Dysfunction of the dystrophin-glycoprotein complex (DGC) in cancer cachexia

K. Swiderski,[1] K.T. Murphy,[1] N.A. Williamson,[2] C.J. Brock,[1] D.M. Baum,[1] A. Chee,[1] T. Naim[1] and G.S. Lynch,[1]
[1]Basic and Clinical Myology Laboratory, Department of Physiology, The University of Melbourne, VIC 3010, Australia, and [2]Mass Spectrometry and Proteomics Facility, Bio21 Molecular Science and Biotechnology Institute, 30 Flemington Road, The University of Melbourne, VIC 3010, Australia.

Cancer cachexia describes the progressive muscle wasting and weakness occurring in up to 80% of patients with advanced cancer, which accounts for more than 20% of all cancer-related deaths. There is a profound unmet need for therapies that can ameliorate cancer cachexia. One approach may come from better understanding the molecular physiology of key proteins implicated in the maintenance of skeletal muscle proteostasis, such as dystrophin. Dystrophin forms a sarcomeric complex called the dystrophin-glycoprotein complex (DGC) that links the sarcolemma to the actin cytoskeleton to transmit the forces of contraction. Dystrophin protein levels decrease in Colon-26 (C-26) tumour bearing mice (a model of cancer cachexia) which is hypothesised to result from increased protein degradation (Acharyya *et al.*, 2005). Amino acid phosphorylation marks proteins for degradation. *In vitro* studies show that multiple kinases phosphorylate dystrophin which affects interactions between dystrophin and actin or the syntrophins. We have shown that endogenous dystrophin is phosphorylated on multiple amino acids *in vivo* and that phosphorylation can modulate the association between dystrophin and β-dystroglycan (Swiderski *et al.*, 2014). However, whether dystrophin amino acid phosphorylation is altered during the progression of muscle wasting, like that in cancer cachexia, and how this affects the function and/or protein interactions within the DGC remains to be determined.

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 cancer cells into the right flank. At 3, 7, 14, or 21 days post-injection mice were anaesthetized deeply with sodium pentobarbitone (Nembutal, 60 mg/kg, *i.p.*) prior to excision of the *quadriceps*, *gastrocnemius*, and *tibialis anterior* muscles. The mice were then killed by cardiac excision while still anaesthetized deeply. Analysis of protein expression was performed by western immunoblotting and immunofluorescence staining. For identification of phosphorylated residues, dystrophin protein was immunoprecipitated from the combined homogenate of the *quadriceps* and *gastrocnemius* muscles and subjected to SDS-PAGE. Dystrophin bands were excised from the gel and subjected to in-gel proteolytic digest after which the resultant peptides were analysed by Liquid Chromatography-Mass Spectrometry (LC-MS/MS) to identify phosphorylated residues, as described previously (Swiderski *et al.*, 2014).

As we have reported previously, injection of C-26 cells reduced body and muscle mass, as well as epididymal fat mass (Murphy *et al.*, 2012; $P < 0.05$). Whole body mass and individual muscle masses were reduced significantly between 14 and 21 days post-injection ($P < 0.05$). Western immunoblotting revealed expression levels of the dystrophin protein increased significantly at 7 and 14 days post-injection ($P < 0.05$) and returned to basal levels by 21 days. Phosphorylation of the β-dystroglycan protein was increased significantly at 14 and 21 days post-injection indicating a disruption in the binding of dystrophin to β-dystroglycan at these times ($P < 0.05$). LC-MS/MS revealed that multiple amino acids within the dystrophin protein were phosphorylated and that the presence of phosphorylation sites was altered at each time-point.

These studies demonstrate that the expression of dystrophin and other DGC proteins is altered and that phosphorylation events within the dystrophin protein change with the progression of cancer cachexia. Further investigation into the effect of phosphorylation of specific dystrophin amino acids on protein-protein interactions and DGC function may lead to the identification of novel therapeutic targets for cancer cachexia.

Acharyya S, Butchbach ME, Sahenk Z, Wang H, Saji M, Carathers M, Ringel MD, Skipworth RJ, Fearon KC, Hollingsworth MA, Muscarella P, Burghes AH, Rafael-Fortney JA, Guttridge DC. (2005) *Cancer Cell* **8**: 421-32.

Murphy KT, Chee A, Trieu J, Naim T & Lynch GS. (2012) *Disease Model Mechanisms.* 5: 533-45.

Swiderski K, Shaffer SA, Gallis B, Odom GL, Arnett AL, Edgar JS, Baum DM, Chee A, Naim T, Gregorevic P, Murphy KT, Moody J, Goodlett DR, Lynch GS & Chamberlain JS. (2014) *Human Molecular Genetics* (in press, July 31).

Supported by an Early Career Development grant from The University of Melbourne (Australia).
K. Swiderski is an NH&MRC Early Career Fellow.