Hyperpolarized 13C diffusion MRS of copolarized pyruvate and fumarate detects evidence for increased lactate export in 8932 pancreas carcinoma cells compared to MCF-7 cells

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Introduction and Purpose:

Upregulation of glycolysis in tumors results in increased lactate concentrations in both intra- and extratumoral areas, the latter leading to an acidification of the tumor microenvironment. Hyperpolarized ^{13}C-labelled metabolic tracers can be used to probe fast metabolic pathways in real-time, however little has been known from these measurements about their presence in intra- or extracellular compartments, a distribution that is largely influenced by the expression of monocarboxylate transporters (MCT). Several studies have shown that diffusion NMR and MRI can provide information about the metabolites' microenvironment as well as about the intactness of the plasma membrane (grade of necrosis) and would be suitable even in a clinical setup.[1,2,3]

The aim of this study is to examine whether diffusion NMR can be used to pick up differences in lactate export between different tumor cell lines. In addition, fumarate is copolarized to provide additional information on the grade of necrosis and to compare the sensitivity for diffuse necrosis between ADC measurements and enzymatic conversion from fumarate to malate.

Methods:

ADCs of ^{13}C-metabolites were measured in MCF-7 tumor cells and 8932 pancreas carcinoma cells on a 14.1T NMR spectrometer (Bruker, Ettlingen, Germany). After injection of hyperpolarized ^{13}C-labeled [1-^{13}C]pyruvate and [1,4-^{13}C]fumarate at a final concentration of 2 mM each the metabolites are taken up by the cells. The hyperpolarized label from pyruvate is intracellularly exchanged to lactate by LDH activity. In cells that are subject to necrosis fumarate is metabolized to malate. The cell lines were chosen based on the observation that both pancreas carcinoma cells and MCF-7 breast cancer cells are highly proliferative but origin from different metabolic microenvironmental surroundings. An established diffusion-weighted NMR pulse sequence based on a pulsed gradient spin echo (PGSE) sequence in combination with an 8 mm NMR tube in-vitro setup was used for measuring ADCs of hyperpolarized ^{13}C nuclei.[3] Cells were taken of the incubator directly before the NMR measurement and a cell number of approximately 20 Mio cells was used for each experiment. Some cultures of 8932 tumor cells were incubated with 1 % Triton X-100 in the assay medium in order to obtain a necrotic stage. The number of dead cells was accessed by Trypan blue staining and counting of viable and dead cells with a Neubauer cell chamber.

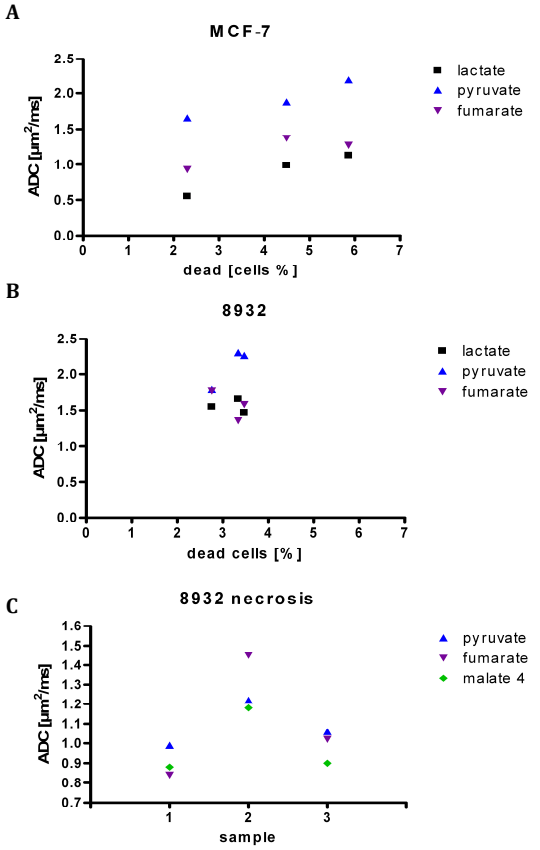

Fig. 1: ADCs of pyruvate, lactate, and fumarate in (A) MCF-7 breast cancer cells (B) 8932 pancreatic cancer cells and (C) necrotic 8932 pancreatic cancer cells which were treated with membrane permeabilizing Triton X-100.

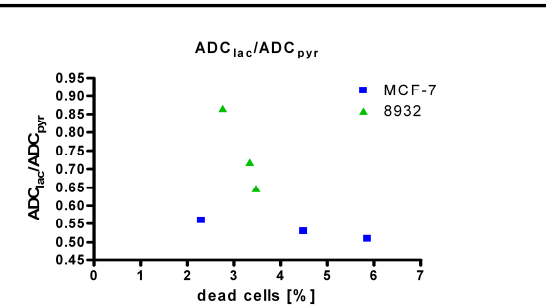

Fig. 2: ADC_{lac}/ADC_{pyr} ratio of MCF-7 breast cancer cells and 8932 pancreatic cancer cells

Results and Discussion:

ADCs of pyruvate, lactate and fumarate were detected in both breast cancer and pancreas carcinoma cells in-vitro and correlated to the degree of necrosis (Fig. 1 A,B). In viable cells lactate ADC is lower than pyruvate ADC for both cell lines reflecting its intracellular origin, whereas pyruvate at the given high dose is predominantly distributed extracellularly. The ADC_{lac}/ADC_{pyr} ratio showed differences between the two cell lines with the ratio being higher in 8932 cells (range: [0.64:0.86]) compared with MCF-7 (range: [0.51:0.56]) (Fig. 2). This might reflect a lower MCT transport of lactate out of the cell in MCF-7 cells as compared to the pancreatic cancer cells which are known for their fast export of lactate.[4] Fumarate was only converted to detectable amounts of malate in cells that were entirely necrotic after addition of Triton X-100 (Fig. 1 C). In this state, no lactate has been observed. Most likely LDH activity is hindered by the dilution of the coenzyme NADH. At the same time the ADCs of all metabolites were showing no large differences reflecting a blending of compartments. Thus, by using this co-polarized agent two essential states of a cell can be characterized simultaneously: 1) The ADC_{lac}/ADC_{pyr} ratio reveals information about the microenvironmental differences between lactate and pyruvate which is being influenced by lactate transport rates 2) malate detects a quick an instantaneous processes of cell death.

Conclusion:

The combination of copolarized C-13 labeled pyruvate and fumarate with ADC measurements holds promise for localizing necrosis and assessing lactate export rate, a parameter that has been shown to correlate with tumor aggressiveness. As these techniques can be extended to spatially resolved metabolic imaging, they might be valuable for clinical tumor response evaluation in the future.

References:

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