translocation frequently observed in individuals with chronic myeloid leukemia. Gleevec also effectively blocks the kinase activity of various activated Abl-family members, the platelet-derived growth factor receptor and the Kit receptor[1–3]. Although the drug inhibits a variety of Abl-family tyrosine kinases, it does not inhibit Src-family kinases.

Abl-family inhibition alone did not inhibit actin-based motility, but—surprisingly—did block the release of the extracelluar virus (EEV) from infected cells. This result indicates that the disengagement of EEV from infected cells is under the strict control of Abl-family kinases, unlike the actin tail polymerization, which is triggered by both Abl- and Src-family kinases (**Fig. 1**). The authors also report that Gleevec treatment reduced vaccinia spread from the mouse peritoneum to the ovaries and protected mice from a lethal intranasal vaccinia challenge.

This is not the first example of poxvirus inhibition by kinase inhibitors. For example, the Erk inhibitor U1026 blocks vaccinia replication in cultured cells[9] and the ErbB-kinase inhibitor CI-1033 can rescue mice from lethal vaccinia challenge[10], but Gleevec is the first kinase-targeted drug approved for use in humans that exhibits antiviral properties.

To date, only three kinase inhibitors have been cleared by the US Food and Drug Administration for use against cancer in humans (Gleevec and the EGF receptor kinase inhibitors gefitinib and erlotinib), and so it would be most instructive to know whether any of these drugs might have antiviral properties against any other 'off-target' viruses of clinical importance. For poxviruses, there is a growing appreciation that host preference is profoundly influenced by virus modulation of cellular signaling pathways[11] and it is likely that many other viruses can also be inhibited by strategies that block key host signaling pathways, as opposed to drugs that target classic viral replication enzymes. Currently, the only marketed drug that has inhibitory effects on poxviruses is cidofovir, which targets viral DNA replication, but it is toxic to patients' kidneys, whereas Gleevec causes far fewer clinical complications[1,3].

This study also raises a number of questions. The PD-family Src/Abl inhibitors block actin-based motility of vaccinia, whereas the Abl-specific Gleevec inhibits only the later EEV release step, suggesting that there may be other viral substrates for host kinase phosphorylation in addition to Tyr112 of A36R (ref. 6). Indeed, the mechanism by which EEV is released from vaccinia-infected cells is not at all clear, and there is some evidence that EEV shedding can sometimes bypass the actin tail polymerization step[5]. Whatever the exact mechanism, however, there are derivatives of Gleevec that have much higher affinity for Abl-family kinases[12], and some of these might be amenable for further development as next-generation antiviral reagents as well.

The next step will be to test Gleevec in individuals with poxvirus infections, such as disseminated vaccinia or monkeypox. It will be important to see whether vaccinees who are prone to vaccinia virus complications express Gleevec-sensitive Abl kinases, or whether immunocompromised individuals can clear systemic poxvirus infections even in the presence of drugs like Gleevec. In any event, it would be worthwhile to now test Gleevec in some of the surrogate animal models for smallpox in humans, such as extromellia virus in mice or monkeypox in nonhuman primates.

The approach of using host factors rather than viral proteins as drug targets offers real promise for the future. Indeed, the end game of this strategy is not to block viral replication dead in its tracks, but instead to reconfigure the overt manifestations of viral pathogenesis into a less virulent subclinical infection that more closely mimics vaccination with an attenuated live vaccine. In fact, it would make considerable sense to embark now on a campaign to systematically screen other host signaling inhibitors for antiviral properties, against a broad spectrum of potential virus pathogens as targets, rather than waiting for classic drug discovery or vaccine initiatives to come to our rescue after the next viral pandemic strikes.

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Sizing up miRNAs as cancer genes

Carlos Caldas & James D Brenton

Findings over the last year or so have built the case that microRNAs might contribute to cancer. Three studies now definitively show this to be the case and also suggest that these small RNAs could be used to categorize tumors.

