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

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**Introduction:** Prostate cancer is the second-leading cause of cancer death 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,\(^1\) 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 \textit{in vivo} MRSI of hyperpolarized [1-13C]-ketoisocaprate (11-13C-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.\(^3\) 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 [1-13C]-KIC in human prostate cancer cell-line-derived xenografts.

**Methods:** The synthesis of [1-13C]-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, [1-13C]-ketoisocaprate acid (Cambridge Isotopes, Andover, MA). \textit{In vitro} studies were performed with the four different human prostate cancer cell lines: PC-3, DU-145, LNCaP and LAPC-4. 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 TRAMP prostate tissue) were subjected to the BCAT enzymatic assay protocol: PC-3, DU-145, LNCaP and LAPC-4. In these \textit{in vivo} experiments, the human prostate cancer cell line, PC-3 and PC-3M xenografts after each injection of hyperpolarized [1-13C]-KIC as a powerful tool for assessing BCAT enzyme activity in various sources of prostate cancer.

**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.\(^4\) 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 \textit{in vivo} MRSI studies with [1-13C]-KIC. In these \textit{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 order 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: the parental cell line (PC-3) and a liver metastasis line (PC-3M). Tumors were induced on either flank of nude 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 \textit{in vivo} and a steady growth rate of 5 mm/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.

\textit{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 each mouse was placed in a custom-build dual-tuned 1H 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 [1-13C]-KIC (dose of 0.3 ml, 40 m\(^3\) at an injection rate of 0.025 ml/s) followed 1.5 hrs later by another injection of [1-13C]-KIC (0.3 ml, 40 m\(^3\)) containing unlabelled leucine in the dissolution buffer. Fast 3D spiral MRSI was used to acquire the volume of interest.

**Figure 1.** BCAT enzyme activity in various sources of prostate cancer.

**Figure 2.** Image of hyperpolarized [1-13C]-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.


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