# METABOLIC PROFILING OF PREINVASIVE AND INVASIVE CERVICAL CANCER USING 1H AND 31P MAGIC ANGLE SPINNING MRS

S. S. De Silva[1], G. S. Payne[1], P. Soutter[2], N. M. Desouza[1]

[1]Clinical Magnetic Resonance, Institute of Cancer Research, Sutton, Surrey, United Kingdom, [2]Obstetrics and Gynaecology, Hammersmith Hospital, London, United Kingdom

**INTRODUCTION:** Cervical cancer is a useful model for studying early events in the biology of epithelial malignancies as it has a well-defined preinvasive stage. The technique of magic angle spinning (MAS)-MR allows spectral information to be obtained from intact cells within a tissue biopsy, and metabolic alterations such as elevated choline resonances have been identified in a number of tumors including uterine cervix [1]. Choline resonances observed with $^{1}$H MRS comprise phosphocholine (PC), glycerophosphocholine (GPC) and choline (Cho), with the former being preferentially elevated in cancer tissue. The purpose of this study was to determine the metabolic profiles of preinvasive and invasive cervical cancer on ex vivo $^{1}$H magic angle spinning (MAS)-MRS, and to further characterize the choline resonances using $^{31}$P MAS-MRS (2).

**METHODS:** ***Tissue Collection:*** Fourteen cervical punch biopsies have been obtained of which 6 have been analysed to date (3 from patients with cervical dyskaryosis ("CIN") and 3 from patients with cervical cancer ("tumour")). Samples were collected from the visible areas of the tumor or areas showing dyskaryosis, washed in phosphate buffered saline (PBS) to remove excess blood and frozen within 10 minutes of resection.

***MAS:*** Tissue samples (22±11 mg) were thawed, washed in PBS, weighed and loaded into 40 μ l sample inserts inside a 4 mm $ZrO_2$ rotors and 5 μ l $D_2O$ was added as a field frequency lock. Measurements were performed in a high-resolution MAS probe in a Bruker avance 11.74T spectrometer (500 MHz for $^{1}$H, 202 MHz for $^{31}$P). Rotors were spun at 3 kHz and maintained at 4$^{o}$C. $^{1}$H HR-MAS spectra were obtained with 512 transients, TR = 4.8s, water presaturation and a Carr-Purcell-Meiboom-Gill (CPMG) sequence, echo time 134 ms. $^{1}$H-decoupled $^{31}$P HR-MAS spectra were obtained with 512 transients, TR = 3.35 s and $^{1}$H decoupling. Peak assignments were based on chemical shift. Peak areas were measured using the AMARES algorithm (3) included in the jMRUI software package (4). $^{1}$H spectral peaks were normalised to the peak from creatine at 3.04 ppm. Concentrations from $^{31}$P spectra were obtained using the peak area of a reference compound in a separate measurement (40 μ l of 20 mM methylene diphosphoric acid (MDPA)), and corrected for the weight of each sample.

**Results and Discussion:**

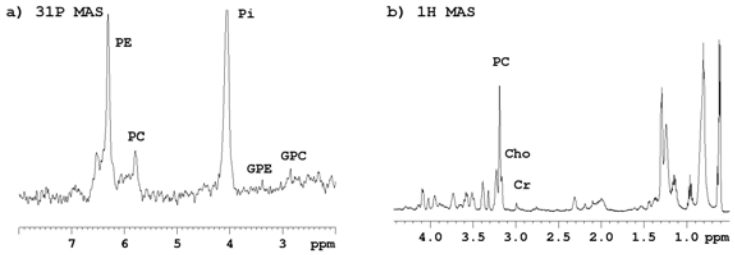


**Figure 1** $^{31}$P MAS (a) and $^{1}$H MAS (b) of a tumour sample using MAS with NS = 512.

| | Cho/Cr | PC/Cr | PC/Cho |
|---|---|---|---|
| **CIN (N=3)** | 2.47±1.36 | 0.8±0.44 | 0.33±0.07 |
| **Tumour (N=3)** | 2.65±3.28 | 6.46±6.98 | 2.68±1.26 |

**Table 1.** Choline metabolite and creatine ratios for CIN and tumour tissue samples (mean ± sd; N=6) obtained from $^{1}$H MAS spectra

| | PE | PC | Pi | GPE | GPC |
|---|---|---|---|---|---|
| **CIN (N=1)** | 0.39 | N.D. | 3.40 | N.D. | N.D. |
| **Tumour (N=3)** | 2.81 ± 1.85 | 1.15 (N=1) | 5.12±2.15 | 0.10 ± 0.09 | 0.18 ± 0.16 |

**Table 2.** Calculated concentrations (μ mol/g wet wt) in tumour tissue samples (mean ± sd). PC was seen in only 1 sample. N.D. = Not detected

Example $^{1}$H and $^{31}$P spectra from a tumour sample are shown in figure 1, while the results from all the data are shown in Tables 1 and 2. While there is no difference in the Cho/Cr ratio between CIN and tumour, there is a very substantial increase in PC, in common with many other tumour types. Other spectral differences are also present, but have not yet been evaluated.

Two of the CIN samples were too small (3 mg and 12 mg) for the $^{31}$P MAS to yield quantifiable spectra, so the results from the one quantifiable data set are quoted. PC is not detectable in this one CIN sample, and is seen in only one tumour sample, showing the advantage of the greater sensitivity and presence of 9 equivalent $^{1}$H nuclei for detecting this peak using $^{1}$H MRS. The limit of detection of PC in these $^{31}$P MAS spectra is estimated to be approximately 0.5 μ mol/g. However all $^{31}$P MAS spectra showed large peaks from PE, but with a much higher concentration in tumour compared with CIN. PE is not readily observed in $^{1}$H spectra. GPE and GPC peaks were very sharp, and again present in higher concentrations in the tumour compared with the preinvasive CIN. Inorganic phosphate (Pi) is present at comparable concentrations in all samples.

**Conclusion:** This preliminary study has used $^{1}$H and $^{31}$P magic angle spinning to investigate the metabolic profiles of preinvasive and invasive cervical cancer on ex vivo tissue sample. In common with many other cancers we have found high levels of PE compared with PC, and that both PC (from the $^{1}$H spectra) and PE (from the $^{31}$P spectra) increase from pre-invasive to invasive cancer. Evaluation of additional samples will enable these conclusions to be validated. While the underlying mechanism and significance remain to be elucidated, the findings may be useful to aid diagnosis and patient management.

**References:** 1. NM deSouza NMR in Biomedicine 2004; 17: 144-153. (2) GS Payne ISMRM 2004 p68 (3)L Vanhamme JMR**129**:35(1997) (4)A Naressi MAGMA**12**:141 (2001)

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