DETECTION OF CANCER IN CERVICAL TISSUE BIOPSIES USING MOBILE LIPID RESONANCES MEASURED WITH DIFFUSION-WEIGHTED 1H MAGNETIC RESONANCE SPECTROSCOPY

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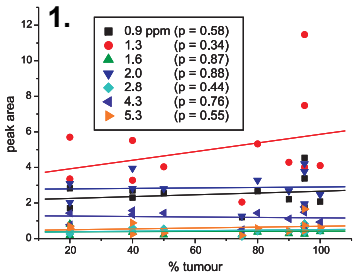
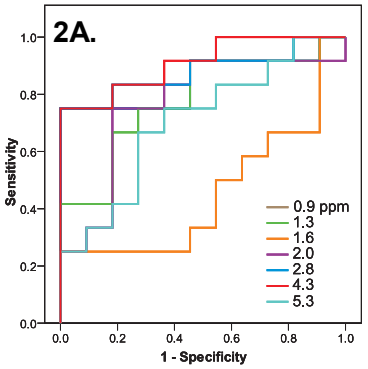
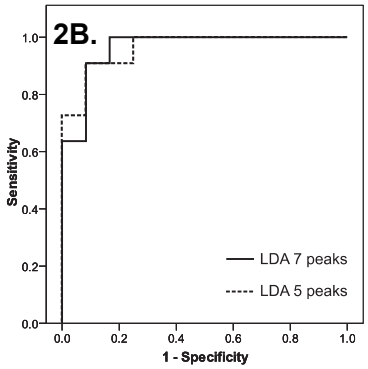
Background: In tissue studies, metabolic profiling of cervical biopsies[1-3] and other cancers[4,5] with magnetic resonance spectroscopy has been previously described, but measurement of mobile lipid resonances (MLR) has been confounded by signals from low molecular weight metabolites which complicate peak assignment and quantification. Therefore the aim of this study was to use a diffusion-weighted sequence[6] (DW) for visualisation of MLR using high resolution magic angle spinning (HR-MAS) ^{1}H MRS in order to establish, whether differences existed between tissues from patients with cervical carcinoma that were containing cancer from those that were not.

Methods: 23 cervical punch biopsies, additional to those for diagnostic histopathology, were taken from patients diagnosed with cervical cancer, subjected to *ex vivo* HR-MAS measurements and subsequently examined histologically. Samples were collected from apparently 'visible' areas of tumour, and frozen within 5 minutes of excision and stored at -80°C prior to HR-MAS analysis. Tissues were thawed, washed in phosphate-buffered saline (PBS) made in D_2O, loaded into MAS rotor inserts and placed in 4 mm ZrO_2 rotors. Following HR-MAS, samples were fixed in formalin (Sigma, UK), stained with hematoxylin + eosin and classified in a binary fashion, by a gynaecological pathologist as containing cancer or 'no-cancer'. Diffusion-weighted spectra of tissue biopsies were acquired using a stimulated echo sequence with bipolar gradients[6] using a repetition time (TR), 4.76 s; echo time (TE), 10.21 ms; time between diffusion gradients (Δ), 100 ms; diffusion gradient length (δ), 10 ms; gradient amplitude 520 mT/m; spectral width, 10,000 Hz; data size, 32 K; 128 transients. All the integrated MLR peaks were normalised to sample weight. SPSS software (version 15, SPSS Inc, USA) was used to perform statistical analysis of the data including Receiver Operating Characteristic (ROC) curves generation. Linear Discriminant Analysis (LDA) was used to find the optimum separation of the two classes (containing cancer or 'no-cancer') using first the areas of the 5 most significant peaks and then all 7. The multiple variables were projected onto an optimum one-dimensional discriminate coordinate using a routine written in Matlab (The Mathworks). The resultant scores were used to generate ROC curves and calculate area under the ROC curve (AUC).







