

## CLINICAL IMPLICATIONS OF BASIC RESEARCH

## MicroRNA and Lung Cancer

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Pioneering work on the nematode *Caenorhabditis elegans* has yielded a wealth of insight into signaling pathways, revealing regulatory mechanisms that are critical to both developmental biology and tumorigenesis. For example, studies of vulval development in the worm were instrumental in identifying components of RAS–mitogen-activated protein kinase signaling. These are highly conserved across species and regulate the growth of normal and malignant cells in mammals. The study of *C. elegans* facilitated another important discovery: the existence of non-coding microRNAs. These tiny fragments of RNA (about 22 nucleotides long) regulate gene expression by hybridizing to complementary sequences in the 3' untranslated region (3'UTR) of target messenger RNA (mRNA). They can thereby repress the translation of mRNA through an unknown mechanism or increase the instability of mRNA. (RNA interference — a technique that has enjoyed extraordinary success recently as a laboratory tool for manipulating gene expression — uses some of the same molecular machinery.) A recent study<sup>1</sup> by Johnson and colleagues engages both lines of research and may suggest a potential strategy for treating lung cancer in humans.

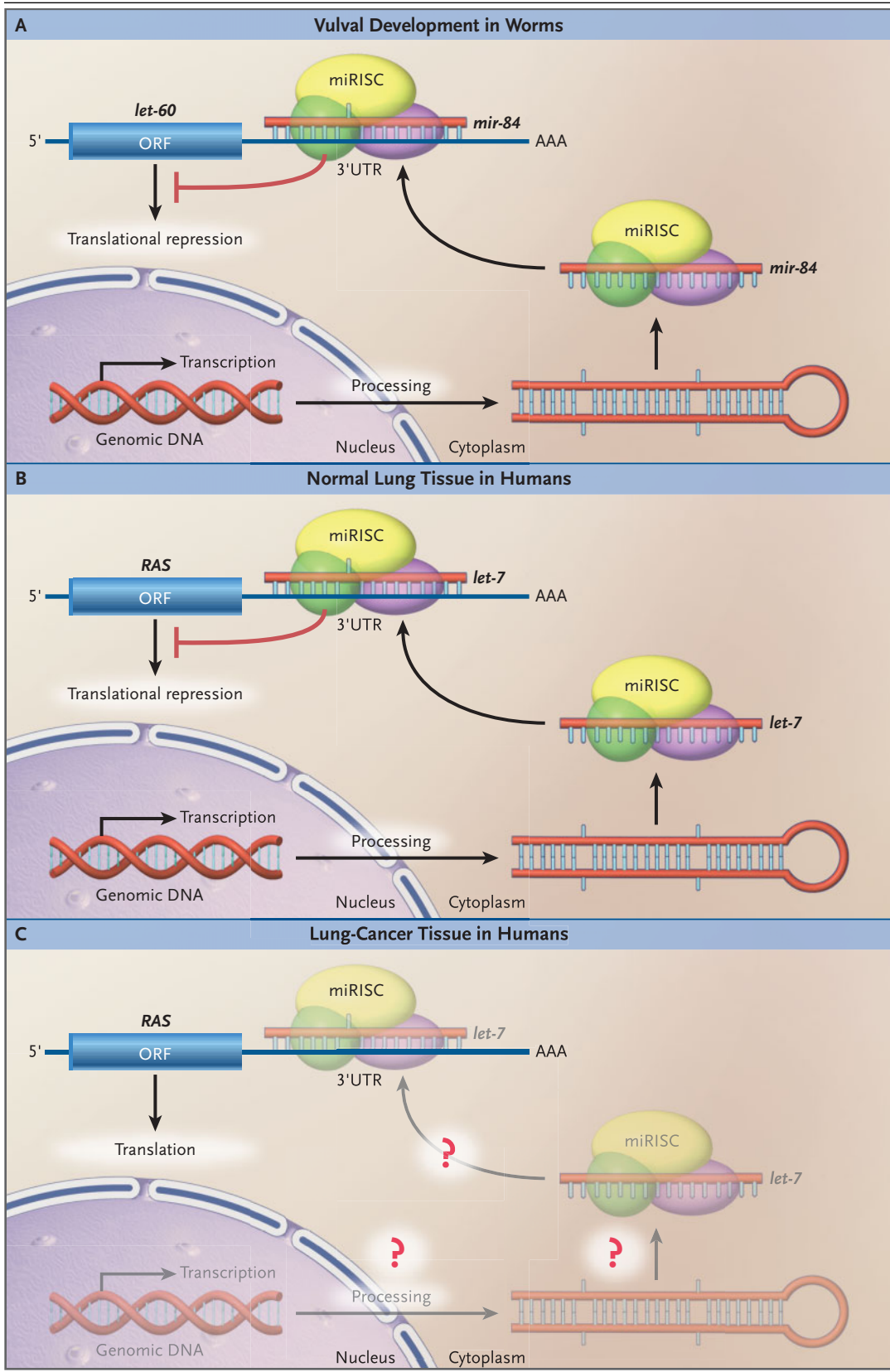
Johnson et al. first set out to identify new RNA targets of microRNAs in the *let-7* family, which includes *let-7* itself. They used a computer-based screen to identify genes encoding mRNAs with 3'UTRs containing multiple *let-7* complementary sites and homed in on four genes: the nematode *RAS* gene (called *let-60*) and the human *KRAS*, *HRAS*, and *NRAS* genes. They then showed that the expression of a reporter construct is controlled by the *let-60* 3'UTR and by *let-7*. They went on to show that mutations in *let-60* and *let-7* microRNAs can complement each other. Ablating the expression of *let-7* tends to kill the worm; simultaneously ablating *let-7* and repressing *let-60* results in a lower rate of death. Similarly, overexpression of *mir-84* (a member of the *let-7* family) inhibits the development of multiple vulvae caused by a gain-of-function mutation in *let-60*. These data strongly suggest the existence of a

