Importance of functional and metabolic impairments in the characterization of the C-26 murine model of cancer cachexia

K.T. Murphy, A. Chee, J. Trieu and G.S. Lynch, Basic and Clinical Myology Laboratory, Department of Physiology, The University of Melbourne, VIC 3010, Australia.

Cancer cachexia describes the progressive skeletal muscle wasting and weakness associated with many cancers. Cachexia impairs quality of life and accounts for more than 20% of all cancer-related deaths. One of the primary developmental issues for treating cancer cachexia has been the lack of standard and appropriate end points for preclinical studies (Murphy & Lynch, 2009). The main outcome of cancer cachexia that affects quality of life and mortality is skeletal muscle function and therefore, animal models of cancer cachexia should exhibit the functional impairments seen in the clinical condition to maximize translational outcomes. Mice bearing Colon-26 (C-26) tumours are a commonly used model of cancer cachexia and our aim was to characterize their functional impairments and determine their suitability as a preclinical model. Since metabolic dysfunction is associated with cancer cachexia, we also investigated whether C-26 tumour-bearing mice exhibited similar metabolic impairments.

All experiments were approved by the Animal Ethics Committee of The University of Melbourne and conducted in accordance with the codes of practice stipulated by the National Health and Medical Research Council (Australia). Anaesthetized (Ketamine, 100 mg/kg; Xylazine, 10 mg/kg, *i.p.*) 12-wk old CD2F1 mice received a *s.c.* injection of Phosphate Buffered Saline (PBS, control) or C-26 tumour cells. After 18-20 days, assessments were made of grip strength, rotarod performance, locomotor activity, whole body metabolism and contractile properties of *tibialis anterior* (TA) muscles and diaphragm muscle strips. Using procedures we have described in detail previously (Murphy *et al.*, 2011), animals were anaesthetized deeply with sodium pentobarbitone (Nembutal, 60 mg/kg, *i.p.*) prior to *in situ* assessment of muscle contractile properties and were later killed as a consequence of cardiac excision while still anaesthetized deeply.

Injection of C-26 cells reduced body and muscle mass, as well as epididymal fat mass ($p<0.05$). C-26 tumour-bearing mice exhibited a 22% and 55% lower grip strength and rotarod performance, respectively ($p<0.05$). Locomotor activity was impaired following C-26 injection, with reductions in movement distance, duration and speed compared with controls ($p<0.05$). TA muscles from C-26 tumour-bearing mice had lower maximum absolute force (−27%) and force during fatiguing stimulation ($p<0.01$). Maximum specific (normalized) force of diaphragm muscle strips was reduced (−10%) in C-26 tumour-bearing mice, and force during fatiguing stimulation was also lower ($p<0.01$). The C-26 tumour-bearing mice exhibited reduced carbohydrate oxidation and increased fat oxidation compared with controls ($p<0.01$) as determined from oxygen uptake and carbon dioxide production.

The range and consistency of functional and metabolic impairments in C-26 tumour-bearing mice confirm their suitability as a preclinical model for cancer cachexia. Appropriate evaluations of functional and metabolic parameters will help maximize the translation of findings to better identify effective treatments for cancer cachexia.

Murphy KT & Lynch GS. (2009) *Expert Opinion on Emerging Drugs* **14**: 619-632.

Murphy KT, Chee A, Gleeson BG, Naim T, Swiderski K, Koopman R & Lynch GS. (2011) *American Journal of Physiology - Regulatory Integrative and Comparitive Physiology* **301**: R716-R726.

Supported by early career development grants from the Victorian Cancer Agency (Australia) and ADInstruments (USA).