Investigation of mobile lipid resonances in cervical tissue biopsies and correlation with cytoplasmic lipid droplets.

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Background: Increased levels of mobile lipid resonances (MLR) have been documented *in vivo* in a variety of tumours including cervical cancer (1) and have been shown to be involved in tumour cell physiology, growth and metastasis (2). Initially, mobile lipid signals were believed to originate from triglycerides in membrane-associated globular microdomains, more recently, intracellular lipid bodies, either adjacent to the plasma membrane or within the cytoplasm have been suggested as the location for mobile lipid signals (3). In this study, diffusion-weighted (DW) ^{1}H HR-MAS was used to characterise the major saturated and unsaturated lipid peaks in normal epithelium & stroma, dysplastic epithelium (low grade CIN) and cancer-containing tissue, compare them between tissue types and correlate them with the presence and number of cytoplasmic lipid droplets.

Methods: 27 cervical punch biopsies from patients with cervical cancer additional to those required for diagnostic histopathology were used for *ex vivo* HR-MAS measurements. The tissue composition of each sample was subsequently confirmed on histopathology by a pathologist who identified presence of epithelium and stroma, the type of mucosa present and whether normal tissue, CIN, or tumour could be identified. Diffusion-weighted spectra of tissue biopsies were acquired using a stimulated echo sequence with bipolar gradients as described previously (4). All MLR peak areas were calculated. For one slide per biopsy Nile red staining was done and examined by fluorescence microscopy (5). Five random histological images for each section were captured at x 20 magnification. Neutral lipids were seen as gold-red fluorescent structures. Mean droplet number per image was calculated. MLR peak areas (for saturated lipids at 0.9 ppm & 1.3 ppm polyunsaturated lipids at 2.8 ppm, triglycerides at 4.3 ppm, unsaturated lipid at 5.3ppm) were plotted against average droplet number (per image) for 'no-cancer' epithelium stroma, low grade CIN and cancer-containing tissue. A correlation between MLR peak areas and lipid droplet content was investigated using linear correlation and the Spearman rank correlation coefficient (*R*).

Results: 9 samples did not contain cancer histologically, 7 were classified as low grade CIN and 11 samples contained cancer. Percentage of cancer in cancer-containing samples was 5 - 95 % (median 65%, quartiles 12.5% and 82.5 %). The highest MLR intensities for peaks at 0.9, 1.3, 1.6, 2.0, 2.3 and 5.3 ppm were seen in the 'low grade CIN' group. The cancer group showed most intense MLR peaks at 2.8, 4.1 and 4.3 ppm (example spectrum: Figure 1, Table 1). Single peak analysis of individual MLR did not reveal statistically significant differences in relative intensities of MLR peaks between biopsy classes. Lipid droplet numbers were: 50.4 ± 23.8 for 'no-cancer'epithelium & stroma, 71.3 ± 7.2 for low grade CIN, and 87.7 ± 53.9 for cancer (mean ± standard deviation). The highest numbers of droplets (averaged) were seen in cancer biopsies compared to 'no-cancer' epithelium & stroma and low grade CIN tissues. However, in each tissue class the droplets were located in random areas of the section. Subjectively there were more areas with visible droplets in cancer and low grade CIN tissues compared with 'no-cancer' epithelium & stroma. Figure 2 shows scatter plots of MLR peak areas for all biopsies (27) against average number of droplets per image. Significant correlations between droplet number and MLR peaks at 0.9, 1.3 and 2.8 ppm were seen. When subdivided by histological class the only significant correlations were seen for 0.9 (R = 0.729, p = 0.011) and 2.8 ppm (R = 0.727, p = 0.011) in the cancer containing group and 1.3 ppm (R = 0.75, p = 0.02) for no-cancer epithelium and stroma class.