reciprocal interaction between *let-7* microRNA species and *let-60* expression in *C. elegans* (Fig. 1).

The authors then investigated the role of *let-7* in the context of human cancer, because the RAS signaling pathway critically affects a cell's propensity for becoming cancerous. Using two different cell lines, they obtained further evidence of a reciprocal interaction: overexpression of *let-7* represses — and inhibition of native *let-7* enhances — the expression of RAS protein. Furthermore, Johnson and colleagues found that *let-7* complementary sites in human *NRAS* and *KRAS* 3'UTRs specifically mediate *let-7*–dependent repression (Fig. 1), which can be abrogated by *let-7* inhibitors. Supporting the relevance of this finding is the fact that the position of genomic regions commonly deleted in lung cancer (which RAS signaling is believed to help initiate) coincides with several human *let-7* genes. Accordingly, microarray analysis of microRNAs revealed specific down-regulation of *let-7* expression in sam-

**Figure 1 (facing page). MicroRNA-Mediated Regulation of RAS Expression in *Caenorhabditis elegans* and Humans.**

In specific vulval precursor cells from worms (Panel A) and in normal human lung tissue (Panel B), *mir-84*, a member of the *let-7* microRNA family, and *let-7*, respectively, are transcribed, and the transcripts, which have characteristic hairpin structures, are processed into mature microRNAs. These are then incorporated into a silencing complex (called miRISC). MicroRNA species guide miRISC to target mRNAs by hybridizing to complementary sequences in the 3'UTRs of the mRNAs and thereby prevent their translation. A recent study by Johnson and colleagues<sup>1</sup> showed that members of the *let-7* family repress the expression of RAS genes and that this mechanism is potentially relevant to the pathogenesis of lung cancer (Panel C). The question marks in Panel C indicate that the reduced expression of *let-7* microRNA in lung cancer may be due to alterations in transcription, processing, or maturation. The abrogation of translational repression results in the overexpression of RAS proteins in lung-cancer cells. Additional details of the mechanism by which microRNA represses gene expression are available elsewhere.<sup>2</sup> ORF denotes open reading frame.



ples of lung but not of breast or colon cancer, as compared with normal adjacent tissue. Finally, direct comparison of three samples of squamous-cell carcinoma of the lung and adjacent normal tissue revealed reduced expression of *let-7* microRNA and concomitant overexpression of *RAS* in the lung carcinomas.

These data are in line with findings from earlier studies demonstrating reduced expression of microRNA in various cancers, such as chronic lymphocytic leukemia and colorectal cancer. In particular, reduced expression of *let-7* in lung cancer indicates a poor prognosis.<sup>3</sup> So, what next? The expression of *RAS* and *let-7* must be analyzed in additional tumor samples, and the relevance of these findings with respect to different subtypes of lung cancer should be investigated. The extent to which altered *let-7* or *RAS* expression tips the balance toward carcinogenesis and tumor survival should be

determined. Mechanisms other than genomic rearrangements or deletions that reduce or ablate *let-7* expression, including defective microRNA processing and maturation, should be explored. Although we are far from the point at which we can judge whether augmenting *let-7* expression in lung-cancer cells might prove therapeutic, the findings of Johnson et al. provide inspiration for further work toward this end.

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  3. Takamizawa J, Konishi H, Yanagisawa K, et al. Reduced expression of the *let-7* microRNAs in human lung cancers in association with shortened postoperative survival. *Cancer Res* 2004;64:3753-6.
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