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Using gene transfer technology to study muscle diseases

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Many of the world's most serious medical conditions are caused or exacerbated by the loss of striated muscle function, or resultant metabolic disturbance (Booth & Lees, 2007). The prevention and treatment of muscle-related illness could significantly improve human health, but requires a more complete understanding of the mechanisms that govern muscle adaptation in health and disease. To this end, the advent of gene delivery technology is poised to revolutionize the study of muscle, and accelerate the development of innovative therapeutic interventions for muscle-related disease (Gregorevic *et al*., 2004a). As an example, recombinant viral vectors derived from non-pathogenic adeno-associated viruses (rAAV vectors) offer a means of readily delivering "gene expression cassettes" and transcription-regulating elements to mammalian striated muscle, to achieve sustained, highly-specific transgene expression (Blankinship *et al.*, 2004). For research, these vectors offer the opportunity to dissect the intracellular mechanisms underlying the phenotypic adaptation of muscle *in vivo* with a level of precision and speed otherwise unachievable. Local intramuscular administration of vectors can elicit robust gene expression within days of treatment, and the ensuing gene expression can be maintained for years without further interventions if desired. Furthermore, modes of vector administration can be utilized to transduce musculature body-wide (especially in murine models) *via* intravascular administration (Gregorevic *et al.*, 2004b).

As a prospective medicine, vector-mediated gene modulation heralds the means with which to correctively restore the expression of defective genes, or drive compensatory expression of other genes for therapeutic gain. Various modes of rAAV-mediated gene delivery have already established proof of "therapeutic concept" in models of disease (Gregorevic *et al*. 2004b, 2006) and in some instances have commenced transition to clinical trials. As an example of muscle-focused gene therapy showing promise for therapeutic application, administration of rAAV6 vectors carrying an engineered dystrophin-based construct can restore organisation of sarcolemmal protein structures throughout the muscles of mice that model a severe form of muscular dystrophy, to achieve whole body amelioration of pathology, increasing muscle function and resulting in extended lifespan (Gregorevic *et al.*, 2004b, 2006). Work is underway to evaluate the feasibility of developing this approach for clinical application. These findings and related work demonstrate that the combination of gene delivery technology with established and developing analytic and therapeutic methods holds truly exciting prospects for a new era in muscle research and medicine.

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Muscle hypertrophy and IGF-1 isoforms: is bigger better?

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Insulin like growth factor-1 (IGF-1) plays a central role in muscle hypertrophy and muscle wasting. IGF-1 exists as different isoforms due to different exon splicing (Shavlakadze *et al*., 2005). IGF-1 isoforms that initiate from exon 1 are termed Class 1 (C1) isoforms, while isoforms that initiate from exon 2 are termed Class 2 (C2) isoforms. It has been proposed that different IGF-1 isoforms have different biological effects and may act through different signalling pathways. Previous studies show that over-expression of the Class 1 IGF-1 Ea (IGF-1:C1) causes skeletal muscle hypertrophy, slows the rate of myofibre atrophy following denervation and delays onset of necrosis in skeletal muscles of dystrophic *mdx* mice. Novel strains of non-dystrophic (normal) and *mdx* transgenic mice that over-express the Class 2 IGF-1 Ea (IGF-1:C2) isoform have muscle specific increase in total IGF-1 levels (~5 times higher compared to non-transgenic controls) and show more consistent muscle hypertrophy compared to the IGF-1:C1 mice.

Over-expression of the IGF-1:C2 resulted in a significant increase in quadriceps muscle mass in male and female IGF-1:C2 and *mdx*/IGF-1:C2 mice compared to their wild type littermates. Muscle hypertrophy in transgenic mice was more pronounced at 12 months of age compared to 3 months of age. Myofibre cross sectional area (CSA) was also examined in IGF-1:C2 and *mdx*/IGF-1:C2 mice. Average myofibre CSA was larger in IGF:C2 mice compared to the wild type littermates at both 3 and 12 months. In muscles from dystrophic *mdx*/IGF-1:C2 mice the average myofibre CSA was increased at 3 months but not at 12 months. The reduction in myofibre CSA in 12 month old *mdx*/IGF-1:C2 mice was due to myofibre splitting or branching. Diaphragm width was increased in 12 month but not in 3 month old male and female *mdx*/IGF-1:C2 mice. Despite the increased muscle mass, IGF-1:C2 did not increase specific force in muscles from non-dystrophic or *mdx* muscles and did not reduce myofibre necrosis in sedentary and treadmill exercised *mdx* mice. In adult (non-dystrophic and *mdx*) muscles, IGF-1:C2 over-expression does not coincide with the up-regulation of the Akt/mTOR signalling. However, striking activation of Akt signalling was observed in growing muscles of young 3 week old IGF-1:C2 mice. This study compared signalling activated by the C1 and C2 IGF-1 Ea isoforms and emphasized the impact of muscle growth. It also critically evaluated medically relevant scenarios where IGF-1 induced muscle hypertrophy might have beneficial effects.

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Novel regeneration in nemaline myopathy

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Nemaline myopathy (NM) is the most common of the congenital myopathies, presenting at birth or in early childhood as hypotonia and muscle weakness. A defining feature of this condition is the presence of electron dense rod-shaped structures in the sarcomeres termed nemaline rods. So far six genes with NM-causing mutations in humans have been identified. These genes code for proteins that form the thin filament of the sarcomere (α-skeletal actin, β-tropomyosin, α-tropomyosin$_{slow}$, nebulin, troponin T slow and cofilin-2).

