Dysfunction of the dystrophin-glycoprotein complex in cancer cachexia

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Cancer cachexia describes the progressive muscle wasting and weakness in up to 80% of patients with advanced cancers and accounts for more than 20% of all cancer-related deaths. There is a profound unmet need for therapies that can ameliorate cancer cachexia and one approach may come from better understanding the molecular physiology of key proteins implicated in muscle proteostasis, such as dystrophin. Dystrophin protein levels decrease in cachectic Colon-26 (C-26) tumour-bearing mice presumably from increased protein degradation (Acharyya *et al*, 2005). Protein stability and turnover is influenced by phosphorylation and endogenous dystrophin is phosphorylated *in vivo* and phosphorylation of the dystrophin protein can alter its function (Swiderski *et al.*, 2014). Whether dystrophin amino acid phosphorylation is altered during muscle wasting in cancer cachexia and how this affects the function and/or protein interactions within the dystrophin-glycoprotein complex (DGC) has not been determined. We hypothesize that phosphorylation of the dystrophin protein modulates its function within the DGC during cancer cachexia.

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 C-26 cells into the right flank. After 3, 7, 14 or 21 days, mice were anaesthetized deeply with sodium pentobarbitone (60 mg/kg, i.p.) and contractile properties of *tibialis anterior* (TA) muscles investigated *in situ* (Murphy *et al*, 2011). 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).

Whole body mass and individual muscle masses were reduced significantly between 14 and 21 days post-injection ($P < 0.05$). Peak tetanic force was only significantly lower than PBS control at 21 days of tumour-bearing (P *0.05). Gene expression of the inflammatory cytokines IL-6* and *TNF*-α and the atrophy markers *MuRF-1* and Atrogin-1 were significantly increased only at 21 days post-injection. Dystrophin protein expression increased significantly at 7 and 14 days post-injection (3-4 fold increase; $P < 0.05$) but was similar to control by 21 days. Phosphorylation of the β-dystroglycan protein was increased significantly at 21 days post-injection, indicating disruption of the binding of dystrophin to β-dystroglycan (4-fold increase; $P < 0.05$). Liquid Chromatography-Mass Spectrometry revealed that S292, S293, S3059, S3476, and S3616 within the dystrophin protein were phosphorylated during tumour bearing and their phosphorylation state varied during tumour bearing.

Expression of dystrophin and other DGC proteins is altered and phosphorylation events within the dystrophin protein change during the progression of cancer cachexia. Reductions in skeletal muscle function and increased expression of inflammatory and muscle atrophy markers are not observed until 21 days post-injection, which correlates with a decrease in dystrophin expression and disruption of the interaction between dystrophin and β-dystroglycan. Identifying the effect of phosphorylation of specific dystrophin amino acids on protein-protein interactions and DGC function may lead to novel therapeutic targets for cancer cachexia.

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