Effect of Androgens on Intracellular Polyamine Levels in Androgen-dependent and Androgen-independent Prostate Cancer Cell Lines

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Introduction: Current therapy for prostate cancer is based initially on androgen-deprivation, although the sensitivity to androgen deprivation may be lost over time. Transformation from an androgen-dependent to an androgen-independent status has been associated with aberrant polyamine metabolism and measurement of polyamines may predict hormonal escape [1]. Polyamine levels may be measured using magnetic resonance spectroscopy (MRS) and several studies have demonstrated the benefit of incorporating polyamine levels to aid MRS detection of prostate cancer *in-vivo* [2-3]. However, the relationships of polyamine levels to changes in tumour cell growth in androgen-dependent and androgen-independent tumours in androgen deplete and replete conditions have not been established. This study compares intracellular polyamine levels in androgen-dependent and androgen-independent prostate cancer cell lines and examines their response to androgen deprivation. The MRS measurement was validated with high performance liquid chromatography (HPLC) in the same samples.

Method: In vitro prostate cell cultures were seeded at $2x10^6$ 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 ~70% confluence. Cells were harvested by trypsinising and then extracted in 250µl 12% perchloric acid. Extracts were pH neutralised with KOH and freeze dried overnight. Residues were made up in 630µl D_20 with a final concentration of 0.794 mM TSP used as an internal reference (for metabolite quantification and 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° pulse-and-acquire sequence, with water presaturation, 256 scans, 512 receiver gain, TR = 5.7 seconds. Polyamines were quantified using XWINNMR software (Bruker). Integrals were calculated relative to the TSP peak at 3.19-3.024 (Pk1) and 1.851-1.742 ppm (Pk2) (see figure 1). At 3.19-3.024 ppm a user defined spline-correction gave intensities of overlapping peaks which were subtracted to leave an estimated peak area ascribed to polyamines. At 1.851-1.741 ppm the polyamine peak was well distinguished. A volume of 50µl was taken from each cell extract for HPLC separation of the polyamines after derivatisation with dansyl chloride as described previously [5].

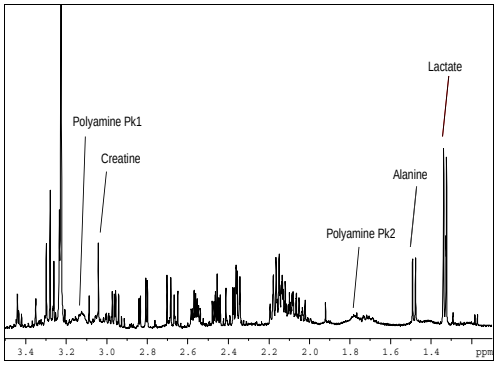

Figure 1: MR spectrum of a perchloric acid extract of PC-3 cells showing assignment of polyamine peaks, Pk1 and

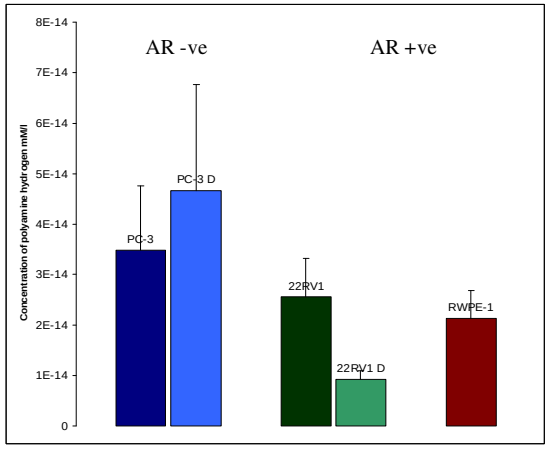

Figure 2: Concentration of polyamine hydrogens in Pk2 of perchloric acid extracts of four prostate cell lines (grouped based on androgen-receptor status). PC-3 and 22RV1 were also grown in androgen deprived medium.

Results and Discussion: The polyamines putrescine (Put), spermidine (Spd) and spermine (Spm) all have peaks at approximately 3.1 and 1.8 ppm and therefore cannot be distinguished by one dimensional MRS. Putrescine, spermidine and spermine contribute 4, 8 and 12 ^1H to the 3.1 ppm peak but all contribute 4 x ^1H to the 1.8 ppm peak. Androgen-responsive cell lines, 22RV1, showed lower polyamine levels compared to androgen-independent PC-3 (AR status negative) cell lines. In androgen-deprived media, there was a decrease in polyamine protons for 22RV1 but not for the androgen independent PC-3 cell line. RWPE-1 (normal prostate epithelial cell line) showed similar levels to 22RV1 grown in normal androgen conditions. Total HPLC polyamine concentrations (figure 3) also showed similar concentration for 22RV1 and RWPE-1 and the lack of change in PC-3 polyamine levels when grown in androgen deprived conditions. Individual polyamine contributions to the total intracellular amount as determined by HPLC are shown in figure 4.

Conclusion: This study demonstrates the potential of MRS in the analysis of polyamine metabolism in-vitro models of prostate cancer. Some differences in intracellular levels and in response to depletion of androgens in medium were seen. Work is currently in progress to include further cell lines in the study and to correlate the changes in polyamine levels with enzyme expression.

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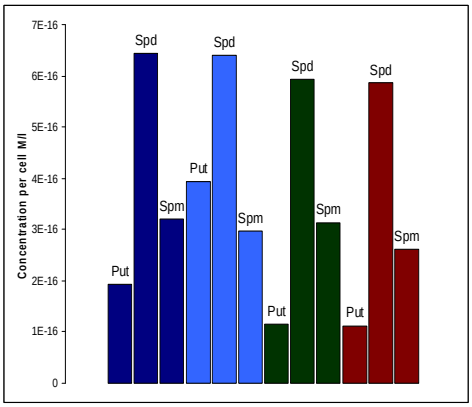

Figure 4: Ratio of polyamines putrescine, spermidine and spermine found PC-3, PC-3 D, 22RV1 and RWPE-1 with HPLC

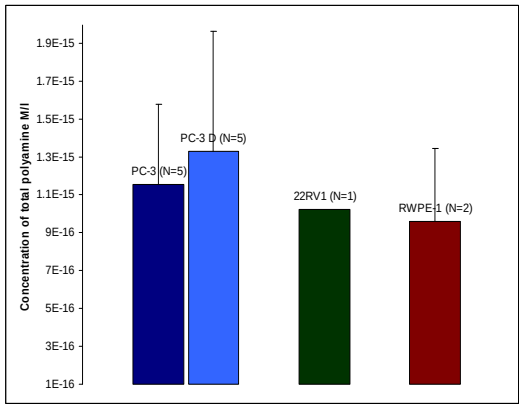

Figure 3: HPLC measurement of total polyamine concentration