Don't shoot the messenger

Jo Whelan, freelance writer

A novel approach to gene therapy has moved a step closer to reality. Researchers at Vanderbilt University Medical Center (http://www.mc.vanderbilt.edu) have shown that an RNA repair technique can restore normal physiological function to defective mutant cells *in vitro*.

Most gene therapy research focuses on treating genetic diseases by inserting normal copies of a mutant gene into the DNA of affected cells. An alternative strategy is to repair the mutant gene, either directly or by repairing the messenger RNA (mRNA) that the faulty DNA generates. One way to repair mRNA is to use ribozymes, RNA molecules that act as 'molecular scissors' by catalyzing the cleavage of mRNA at sequence-specific sites (Figure 1). The Vanderbilt work is the first direct demonstration that ribozymes are a viable tool for correcting the functioning of mutant cells.

Model animal

The researchers, led by Al George, used a dog model of myotonia congenita, a non-lethal condition that is characterized by muscle stiffness and caused by mutations in the chloride channels of skeletal muscle cells. Targeting a mutation discovered in an affected dog named Sparky, they engineered a ribozyme that splices off the mutation-containing 3′ section of the mRNA and replaces it with the wild-type sequence [1]. This technique is called ribozyme-mediated *trans*-splicing. The ribozyme is produced as a DNA template, which is transfected into cells using a vector, in the same way as in standard gene therapy. The template is then transcribed within the cell to create the RNA ribozyme. In theory, the ribozyme is continuously transcribed and is over-expressed, which should mean that enough is present to repair all of the mutant mRNA in the cell.

Function restored

George's group used a plasmid vector to transfect their ribozyme into a cell line with impaired chloride transportation caused by the Sparky mutation [1]. They then assessed the repair efficiency with RT-PCR, but found that the repaired sequence made up only 1.2% of the relevant mRNA in the cell population.

Similar work has been done by other groups. 'What we did that was different was to use a direct functional assay for the protein product of the repaired mRNA,' explains George. 'The remarkable thing was that when we assayed chloride channel function on a cell-by-cell basis, we found cells in which there was complete restoration of normal chloride channel activity [1]. This doesn't necessarily mean there was 100% correction of the mRNA sequence in those cells, but there was enough to give wild-type activity.' Other cells showed a spectrum of intermediate levels of activity.

These results offer the first direct proof that ribozymes can repair mutant mRNA to produce functional wild-type proteins. However, the repair efficiency will have to be improved before it can be useful. The poor efficiency could just be a reflection of poor delivery to the cell nucleus, and the researchers are working on using a viral vector to improve delivery. But this might not

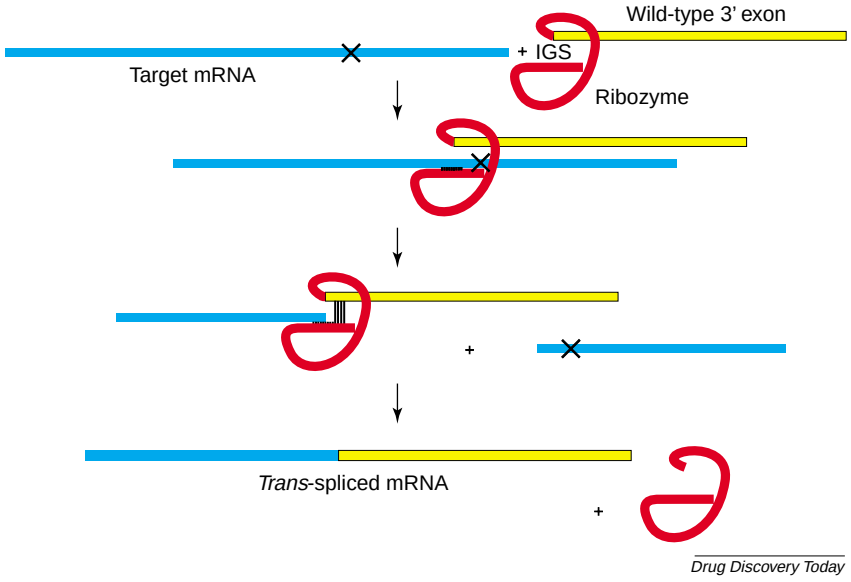

Figure 1. *Trans*-splicing ribozyme mRNA repair reaction. The ribozyme catalytic region (red) contains the internal guide sequence (IGS) which interacts by base pairing to a complementary site on the target mRNA (blue). The ribozyme catalyzes the cleavage of the target mRNA at a point upstream of a mutation (X) followed by ligation of the wild-type 3′-exon (yellow) with the remaining target sequence. Figure courtesy of Al George, Vanderbilt University Medical Center (http://www.mc.vanderbilt.edu)

