Resolving and identifying an unexpected MRS peak – arising from a PUFA – in the "citrate region" of intact human prostate cancer tissue using 1D and 2D 1H-HRMAS NMR

K. Stenman[1], A. Rydh[1], K. Riklund[1], A. Bergh[2], and J. Hauksson[3]

[1]Dept. of Radiation Sciences, Diagnostic Radiology, Umeå, Sweden, [2]Dept. of Medical Biosciences, Pathology, Umeå, Sweden, [3]Dept. of Radiation Sciences, Radiation Physics, Umeå, Sweden

Abstract: High resolution magic angle spinning NMR (HR-MAS NMR) has become an invaluable aid in the field of MRS diagnostics due to the low chemical shift resolution in clinical scanners. While characterizing intact human prostate cancer (PCa) tissue with 1D 1H HRMAS, an elevated signal at 2.8 ppm compared with non-malignant prostate tissue was found. A 2D total correlation spectroscopy (TOCSY) revealed that this signal stems from a polyunsaturated fatty acid (PUFA). "2D gradient enhanced-heteronuclear single quantum correlation (GE-HSQC) spectroscopy" was performed in order to identify this PUFA which in this study is recognized as an n-6 PUFA, ω-6 (18:2(n-6)). This study confirms that this PUFA is present in human PCa tissue concurrently with aspartate which it partially overlaps in the "citrate region". The aspartate multiplet co-resonates with the characteristic double doublet of citrate in healthy prostate tissue. Spectra with severe signal overlap may cause misinterpretations during MRS evaluations in the clinic. This study demonstrates a positive aspect of this technique which allows unexpected metabolites to be detected and identified. This feature may contribute to an improved accuracy using clinical MR scanners.

Method and Subjects: NMR spectroscopy was performed on a Bruker AMX2 NMR spectrometer operating at a ^{1}H frequency of 500.13 MHz (Bruker Biospin GMBH, Karlsruhe, Germany). A 4 mm HRMAS dual band (^{1}H and ^{13}C) Bruker probe and 4 mm zirconia rotors with spherical inserts and Kel-F caps were used. The sample spinning rate was set to 5 kHz. For the 2D GE-HSQC spectra a phase-sensitive improved-sensitivity 2D HSQC experiment using echo-antiecho was employed. The data were acquired with 128 increments and 320 transients per increment. The FID's of the HSQC data were apodized using a Gaussian window function with a Gaussian broadening of 0.02 Hz and an exponential line broadening of -3 Hz in the ^{1}H dimension followed by a 90° shifted sine squared window function in the ^{13}C dimension. The resulting 2D matrix consists of 1024*256 data points. In two patients operated for peripheral zone Gleason score 7 (3+4) unilateral prostate cancers, tumor tissue and non-malignant tissue from contra-lateral tumor-free peripheral zone were immediately dissected from the surgical specimen by a board certified specialist in uro-genital pathology within 20 minutes after surgical removal and frozen in liquid nitrogen.

Result: The GE-HSQC spectra provide a detailed map of the cross peaks contributing to the PUFA. The nonmalignant prostate tissue shows the distinct cross-peaks from citrate and no sign of peaks from a PUFA (fig 1). The spectra from PCa tissue show clearly visible cross-peaks arising from a PUFA; (^{1}H/^{13}C) 2.78/25.93 ppm, 2.06/27.50 ppm, 2.32/34.41 ppm, 2.34/34.53 ppm, 1.61/25.23+25.20 ppm, 1.30/31.85 ppm, 0.885/14.17 ppm, 0.897/14.20 ppm and no sign of cross-peaks from citrate (fig 2). On the basis that the cross-peak for Fω-1(Δ-1) protons, with the chemical shifts ^{1}H 2.08 ppm and ^{13}C 20.88 ppm characteristic for ω-3, is absent we determine the PUFA to be of n-6 origin; ω-6 (18:2(n-6)) also called linolenic acid. ω-6 is often found in excess relative ω-3 in western societies due to an improperly balanced diet concerning essential fatty acids and is believed to instigate PCa.

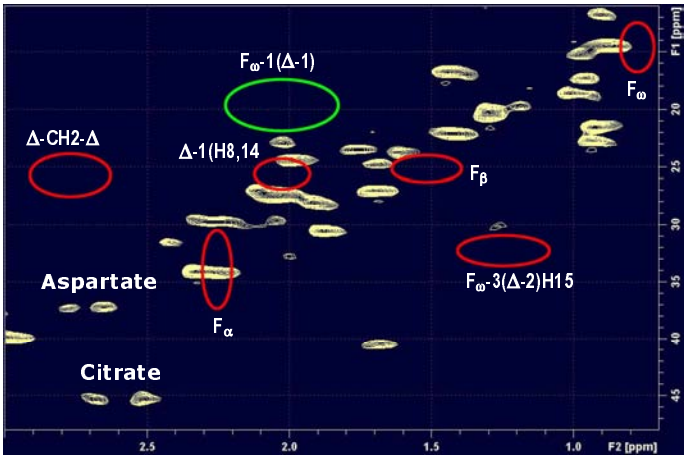

Figure 1: GE-HSQC spectra obtained from nonmalignant human tissue

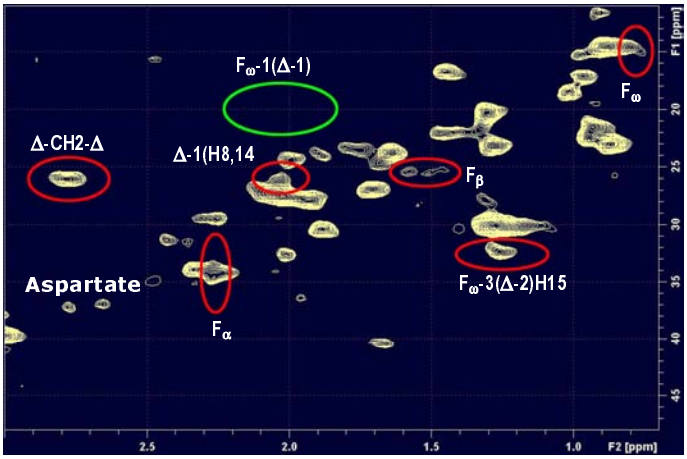

Figure 2: GE-HSQC spectra obtained from PCa tissue

Conclusion: 2D GE-HSQC HR-MAS NMR proves to be an invaluable tool for detailed metabolic elucidations. Overlapping and/or unexpected MRS peaks can be resolved and identified. This is of great significance when building a robust metabolic profile aimed to be applied clinically using MRS.