Discussion: There was a large heterogeneity in the lipid content among samples in all three classes which resulted in no significant differences in MLR intensities between them. Biopsies classified as low grade CIN generally displayed more MLR (apart from the polyunsaturated peak at 2.8 and triglyceride at 4.1 and 4.3 ppm), which may reflect increased proliferation in these samples, and resultant synthesis of lipids. In each tissue class the distribution of droplets was non-uniform over the section, therefore acquired images contained heterogeneous numbers of droplets (including areas without any droplets), but this variable distribution was not related to presence of epithelial or stromal components (based on histology). It may be related to tissue stress such as hypoxia, as previously reported in human cervical tumours (6-8). Relatively good correlations were seen between MLR peaks and lipid droplet number for intense peaks where SNR was higher; poorer correlation was usually seen for smaller MLR peaks where SNR is lower (Figure 2). The best correlations were seen for the 0.9 and 1.3 ppm resonances. The location of trendlines and resulting correlation coefficients best-fit equations do not take into the account the size of droplets which is important as smaller droplets were probably overlooked due to the low resolution used. Also the number of samples in this study is relatively low and it would be important to confirm these observations on a larger number of biopsies.

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Figure 1

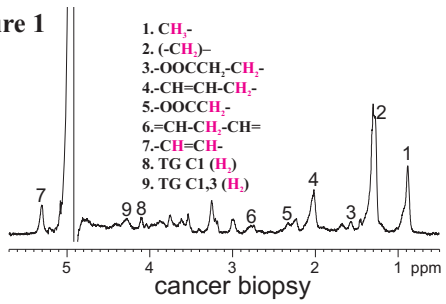

Figure 2

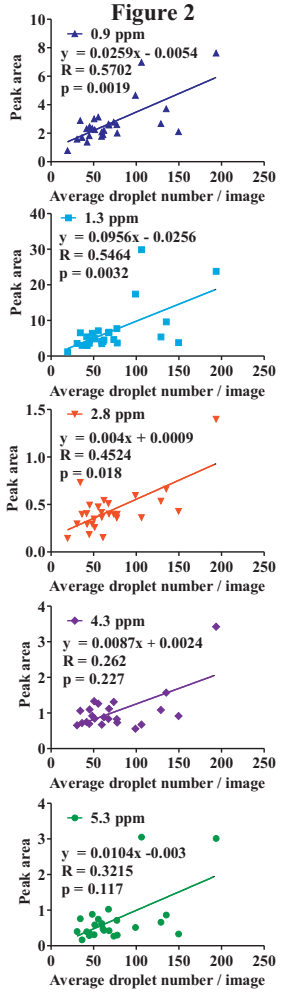

Table 1

Peak	Tissue	N	Peak area Mean ± SD
0.9 ppm	no-cancer epith/stroma	9	2.33 ± 1.11
	low grade CIN	7	3.10 ± 1.63
	cancer	11	2.86 ± 1.69
1.3 ppm	no-cancer epith/stroma	9	5.95 ± 4.72
	low grade CIN	7	9.08 ± 8.61
	cancer	11	6.61 ± 5.95
1.6 ppm	no-cancer epith/stroma	9	0.59 ± 0.46
	low grade CIN	7	0.69± 0.63
	cancer	11	0.51 ± 0.46
2.0 ppm	no-cancer epith/stroma	9	2.17 ± 0.85
	low grade CIN	7	3.04 ± 1.81
	cancer	11	2.81 ± 1.07
2.3 ppm	no-cancer epith/stroma	9	0.77 ± 0.39
	low grade CIN	7	0.99 ± 0.42
	cancer	11	0.98 ± 0.57
2.8 ppm	no-cancer epith/stroma	9	0.41 ± 0.19
	low grade CIN	7	0.35 ± 0.09
	cancer	11	0.52 ± 0.31
4.1 ppm	no-cancer epith/stroma	9	0.37 ± 0.12
	low grade CIN	7	0.29± 0.13
	cancer	11	0.55 ± 029
4.3 ppm	no-cancer epith/stroma	9	0.82 ± 0.20
	low grade CIN	7	0.95 ± 0.25
	cancer	11	1.25 ± 0.82
5.3 ppm	no-cancer epith/stroma	9	0.51 ± 0.29
	low grade CIN	7	0.92 ± 0.90
	cancer	11	0.74 ± 0.77