

## NEWS AND VIEWS

### Anti-miRNA anti-virals make antisense

Whilst talk of RNA interference may predominate at both academic and industry conferences, the role for DNA antisense oligonucleotide technology has far from been diminished. RNAi based therapeutic strategies laud the appropriation of an endogenous gene regulation pathway as a primary advantage of RNA interference over other anti-mRNA approaches, yet in principle this is also a significant vulnerability of RNAi; disruption or overloading of the small RNA gene regulation network may have unpredictable consequences for cells. Leaman et al (2005) showed that 2'-O-Methyl modified antisense oligonucleotide mediated depletion in *Drosophila* embryos produced marked phenotypes for 96% of miRNAs targeted. Antisense oligonucleotides therefore offer both a simple mechanism for dissecting the role of endogenous small RNAs in gene regulation, and a potential therapeutic tool should miRNAs be discovered that are implicated in human disease.

Although no human pathology specifically due to a miRNA mutation has yet been identified, a number of studies suggest that miRNAs may be useful therapeutic targets. He et al (2005) identified a cluster of microRNAs, the mir-17-92 polycistron, located in a region of DNA that is commonly amplified in human B-cell lymphomas and the RNA levels of which are often substantially increased in these cancers. Overexpression of the mir-17-92 cluster accelerated tumour development in a mouse B-cell lymphoma model, and implicated the mir-17-92 cluster as a potential human oncogene. Cimmino et al (2005) recently discovered two small RNA regulators of the anti-apoptotic factor Bcl2. MicroRNAs miR-15a and miR-16-1 negatively regulate Bcl2 at a posttranscriptional level and repression by these microRNAs induces apoptosis in a leukemic cell line model. B-cell chronic lymphocytic leukemia (CLL) is the most frequent adult leukemia in the Western world and the malignant cells enhance their survival by overexpressing Bcl2. MicroRNAs suppressing Bcl2 overexpression might therefore be

considered tumour suppressors. Dysregulation of this small RNA regulatory network might be involved in tumour progression, and so, manipulation of the levels of specific miRNAs with antisense oligonucleotides might be employed for therapeutic benefit.

A growing body of evidence also implicates miRNAs in viral infection. Dunn et al (2005) show that human cytomegalovirus expresses novel microRNAs during viral infection and predicts that a number of these microRNAs may be involved in suppression of the antiviral response. HIV has been predicted to encode miRNAs that down regulate CD4, CD28 and some interleukins, whilst SV40 infected mammalian cells express miRNAs that reduce their susceptibility to lysis by cytotoxic T cells (Couturier et al, 2005; Sullivan et al 2005). Furthermore, Jopling et al (2005) have identified a liver-specific miRNA that interacts with the 5' NCR of the hepatitis C virus (HCMV) and seems to aid viral replication. Antagonism of miR-122 function using an antisense oligonucleotide resulted in a dramatic decrease of HCMV viral RNA. Many miRNAs are conserved between HCMV strains specific to different species – miRNAs may well represent a common mechanism for inhibiting mRNA targets for minimal investment in viral genomic sequence. As such, antisense oligonucleotides targeting viral miRNAs might produce highly effective anti-viral reagents. High conservation amongst HCMV miRNAs, coupled with easy design and formulation of multiple antisense reagents might reduce the potential for viral escape from inhibition due as oligonucleotides may offer a reduced sensitivity to mutation relative to proteins – a single base mutation in an miRNA might still bind with reasonable affinity to an almost-complimentary oligonucleotide, whereas a single amino acid substitution can radical alter the binding properties of a protein targeting pharmaceutical.

Alnylam Pharmaceuticals (Cambridge, US) and Isis Pharmaceuticals (Carlsbad, US) have both

moved rapidly to capitalize on such findings, announcing a co-exclusive license agreement with Stanford University related to the discovery and development of therapeutic products for hepatitis C virus (HCV) infection by inhibiting a liver-specific microRNA.

With advances in specific systemic delivery of nucleic acids, virus specific reagents might be delivered only to cells expressing viral antigens at the cell surface. The sequence specificity and serum stability of second and third generation oligonucleotides offers to circumvent some of the problems facing siRNA based therapies and offers hope that after 20+ years of research and investment in antisense reagents they still have an important role in a field pre-occupied with RNAi.

## REFERENCES

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