Elevated MuSK expression restores dystrophin-associated proteins to the sarcolemma of mdx muscle fibres

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Muscle-specific kinase (MuSK) is a receptor tyrosine kinase that is essential for the development and maintenance of the neuromuscular junction. Our recent work in the mdx mouse model of Duchenne Muscular Dystrophy (DMD) suggests that experimentally elevating MuSK expression might protect dystrophic muscle fibres against eccentric contraction-induced injury. The dystrophin-associated protein complex (DAPC) links cytoskeletal actin to the extracellular matrix and provides structural support during contraction, reducing mechanical stress by distributing force laterally along the sarcolemma. In the absence of dystrophin, members of the DAPC are unstable and are depleted from the sarcolemma. Loss of particular DAPC members may well explain some of the fragility of dystrophic muscles. For example, dystrophin binds directly to dystroglycan, which, in turn, is linked to the basement membrane surrounding the fibre. Targeted inactivation of the dystroglycan gene in mice destabilises the sarcomere and increases the susceptibility of muscle fibres to contraction-induced injury and force loss: similar to the fragility found in (dystrophin-deficient) mdx mouse muscles (Rader *et al.*, 2016). In our previous work, intramuscular injection of an adeno-associated viral vector (AAV) was used to raise the expression of MuSK and rapsyn in mdx mouse muscle fibres. This was found to reduce the eccentric contraction-induced force loss. Here we have investigated the possibility that MuSK can protect mdx muscles by restoring DAPs to the dystrophic sarcolemma.

Eight-week old male mdx mice were anaesthetized with 4% isoflurane (Cenvet, Australia). After the foot-withdrawal reflex was fully suppressed anaesthesia was maintained with 2-3% isoflurane/oxygen through a nose cone. A total volume of 20µl of 2×10^9 viral genomes of AAV encoding MuSK fused to green fluorescence proteins (MuSK-GFP) in sterile 0.9% sodium chloride was injected unilaterally into the *tibialis anterior* muscle. The contralateral muscle was injected with empty AAV vector, to serve as a control. The mouse was given an intraperitoneal injection of buprenorphine (0.03mg/kg; Reckitt, Benckiser, Australia) for analgesia prior to recovery from anaesthesia. At 12 weeks, transverse muscle cryosections were labelled for DAPs by indirect immunofluorescence and were imaged on a confocal microscope. The relative intensity of sarcolemmal immunofluorescence in MuSK-GFP-expressing mdx muscles and contralateral control muscles was quantified using ImageJ software. Sarcolemmal labelling intensities were normalized to (untreated) C57BL10 (genetic background) control muscles.

Muscles expressing MuSK-GFP displayed a 30% increase in the intensity of sarcolemmal beta-dystroglycan (P=0.02; paired Student's t-test). Utrophin, a dystrophin homologue was also expressed at higher levels in the sarcolemma of mdx muscles that expressed MuSK-GFP, compared to contralateral control muscles. Analysis of the intensity of anti-beta-dystroglycan and anti-utrophin labelling by individual muscle fibres revealed a positive correlation between the level of expression of MuSK-GFP and the intensity of the DAP within the same fibre sarcolemma. These results show that elevating the expression of MuSK in mdx muscle fibres can help restore expression of at least two components of the DAPC in dystrophic muscles. They provide a potential explanation for the protective effects of MuSK-GFP against eccentric contraction-induced injury.

Rader EP, Turka R, Willera Y, Beltrána D, Inamoria K, Petersona TA, Englea J, Proutya S, Matsumuraf K, Saitof F, Andersona ME & Campbell KP. (2016). Role of dystroglycan in limiting contraction-induced injury to the sarcomeric cytoskeleton of mature skeletal muscle. *Proc Natl Acad Sci USA* **113**, 10992–10997.