A Metabolically Accurate Prostate Tissue Culture Model for ^{13}C Labeling Studies

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Introduction

While the current commercially available combined anatomic imaging (MRI) and proton spectroscopic imaging (MRSI) exam has improved the assessment of prostate cancer (1), it does not provide an assessment of all the changes in metabolic fluxes through glycolysis, citric acid cycle and fatty acid synthesis. However, it may be possible to further assess these pathways with the use of ^{13}C labeled substrates, such as ^{13}C labeled pyruvate and acetate. Since all three of these pathways have been shown to have metabolic perturbations associated with the evolution and progression of prostate cancer (2), metabolic markers from these pathways may help diagnosis smaller lesions or discriminate cancer from other benign conditions. Due to improved control over the delivery and concentration of ^{13}C labeled substrates, cell culture models of prostate cancer can facilitate the identification of the key metabolic products of ^{13}C labeled substrates and determine the kinetics of incorporation of ^{13}C labels into these metabolites. Unfortunately, due to the importance of surrounding stroma to glandular cell function and metabolism, pure cell cultures do not provide good model systems for healthy prostate tissue metabolism. A recently developed tissue culture technique solves this problem by maintaining tissue slices from human radical prostatectomy samples in culture for up to 48 hours (3). The purpose of this study is to determine if the metabolism of these tissue slices truly reflects the in vivo metabolism and to demonstrate that the tissue slices will metabolize ^{13}C labeled substrates placed in the medium.

Methods

A tissue core 8mm in diameter was taken from a human prostate after prostatectomy. The core was sectioned into 300 m thick slices and incubated at 37℃ and 5% CO_2. Every fifth slice was embedded and saved for subsequent histopathological analysis of the sample. At the desired time, approximately 12 slices are collected into 20-28mg samples and analyzed using an 11.7Tesla Varian HR-MAS spectrometer equipped with a ^{1}H nanoprobe. For the carbon labeling experiments, the tissue was cultured for 60 minutes in medium with 11mM ^{13}C-3 Pyruvate substituted for the original pyruvate. A carbon-decoupled proton ($^{1}H[^{13}C]$) spectrum was acquired before and after a Heteronuclear Single Quantum Coherence (HSQC) spectrum was acquired for each sample with ^{13}C-2 labeled glycine added for referencing. The percentage of ^{13}C enrichment was calculated by comparing the metabolite peak heights relative to the labeled glycine in the $^{1}H[^{13}C]$ spectrum to the relative metabolite peak heights in the HSQC.

Results

Similar to ex vivo HR-MAS spectra of fresh prostate tissues, the $^{1}H[^{13}C]$ spectrum from a cancerous prostate tissue culture in Figure 1 shows a decrease in citrate and polyamines and an increase in the choline region as compared to the healthy tissue sample. The ^{13}C labeling assay yielded ^{13}C enrichment in Lactate, Alanine, and Glutamate as summarized in Table 1.

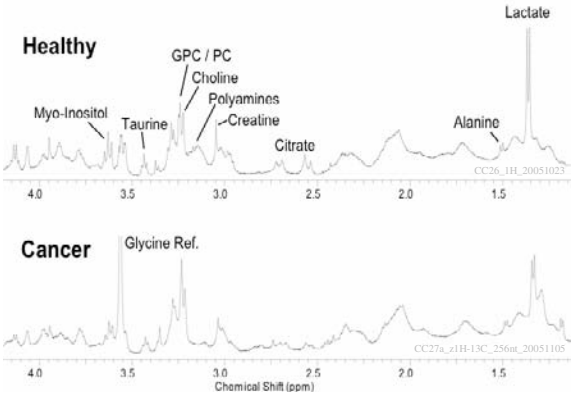

Figure 1 $^{1}H[^{13}C]$ spectra from a healthy (TOP) and cancerous (BOTTOM) prostate tissue culture sample.

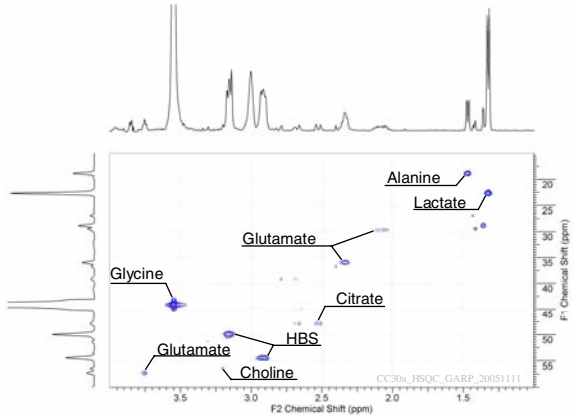

Figure 2 An HSQC performed on a 30mg prostate tissue culture sample after ^{13}C enrichment with 3-^{13}C Pyruvate. The HSQC was acquired using 32 averages for each increment.

Metabolite	^{13}C Enrichment
Lactate	29%
Alanine	15%
Glutamate	15%

Table 1 Calculated ^{13}C enrichment achieved on a 30mg healthy prostate tissue culture sample after exposure to ^{13}C-3 pyruvate for 60 minutes.

Discussion

The prostate tissue culture model system exhibits the signature metabolic profile of healthy glandular prostate tissue with citrate, polyamines, and free choline readily apparent in the $^{1}H[^{13}C]$ spectrum (Figure 1). In addition, when using cancer prostate tissue, the model yields the characteristic reduction in citrate and polyamines and increase in the choline compounds (Figure 1). Thus, the model system coupled with metabolic imaging may be useful for preclinical evaluation of new therapeutic drugs or for studying the mechanisms leading to the development of prostate cancer. The ^{13}C enrichment results shown in Figure 2 and Table 1 indicate the prostate tissue culture model system does incorporate ^{13}C labeled compounds that have been added to the medium. Therefore, by adding ^{13}C labeling to the tissue culture model system, it will be possible to investigate the cellular bioenergetic changes that occur with prostate cancer progression. Finally, ^{13}C labeling in this model system could help identify new metabolic markers that improve prostate cancer diagnosis and/or evaluation of therapeutic efficacy. In the near future, these markers may be clinically relevant because of recent advances in ^{13}C hyperpolarization techniques and development of high field MR scanners.

References

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