# Modulations of intra and extracellular pH in tumor variants defective in either respiration or glycolysis, observed by in vivo MRS

**N. W. Lutz[1], J. Chiche[2], Y. Le Fur[1], C. Vilmen[1], F. Frassineti[3], L. Daniel[3], J. Pouysségur[2], and P. J. Cozzone[1]**
[1]CRMBM UMR 6612 CNRS, Aix-Marseille University, Medical School, Marseille, PACA, France, [2]Institute of Developmental Biology and Cancer Research CNRS UMR 6543, Centre A. Lacassagne, Nice, [3]Inserm UMR 911-CRO2, Aix-Marseille University, Medical School

## Introduction

HIF-induced tumor neovascularization has become an important target of anticancer therapy. While the current use of angiogenesis inhibitors aims at converting cancer to a chronic manageable disease [1], further modifications of the hypoxic tumor microenvironment are needed to achieve complete tumor regression. It has previously been proposed to take advantage of the increased production of protons under drug-enhanced hypoxia [2]. Thus, the central idea is to treat tumors with additional drugs designed to decrease intracellular and increase extracellular pH.

The purpose of the current study is to contribute to the validation of the suggested treatment method. Effects of modulations of multiple mechanisms of glycolytic activity and pH regulation on intracellular and extracellular pH ($pH_i$, $pH_e$) are being investigated. To this end, we chose a model system (ras-transformed fibroblasts) in which several mutations where engineered to manipulate glycolysis and pH. Effects of these mutations were studied in xenograft models of nude mice, using in vivo magnetic resonance spectroscopy and imaging. Intra and extracellular pH were determined simultaneously, in conjunction with tumor morphology and necrosis, and were complemented with histological data.

## Methods

Tumors were induced in the thighs of nude mice by subcutaneous inoculation of 1-2x$10^6$ ras-transformed CCL39 hamster fibroblasts. Three CCL39 variants have been compared so far: (i) wild-type, (ii) glycolysis-suppressed (DS7; defective in phosphoglucose isomerase, pgi-), and (iii) respiration-suppressed (GSK3; res-) cells. These mutants were selected as described previously [3]. At 4-5 weeks post inoculation tumors were subjected to $^1$H MRI and $^{31}$P MRS using a Biospec 4.7 T imager/spectrometer (Bruker Biospin, Ettlingen, Germany), following i.m. injection of ca. 70 µl ketamine/domitor for anesthesia, and i.p. injection of 0.8-1.0 ml of a 245 mM solution of 3-aminopropylphosphonate (3-APP), an extracellular pH indicator. Localizer images were followed by spin-echo images covering the entire tumor (between 12 and 16 1-mm slices, 128x128 matrix, TR =1s, TE=15 ms), using a volume coil. $^{31}$P MR spectra were acquired using a surface coil and 5 to 7 outer-volume saturation bands for localization (TR = 8s, SW = 80 ppm, NS = 500-640). Images and spectra were processed using our IDL-based DISPIMAG and CSIAPO software, respectively.

## Results

Intra and extracellular pH maxima are presented as a function of the cell variant used to generate tumor xenografts in nude mice (Figures 1 and 2).

**Figure 1**

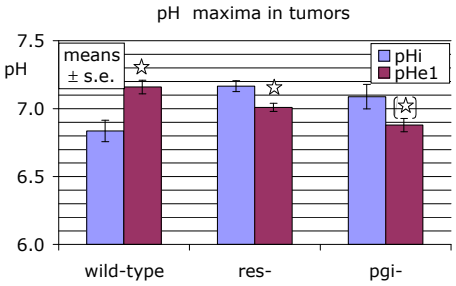
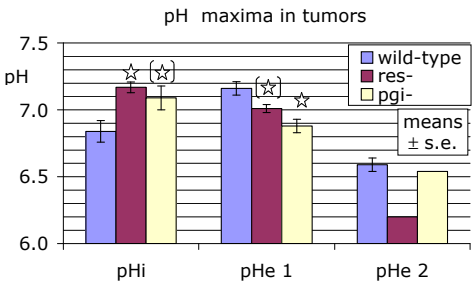



**Figure 2**




For $pH_i$, only one maximum was detected in each tumor, based on the peak of inorganic phosphate ($P_i$). For $pH_e$ based on the 3-APP signal [4], one pH maximum close to or above 7.0 was detected in all three variants ($pH_{e1}$ in Figs.1 and 2). However, each wild-type tumor, and one tumor of each res- and pgi- group, presented a second maximum ($pH_{e2}$) around or below 6.6. In comparison to wild-type tumors, $pH_i$ was slightly increased in tumors from glycolysis-deficient cells, and was more increased ($p<0.05$) for respiration-deficient tumors (Fig.2). In contrast, the values for the consistently present $pH_{e1}$ maxima were significantly decreased ($p<0.05$) for tumors from glycolysis-deficient vs. wild-type cells, but to a lesser extent for respiration-deficient tumors. As a consequence, $pH_{e1}$ was significantly above $pH_i$ in wild-type tumors (Fig. 1), but was lower than $pH_i$ in both res- and pgi- groups. Interestingly, we found a significant linear correlation ($r=0.98$) between the area fraction of the "$pH_{e2}$ peak" (as percent of total "$pH_{e1}$+$pH_{e2}$ areas"), and the volume fraction of necrotic tumor tissue (as percent of total tumor volume; determined by MRI). Necrosis was confirmed by histology. The overall energetic status of the tumors, as judged by NTP/$P_i$ ratios, was best for the res- group, and worst for the pgi- group. Asterisks denote a significant difference vs. the first value in each group of 2 or 3 data bars ($p<0.05$, Mann-Whitney U test). (*) denotes borderline significance ($0.05<p<0.10$).

## Discussion

Increased $pH_i$ in glycolysis-deficient vs. wild-type tumors may be explained by reduced lactic acid production. However, increased $pH_i$ in respiration-deficient tumors may appear counterintuitive, unless it is assumed that these cells upregulate acid export into the extracellular environment along with glycolysis. Indeed, $pH_{e1}$ values were somewhat decreased in res- tumors compared to wild-type tumors. On the other hand,, it should be noted that wild-type tumors were characterized by additional low-$pH_e$ peaks, varying in size, that were almost entirely absent from the $^{31}$P NMR spectra of the slower-growing res- and pgi- tumors. Thus, it can be assumed that there are at least two characteristic extracellular environments, based on distinct $pH_{e1}$ and $pH_{e2}$ values. Further experiments are currently underway to determine the spatial distribution of $pH_{e1}$ and $pH_{e2}$ regions in tumors.

## References

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