Combinatorial gene therapy using viral vector technology to treat Duchenne muscular dystrophy

T.D. Colgan,[1,2] K.T. Murphy,[1] G.S. Lynch[1] and P. Gregorevic,[2] [1]Basic and Clinical Myology Laboratory, Department of Physiology, The University of Melbourne, VIC 3010, Australia and [2]Laboratory for Muscle Research & Therapeutics Development, Baker IDI Heart and Diabetes Institute, PO Box 6492, St Kilda Road Central, VIC 8008, Australia.

Duchenne muscular dystrophy (DMD) is a severe and progressive muscle wasting disorder that results in ambulatory reduction of affected children and premature death from cardiac and/or respiratory failure. DMD is caused by a variety of mutations that result in the loss of, or the production of an aberrant dystrophin protein. As DMD is a single gene disorder, gene therapies have been pursued with the intention of restoring dystrophin expression in order to ameliorate the dystrophic pathology. Gregorevic *et al.* (2006) demonstrated the efficacy of a recombinant adeno-associated virus serotype 6 (rAAV6) systemic delivery of microdystrophin. Though the truncated dystrophin gene increased muscle strength and longevity of treated dystrophin$^{-/-}$:utrophin$^{-/-}$ (double knockout: *dko*) mice when compared to their untreated littermates, wild-type levels of strength and lifespan were not obtained. Alternate strategies that increase muscle mass and strength have subsequently been pursued. Follistatin binds and inhibits TGF-β ligands myostatin and activin, which are negative regulators of muscle mass. Follistatin has been recently confirmed to mediate increases in muscle growth and strength after systemic delivery (Winbanks *et al.*, 2012). We tested the hypothesis that intramuscular co-delivery of follistatin with microdystrophin would ameliorate the dystrophic pathology to a greater extent than either gene delivered in isolation.

We identified that intramuscular delivery of follistatin had a negative correlation between muscle mass and the severity of the dystrophic pathology. Biochemical analysis revealed a reduction in follistatin expression in dystrophic muscles which we hypothesise to be due to increased fibre turnover. While we identified that dystrophic muscles receiving a combinatorial delivery had more follistatin than those treated with follistatin in isolation, mass and fibre diameter was not increased. These results contradicted with our inital hypothesis and aspects deviated from prior experimental findings. We have since conducted intravenous delivery of the genes in isolation and combination, with the hypothesis that a systemic delivery would be more effective than an intramuscular delivery and have greater efficacy when both genes were administered together.

All experiments were conducted in accordance with the code of practise for the care and use of animals for scientific purposes, as stipulated by the NHMRC. To test our hypothesis cohorts of *dko* mice received an intravenous injection via the tail vein, of either rAAV6:microdystrophin (microdystrophin), rAAV6:FST317 (follistatin), a combination of both, or a control vector (rAAV6:MCS). Five weeks post-injection we noted that while the body condition of treated animals vastly improved, and coincided with an observed reduction in histopathology and increase in muscle fibre diameter, we identified that dystrophic mice receiving follistatin in isolation were less likely to meet their experimental end-point compared to their siblings receiving a control vector. To develop a better treatment for muscular dystrophy, we must now identify the mechanism by which follistatin can increase premature death in this dystrophic model and if this can be circumvented to still yield the therapeutic benefit provided by the combinatorial delivery. These findings place emphasis on a number of critical factors that will need to be considered if these therapeutic genes are to be tested in boys with muscular dystrophy and highlights the potential for a combinatorial gene therapy to ameliorate the dystrophic pathology.

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