Mitochondrial skeletal muscle uncoupling in a murine cancer cachexia model

Valeria Righi, Cibely Cristina Fontes De Oliveira, Alexander. A. Shestov, Dionysios Mintzopoulos, Nikolaos Psychogiou, Caterina Constantinou, Silvia Busquets, Francisco J. Lopez-Soriano, Sylvain Milot, Francois Lepine, Michael N. Mindrinos, Laurence G. Rahme, Josep M. Argiles, and Ari A. Ticha

NMR Surgical Laboratory, Department of Surgery, Massachusetts General Hospital and Shriners Burn Institute, Harvard Medical School, Boston, Massachusetts, United States, Department of Science for Life Quality, University of Bologna, Rimini, Rimini, Italy, Cancer Research Group, Department of Bioquímica y Biología Molecular, Facultat de Biologia, Universitat de Barcelona, Barcelona, Barcelona, Spain, Center for Magnetic Resonance Research, Department of Radiology, University of Minnesota Medical School, Minneapolis, Minnesota, United States, Department of Radiology, Athinoula A. Martinos Center of Biomedical Imaging, Boston, Massachusetts, United States, Molecular Surgery Laboratory, Department of Surgery, Massachusetts General Hospital and Shriners Burn Institute, Harvard Medical School, Boston, Massachusetts, United States, INRS-Institut Armand-Frappier, Université du Québec, Laval, Quebec, Canada, INRS-Institut Armand-Frappier, Université du Québec, Laval, Quebec, Canada, Department of Biochemistry, Stanford University School of Medicine, Stanford, California, United States

Target Audience: Radiologists, Oncologists, Cancer Scientists.

Purpose- About half of all cancer patients, particularly those with cancers in the gastrointestinal tract and lung, present with cachexia in which disease-associated metabolic changes lead to a severe loss of skeletal muscle mass resulting in a body weight reduction of 30% or more [1]. We tested the hypothesis that cancer promotes mitochondrial uncoupling in skeletal muscle. Several clinically relevant animal models resulting in a cachectic state are characterized by profound muscle wasting, but there is no agreed model for preclinical testing of cachexia drug therapy. In this study we used the Lewis lung carcinoma [2]. Here, we used in vivo NMR spectroscopy and ex-vivo by Gas Chromatography Mass Spectrometry (GC-MS) technique to assess ATP synthesis rate and TCA cycle flux respectively, and to determine if their ratio, which provides an index of mitochondrial coupling, is affected in the skeletal muscle in cancer cachexia. Our results were complemented by genomic analysis and electron microscopy and suggest that mitochondrial uncoupling could be pharmacologically targeted to treat cancer cachexia.

Materials and Methods— Mice were randomized into tumor-free control (C) and tumor-bearing (TB). TB-mice received an intramuscular (right hind leg) inoculum of 4 × 10^5 Lewis lung carcinoma cells obtained from exponential tumors. $^{31}$P NMR spectroscopy. NMR spectra of hind limb were acquired 14 days after intramuscular (hind leg) inoculum. All NMR experiments were performed in an 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) and phosphocreatine (PCr) peaks, and 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. NMR Data analysis. $^{31}$P NMR spectra were analyzed using the MestReNova NMR software package (Mestrelab Research S.L., v. 6.2.1 NMR solutions, Website: www.mestrec.com).  The T1obs relaxation time for Pi and PCr was calculated by fitting the function $y = A_0 (1 - A_2e^{-\gamma(Tobs)})$ to the inversion recovery data, where $y$ is the y magnetization and $t$ is the inversion time. Calculation of ATP concentration: ATP concentration was measured using a Bioluminescence Assay Kit CLS II, Cat# 1699695 (Roche Diagnostics Corporation, Indianapolis, IN 46250-0414, USA). Calculation of ATP synthesis rate: For the calculation of the ATP synthesis rate, information from the $^{31}$P NMR spectra and the previously mentioned biochemically measured concentration of ATP was used, as described by Forsen and Hoffman [3]. TCA flux assessment: TCA cycle flux was calculated from the time course of $^{13}$C enrichment in C-4 and C-2 of glutamate (mass-isotopomers M+1 and M+2 of glutamate) during an infusion of 2-13C acetate. Plasma acetate concentration and C-4 enrichment of glutamate in the gastrocnemius muscle were obtained by GC/MS, as described elsewhere [4]. A novel “fragmented mass-isotopomer” approach for dynamic analysis of 13C mass-isotopomers data measured ex-vivo by Gas Chromatography Mass Spectrometry (GC-MS) technique was used to evaluate skeletal muscle TCA flux at different conditions [5]. Transmission Electron Microscopy (TEM). For mitochondrial morphology analysis, the muscles were extracted and dissected in small pieces of 1 mm2 approximately and transferred to glass vials filled with 2% parafomaldehyde and 2.5% glutaraldehyde in cacodylate buffer (Fixative solution). Pieces of 1 mm2 were examined by electron microscopy with an automatic TEM (Transmission Electron Microscope) and the serial sections were photographed at 6000× magnification. TCA cycle flux in normal and cancer-bearing mice was measured ex-vivo by Gas Chromatography Mass Spectrometry (GC-MS) technique and used to evaluate skeletal muscle TCA flux at different conditions [5]. Transmission Electron Microscopy (TEM). For mitochondrial morphology analysis, the muscles were extracted and dissected in small pieces of 1 mm2 approximately and transferred to glass vials filled with 2% parafomaldehyde and 2.5% glutaraldehyde in cacodylate buffer (Fixative solution). Pieces of 1 mm2 were examined by electron microscopy with an automatic TEM (Transmission Electron Microscope) and the serial sections were photographed at 6000× magnification. TCA cycle flux in normal and cancer-bearing mice was measured ex-vivo by Gas Chromatography Mass Spectrometry (GC-MS) technique.