Immunosuppressants Pave the Way for Lasting Gene Therapy

July 16, 2012

EM Scherer

Duchenne muscular dystrophy (DMD) is a lethal X-linked disorder caused by mutations in the dystrophin gene. It is the most common and severe form of muscular dystrophy, affecting approximately one in 3500 boys at birth worldwide.

Multiple strategies have been proposed to replace functional dystrophin in individuals suffering from DMD, including muscle stem cell transplants to engraft healthy donor muscle cells or gene therapy to stimulate exogenous dystrophin expression in affected tissues. Researchers including Drs. Zejing Wang, Dusty Miller, Rainer Storb and Stephen Tapscott in the Human Biology and Clinical Research divisions, in collaboration with Dr. Jeffrey Chamberlain from the University of Washington, are actively pursuing these approaches.

Adeno-associated virus (AAV) has been extensively used as a gene delivery agent for gene therapy because most of its genome can be replaced with therapeutic genes, and such genes can be expressed for long periods without integration into the host genome. In addition, AAV is non-pathogenic to humans and animals and infects replicating and non-replicating cells alike. Despite these advantages, however, initial attempts to treat DMD by AAV-mediated dystrophin gene delivery have failed. This has been attributed in part to host immune responses to AAV and/or its gene products, given the observed accumulation of T cell infiltrates at the sites of intramuscular AAV administration.

Therefore, to improve gene delivery by preventing unwanted host immune responses to AAV-dystrophin, Wang and colleagues previously evaluated the levels of dystrophin expression following combined immunosuppression and gene therapies in a canine model of DMD (Wang *et al. Molecular Therapy* 2007).

The authors were the first to demonstrate that a triple-drug cocktail can partially restore dystrophin levels out to 30 weeks post-AAV therapy (12 weeks after cessation of immunosuppression). This immunosuppression regimen included anti-thymocyte globulin (ATG), a T cell depletion agent, and the T cell inhibitors cyclosporine (CSP) and mycophenolate mofetil (MMF) – all of which are routinely used for bone marrow and solid organ transplants.

In their most recent study, Wang and colleagues evaluated this immunosuppression regimen for a longer duration (24 weeks versus 18 in the previous study) with a large scale infusion of higher does of the AAV-dystrophin vector into a group of muscles in the hind limbs of two DMD-affected animals.

Remarkably, in the treated limb of one individual they found near uniform dystrophin expression by immunohistochemical staining two years after gene therapy and 18 months after immunosuppression. In contrast, dystrophin expression was not observed in the contralateral control limb that did not receive gene therapy. Moreover, the muscle histology of the treated limb was significantly improved with no obvious T cell infiltrates observed in the earlier or later biopsies. In the second individual, dystrophin expression was less uniform, but still stable, in the treated limb out to 19 months. Interestingly, only this individual possessed readily detectable serum neutralizing antibodies against AAV prior to gene therapy, whereas both individuals developed peak AAV-neutralizing antibody responses to AAV following AAV-dystrophin delivery.

These provocative results demonstrate that combining transient immunosuppression with gene therapy could provide unprecedented treatment efficacies for DMD. It also suggests that the success of these treatments will depend on the level of pre-existing immunity to AAV or other gene delivery vectors.

Wang Z, Storb R, Halbert CL, Banks GB, Butts TM, Finn EE, Allen JM, Miller AD, Chamberlain JS, Tapscott SJ. 2012. Successful regional delivery and long-term expression of a dystrophin gene in canine muscular dystrophy: a preclinical model for human therapies. *Molecular Therapy,* Epub ahead of print, doi: 10.1038/mt.2012.111

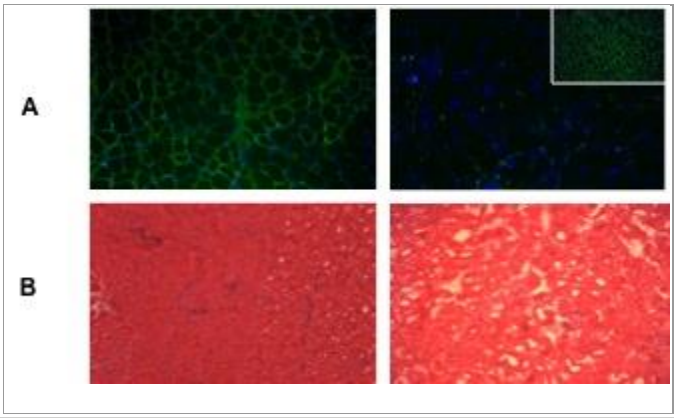

Image courtesy of Dr. Zejing Wang

Left two panels show a biopsy from the treated limb of an individual with DMD exhibiting nearly uniform dystrophin expression following combined immunosuppression and gene therapy. Right two panels show a biopsy from the contralateral control limb in the same individual, which did not receive gene therapy. Top and bottom panels show: A) immunohistochemical staining of dystrophin, where inset shows a healthy animal control without DMD, and B) hematoxylin and eosin staining of nuclei and cell cytoplasm, respectively. Bar represents 100 micrometers.