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A genome-wide view of antisense

To the editor:

The ability to study gene expression on a genomic scale is allowing a deeper understanding of specificity of antisense inhibition. Although concerns about nonsequence specific antisense effects have been raised[1], few genome-wide studies have systematically investigated these effects. We draw readers' attention to one example from our laboratory where antisense treatment has clearly been shown to alter expression of genes that appear to have no relationship to the intended antisense-targeted gene.

Antisense inhibition of gene expression relies on the simple rules of Watson-Crick base pairing. A synthetic small single-stranded oligonucleotide (generally a 13–25-mer) that is complementary to a specific gene is introduced into cells, binds with mRNA, and inhibits translation. Hybridization of the antisense oligonucleotide with the target mRNA can physically block the translation machinery or activate RNase H cleavage at the RNA–DNA duplex site[2–4]. Targeting gene expression at the RNA level allows protein production to be turned off, even if RNA is abundant. When the protein product of translation is important for cell growth and/or viability, antisense inhibition of gene expression can produce a lethal phenotype. Because a particular 15- to 17-mer sequence has been estimated to occur only once in the entire human genome[1], theoretically, antisense inhibition of gene expression should be exquisitely specific.

High-density cDNA microarrays enable parallel analysis of the expression of thousands of genes in a single hybridization for complex biological systems[5]. We have previously examined genomic effects of antisense inhibition of protein kinase A RIα expression in tumor cells using cDNA microarrays[6]. Using *in vivo* tumor models of PC3M human prostate carcinoma grown in nude mice, the specificity of antisense effects on gene expression signatures was critically assessed using three oligonucleotides that differed in sequence or chemical modification: an immunostimulatory phosphorothioate oligonucleotide directed against human RIα, a second-generation immunoinhibitory RNA-DNA mixed-backbone oligonucleotide, and a non-immunostimulatory phosphorothioate oligonucleotide targeted to mouse RIα (this oligonucleotide cross-hybridizes with human RIα).

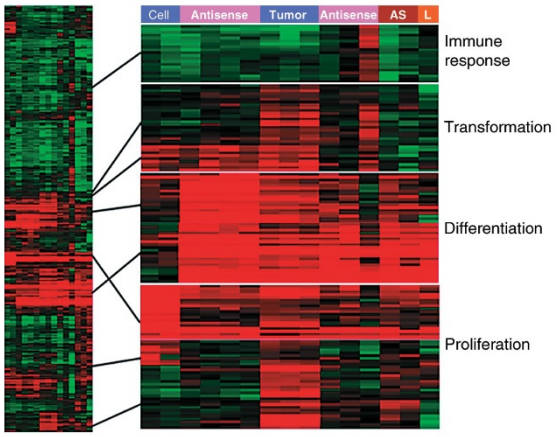

Figure 1. Molecular portrait of the prostate carcinoma reverted phenotype (reproduced from ref. 6). cDNA microarray analysis of the RIα antisense-induced expression profile shows up- and downregulation of coordinately expressed gene clusters that produce the molecular portrait of a reverted tumor-cell phenotype. These studies also reveal that antisense modulates many genes that appear to have little relationship with the targeted gene (RIα).

Antisense treatment was found to affect one cluster, or signature, of genes involved in proliferation and another involved in differentiation (Fig. 1)[6]. These expression signatures were quiescent and unaltered in the livers of antisense-treated animals, indicating that distinct cAMP signaling pathways regulate growth for normal and cancer cells.

A careful analysis of the microarray data reveals that the antisense modulates many genes that appear to have a tenuous or no relationship with the targeted gene (RIα)[6]. A few examples include remarkable upregulation of genes encoding Cdc42, RAP1A, and cytoskeleton regulatory proteins, and marked downregulation of genes coding for MAP kinase 5, collagen type 4, catalase, and M-phase inducer phosphatase 2.

While recent clinical success with Genta Pharmaceuticals' (Berkeley Heights, NJ) Genasense against BCL-2 demonstrates the promise of antisense as an adjunct to more conventional chemotherapeutics, our results demonstrate that downregulating a specific protein will likely have unforeseen consequences in multiple cellular signaling pathways, at least with phosphorothioate oligonucleotides. It is therefore crucial to examine the antisense effect at the genomic level rather than at the level of a single target gene. Our results indicate that microarray studies can facilitate the study of oligonucleotide pharmacokinetics, sequence specificity, non-sequence-specific effects, and toxicity. Further adoption of this technology will facilitate development of nucleic acid medicines with higher target specificity and minimized side effects.

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Delivering zinc fingers

To the editor:

In his News & Views on advances in the use of zinc-fingers as DNA binding modules in artificial transcription factors in the March issue (*Nat. Biotechnol.* **21**, 242–243, 2003), Ansari mentions "the loftier goal of using artificial transcription factors as therapeutic agents." In this context, he states several challenges, including the need to evade the immune system, delivery, and the ability to regulate their function based upon intra- and extra-cellular signals.

For the delivery issue, transgenes encoding chimeric transcription factors can be transferred via retroviral gene therapy, although this method suffers from the