Elevated phosphocholine as a marker of carcinogenesis in the woodchuck model of hepatocellular carcinoma

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Background. An estimated 350million people worldwide are currently infected with hepatitis B virus; approximately 17.5million of those will develop hepatocellular carcinoma, an aggressive and often fatal form of liver cancer. Woodchucks chronically infected with woodchuck hepatitis virus will develop hepatocellular carcinoma within 2 years post-infection, mimicking the 20-year disease progression in humans chronically infected with hepatitis B virus. The aim of this study is to use the woodchuck model to determine potential biomarkers in the 31P spectrum that correlate with tumour growth.

Methods. Changes to liver tissue composition over time were followed *in vivo* using two-dimensional 31P chemical shift imaging (2D-CSI, 16x16 data matrix, 24x24x2cm, 1024 data points, 16 averages) acquired monthly in a 7T horizontal bore MRI using a 1H/31P doubly tunable volume coil (1,2). Tissues obtained at necropsy were frozen in liquid nitrogen and prepared for analysis by 31P-NMR (145.865MHz, SW=11574.07Hz, 1024 scans) by a double perchloric acid extraction (3). The relative concentrations of phosphocholine (PC) and phosphoethanolamine (PE) in the *ex vivo* extracts were quantified to an external phenylphosphate reference (8.8mM) (4). All results are reported as mean +/- standard error. The results were compared using a Student's t-test and were considered significant when $p<0.05$.

Results. Hepatocellular carcinoma (HCC) was detected *in vivo* by 1H-MRI only in infected woodchucks. By analyzing the 31P-MRS spectrum of livers of infected woodchucks in the earliest stages of chronic infection, changes in tissue composition due to HCC development could be detected prior to visualization on the 1H-MR image. The tumours were detected and followed over time by their significantly elevated phosphomonoester resonances (PME) ($p<0.0005$) at 7.5-8.0ppm. PME is composed of resonances from PC and PE, therefore 31P-NMR was performed on perchloric acid extracts to determine the relative contribution to the PME signal from both molecules. The *in vivo* findings were confirmed when significant increase in PC/PE ratio was detected in HCC tissue from infected woodchucks (n=11) compared to normal liver tissue from uninfected control woodchucks (n=5), Histological examination of the tissues also confirmed that HCC was present only in infected woodchucks.

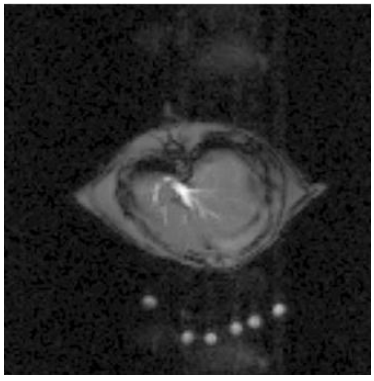

Figure 1. Representative axial 1H-MRI of woodchuck with HCC

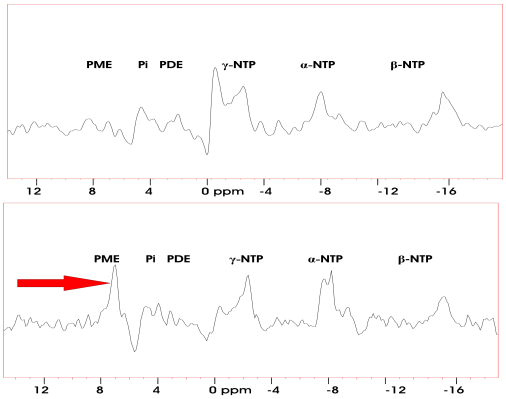

Figure 2. Representative *in vivo* 2D-CSI 31P-MRS spectrum of control (top) and hepatocellular carcinoma (bottom) voxels from woodchuck livers

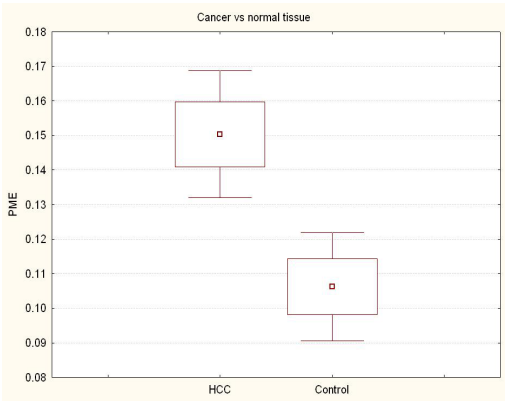

Figure 3. Significant elevations in PME resonances in cancer (HCC) compared to control tissue using 2D-CSI 31P-MRS ($p<0.0005$)

Discussion. The PME resonance represents increased cellular proliferation within the tumour. This study demonstrates that elevations in the PME resonances detected *in vivo* are due to increased phosphocholine in tumour tissue. Elevated choline resonance has previously been used a marker for breast and prostate cancer. This is the first study to detect tumour growth in the liver comparing the results from both *in vivo* and *ex vivo* phosphorus spectroscopy or 1H-MRI in chronically infected woodchucks (3). Noninvasive (MRI/CSI) monitoring of changes in PME resonances could potentially be used to follow tumour growth or treatment response in human cases of hepatocellular carcinoma, thereby improving the detection and outcome of clinical cases.

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