required for their synthesis. It may also be possible to find nucleic acid ligands for certain poorly immunogenic targets. In addition, antibodies are subject to animal to animal variation, whereas nucleic acid ligands can be reproduced accurately anywhere in the world on the basis of their sequence. For applications and detection methods where the bulk of an antibody is a limitation, the small size of nucleic acid ligands also offers an advantage.

In conventional nucleic acid-based diagnostics, one can easily identify probes that characterize a pathogenic species or strain using a knowledge of the nucleotide sequence of relatively invariant regions of genomes. Similarly, finding antibodies for conventional immunoassays is also simple, because all one needs is an antigen, a syringe, and an animal. On the basis of the diversity of ligands available through SELEX, it is possible that nucleic acid ligands will also prove to be as general and easy to find as conventional nucleic acid probes and antibodies. Thus far, however, most of the effort in SELEX has been directed at developing therapeutic molecules, ribozyme variants, and ligands for other research applications. As more DNA ligands are developed for diagnostic purposes, the generality of this approach will become clear.

1. Drolet D.W., Moon-McDermott L., and Roming T.S. 1996. *Nat. Biotechnol.* **14**:1021–1025.
2. Mills D.R., Peterson R.L., and Spiegelman S. 1967. *Proc. Natl. Acad. Sci. USA* **58**:217–224.
3. Tuerk C. and Gold L. 1990. *Science* **249**:505–510.
4. Gold L., Polisky B., Uhlenbeck O., and Yarus M. 1995. *Annu. Rev. Biochem.* **64**:763–797.
5. Green L.S. et al. 1995. *Chem. Biol.* **2**:683–695.

Adenoviruses get safe, effective, and specific

James Kling

In his recent report in *Science* (272: 1470–1473, 1996), Thomas Shenk of the Howard Hughes Medical Institute (Princeton University, Princeton, NJ) sent a clear warning to gene therapy researchers working with adenoviruses. Unmodified adenoviruses contain a sequence that could be correlated with oncogenesis. Shenk has shown that the adenovirus protein E4orf6—which is produced by many adenoviral vectors in clinical trial—blocks the transcriptional activation of cellular growth control proteins by the p53 tumor suppressor, an event that might lead to the uncontrolled growth of transfected cells. Furthermore, over recent weeks, a number of researchers around the United States have reported on other adenovirus imperfections.

First, there is the issue of adenovirus oncogenicity, which Shenk explored. He cotransfected p53-deficient SAOS-2 cells with a p53-producing plasmid and another plasmid containing a reporter gene containing two copies of the p53 binding site. The cells showed a sixfold increase in production of the cellular growth proteins over cells transfected with the reporter gene plasmid alone. A third plasmid containing E4orf6 nullified the expression enhancement provided by p53, probably as a consequence of the formation of an E4orf6-p53 complex which Shenk later isolated in vitro. The suggestion, of course, is that all adenovirus strains used in gene therapy should be made E4orf6-deficient.

Cell selectivity is another problem of adenoviral vectors. The protruding fiber protein is relatively unchoosy in mediating viral attachment to cell receptors. David Curiel at the University of Alabama (Birmingham, AL) has developed a method of constructing more selective fibers. This extends earlier work (*Gene Ther.* 2:660–669, 1995), wherein ligands that bound only to receptors on target cell types were incorporated physiologically into the fiber. In their latest work, Curiel's group combine a fiber-deleted, propagation-defective rescue plasmid and a shuttle plasmid encoding a variant fiber gene (which encodes the ligand) to allow rapid construction of fiber-modified adenoviruses.

Curiel's group is developing another strategy that should reduce selectivity. They used a Fab antibody fragment directed against the terminal "knob" region of the viral fiber—the primary cellular binding region—and conjugated it to the vitamin folic acid. In doing so, they constructed a vector that could be targeted at the folate receptor. KB human cancer cells, which express the folate receptor, were treated with folate-targeted adenovirus carrying a gangciclovir-mediating gene. Upon subjection to gangciclovir, 73% of the transfected cells died; the control cells, treated with gangciclovir alone, suffered a mortality of 8%.

Thomas Wickham's group at Genvec, Inc. (Rockville, MD) has also employed antibody targeting. Rather than folate, his group focused on cellular α_V integrin receptors that take part in secondary binding interactions with a component of the viral fiber—the RGD sequence in the adenovirus base coat protein. They first engineered an adenovirus that encoded β-glucuronidase to express the FLAG peptide epitope (DYKDDDDK) in the penton base protein. Using a bispecific antibody—complementary to the α_V integrin receptor at one end and the FLAG peptide at the other—they could increase the expression of β-glucuronidase 7–9-fold in transduced human venule endothelial cells relative to controls with no antibody. The virus's natural target selection should be eliminated because the proximity of the FLAG sequence to the protein's RGD sequence should prevent binding.

Another adenovirus problem has been immunogenicity. A research team headed by Douglas Brough, also at Genvec, has attempted to reduce immunogenicity by removing not only the E1 regions of the viral genome, but also by removing the E4 region. In previous work, the removal of E4 genes lowered viral titers, in part at least because the deletion lowers the production of the viral coat fiber. However, Brough's group found that when they introduced a spacer between the L5 gene—which encodes the fiber—and the E4 promoter, the E1⁻/E4 vector expressed fiber at near normal level. Without the spacer, it appeared that antisense sequences to the fiber gene are produced, crippling viral production. The spacer restored the kinetic profile and virus yield of E1⁻ vectors.

Another recent report (*Proc. Natl. Acad. Sci. USA* 93:5731–5736, 1996) demonstrates the potential of adenoviruses. Thomas Caskey and colleagues at Baylor College of Medicine (Houston, TX) successfully transfected an unusually large 28.2 kb expression cassette that included a *lacZ* reporter construct and murine dystrophin cDNA under control of a muscle-specific promoter. The target cells, dystrophin-deficient muscle cells, independently expressed both the dystrophin and the *lacZ* reporter genes. The transfer of such a large fragment was possible because the adenovirus contained no viral DNA whatsoever.

The team employed a helper-virus strategy. Using adenoviral mutant SV5—designed to enhance propagation of the gene transfer vector and allow separation of the two viruses—they produced a titer of the gene transfer vector 100-fold higher than the titer of the contaminating helper virus. The helper virus itself is defective and is similar to first-generation adenoviral vectors used previously in clinical work, so even low-level impurities in the final vector preparation are probably safe. ///

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