MicroRNAs (miRNAs), a family of mature noncoding small RNAs 21–25 nucleotides in length, negatively regulate the expression of protein-encoding genes. Therefore, it is not surprising that studies directly implicating miRNAs in cancer are emerging, because cancers ultimately arise because of disrupted gene expression. Such findings are epitomized by three reports in a recent issue of *Nature*[1–3]. The new work describes miRNAs with oncogenic and tumor suppressor activity and unveils a new molecular taxonomy of human cancers based on miRNA profiling.

miRNAs are processed sequentially from primary miRNA (pri-miRNA) precursor transcripts, and regulate gene expression at the post-transcriptional level[4–6]. They work either by direct cleavage of the target mRNA using the RNA interference machinery or by inhibition of protein synthesis. The expression of miRNAs is highly specific for tissue and developmental stage, but little is known about how these expression patterns are regulated[4–6].

The founding miRNA family members (*lin-4* and *let-7*) were identified as loss-of-function mutations in *C. elegans* that cause defects in developmental timing[7,8]. *lin-4* and *let-7* encode noncoding RNAs 21–22 nucleotides in length that are complementary to conserved sites in the 3′ untranslated region of their target genes.

Subsequently, miRNAs were found to be an abundant class of transcripts in all metazoans. Bioinformatic approaches identified 200–255 human miRNA genes, but more recent work has predicted the number to be closer to 1,000 (ref. 9). This makes the miRNA genes one of the most abundant classes of regulatory genes in mammals.

So which and how many mRNAs are regulated by miRNAs? Current estimates suggest that about one-third of human mRNAs appear

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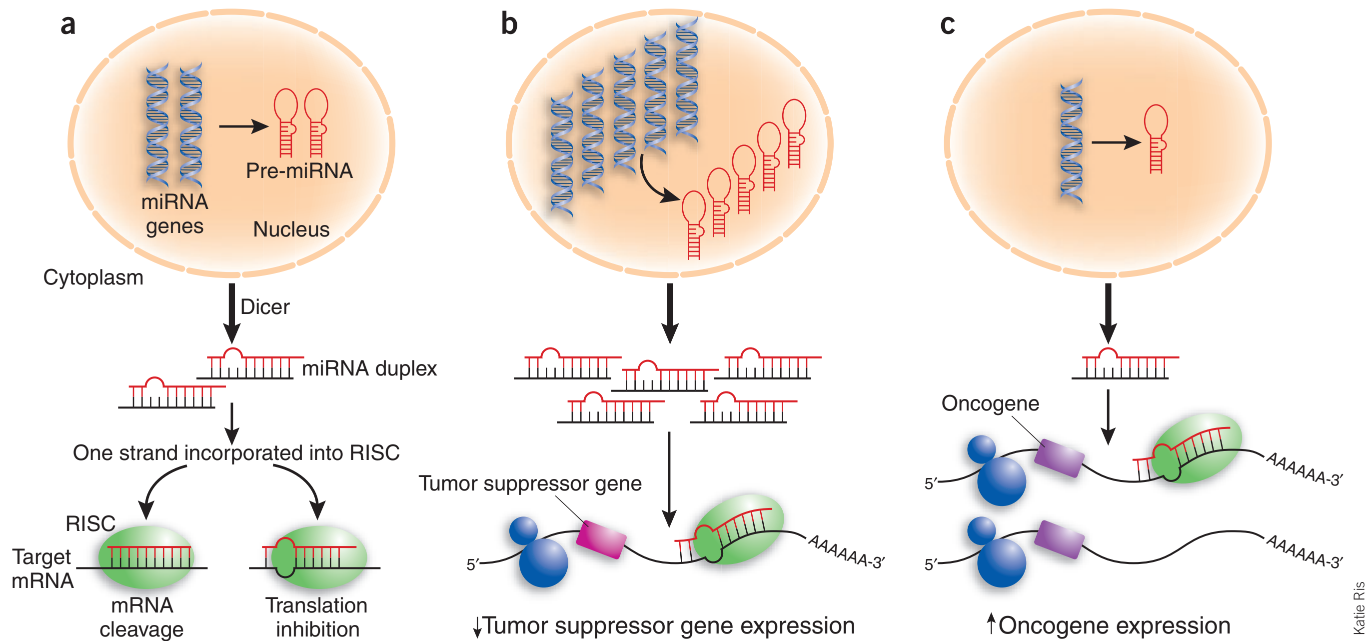

Figure 1 A model of miRNA involvement in cancer by modulation of expression of tumor suppressor genes and oncogenes. (**a**) pri-miRNAs are processed into pre-miRNAs in the nucleus. pre-miRNAs are exported to the cytoplasm and are processed by the enzyme Dicer to yield miRNAs. These miRNAs operate by either cleaving mRNA or inhibiting translation in concert with RISC (RNA-induced silencing complex). (**b**) Overexpression of miRNAs—for instance, by amplification of the miRNA-encoding locus—could decrease expression of the target, such as a tumor suppressor gene. (**c**) Underexpression of miRNAs—for instance, by deletion or methylation of the miRNA locus—could result in increased expression of a target such as an oncogene.