Recent gene profiling studies on nemaline patients and the α-Tm$_{slow}$(Met9Arg) transgenic mouse model have shown that focal muscle repair and altered regeneration are previously unrecognised features of NM. Affymetric oligonucleotide array analysis of muscle from a heterogeneous group (i.e., various mutations) of patients with NM showed increased expression of genes associated with proliferating myoblasts and satellite cells (*NCAM1* and *CDK4*). This was confirmed immunohistochemically using a satellite-specific marker, Pax7, where there was a 10-fold increase in satellite cell abundance in the nemaline patient samples compared to normal healthy muscle. This is consistent with data from the mouse model where markers of satellite cell number, activated satellite cells and immature fibers (M-Cadherin, MyoD, desmin, Pax7 and Myf6) were elevated by Western-blot and immunohistochemical analysis. This study showed direct evidence of focal muscle repair in a number of muscles from the nemaline mouse (segmental regeneration with centrally-located nuclei) by electron microscopy. In keeping with ongoing repair, there was an increase in the number of fibres with centralised nuclei compared to wild-type mice. The number of central nucleated fibres was rather low (7-12%) compared to diseases characterized by overt regeneration (e.g. muscular dystrophies), which may explain why this feature had not been reported previously for NM. A novel regenerative process was also observed in a previous study on this mouse model, regeneration with a relative lack of centralised nuclei in response to chronic over-stretch.

Taken together, these studies suggest that there is a process of ongoing focal repair in nemaline muscle. This repair is distinct from the classical form of muscle regeneration as occurs in the muscular dystrophies where there is myonecrosis and extensive numbers of regenerating myofibers with centralized nuclei. The focal repair in nemaline myopathy maybe specific to diseases of the sarcomeric thin filament and are distinct from sarcolemmal repair in muscular dystrophy.

Acute Quadriplegic Myopathy and myosin loss in ICU patients: Underlying mechanisms, improved diagnostics and a specific intervention strategy

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Severe muscle wasting and impaired muscle function accompany critical illness in intensive care unit (ICU) patients with negative consequences for recovery from primary disease and weaning from the respirator. While ICU outcome has traditionally focused simply on survival, modern critical care also addresses post-ICU complications and quality of life. Several recent studies show unambiguously that neuromuscular dysfunction, resulting in muscle wasting and weakness, is the most persistent and debilitating of problems for survivors from the ICU for as long as two years after hospital discharge (Herridge *et al*., 2003; Cheung *et al*., 2006). There is accordingly a significant need for more research focused on the mechanisms underlying the muscle wasting and weakness in ICU patients. Primary disease, sepsis and multiorgan failure undoubtedly contribute to the impaired muscle function, but there is heterogeneity of underlying disease and pharmacological treatment among patients with similar outcomes. Thus, it is highly likely that the common components of ICU treatment *per se*, such as bed rest, muscle unloading, mechanical ventilation, neuromuscular block, and corticosteroids are directly involved in the progressive impairment of muscle function during long-term ICU treatment.

Acute Quadriplegic Myopathy (AQM) is considered a consequence of modern treatment in anesthesiology and intensive care. The first AQM case report was published three decades ago by MacFarlane and Rosenthal (1977). Patients with AQM are characterized by weakness/paralysis and preferential myosin losses in spinal nerve innervated muscles with craniofacial muscles being spared or less affected, and intact cognitive and sensory function. Prognosis is typically good if the patient survives the primary disease, but full or near full recovery may take as long as 10-12 months. Besides AQM, this disease has been given a number of different descriptive titles, such as critical illness myopathy, thick filament myosin myopathy, acute myopathy in severe asthma, and myopathy of intensive care. While AQM was initially thought to be a rare event, we now know that neuromuscular dysfunction is found in up to 30% of the general ICU population and 70-80% of certain sub-groups. This potentially lethal condition prolongs the recovery of critical care patients, thereby increasing the median ICU treatment costs three-fold per patient. Additional substantial costs are associated with the subsequent extended rehabilitation requirements and drastically impaired quality of life.

The understanding of basic mechanisms underlying AQM in the clinical setting is poor, in part due to the fact that the generalized muscle weakness is complicated by different underlying disease, polypharmacy, age, gender, and collection of muscle samples several weeks after admission to the ICU. There is, accordingly, compelling need for experimental animal models mimicking the ICU conditions. In an attempt to mimic the ICU condition, we have used novel large (porcine) and small (rodent) experimental ICU models in time resolved studies (hours to 3 weeks) in parallel with clinical studies in ICU patients. Specific interest is focused on regulation of myofibrillar protein synthesis and degradation at the gene level, protein expression at the muscle fibre level, and regulation of muscle contraction at the single muscle fibre level. In addition, different methods for improving monitoring and diagnosis are presently being evaluated in the clinical studies and specific intervention strategies are tested in the rodent ICU model. Preliminary results demonstrate specific pathways controlling protein synthesis/degradation and a positive effect of mechanical loading on muscle structure and function has been observed in pharmacologically paralysed limb muscles.

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