Abstract: 15O

Interrogating the biological roles of dystrophin and utrophin in dystrophic muscle adaptations to exercise

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Duchenne muscular dystrophy (DMD) is a progressive and severe muscle wasting disease caused by mutations or deletions in the dystrophin gene, for which there is still no cure or effective treatment. In patients with DMD and in two well-characterised murine models lacking dystrophin (*mdx*) and dystrophin/utrophin (*dko*), muscles are fragile, injury prone and compromised in their regenerative capacity. Having recently identified novel roles for dystrophin and utrophin in the metabolic remodelling of dystrophic skeletal muscle to chronic low-frequency electrical stimulation (LFS, 10 Hz, 12 h/d, 28 d) (Hardee *et al*., 2021), we sought to determine how these proteins are implicated in the coupling of cell signalling and gene expression in response to muscle contraction.

All experiments were approved by the Animal Ethics Committee of The University of Melbourne and conducted in accordance with the Australian code for the care and use of animals for scientific purposes (8th ed. Canberra: NHMRC). Wild-type (C57BL/10) and dystrophin/utrophin-deficient *dko* mice were anaesthetised with ketamine/xylazine (100 mg/kg ketamine, 10 mg/kg xylazine, i.p.) and microelectrodes implanted surrounding the sciatic nerve to facilitate unilateral, wireless stimulation of the lower hind limb muscles. Mice were subjected to a single bout of LFS (10 Hz, 350 µs pulse duration, 12 h) with the right leg being stimulated and the unstimulated left leg serving as the contralateral control. Muscles were examined 0 and 3 h post-stimulation via phosphoproteomics and quantitative PCR, respectively.

Similar to our previous observations with chronic LFS, dystrophin/utrophin deficiency in *dko* mice impaired the activation of metabolic genes (e.g., *Pdk4*, *Hk2*) 3 h post-stimulation. Label free phosphoproteomics was performed to understand how signalling contributed to this impaired response. A total of 1622 phosphosites (866 phosphoproteins) were significantly regulated by contraction in wild-type mice, while only 302 phosphosites (241 phosphoproteins) were significantly regulated in *dko* mice. Functional annotation and gene ontology analyses revealed that contraction regulated phosphoproteins localised to the cytoplasm, z disc and cytoskeleton in muscles of wild-type mice, with the enrichment of these terms annotated significantly less in these muscles from *dko* mice. Kinase-substrate enrichment analysis revealed increased activity of AMPKA1, Akt1, ERK2, PKACA, and mTOR after contraction in wild-type mice. In contrast, absence of dystrophin/utrophin further increased the activity of AMPKA1 and Akt1, impaired the activation of PKACA and mTOR, and decreased the activity of ERK1, ERK2, and CDK1 in *dko* mice.

The findings reveal how absence of dystrophin and utrophin uncouples mechano-metabolic signalling and the transcriptional activation of metabolic genes and identify novel biological targets for restoring adaptive remodelling to muscular contraction in DMD.

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