

## Metabolic changes associated with HPV infection in cancer cells observed with <sup>1</sup>H MRS

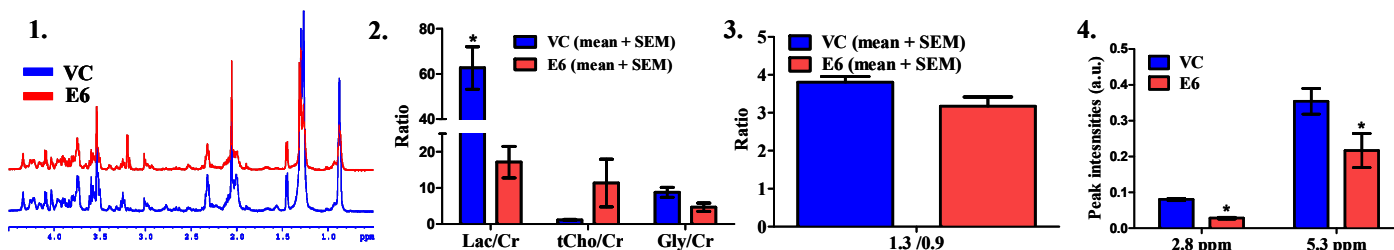
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**Background:** ‘High risk’ types of human papilloma virus (HPV) can increase the risk of developing cervical cancer with types 16 and 18 causing about 70% of cancers of the cervix<sup>1</sup>. The metabolic effects of HPV infection are unknown and it is unclear whether HPV infection causes metabolic changes within the cell. Therefore the aim of this study was to use <sup>1</sup>H MRS to test for changes in a related model consisting of isogenic *HPV-16 E6*-transfected derivatives (E6 is the key cancer-causing protein expressed by the HPV) of the A2780 human ovarian cancer cell line that differ only in p53 status<sup>2</sup>.

**Methods:** An ovarian A2780 cell line transfected with *HPV-16 E6* (E6) protein or an empty vector (VC) as control<sup>2</sup> were cultured as adherent cells at 37°C in an incubator with 5% CO<sub>2</sub> in McCoy’s 5A medium (Sigma, UK), supplemented with 10% fetal calf serum and 1 µg/ml puromycin. In all experiments (n=3) for each variant attention was given to ensure that cells were not deprived of nutrients at any stage of growth. Cells were harvested on the day 7 of the culture (confluence), pelleted ( $1 \times 10^7 \pm 0.1 \times 10^7$  cells per sample) and spectra were acquired using a Bruker Avance 11.74T spectrometer (Bruker BioSpin, Germany), <sup>1</sup>H frequency of 500 MHz, equipped with a 4mm triple resonance <sup>1</sup>H/<sup>31</sup>P/<sup>13</sup>C HR-MAS probe with a gradient aligned along the magic angle axis. <sup>1</sup>H HR-MAS spectra were acquired with water presaturation using a Carr–Purcell–Meiboom–Gill (CPMG) sequence (TE 135ms; time between each 180° 333.33 µs). A total of 512 scans were collected using 16k data points, a repetition time of 4.8 s, with total acquisition time of 41 minutes. Diffusion-weighted spectra were also acquired using a stimulated echo sequence with bipolar gradients<sup>3</sup> with 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. Cells were counted, viability and size was measured using Vi-Cell Viability Analyzer (Beckman Coulter, Inc.,USA).

**Results:** The doubling times of E6 transfected cells (~24h) were 33% longer than VC A2780 cells (±18h; p<0.007), and on the day 7 of the culture resulted in significant (p<0.05) decrease in the average diameter of VC cells (10.6 ± 0.5µm) compared with E6 transfects (12.0 ± 0.7 µm). However it did not affect the viability of the cells, which in both cell lines was around 90% ± 3%. Figure 1 shows <sup>1</sup>H HR-MAS CPMG spectra of VC and E6 transfected cells. Differences in the E6 transfected cells include lower lactate (1.32 ppm) and glycine (3.56 ppm) and higher cholines (3.2 ppm) Changes in lactate, total choline and glycine normalised to creatine peak at 3.03 ppm are shown in figure 2. Differences in lactate / creatine ratio are significant (p<0.02), those in total choline are not, but the data suggests a trend. Diffusion-weighted (DW) spectra revealed lower lipid content in E6 transfected and also a change in the methylene (1.3 ppm) over methyl (0.9 ppm) ratio (figure 3). Significant differences were observed in the levels of polyunsaturation of lipids reflected by the intensities of peaks at 2.8 (p<0.00002) and 5.3 ppm (p<0.02; figure 4).



**Discussion:** Proton NMR spectroscopy reveals metabolic changes associated with HPV infection likely to be associated with altered signalling which affects metabolic pathways as well as p53 expression. The observed metabolic changes may be related to changes in the proliferation rates: decrease in lactate in E6 cells suggests decreased glycolysis, while decreased lipid suggest decreased membranes turnover with changes in lipid (poly-) unsaturation. Similar changes may also apply to HPV infection in the cervix. Documenting these changes may provide further insights to understand the metabolic ‘field effect’ often observed around cervical tumor (and could be caused by HPV infection alone) and into viral oncogenesis itself.

**Acknowledgements:** We thank Dr M. Walton (from The Institute of Cancer Research) for isogenic *HPV-16 E6*-transfected derivatives of the A2780 human ovarian cancer cell line. This work was funded by the EC FP6 Marie Curie Action: Early Stage Training (contact No. 020718). We also acknowledge the support received for the CRUK and EPSRC Cancer Imaging Centre in association with the MRC and Department of Health (England) (grant C1060/ A10334) and NHS Funding to the NIHR Biomedical Research Centre.

**Refs:** 1. Cancer Research UK: What is the HPV virus? (<http://www.cancerhelp.org.uk/about-cancer/cancer-questions/what-is-the-hpv-virus#cervical>), 2. Pestell et al., Molecular Pharmacology, 2009; 57: 503–511, 3. Jerschow and Muller Journal of Magnetic Resonance 1997; 125: 372–5