Metabolic Characterization of the Cachectic Phenotype

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Introduction: Cachexia exists in approximately 50% of cancer patients and accounts for at least 20% of deaths from cancer. It is characterized by progressive weight loss, metabolic alterations, depletion of lipid stores, and severe loss of skeletal muscle protein, all of which significantly impair quality of life and response to treatment. Currently, there is no known cure for cachexia, since mechanisms underlying its manifestation are not defined clearly enough to identify and design effective therapeutic strategies. The complexities of cancer, and cachexia induced by cancer, dictate the necessity of studying this disease in the context of its microenvironment as well as in the context of interactions between the tumor and the body, *i.e.*, the 'macroenvironment'. We are applying molecular and functional imaging to understand cancer cachexia and develop clinically translatable indices for early detection of this condition. In our efforts to better characterize the metabolism of cachetic tumor, we performed *in vivo* ^{1}H MRSI and detected a high level of total choline in cachectic tumors compared to non-cachectic ones.

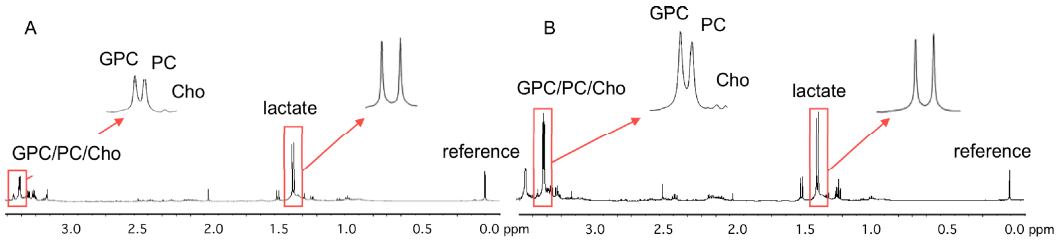

Figure 1: ^{1}H MR high-resolution spectra from water soluble extracts of (A) non-cachectic MAC13 and (B) cachectic MAC16 tumors obtained at 11.7T. 3-(trimethylsilyl) propionic-2,2,3,3,-d4 acid was used as a reference and internal concentration standard.

High-resolution ^{1}H MRS analysis of tumor extracts confirmed the higher total choline level in cachectic tumor bearing mice. The total choline signal consists of free choline (Cho), phosphocholine (PC) and glycerophosphocholine (GPC). Interestingly, we did not observe significant differences in lactate levels between cachectic and non-cachectic tumor (**Figure 1**). To further characterize glucose metabolism in cachexia, [^{18}F]fluorodeoxyglucose (FDG) positron emission tomography (PET) studies were performed. To detect the early onset of cachexia-inducing signals, we have created a cell-based optical biosensor using genetically engineered myoblasts that are injected into the gastrocnemius skeletal muscle. Glucocorticoids induce the expression of a muscle specific ubiquitin ligase (MuRF1) [1, 2], which has been shown to be involved with the catabolism of muscle proteins during cachexia [3]. The up-regulation of MuRF1 occurs by the specific activation of the glucocorticoid receptor (GR) upon hormone binding. Activated GR then translocates to the nucleus and directly binds to its response element (GRE) within the MuRF1 promoter. Importantly, increased expression of MuRF1 has been shown to be an early event in cachexia processes [1]. We have constructed a fluorescent protein reporter gene that is linked to a portion of the MuRF1 promoter containing the GRE, to report on the initial stages of the induction of cachexia. This reporter system was tested and optimized in mouse C_2C_{12} myoblast cells, a well-established muscle cell model system.

Methods: Cachectic (MAC16) and non-cachectic (MAC13) murine colon adenocarcinoma cells were used in the present study. MAC16 tumors induce extensive weight loss in tumor-bearing animals, whereas MAC13 tumors, although histologically similar, do not induce weight loss. For PET imaging, mice fasted overnight were injected with 200 μCi of FDG. At 60 min post injection, a 15 min static image was acquired over the tumors. Images were decay corrected and reconstructed using 2D OSEM (Ordered Subset Expectation Maximization). Image analysis was performed using AMIDE software. C_2C_{12} myoblast cells were transfected with constitutively expressed green fluorescent protein (GFP) and MURF1 promoter driven tdTomato red fluorescent protein (RFP). Transfected cells were selected with G418, grown to 100% confluence, and differentiated in 2% charcoal/dextran treated FBS containing differentiation medium. RFP expression was induced by dexamethasone treatment through MURF1 promoter transactivation by GR.

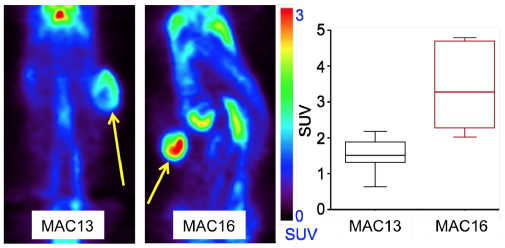

Figure 2: ^{18}FDG PET images of MAC13 and MAC16 tumor bearing mice. Mean standard uptake values (SUV) are represented (n=8 and n=5 respectively) $P < 0.005$. Images were scaled at the same maximum values. Tumors are marked by arrows.

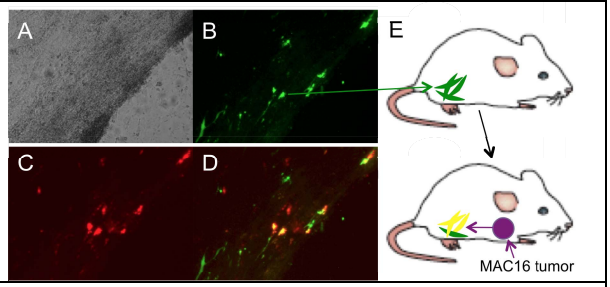

Figure 3: (A) Bright field image of C_2C_{12} myotubes, (B) GFP expression within these cells, (C) RFP expression after activation with dexamethasone, (D) (B) and (C) merged and (E) schematic representation of *in vivo* fluorescence detection of cachexia. Stably transfected myoblasts will be injected into the gastrocnemius muscle, viability tracked by GFP expression, and induction of cachexia tracked by RFP expression.

Results and Discussion: Despite the absence of differences in lactate concentrations between both tumor types, ^{18}FDG PET imaging revealed a significant increase in glucose uptake in the cachectic MAC16 tumors compared to the non-cachectic MAC13 tumors (**Figure 2**). C_2C_{12} myoblasts were stably transfected to express GFP as shown in **Figure 3B**. Expression of RFP was induced by dexamethasone (**Figure 3C**). Stably transfected C_2C_{12} myoblasts will be used as cell-based optical biosensors. Regulatory changes occurring in engrafted C_2C_{12} muscle cells in mice inoculated with cachexia inducing tumor cells are being compared to analogous grafts on non-cachexia tumor bearing mice to identify the sequence of events in the cachexia-cascade. These studies are part of our ongoing work to obtain a comprehensive characterization of the 'cachectic phenotype' using noninvasive multimodality imaging that will allow us to detect cancer-induced cachexia and identify new targets to prevent or reverse this condition.

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References: 1. Waddell DS *et al.*, Am J Physiol Endocrinol Metab. 2008; 2. Bodine SC *et al.*, Science. 2001; 3. Auclair D *et al.*, Am J Physiol. 1997.