

was not a substantial change in KRAS mRNA in KRAS-variant triple-negative tumours, Paranjape and colleagues reported an enrichment of both the NRAS mutant and MAP-kinase-activation signatures in tumours that had the polymorphism. Moreover, in line with previous reports in non-small-cell lung cancer, expression of *let-7* family members was lower in KRAS variant triple-negative breast cancer than it was in non-KRAS variant cancer. Therefore, KRAS-variant triple-negative breast cancer shows substantial changes in RAS and *let-7* pathway activity.

These findings provide valuable insights into the relation of *let-7* with subtype-specific risk of breast cancer. Analyses of this scale and depth raise several important questions. First, what is the role of individual *let-7* targets (eg, KRAS, HMGA2, and CMYC) in triple-negative breast cancer? Is there a functional relation between *let-7* expression and BRCA1 expression or function, such that reduced concentrations of *let-7* can bypass the need for BRCA1 mutation in triple-negative breast cancer? If so, might such an association help with development of therapeutic strategies targeting homologous recombination repair deficiency? In this regard, does the enrichment of the MAP-kinase activation signature suggest that KRAS-variant triple-negative breast cancer has enhanced signalling through the MAP-kinase pathway or particular sensitivity to MAP-kinase inhibitors? Finally, and perhaps most importantly, the odds ratio for risk of development of triple-negative breast

cancer in one cohort of premenopausal women was 2.307 (95% CI 1.261–4.219). Equivalently strong odds ratios were reported for the KRAS variant in a cohort of smokers with non-small cell lung cancer and fewer than 40 pack-years smoking history.<sup>4</sup> Although these studies need validation in independent cohorts, the magnitude of the association of the KRAS-variant and triple-negative breast cancer suggests basic research on miRNA–mRNA target interactions might contribute clinically to risk stratification of premenopausal women within breast-cancer-screening trials.

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## MicroRNA therapeutics in preclinical cancer models

Substantial progress has been made in the past decade that links the expression of various microRNAs (miRNAs) with cancer. Despite their small sizes at about 22 nucleotides, these endogenous non-coding RNAs have an enormous effect on protein-coding-gene expression, and regulate various cellular events including proliferation, apoptosis, and differentiation. As would be expected, aberrant miRNA expression can have adverse effects on these processes, ultimately contributing to tumour initiation, promotion, and progression.<sup>1</sup>

Because abnormal expression of miRNAs has been reported in cancer, reconstituting their expression either by restoration of silenced miRNAs or suppression of

overexpressed miRNAs might have clinical application. Although miRNAs are not presently used as cancer therapeutics, successful in-vivo studies support the notion that they could be used as innovative therapeutics to address unmet needs.

The first reported use of miRNAs as an in-vivo therapeutic technique exploited the tumour-suppressive property of one miRNA, *let-7*, to prevent and treat lung tumours in mice. In these pioneering studies, *let-7* impaired cellular transplantation in a xenograft system and reduced the tumour burden in an **autochthonous** mouse model of non-small-cell lung cancer.<sup>2,3</sup> However, during the course of treatment, complete rescue of tumour formation was only partial in both systems.

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**Autochthonous**  
A tumour that is formed in the place where it is originally found



Brian Bell/Science Photo Library

Esquela-Kerscher and colleagues<sup>2</sup> suggested that more than one stable dose of *let-7* is necessary to inhibit growth. Kumar and colleagues<sup>3</sup> speculated that either tumour-cell resistance or suppression of additional *let-7* targets other than *KRAS* might prevent complete rescue. Nonetheless, miRNA therapeutics might aid in sensitisation of cells to more traditional anticancer treatments such as radiotherapy or chemotherapy, warranting the continued development of miRNA therapeutics for use in vivo.

In addition to the ability of *let-7* to inhibit tumour initiation in non-small-cell lung cancer, its therapeutic effect on tumour progression was assessed in established tumours. In an autochthonous mouse model of non-small-cell lung cancer, intranasal delivery of lentiviral *let-7*<sup>4</sup> or intravenous delivery of lipid-encapsulated *let-7*<sup>5</sup> caused regression of preformed lung tumours. Tumour regression was attributed to decreased cellular proliferation and induction of necrosis.

Similar to non-small-cell lung cancer treated with *let-7*, *MYC*-driven hepatocellular carcinomas responded to administration of miR-26a.<sup>6</sup> Liver-tumour burden was reduced in mice after miR-26a treatment chiefly through inhibition of tumour-cell proliferation and induction of tumour-specific apoptosis by downregulation of cyclin D2 and cyclin E2. Whereas delivery of small hairpin RNAs (shRNAs) can cause acute toxic effects in the liver, this study emphasised the effectiveness and non-toxic delivery of miR-26a. Collectively, these preclinical studies show that restoration of miRNA concentrations in tumours might be a beneficial therapeutic technique that inhibits tumour initiation and promotion.

