

Metabolic activity of diseased and healthy prostate cells investigated as lactate formation from hyperpolarized ^{13}C -pyruvate

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Introduction: A biopsy is the only conclusive method to diagnose prostate cancer, resulting in many false negatives. Studies in a rat cancer model *in vivo* have shown that injected hyperpolarized ^{13}C -pyruvate produces a strong ^{13}C -lactate MR signal in tumor tissue, making this a new potential diagnostic method [1]. In the present study we investigate further on this anaerobic conversion of pyruvate to lactate by using an *in vitro* model with four different prostate cell lines, PNT 1A, DU-145, PC-3 and BPH-1. The enzyme catalyzing the conversion from pyruvate to lactate, lactate dehydrogenase (LDH), is a tetrameric enzyme with five isoforms, designated LDH1-5. LDH5 has a high affinity for pyruvate and is highly up-regulated in prostatic carcinoma [2]. We found a clear increase in pyruvate to lactate metabolism in diseased compared to normal tissue, which could be correlated to an up regulation of LDH5.

Methods: PNT 1A is described by the supplier (ECACC) as a normal cell line, whereas BPH-1 (DSMZ) is described as a benign prostate hyperplasia. PC-3 and DU-145 (both DSMZ) are isolated from metastases of prostate carcinoma. For gel electrophoresis, cells were lysed by sonication. Total LDH activity in supernatants was determined spectrophotometrically using a cytotoxicity detection kit (Roche #1644793). LDH isoforms were separated on a Paragon system from Beckman Coulter and activity was visualized by a formazan color reaction. ^{13}C -Pyruvic acid was hyperpolarized in solid phase as described earlier [3]. After dissolution, ^{13}C -pyruvate was added to 1 ml cell suspension in a 10 mm NMR tube to a final concentration of 1 mM. Spectra were acquired on a 9.4 T Varian spectrometer with 3 s intervals (15° flip angle) during 27 s, followed by a 90° pulse after 30 s.

Results and Discussion: The ^{13}C -lactate formation is significantly higher in the cancer cells compared to the normal prostate cells, during the 27 s observation period, confirming a higher LDH activity in cancer cells (fig. 1). The hyperplasia cells produce more lactate than both the cancer lines, which would indicate a diseased state also in this cell line. The total LDH activity in each respective cell line follows the same pattern as the lactate formation, with the lowest activity in PNT 1A and the highest in BPH-1 (fig. 2). The distribution of total activity between the different LDH isoforms is presented in figure 3. The LDH5/total LDH activity quote varies from 5% in the non-diseased cell line PNT 1A to 55% in the carcinoma cell line PC-3. Also in this case, BPH-1 presents a very cancer-like behavior. To conclude, a high ^{13}C -lactate formation after administration of hyperpolarized ^{13}C -pyruvate to cancer cell suspensions correlates a new potential cancer diagnosis method, DNP-MR, to established diagnostic methods, using blood serum and biopsy analysis.

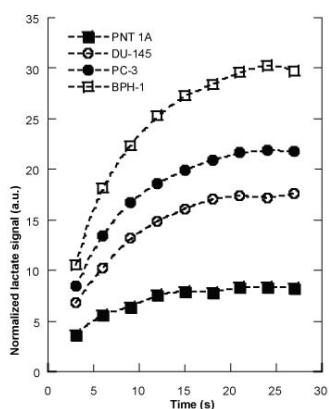


Figure 1. Relative ^{13}C signal from lactate formed from hyperpolarized pyruvate during 27 s. Results represent a subset of four experiments for each cell line.

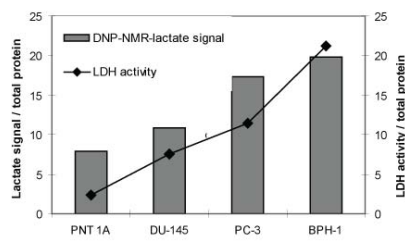


Figure 2. Relative ^{13}C -lactate signal in cell suspension after 30 s incubation with pyruvate, measured with a 90° NMR flip angle (grey bars), correlated to total LDH activity, measured spectrophotometrically. n=6 (PNT 1A), 5 (DU-145), 4 (PC-3) and 6 (BPH-1).

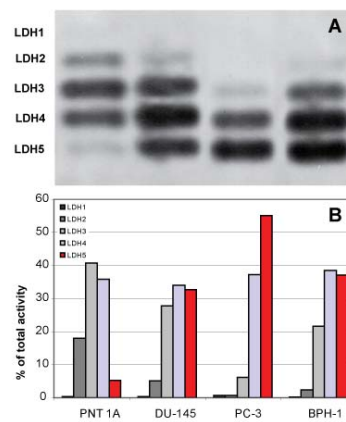


Figure 3. A. LDH gel showing distribution of enzymatic activity between isoenzymes in the four prostate cell lines and B. Visualization of the same isoenzyme pattern as percentage of total activity.

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References: [1] Golman *et al*, Cancer Res 66(22):10855. 2006, [2] Srinivasan *et al.*, Inv Urol 11(3):244, 1973, [3] Ardenkjær-Larsen *et al.*, PNAS 100:10158, 2003.