not be surprising that only patients with an inflammatory phenotype would have a response to immune-modulating interferon therapy. Having a strong and relatively simple assay, such as low miR-26 expression, to predict the response to adjuvant interferon might provide a useful way to determine which patients should receive this intervention. In the future, the subtyping of cancers on the basis of microRNA signatures could provide a useful method to stratify patients for clinical studies.

The study by Ji et al. suggests a strong association between low miR-26 expression and both prognosis and response to interferon therapy in patients with hepatocellular carcinoma, but the association does not necessarily indicate a causal role of low miR-26 expression in such patients. However, a recent study by Kota and colleagues[10] that was performed in tumor-prone mice expressing the *MYC* oncogene in liver cells indicated not only that low miR-26 expression had a causal role in tumor formation but also that replacing miR-26 in liver tumors with the use of gene therapy could have potent antitumor effects. The investigators singled out miR-26a, because although this microRNA is widely expressed in most tissues, including the liver, its expression is substantially reduced in MYC-induced hepatocellular carcinoma. They also found how *miR-26a* might act as a tumor-suppressor gene, since in normal tissues it blocks cell proliferation by silencing two cyclins needed for DNA replication, a first step in cell division. The importance of miR-26a in suppressing MYC-induced hepatocellular carcinoma was verified when tumors dramatically regressed in mice that were injected with adeno-associated virus that was engineered to express miR-26a.

The study by Kota et al. illustrates the potential promise of manipulating microRNA expression in cancer therapy.[11] Because microRNAs suppress hundreds of genes to regulate whole programs of gene expression, it may be difficult for tumors to escape from the effect of microRNA drugs by mutating a few sequences. Since miR-26 is expressed by most normal cells, such replacement therapy is unlikely to be toxic to normal cells. The major obstacle to therapies that are based on RNA interference is delivering these oligonucleotides inside cells. Because of its filtering role, the liver traps and internalizes both small RNA drugs and gene-therapy viruses, making it an ideal testing ground for this new approach to treating cancer.

Dr. Lieberman reports serving on an advisory board for, receiving consulting fees from, and having an equity interest in Alnylam and Cequent; receiving consulting fees from Baxter; receiving lecture fees from Asuragen, Genentech, Merck, Wyeth, Pfizer, Baxter, and MedImmune; receiving grant support from an alliance between GlaxoSmithKline and the Immune Disease Institute; and holding a patent and being named on patent applications for delivery of small interfering RNA molecules and the treatment of viral infections. No other potential conflict of interest relevant to this article was reported.

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