Phosphorylation of dystrophin S3059 protects against skeletal muscle wasting

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The dystrophin-glycoprotein complex (DGC) is a multi-protein structure required to maintain integrity of the muscle fibre membrane, and transmit force by linking the actin cytoskeleton with the extracellular matrix. The DGC also plays a critical role in the signalling mechanisms that maintain muscle homeostasis. Membrane localisation of dystrophin is perturbed in muscles wasting as a consequence of cancer cachexia, tenotomy and advanced ageing (Acharyya *et al*., 2005; Hord *et al*., 2016), which are all associated with low level, chronic inflammation. Through proteomics and mutagenesis studies, we identified novel phosphorylated residues within endogenous dystrophin, and that phosphorylation at serine 3059 (S3059) enhanced interaction between dystrophin and β-dystroglycan, another key DGC protein (Swiderski *et al.*, 2014). In addition, our mass spectrometric analysis of muscles from mice with cancer cachexia identified a link between loss of dystrophin S3059 phosphorylation and DGC destabilisation. We hypothesized that dystrophin S3059 phosphorylation is fundamental to the aetiology of muscle wasting and investigated the role of S3059 phosphorylation on DGC protein interactions and muscle cell size *in vitro*.

All experiments were approved by the Animal Ethics Committee of The University of Melbourne and conducted in accordance with the Australian code of practice for the care and use of animals for scientific purposes (NHMRC). Male CD2F1 mice were anaesthetized (ketamine, 100 mg/kg; xylazine, 10 mg/kg, *i.p.*) and given either a subcutaneous injection of phosphate buffered saline (PBS; control) or Colon-26 (C-26) cancer cells into the right flank. After 3, 7, 14 or 21 days, mice were anaesthetized deeply with sodium pentobarbitone (60 mg/kg, *i.p.*). The *quadriceps*, *gastrocnemius*, and *tibialis anterior* muscles were excised and mice were subsequently killed by cardiac excision. Muscle protein expression was assessed from western immunoblotting and gene expression analyses by qPCR. Phosphorylated amino acids were identified following immunoprecipitation of dystrophin from skeletal muscle lysates by mass spectrometry of chymotryptic peptides (Swiderski *et al.*, 2014). To test the contribution of amino acid phosphorylation to muscle fibre size changes, phospho-null (mutation to alanine) and phosphomimetic (mutation to glutamine) mutations were made in dystrophin constructs which were transfected into C2C12 muscle cells or AAV-293 cells to assess effects on myotube diameter and protein function.

Mass spectrometric analysis of dystrophin phosphorylation in skeletal muscle from tumour-bearing mice revealed that loss of S3059 phosphorylation may be linked to muscle atrophy, with an absence of S3059 phosphorylation correlating with functional decline of the hind limb muscles and altered gene expression profiles of pro-inflammatory cytokines. Over-expression of a dystrophin construct unable to be phosphorylated at S3059 (S-A) reduced myotube size in C2C12 cells ($P < 0.05$). Furthermore, over-expression of a dystrophin construct with a phosphomimetic mutation at S3059 (S-E) was able to attenuate myotube atrophy in the presence of co-culture with C-26 cells ($P < 0.05$). Therefore, our findings demonstrate a link between loss of dystrophin S3059 phosphorylation and destabilisation of the DGC.

Identifying therapeutic approaches to restore the DGC at the muscle fibre membrane is essential for improving clinical outcomes for patients whose muscles are wasting and seemingly unresponsive to other treatments. Determining the mechanisms underlying post-translational modification of S3059 will identify novel targets to restore DGC interactions to preserve and protect muscles in different conditions of wasting.

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