AAV mediated over-expression of the β2-Adrenoceptor promotes skeletal muscle hypertrophy

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Skeletal muscle wasting is a prominent feature of a number of debilitating diseases including cancer, muscular dystrophy and ageing. The use of β-agonists to stimulate β-adrenoceptors, members of the G-Protein Coupled Receptor family and the signalling pathways that they control has been shown to be effective in the treatment of various animals models of skeletal muscle wasting (Ryall *et al.*, 2004), (Harcourt *et al.*, 2007). Whilst the stimulation of this pathway has yielded positive effects on skeletal muscle growth and functional capacity, off-target effects on the cardiac musculature including cardiac hypertrophy, fibrosis and increased heart rate have hindered the therapeutic potential of β-agonists in skeletal muscle (Gregorevic *et al.*, 2005). This study therefore sought to generate a novel approach to confine β-adrenoceptor signalling to the skeletal muscle as a means to specifically combat skeletal muscle wasting without the associated potential deleterious off-target effects on other tissues.

Here, we describe the specific and highly efficient transduction of skeletal muscle with constructs expressing the β2-adrenoceptor (β2-AR) and the G-Protein subunits: Gα stimulatory (Gαs) and Gα inhibitory 2 (Gαi2) via adeno-associated viral vector (AAV)-mediated delivery. All experiments were approved by the Alfred Medical Research and Education Precinct Animal Ethics Committee in accordance with the current code of practice for the use and care of animals for scientific purposes, as stipulated by the National Health and Medical Research Council. Eight week old male C57Bl/6 mice were anaesthetised via inhalation of isoflurane in oxygen with post-operative analgesia and administered a single intramuscular injection of AAV:β2-AR, AAV:Gαs or AAV:Gαi2, at a dose of $1x10^{10}$ vector genomes, into the tibialis anterior muscle.

AAV:β2-AR promoted β2-AR signalling *in vivo*, causing a 22% increase in muscle mass after four weeks, associated with a significant increase in myofibre diameter. This was comparable to the muscle hypertrophy observed with administration of the β-agonist formoterol. In addition, we found that signalling associated with the β2-AR could be modulated by increasing the number of G-protein subunits made available to the receptor. Combinatorial delivery of AAV:β2-AR and AAV:Gαi2 resulted in a complete abrogation of the hypertrophic effect of AAV:β2-AR four weeks post vector administration. We also identified that AAV:Gαs promoted adrenergic signalling in a similar fashion to AAV:β2-AR, resulting in a 20% increase in muscle mass four weeks post vector administration.

Our data demonstrate that the over-expression of β2-ARs and associated G-Protein subunits potently regulate muscle mass. We propose that a vector-mediated approach to modulate β-adrenoceptor signalling in muscle represents a gene therapy based strategy to combat muscle wasting. Molecular manipulation of adrenergic signalling offers a safe, effective and efficient basis to develop therapeutics without adverse effects associated with pharmacological interventions.

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