Mitochondrial energy metabolism in skeletal muscle in a murine cancer cachexia model

C. F. De Oliveira1, D. Mintzopoulos2,3, C. Constantiniou2,4, V. Righi2, N. Psychogios2, M. N. Mindrinos4, Y.-M. Yu1, A. A. Shestopov6, R. G. Tompkins7, F. Lepine6, L. G. Rahme8, J. M. Argiles4, and A. T. Zinka2

1Cancer Research Group, Departamento de Bioquímica, Facultat de Biologia, Universitat de Barcelona, Barcelona, Spain, 2NMR Surgical Laboratory, Department of Surgery, Massachusetts General Hospital and Shriners Burn Institute, Harvard Medical School, Boston, MA, United States, 3Department of Radiology, Athinoula A. Martinos Center for Biomedical Imaging, Massachusetts General Hospital, Charlestown, MA, United States, 4Molecular Surgery Laboratory, Massachusetts General Hospital and Shriners Burn Institute, Harvard Medical School, Boston, MA, United States, 5Department of Biochemistry, School of Medicine, Stanford University, Stanford, CA, United States, 6Department of Surgery, Massachusetts General Hospital and Shriners Burn Institute, Harvard Medical School, Boston, MA, United States

Introduction—Development of cachexia is the most common manifestation of advanced malignant disease that occurs in the majority of cancer patients before death and, according to Warren, is responsible for the death of 22% of cancer patients (1). Implantation of a fast-growing tumor to mice (Lewis lung carcinoma) results in a clear cachectic state characterized by profound muscle wasting (2). In vivo NMR spectroscopy allows measurements of physiological biomarkers in intact systems (3,4), and has recently shown mitochondrial dysfunction in burns (5), which is also characterized by severe muscle wasting.

Materials and Methods—NMR spectra of hind limb were acquired 14 days after intramuscular (hind leg) inoculum of 4 x 10^6 Lewis lung carcinoma cells obtained from exponential tumors. All NMR experiments were performed in a horizontal bore magnet (proton frequency 400 MHz, 21 cm diameter, Magnex Scientific) using a Bruker Avance console. A 90° pulse was optimized for detection of phosphorus spectra (repetition time 2 s, 400 averages, 4K data points). Saturation 90°-selective pulse trains (duration 36.534 ms, bandwidth 75 Hz) followed by crushing gradients were used to saturate the γ-ATP peak. The same saturation pulse train was also applied downfield of the inorganic phosphate (Pi) resonance, symmetrically to the γ-ATP resonance. T1 relaxation times of Pi and phosphocreatine (PCr) were measured using an inversion recovery pulse sequence in the presence of γ-ATP saturation. An adiabatic pulse (400 scans, sweep with 10 kHz, 4K data) was used to invert Pi and PCr, with an inversion time between 152 ms and 7651 ms. Biopsies were harvested from the left gastrocnemius muscle. RNA was extracted, purified, and quantified and genomic analysis was performed following standard Affymetrix protocols(Affymetrix, CA, USA). (TCA) cycle flux (calculated from the time course of 13C enrichment in C-4 and C-2 of glutamate during an infusion of [2-13C]acetate) (6).

Results—Table 1 shows that ATP synthesis rate by P31 NMR and TCA cycle flux by mass spectrometry were significantly reduced by 47% and 25% respectively in cancer-bearing mice (P<0.03; t-test). The ratio of ATP synthesis rate to the TCA cycle flux, which provides an index of mitochondrial coupling, was 30% less in cancer-bearing mice (P<0.05; t-test), (Table 1). Fig. 1 shows images of gastrocnemius muscle micrographs from the contralateral to the TB leg revealed disorganization of myofibrils (Fig. 1D), giant mitochondria (Fig. 1E-F) and lipid accumulation (Fig. 1E-F). Gene expression analysis showed that upregulating protein 3 (UCHP3), and forkhead box O 3 alpha (Fox03alpha) expressions were significantly upregulated (P=0.00003 and P=0.0002 respectively) and this corroborated in part previous findings (7,8).

Discussion—The present novel results demonstrate that cancer-induced cachexia causes a profound functional and structural disorganization of skeletal muscle as indicated by reduced mitochondrial coupling and the observed fiber disruption, band disarrangement, and dilated sarcoplasmal reticulum in the cachectic gastrocnemius muscle from TB mice. In addition, the area of cachectic gastrocnemius intermyofibrillar mitochondria is characterized by the presence of giant mitochondria, which could be attributed to their inability to fuse with each other or with normal mitochondria (9) indicating a causal effect of structural mitochondria dysfunction on cancer-induced muscle wasting. The observed increased IMCLs can be attributed to defective intracellular lipid metabolism as it is shown by our preliminary genomic data (Fig. 1). Meanwhile, increased IMCL in muscle wasting have also been suggested to signal apoptosis (10), a procedure known to contribute in cancer-induced muscle wasting and observed to be induced in our experimental murine model of cancer-induced cachexia.

References