Branched-Chain Amino Acid Metabolism in Prostate Cancer: Hyperpolarized [1-13C]-Ketoisocaproate as a Novel Molecular Probe

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Introduction: Prostate cancer is the second-leading cause of cancer deaths among American men,[1] but in recent years, the validation of novel biomarkers has transformed the detection, prognostication and treatment of the disease.[2] Unfortunately, despite these advancements, nearly 20% of current prostate biopsies result in false negatives,[3] and there remains no reliable indicator for establishing the aggressiveness of a particular prostate tumor.[4] These deficiencies have resulted in painful biopsies, over-treatment and undesired side effects (e.g. impotence) for patients that possess tumors that will not be a health risk in their lifetime. Conversely, aggressive neoplasms may not be properly treated until they reach an advanced stage. The accurate characterization of prostate cancer via a non-invasive method would address these major clinical issues. The primary objective of this research effort is to develop a novel, non-invasive imaging technique that distinguishes malignant from healthy prostate tissue based upon their distinctive metabolic profiles. To this end, the strategy is to validate *in vivo* MRSI of hyperpolarized [1-^{13}C]-ketoisocaproic acid ([1-^{13}C]-KIC) as a transformative method for guiding treatment and regulation of prostate cancer. This approach relies upon the ability of KIC to interrogate pathways of branched-chain amino acid (BCAA) metabolism, which are known to be modified in the tumor-bearing state.[5] In this work, we investigate the relative branched-chain amino transaminase (BCAT) activity found in various sources of prostate cancer and explore the feasibility of imaging the metabolism of hyperpolarized [1-^{13}C]-KIC in human prostate cancer cell line-derived xenografts.

Methods: The synthesis of [1-^{13}C]-KIC free acid was adapted from a previously published procedure,[6] and the compound was prepared in a spectroscopically pure form in a 96% yield from the commercially available sodium salt, [1-^{13}C]-ketoisocaproic acid (Cambridge Isotopes, Andover, MA). *In vitro* studies were performed with the four different human prostate cancer cell lines: PC-3, DU-145, LNCaP and LAPC-4.[7] Cells were cultured with media supplemented with FBS/penicillin/streptomycin and grown to 80% confluence prior to experimentation. BCAT activities in the above cell lines and tissue extracts (human and TRansgenic Adenocarcinoma of Mouse Prostate (TRAMP) mouse model) were determined spectrophotometrically via a previously reported protocol that was adapted to 96-well plate setup.[8] The protein content was determined spectrophotometrically via the Bradford assay. Two strains of the PC-3 cell line were utilized in the preparation of xenografts: the parental cell line (PC-3) and a liver metastasized line (PC-3M). Tumors were induced on either flank of nu/nu nude mice through the subcutaneous injection of 2-5 million cells in a PBS/matrigel (50:50) medium. PC-3-based xenografts showed relatively slow tumor progression *in vivo* and a steady growth rate of 5 mm^3/day was observed. Tumors were imaged upon reaching a size of roughly 200 mm^3 (circa 6 weeks post injection). PC-3M-derived xenografts displayed instability for sustained tumor development. In two of three mice examined, tumor regression was observed after two weeks, and only one mouse provided a tumor >150 mm^3 for further analysis.

In vivo MRS studies were conducted on the human prostate cancer cell line (PC-3 and PC-3M) xenografts utilizing a clinical 3T GE Signa MRI scanner equipped with a high-performance insert gradient set optimized for small animal imaging. The mice were anesthetized with 1-3% isoflurane in oxygen (~1.5 l/min), a tail vein catheter was inserted into each mouse, and the animal placed in a custom-build dual-tuned ^{13}C/^{1}H quadrature birdcage RF coil (50-mm diameter) centered in the scanner bore. Body temperature was monitored using a fiber optic rectal probe and controlled using a temperature-controlled forced air heating system. In addition, breathing was monitored using a small animal respiratory monitoring hardware and software system with heart rate and O_2 saturation recorded using a pulse oximeter. Within a given scanning session, each mouse received one bolus injection of DNP hyperpolarized [1-^{13}C]-KIC (dose of 0.3 ml, 40 mM at an injection rate of 0.025 ml/s) followed 1.5 hrs later by another injection of [1-^{13}C]-KIC (0.3 ml, 40 mM) containing unlabelled leucine in the dissolution buffer. Fast 3D spiral MRSI was used to acquire the volume of interest.