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to be miRNA targets[10]. Vertebrate miRNAs target about 200 transcripts each and more than one miRNA might coordinately regulate a single target[11]. This offers an enormous level of combinatorial possibilities and suggests a model of highly managed regulation of gene expression. Indeed, miRNAs have now been suggested to have a role in developmental timing, neuronal cell fate, cell death, cell proliferation, regulation of insulin secretion, hematopoietic cell fate and stem cell division[4–6].

The first evidence for direct involvement of miRNAs in cancer was the finding that *miR-15* and *miR-16* are located within a 30 kb deletion in chronic lymphocytic leukemia, and that both genes were deleted or underexpressed in most cases of this cancer[12]. Moreover, a potential mRNA target of *miR-16*, the gene encoding arginyl-tRNA synthetase (*RARS*), was overexpressed in samples that underexpressed *miR-15* and *miR-16*. One year later, reduced expression of *miR-143* and *miR-145* in colorectal neoplasia was reported[13].

Next followed a quick succession of findings[4–6]: more than half of mapped miRNAs are located at fragile sites and genomic regions involved in cancers; chromosomal translocation t(8;17) in aggressive B-cell leukemia results in fusion of *miR-142* precursor and a truncated *MYC* gene; *miR-26a* and *miR-99a* are underexpressed in lung cancer cell lines; a region of DNA that contains the *miR-17-92* polycistron is the target for 13q31-32 amplification in malignant lymphoma; *miR-155* is overexpressed in Burkitt lymphoma; and *let-7* expression is reduced in human lung cancers. Finally, this spring, a study showed that *let-7* miRNA underexpression in lung cancer correlates inversely with expression of the oncoprotein RAS and the *let-7* miRNA family negatively regulates RAS in *C. elegans* tissues and human cell lines[14]. The three reports in *Nature* come on the heels of this flurry.

He *et al.*[1] used a custom microarray to profile 191 miRNAs in B-cell lymphoma cell lines previously shown to have the 13q31-q32 amplicon. Five miRNAs from the *miR-17-92* polycistron were upregulated and correlated with DNA copy number. *pri-miR-17-92* was also overexpressed in human lymphoma samples.

The authors next took advantage of a well-characterized mouse model of B-cell lymphoma development in which the transcription factor Myc, one of the most common oncogenes in human cancer, is overexpressed. Hematopoetic stem cells overexpressing both *Myc* and *mir-17-92* were used to generate mosaic animals. Strikingly, these mice developed cancers earlier than mosaics generated with *Myc* alone and the lymphomas were more aggressive, with increased mitotic rate and less apoptosis. The experimental tumors were predominantly of the pre-B-cell type, suggesting transformation of early progenitor cells.

These results show that these miRNAs are overexpressed in human cancer and can augment the oncogenic effects of *Myc* in a mouse model. But the mechanism is likely to be complex, as no single individual miRNA from the cluster could accelerate the development of tumors as efficiently as the intact polycistron. Moreover, the experiments did not identify the targets of *miR-17-92*.

Independently, O'Donnell *et al.*[2] asked whether MYC, as a prominent transcription factor, could directly regulate expression of miRNAs. Cell-based experiments with an inducible *MYC* transgene showed that MYC directly binds to and upregulates the *miR-17-92* locus. The authors then investigated the transcription factor E2F1, which is induced by MYC and promotes cell-cycle progression and induces apoptosis, depending upon the context of its expression[15].

E2F1 had been predicted to be a target of *miR-17-92* using bioinformatic methods[10]. In a series of elegant experiments, the authors showed that the miRNAs specifically regulated E2F1. For instance, they showed that transfection of a transgene containing the entire *miR-17-92* cistron strongly repressed E2F1 protein, without a marked effect on *E2F1* mRNA. In the cell line where MYC was induced, there was a marked increase in *E2F1* mRNA without a corresponding rise in E2F1 protein, although its levels increased slightly. Thus MYC expression induces *miR-17-92*, and this dampens increase of E2F1 protein by post-transcriptional repression. Hence, *miR-17-92* may act as a rheostat to fine-tune E2F1 translation in a temporal manner.