Results: Cancer was identified in 12 samples which contained between 20% and 100% cancer (median 77,5 %, quartiles 40% and 95%).The independence of peak amplitude on tumour load in the cancer containing group is illustrated in Figure 1. Statistically significant differences in MLR at 0.9, 1.3, 2.0, 2.8 and 4.3 ppm were seen between cancer containing and 'no-cancer' biopsies (Table 1). ROC curves show the significance of individual MLR in separating these groups (Figure 2A). Table 1 shows that AUC > 0.7 for all peaks except 1.6 ppm. Combining information from all peaks using LDA gave almost complete separation of cancer-containing cervical tissues. Discriminant coordinates were found to be weighted such that peaks at 1.3, 2.8 and 4.3 ppm were more important in defining a cancer-containing tissue cluster and 0.9 and 2.0 were more important in defining a 'no-cancer' tissue cluster. The ROC curve generated by projecting the 5 significant peak areas (Figure 2B) on these coordinates shows improved AUC of 0.962.

Discussion: The improvement in the spectral resolution of the DW spectra allowed observation of unsaturated lipids and of protons from the glycerol backbone of triglycerides. Contribution of the less commonly characterised unsaturated lipids (at 2.8 and 5.3 ppm) and triglyceride (at 4.3 ppm) content therefore could be used to identify biopsies containing cancer based on these peaks as well as more commonly characterised methyl (0.9 ppm)and methylene (1.3 ppm) peaks. A clear difference in the mean peak areas of most of the MLR between samples containing histologically-identified cancer, and those that did not (Table 1) was observed although there was no significant detectable dependence of MLR signals on tumour load (samples contained 20-100% tumour). This suggests a "field effect" whereby the presence of cancer changes the metabolism of immediately adjacent non-cancer cells making their MLR profile more like that of cancer. The best separation of cancer-containing biopsies was based on the doubling in intensity of the 4.3 ppm triglyceride peak, suggesting that triglyceride content is increasing in cervical tissue containing tumour. Also levels of (poly-) unsaturated lipids were increased in the cancer-containing tissues; this increase was more significant than the corresponding general increase in saturated lipids at 0.9 and 1.3 ppm and could be utilised using the LDA model.

Conclusion: Diffusion-weighting of HR-MAS spectroscopic sequences is a useful method for characterising MLR in cancer tissues and displays an accumulation of lipids arising during tumourigenesis and an increase in the unsaturated lipid and triglyceride peaks with respect to saturated MLR. It enables discrimination of cancer-containing samples with an ROC-AUC of 0.962.

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References: 1) Mountdord et al., Magn Reson Med 1990; 13: 324-31, 2) Mahon et al., NMR in biomedicine 2004; 17: 144-53, 3) Sitter et al., Magma 2004; 16: 174-81, 4) Cheng et al., FEBS letters 2001; 494: 112-6, 5) Sitter et al., NMR in biomedicine 2006; 19: 30-40, 6) Jerschow and Muller Journal of Magnetic Resonance 1997; 125: 372-5

Table 1.

Peak	Tissue	N	Mean ± SE	Significance (ttest)	Area under the curve
0.9 ppm*	no-cancer	11	1.63 ± 0.12	0.00598	
	cancer	12	2.50 ± 0.25		0.848
1.3 ppm*	no-cancer	11	3.28 ± 0.32	0.03826	
	cancer	12	5.05 ± 0.71		0.780
1.6 ppm	no-cancer	11	0.35 ± 0.03	NS	
	cancer	12	0.40 ± 0.06		0.455
2.0 ppm*	no-cancer	11	2.07 ± 0.20	0.02378	
	cancer	12	2.86 ± 0.25		0.765
2.8 ppm*	no-cancer	11	0.24 ± 0.03	0.00273	
	cancer	12	0.45 ± 0.05		0.789
4.3 ppm*	no-cancer	11	0.61 ± 0.05	0.00015	
	cancer	12	1.21 ± 0.12		0.909
5.3 ppm	no-cancer	11	0.38 ± 0.06	NS	
	cancer	12	0.61 ± 0.11		0.705