Metabolism of hyperpolarized 1, 4-$^{13}C_2$-fumarate in human cancer cell lines

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Introduction: Hyperpolarization of metabolic markers provides a powerful tool to study real time metabolism on a cellular level. Fumarate is a marker, suitable for hyperpolarization, which recently has been identified to play a role in the HIF signaling pathway in several types of cancer [1]. To investigate if hyperpolarized fumarate can be used as a marker to study an altered metabolism in cancer, we studied the conversion of 1,4-$^{13}C_2$-fumarate into 1,4-$^{13}C_2$-malate in four human cancer cell lines; breast adenocarcinoma (MDA-MB-231), chondrosarcoma (H-EMC-SS) and two prostate cancer lines (PC-3 and DU-145). We further investigate the two prostate cancer lines to determine total amount of malate after addition of fumarate.

Methods: 1,4-$^{13}C_2$-fumarate was hyperpolarized in solid phase as described earlier [2]. After dissolution, 1,4-$^{13}C_2$-fumarate was added to a final concentration of 3 mM to 0.5 ml cell suspension of 10 million cells in a 5 mm NMR tube, kept at 37 ^{o}C. Spectra were acquired on a 9.4 T Varian spectrometer with 2.4 s intervals, 15^{o} flip angle and a total of 60 scans. 1,4-$^{13}C_2$-malate formation rate was quantified from the first 40 s of the experiment using jMRUI 3.0. Conversion rates were given per gram of soluble protein in the cell lysate supernatant. In a parallel experiment, the reaction was quenched with perchloric acid after 60 s and total malate concentration was determined using fluorescence spectroscopy.

Results and Discussion: Conversion of 1,4-$^{13}C_2$-fumarate to 1,4-$^{13}C_2$-malate could be seen in all four cancer lines investigated (Table 1). The highest conversion was seen in the breast cancer cell line MDA-MB-231. This cell line is highly invasive and hormone-insensitive and represents a late stage cancer. Formation rate of malate varies greatly between the two prostate cancer lines, with a 7 times higher conversion rate in the more aggressive PC-3 line, compared to DU-145 (Figure 1). Analysis of malate concentration, after addition of fumarate to the DU-145 and PC-3 cell suspensions, reports a 10 times higher concentration in the more aggressive cell line, in agreement with the higher fumarate conversion.

Conclusion: Hyperpolarized 1,4-$^{13}C_2$-fumarate can be used as a real time metabolic marker in cancer cell studies. The observed difference in conversion rate into 1,4-$^{13}C_2$-malate between cell lines of varying aggressiveness opens up for a potential new method to stage cancer.

Cell line	Malate formation rate (µmol min^{-1} g^{-1} protein)
MDA-MB-231	77 ± 19
H-EMC-SS	40 ± 14
PC-3	42 ± 0.5
DU-145	6.± 2

Table 1. Formation of 1,4-$^{13}C_2$-malate from 1,4-$^{13}C_2$-fumarate in the 4 investigated cell lines. n≥3

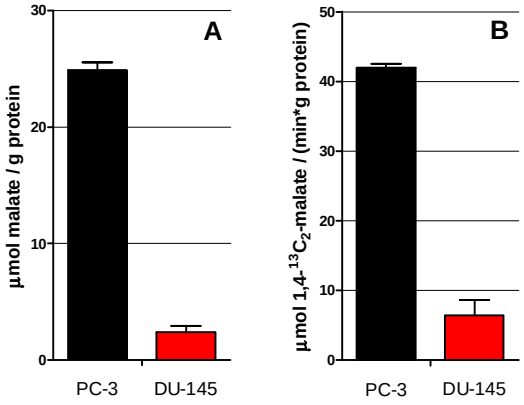

Figure 1. A. Total amount of malate in cell lysates and **B.** 1,4-$^{13}C_2$-malate formation over time in prostate cell lines.

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References: [1] Esteban and Maxwell, Nature medicine 11: 1047-1048, 2005. [2] Ardenkjær-Larsen *et al.*, PNAS 100:10158, 2003.