One interpretation of these two studies is that *miR-17-92* miRNAs are oncogenic but can also have tumor-suppressor effects by decreasing E2F1 translation[16]. This is probably an oversimplification, as E2F1 has been shown to function both as oncogene and tumor suppressor[17]. In line with this notion, decreased E2F1 translation in the *miR-17-92* mouse lymphoma could explain the reduced apoptosis. It is now of great interest to analyze in detail human lymphomas with *miR-17-92* overexpression.

The data from these studies suggest a simple framework to understand the role of miRNAs in cancer: miRNA-mediated tumorigenesis results from either downregulation of tumor suppressor genes or upregulation of oncogenes (**Fig. 1**).

Lu *et al.*[3] asked whether miRNA expression patterns could be used to classify human cancer. To do this reliably, they first developed a sensitive bead-based hybridization technology with higher specificity than microarray methods. They then measured the expression of all 217 known human miRNAs in a panel of 218 human cancer samples.

Hierarchical cluster analysis showed that cancers from similar developmental origins grouped together, with clear partitioning of hematopoietic from epithelial cancers. Gastrointestinal cancers, which arise from endoderm, were also clustered together. In contrast to the miRNA results, data from mRNA microarray profiling did not provide accurate classification.

Stratification within cancer types using miRNA expression was also achieved; as comparison between subtypes of acute lymphoblastic leukemia samples also showed clear partitioning into previously characterized molecular classifications (BCR/ABL, T-cell acute lymphoblastic leukemia and mixed lineage leukemia). Strikingly, cancers in both human and mouse had lower expression of a large proportion of miRNAs as compared to normal tissues. Moreover, using two different cellular systems, they showed upregulation of miRNAs as cells differentiated. This led the authors to suggest that lower levels of miRNAs reflect loss of differentiation, which is a cardinal feature of cancer.

These three studies establish the miRNA-cancer connection: two give us mechanistic insights about how misexpression of miRNA might disrupt tumorigenic networks and the third shows the promise of using miRNA profiling for cancer classification.

The miRNA expression profiling report has profound clinical implications if expression levels of 200 miRNAs can reproducibly classify cancers. The stability of miRNAs and the ability to isolate them intact (even from paraffin-embedded tissues) will prompt many studies to show the prognostic and predictive value of miRNA profiling, given that there are now several high-throughput platforms to do so. Time will tell whether miRNA profiling will supersede mRNA profiling.

An even more exciting prospect is the potential for analysis of patterns of tissue miRNA expression using *in situ* hybridization. This is now possible using special high-affinity RNA analog probes[18] and would facilitate tissue-level studies of preneoplastic lesions, tumor microenvironment, clonal heterogeneity, invasion and metastasis.

As key regulators of gene expression, miRNAs may also have a role in genetic predisposition to disease. The recent description of a sequence polymorphism in a mature miRNA[19] raises the prospect of testing the association of such polymorphisms with cancer. Therapeutic targeting of these tiny RNA molecules is attractive and, in theory, technically possible, using miRNA silencing induced by small interfering RNA[5,6]. Such approaches will have to wait for further understanding of regulation of oncogenic and tumor suppressive networks by miRNAs before their use in the clinic can be contemplated.

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microRNAs manage the heart

Genes encoding microRNAs—already thought to be involved in the control of developmental events—have now found their way into the heart, according to a study in a recent issue of *Nature* (advanced online publication 12 June 2005; doi:10.1038/nature03817).

Yong Zhao *et al.* focused on two related microRNAs expressed in the heart. Shown is the expression pattern of one of these microRNAs, miR-1-1, in the mouse embryo. miR-1-1 is found in the developing heart (dark blue) and in the somites, embryonic structures that give rise to muscle.

The researchers next devised a new algorithm to search for microRNA target sequences in mRNA. Both microRNAs, they found, negatively regulate Hand2, a transcription factor that promotes expansion of ventricular heart muscle precursor cells. In line with that finding, overexpression of the microRNAs in the heart decreased the pool of proliferating precursor cells.

The authors also identified transcriptional regulators that control expression of the microRNAs. These included MyoD and other regulators of muscle differentiation. The data suggest that these heart microRNAs control the balance between differentiation and proliferation during heart development.

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