Gene therapy for muscular disease

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The biggest challenges facing gene therapy involve two main obstacles: getting your vector to its target and achieving therapeutic gene expression. However, gene therapies aimed at correcting muscular dystrophy face the daunting task of reaching every muscle in the body to express the missing dystrophin protein, which is necessary for muscle architecture.

Penetrating muscle cells

'If your goal is gene replacement, then you have to get your gene from the top of your head to the tip of your toes, and all the muscles deep within,' explains Thomas Rando, a neurologist and scientist at Stanford University (http://www.stanford.edu). However, Jeffrey Chamberlain and colleagues at the University of Washington in Seattle (http://www.washington.edu) have devised a way to do just that [1].

The vector in question is adeno-associated virus pseudotype 6 (AAV6) vector. Chamberlain's group selected this particular virus after noticing that AAV6 could latch onto and penetrate muscle cells better than other pseudotypes of the virus, and 100 times better than the most commonly used AAV2. Although smaller than other commonly studied gene therapy vectors, AAV is attractive because it is less immunogenic.

Delivering the vector throughout the body requires injection directly into the bloodstream. However, the walls of the blood vessels pose a barrier to muscle delivery. To overcome this, Chamberlain and his group tried various compounds such as histamine to increase vascular permeability. Vascular endothelial growth factor (VEGF) proved the most successful.

Increasing vascular permeability

'VEGF allows AAV6 to leak out the bloodstream very rapidly,' explains Chamberlain. 'It has a half life of a minute and is a very transient effect, which allows us to pulse it.' After slipping through the vessel walls, the vector then seeks out muscle cells and latches onto them. 'We could now deliver genes to every muscle in the body in a dose-dependent phenomenon,' he says. The vector was also found in heart muscle, a particularly important target in muscular dystrophy because all muscles are affected.

The team next inserted a truncated yet active form of dystrophin, called microdystrophin, into the AAV6 vector and delivered the therapeutic gene into the dystrophin-deficient *mdx* mouse model of Duchenne muscular dystrophy. Six weeks after treatment, extensive dystrophin expression was found throughout their skeletal muscles. Significantly, treated mice showed a 50% reduction in serum creatinine kinase levels, indicating decreased muscle degeneration as a result of treatment. Furthermore, no toxicity was observed, despite doses of vector and VEGF similar to those used for larger animals.

Setting the standard

'This is the first good demonstration of systematic delivery and widespread distribution and expression of a therapeutic gene,' says Rando. 'This is really setting the standards in terms of that. It will certainly set the standards in terms of delivery.'

The findings are encouraging. Treating muscular diseases successfully will require long-term expression of the

therapeutic gene in question and AAV6 is one of the most successful vectors capable of this. Furthermore, the approach could potentially be used to treat a host of other muscle related diseases including heart muscle damage, age-related muscle loss, and hemophilia.

As a potential therapy for muscular dystrophy, Melissa Spencer, a muscular dystrophy researcher at UCLA (http://www.ucla.edu), is encouraged. 'It looks fantastic. It's the most promising gene therapy study I've seen,' she says.

'What is really nice about this is that a single injection can deliver virus to nearly all the muscles, says Elizabeth McNally, a cardiologist and geneticist at the University of Chicago (http://www.uchicago.edu), 'We hope it works just as effectively when we move to humans, but that remains to be seen.' In the meantime, Chamberlain says they are now focusing on lowing dose levels, testing the therapy in other species and planning a Phase I safety trial in humans.

Reference

1 Gregorevic, P. *et al.* (2004) Systemic delivery of genes to striated muscles using adeno-associated viral vectors. *Nat. Med.* 10, 828–834

1359-6446/04/$ – see front matter PII: S1359-6446(04)03237-4