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be the whole story. 'There may be an inherent heterogeneity in the ability of the ribozyme to distribute itself in the cell to effect repair of the targets,' says George. 'We are exploring possible explanations for this, including that it may be related to the different phases of the cell cycle.'

Tissue specific

George does not think that ribozyme-mediated mRNA *trans*-splicing can yet be regarded as superior to other gene therapy approaches. However, it has several potential advantages. mRNA is only expressed in tissues where the gene in question is expressed, so targeting it provides a level of tissue specificity in addition to any specificity conferred by the delivery method. It also addresses concerns about gene regulation. The engineered gene does not have to be under the control of the native promoter because the mRNA produced is regulated in the normal physiological way.

The technique could have important advantages against autosomal dominant disorders such as certain dominant muscular dystrophies. 'In these disorders there is often a dominant-negative disease mechanism,' George explains. 'This implies that there is ample wild-type gene being expressed by the normal allele, but there is a negative impact from the mutant allele. So simply increasing the expression of the wild-type gene in the traditional way might not necessarily be effective. The mRNA repair strategy addresses that because you are eliminating the mutant product, while simultaneously increasing the abundance of the wild-type allele.'

Future promise

The aim is to try and treat myotonia congenita in an affected dog. Before that, the researchers will need to increase the transfection efficiency in cultured cells. Testing in dogs will initially involve localized therapy delivered by injection into the muscle, rather than systemic delivery, which George describes as 'very challenging'. Human trials are a long way off.

Jeffrey Chamberlain, Professor of Neurology at the University of Washington School of Medicine (http://www.washington.edu/medical/som) and a leading researcher into gene therapies for muscular dystrophy, says: 'This work is extremely promising in terms of enabling gene therapy for dominantly inherited diseases.' He continues, 'The major challenges for applying it in the clinic will be delivery of the ribozyme shuttles to the appropriate target cells in the body, and identifying whether the ribozymes will persist, or whether they can be delivered repeatedly to maintain health of the patient.'

Reference

1 Rogers, C.S. *et al.* (2002) Functional repair of a mutant chloride channel using a trans-splicing ribozyme. *J. Clin. Invest.* 110, 1783–1789

Gleevec: tailoring to fit

Thomas S. May, freelance writer

Imatinib mesylate, otherwise known as Gleevec, became an 'overnight success' after Phase I clinical trials, which began in 1998, found that the drug caused remission in all of the 31 patients with chronic myelogenous leukemia (CML) who participated in the trial. Not only was Gleevec (STI-571) extremely effective, but it also appeared to cause remarkably few side effects in the test subjects. Phase II and III trials were also promising and the drug was approved for use in CML patients by the US Food and Drug Administration (FDA) in May of 2001.

Unfortunately, however, a majority of CML patients whose disease has advanced to the 'blast crisis' stage eventually relapse and die of leukemia because of 'Gleevec resistance'. However, according to a new study, resistance to Gleevec can be overcome [1].

Overcoming resistance

Gleevec works by inhibiting the activity of the tyrosine kinase Bcr-Abl, which can cause uncontrolled proliferation of white blood cells. About 95% of CML patients have the mutated Bcr-Abl gene, and Gleevec can halt the progress of leukemia in these patients – if they begin treatment early on in the course of their disease.

A team, lead by Brian Druker of Howard Hughes Medical Institute (http://www.hhmi.org), claim that a compound called PD180970 can stop the activity of several mutations found in patients who develop a resistance to Gleevec. 'Our data indicate that PD180970 is active against several Bcr-Abl mutations that are resistant to imatinib and support the notion that developing additional Abl kinase inhibitors would be useful as a treatment strategy for chronic

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