The third component to oncogenesis involves tumour progression, which often leads to tumour conversion and metastasis. Although metastasis accounts for most cancer mortality, available therapeutics largely reduce primary tumour burden without specifically targeting metastatic lesions. However, two reports<sup>7,8</sup> propose miRNAs as a promising alternative to antimetastatic therapeutics. The first study<sup>7</sup> reported that antiproliferative nature of prostate-cancer cells was positively correlated with high levels of mature, endogenous miR-16.<sup>7</sup> Synthetic miR-16 administration in cells with low endogenous miR-16 substantially reduced their proliferation. Furthermore, when treated with miR-16, a luciferase prostate cancer xenograft

mouse did not form metastatic tumour growth in bone whereas untreated mice developed moderate-to-severe metastatic bone lesions. This study shows impaired metastasis by miRNA replacement therapy; however, whether miR-16 displays an inhibitory role only in metastatic tumours or in primary and metastatic tumours is unknown.

Similarly, miR-31 inhibits metastasis but does not affect the primary tumour.<sup>8</sup> When miR-31 is expressed in an orthotopic mouse model of human breast cancer, local invasion and metastatic burden of spontaneous lung metastasis are inhibited without affecting the growth of the primary mammary tumour. Expression of miR-31 triggers anti-metastatic effects in both established lung and bone metastases, mainly through cell-cycle arrest and apoptosis, modulated by Akt signalling and the proapoptotic Bim protein. These studies suggest that miRNA-replacement therapy could be an effective intervention to specifically treat patients with metastases and possibly lower cancer mortality.

Much the same as forced expression of silenced miRNAs, suppression of overexpressed miRNAs in cancer is also a promising therapeutic strategy. As with the loss of tumour suppressive miRNAs, oncogenic miRNA upregulation is associated with various stages in tumour development. In particular, miR-10b concentrations are increased in metastatic tumours, and expression of an **antagomir** to miR-10b resulted in decreased cellular motility and invasiveness in vitro.<sup>9</sup> Equivalent findings are noted in vivo; systematic delivery of anti-miR-10b in an orthotopic mouse model of breast cancer showed a significant reduction in the number and size of lung metastases, with no obvious effect on primary tumours. However, some small physiological changes, such as changes in liver and spleen size and the concentrations of serum proteins and metabolites due to the antagomir composition (not to the sequence of miR-10b itself) suggested that challenges remain for non-toxic delivery of miR-inhibitors in vivo.

Although experiments are in progress to assess treatment of cancer with miR-inhibitors, one particular miR-inhibitor against hepatitis C virus exemplifies the feasibility of miRNA-targeted therapy in human disease.<sup>10</sup> A locked nucleic-acid-modified anti-miR-122 (Miravirsen, Santaris Pharma, San Diego, CA, USA) effectively treats chimpanzees infected with hepatitis C

**Antagomir**

An oligonucleotide used to silence microRNA

virus without any observable resistance or physiological side-effects. This treatment has advanced to phase 2 clinical trials, which emphasises the strengths of anti-miR-122, including high efficacy and good tolerability without adverse effects.

As the results of years of effort begin to show the effects of miRNAs in cancer, a few candidate miRNAs have emerged as therapeutics to prevent and treat various stages of tumorigenesis. However, the notion of treating cancer with miRNA replacement or miR-inhibitors is still at its infancy and requires more functional in-vivo studies. Safe and efficacious delivery mechanisms also need to be established.

Development of miRNA therapy, which employs miRNA's pleiotropic role in gene regulation, has the potential to overcome the limitations of present cancer therapies. Although targeted therapies such as imatinib and erlotinib have given many cancer patients tremendous benefit by tailoring therapy to a tumour's genetic profile; redundancy and complexity of signal pathways often leads to relapse even with combined targeted therapies. Based on present preclinical trials, combination of miRNA therapy with targeted or traditional therapies might be able to create a synergistic effect for treatment of cancer and become an alternative treatment for cancer. Many biotechnology companies have miRNA therapeutics programmes, and human clinical trials, which should begin in the next few years,

will show whether the high expectations of this novel approach are warranted.

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## Value-based insurance design in oncology

In recent years, great advances have been made in our understanding of cancer, leading to the successful development of many novel drugs. However, this progress has been accompanied by a substantial increase in drug prices, which has affected the availability of these drugs for patients.<sup>1</sup> Additionally, some new drugs have benefits that can be measured in weeks of added life expectancy. In these cases, an alternative view, from the perspective of the health-care system, is that high prices are not justified by the gain in life expectancy that these interventions provide.

Furthermore, as new payment systems in oncology are being developed in the USA,<sup>2</sup> policy makers and involved stakeholders should consider the proven clinical value of interventions. In this context, section 2713 (c) of the Patient

Protection and Affordable Care Act included a notion that allows the development of guidelines to use value-based insurance designs (V-BID) as an approach to improve alignment of a patient's out-of-pocket contribution to the value or cost–benefit tradeoff.<sup>3</sup>

The implementation of V-BID programmes in oncology would be based on three observations. First, high-cost sharing based only on price discourages the use of high-value, potentially life-saving interventions. Second, interventions differ in the clinical benefit that they provide, and one intervention might provide different benefits based on its indication (eg, breast vs colorectal cancer) or the clinical scenario (eg, adjuvant vs palliative setting). Third, the value of a specific intervention might be patient specific, and biomarkers can identify those who would benefit the most.