

Investigation of features related to androgen dependency in prostate cells using ^1H HRMAS MRS and Principal Component Analysis

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Introduction: Standard treatment for prostate cancer includes hormone suppression therapy. Invariably the tumour shows initial response, but eventually escapes anti-androgen control. Non-invasive diagnosis of androgen independence in prostate tumours would aid current treatment by indicating when anti-androgen therapy is no longer beneficial. ^1H HRMAS MRS has potential to provide biomarkers of prostate cancer behaviour with multivariate approaches such as Principal Components Analysis (PCA) offering advantages by efficiently interrogating the whole spectrum with minimal prior knowledge or assumptions required. The purpose of this work was to apply PCA to ^1H HRMAS MRS spectra from a range of androgen sensitive and insensitive prostate cell lines grown in the presence and absence of androgens in order to explore MRS features characteristic of androgen sensitivity.

Methods: *Data acquisition:* In vitro prostate cell cultures were seeded at 2×10^6 cells per 173cm^3 flask and were cultured in media supplemented with either 10% FCS or, 10% dialysed FBS at 37°C in the presence of humidified 5% CO_2 . The medium was changed 48 hours later and the cells harvested three days after seeding when the cells had reached $\sim 70\%$ confluence. Cells were harvested by trypsinising and then extracted in $250\mu\text{l}$ 12% perchloric acid. Extracts were pH neutralised with KOH and freeze dried overnight. Residues were made up in $630\mu\text{l}$ D_2O with a final concentration of 0.794 mM TSP which was used for spectral chemical shift calibration. All experiments were performed on a Bruker 500MHz spectrometer using a 5 mm multinuclear broadband observe (BBO) probe and acquired using a 30 degree pulse-and-acquire sequence, with water presaturation, 256 scans, 512 receiver gain, $\text{TR}=5.7\text{s}$ at 298 K .

Data analysis: Each 16k FID was apodised with a line broadening factor of 2Hz. The frequency spectra were manually phased and aligned to TSP (0ppm). A region ($4.7 - 0.4\text{ ppm}$) was chosen for analysis, with a small subsection relating to a single contaminant peak ($2.2-2.3\text{ ppm}$) removed. Prior to PCA analysis, each spectrum was L2-normalised (divided by the square root of the sum of squares of spectral values). All processing and analysis was performed using in-house MATLAB code.

Results & discussion: Prostate cancer cell lines, PC-3 ($n=5$) and 22RV1 ($n=5$), grown in 10% FBS were compared with a normal prostate epithelial cell line (RWPE ($n=4$)) grown under similar conditions. The PCA scores plot (Figure 1) shows good separation between the cancerous and healthy groups along the first component but also good separation of the cancer cell lines along the second. (Figure 2) shows elevated choline, lactate and alanine characteristic of tumour tissue, but also shows other peaks that could be attributable to tumour. When the same normal spectra were compared to PC-3 ($n=4$) and 22RV1 ($n=5$) grown in absence of androgen, the same scores plot was seen but the first component (not shown) showed greater influence of creatine and a reversal of the alanine coefficients. A comparison of PC-3 and 22RV1 grown both in the presence and absence of androgen proved to cluster more as a result of their cell line of origin than their growth conditions. Therefore, Cr and Ala, were isolated by selecting small spectral regions around them. Alanine did not differentiate well between classes, but the effect of growth medium on creatine (represented by component 2, figure 3) shows that it is reduced when the cells are grown in absence of androgen (figure 4).

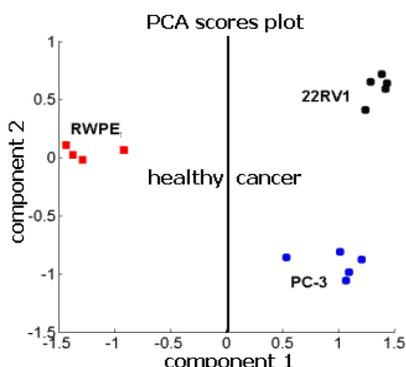


Figure 1: Scores against component 1 can be used to differentiate between cancerous cell lines and normal cell lines. Component 2 separates the cancerous cell lines.

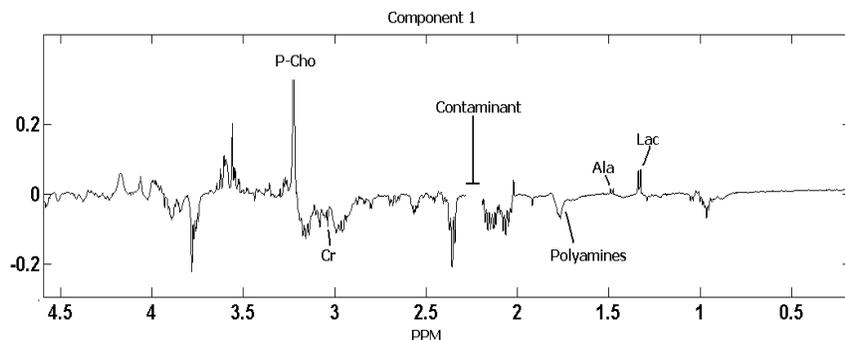


Figure 2: Principal component 1: The negative regions correspond to regions of the spectra that are elevated in the androgen sensitive, non-cancerous cell line -RWPE. The positive regions represent the features that are enhanced in the spectra of the androgen insensitive, cancerous cell lines - 22RV1 and PC-3. In this instance, all were grown in the presence of androgens.

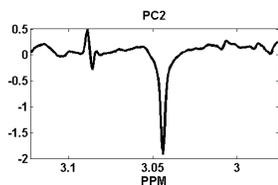
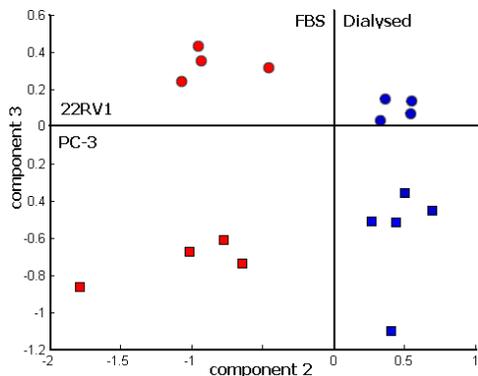


Figure 3: (above): 2nd PC showing coefficients for creatine.

Figure 4: (right): The 2nd component separates based on growth medium (red)10% FBS (blue)10% dialysed FBS. The 3rd component separates based on cell line (circle) 22RV1 (square) PC-3



Conclusion: This pilot application of PCA to prostate cell extracts is revealing clear differences between cell types and presence of androgen. Ongoing work explores whether any of these features can differentiate androgen independent and dependent cell lines in the general case.

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