Results and Discussion: The TRAMP mouse model has become an indispensable tool for characterization of molecular mechanisms involved in the initiation and progression of prostate cancer.[9] A series of enzymatic assays were performed in order to determine whether the TRAMP mouse model mirrored human prostate cancer in terms of BCAT activity and, in turn, would be suitable for *in vivo* MRSI studies with [1-^{13}C]-KIC. In these *ex vivo* experiments, the TRAMP prostate tissue was found to possess an activity of 0.84 ± 0.17 U/gram of protein (Fig. 1). For comparison, protein extracts from human prostate tumor samples were obtained. The corresponding homogenates displayed an increased overall level of BCAT activity (2.37 ± 0.64 U/gram of protein). Although the TRAMP model has been used extensively to study prostate cancer, BCAA metabolism in this model does not appear to mimic human prostate tumors, in respect to elevated BCAT activity. In an effort to obtain an animal model that possesses higher levels of BCAT activity, a series of human prostate cancer cell lines were examined that could be utilized for the preparation of the corresponding xenograft tumor models. Four cell lines were subjected to the BCAT enzymatic assay protocol: PC-3, DU-145, LNCaP and LAPC-4. In these experiments, the human prostate cancer cell line, PC-3, was identified as a potential basis for xenograft preparation as it displayed the highest level of BCAT activity (1.04 ± 0.40 U/gram of protein) (Fig. 1.). In addition, the DU-145 cell line was found to have comparable BCAT activity (0.84 ± 0.35 U/gram of protein) to the TRAMP mouse model. However, only low levels were detected *in vitro* from the LNCaP and LAPC-4 cell lines.

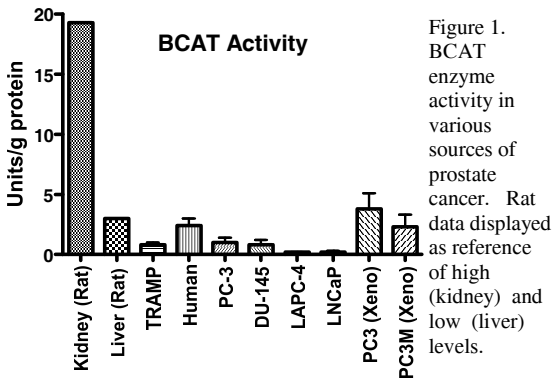

Figure 1. BCAT enzyme activity in various sources of prostate cancer. Rat data displayed as reference of high (kidney) and low (liver) levels.

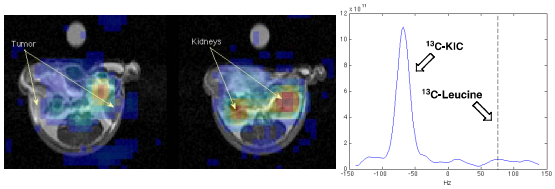

Figure 2. Image of hyperpolarized [1-^{13}C]-KIC in PC-3 xenograft (left) and corresponding spectrum (right). Little KIC signal was seen in the tumor. The bright signal region is from kidney.

Xenograft models of human prostate cancer provide the opportunity to validate the applicability of *in vivo* MRSI of hyperpolarized [1-^{13}C]-KIC as a powerful tool for assessing characteristics of prostate tumor metabolism. Metabolite production was analyzed in the both PC-3 and PC-3M xenografts after each injection of hyperpolarized [1-^{13}C]-KIC. Administration of 40 mM [1-^{13}C]-KIC showed no observable toxicity effects. However, leucine production was not detected in the tumors (Fig. 2). Potentially, the low level of BCAA production could be a result of poor vascularization of the tumors. Thus, only a limited volume of tracer would enter the tumor, leading to a lower level of metabolic product that was below the detection threshold. Following the MRS studies, the mice were euthanized, and the tumors were removed in order to determine BCAT enzymatic activity. These results are summarized in Fig. 1. Interestingly, a greater than 2.5-fold increase in activity was detected from the PC-3-derived xenografts in respect to the *in vitro* results. A similar increase in xenograft BCAT activity has been previously observed.[6] Overall, PC-3-based xenografts displayed a 3.81 ± 1.27 U/gram of protein level of BCAT activity, while PC-3M xenografts also showed high enzymatic levels (2.47 ± 1.01 U/gram of protein) similar to human prostate tumor extracts.

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