Genetic manipulation of proton transport mechanisms generates modulations of intra and extracellular pH and growth characteristics in tumors

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Introduction

The tumor microenvironment is increasingly being explored to develop new avenues of cancer treatment. Some of us have previously proposed to take advantage of the excessive production of lactic acid through glycolysis in tumors [1]. The central idea of this concept is to treat tumors with drugs designed to decrease intracellular pH and to increase extracellular pH. To validate the suggested treatment method, we have previously investigated the effects of modulation of glycolytic activity on intracellular and extracellular pH (pH_i, pH_e). We have demonstrated pH effects in a model system (ras-transformed fibroblasts) in which mutations had been engineered to manipulate glycolysis [2]. These studies were based on xenograft models of nude mice, and on in vivo magnetic resonance spectroscopy and imaging. We now present results obtained for ras-transformed fibroblasts that have been genetically manipulated to modulate two proton transport mechanisms, the sodium/proton exchanger, NHE1, and the monocarboxylate transporter, MCT4. As in the precursor study, pH_i and pH_e were determined simultaneously, in conjunction with tumor morphology, necrosis and growth characteristics, and were complemented with histological data.

Methods

Tumors were induced in the thighs of nude mice by subcutaneous inoculation of 1-2x10^6 ras-transformed CCL39 hamster fibroblasts. Three CCL39 variants have been compared: (i) wild-type (nhe1+, mct4–; CCL39-WT), (ii) MCT4-expressing (nhe1+, mct4+; CCL39-MCT4), and (iii) NHE1-suppressed (nhe1–, mct4–; PS120) cells. At 3-5 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 1.0 ml of a 245 mM solution (pH 7.4) of 3-aminopropylphosphonate (3-APP), an extracellular pH reporter molecule. Scout 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 (OVS) 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 and Discussion

Figure 1

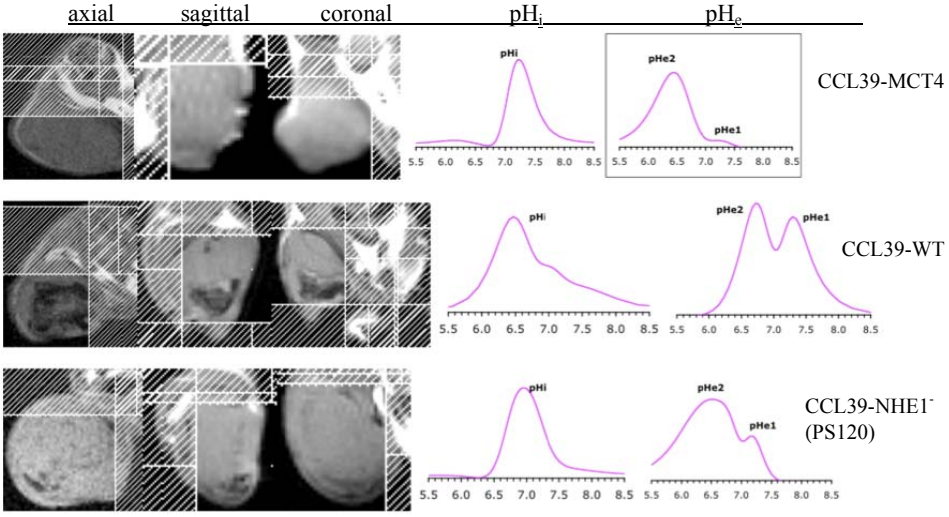

Figure 2

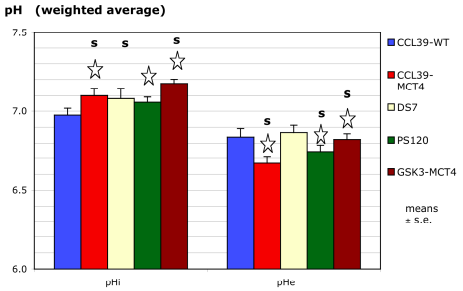

Fig. 1 shows representative T_1-weighted image slices of CCL39 tumors expressing both proton transporters, NHE1 and MCT4 (top row), NHE1 only (center row), or none of these (bottom row), together with their pH_i and pH_e profiles. Major (minor) necrosis is visible in CCL39-WT (CCL-NHE1$^-$) tumors. The ^{31}P spectra used for measuring pH and metabolite profiles were based on voxels selected by OVS (OVS = hatched patterns). Highest weighted-average pH_i ($\overline{pH_i}$) and lowest weighted-average pH_e ($\overline{pH_e}$) were obtained for tumors expressing both proton transporters (CCL39-MCT4), while lowest $\overline{pH_i}$ and highest $\overline{pH_e}$ were observed for tumors expressing NHE1$^-$ only (CCL39-WT). This was confirmed by statistical comparison (Fig. 2); this figure also includes pH data for CCL39 mutants deficient in glycolysis (DS7, mct4$^-$), or deficient in respiration (GSK3-MCT4, mct4$^+$) [* denotes significant difference vs. CCL39-WT, $p<0.05$, Mann-Whitney *U* test; "s" denotes significance based on Dunnett's test]. The presence of both NHE1 and MCT4 allows tumors to efficiently export protons to the extracellular space. Thus, for CCL39-MCT4 tumors $\overline{pH_i}$ and ($\overline{pH_i} - \overline{pH_e}$) values are kept very high, and necrosis is at minimum levels (0.9 % of tumor vol.), despite full glycolytic potential and fastest growth (23 days from cell inoculation to 1-cm diameter of tumor). While the deficiency in one proton transporter (MCT4) in CCL39-WT tumors caused lowest ($\overline{pH_i} - \overline{pH_e}$) values and marked necrosis (5.4 %) at slowed growth (37 days), deficiency in both NHE1 and MCT4 transporters caused a ca. 50 % tumor regression in PS120 tumors. These results suggest that targeting NHE1 and/or MCT4 consistently affects pH_i, pH_e and tumor growth, thus validating the tumor treatment strategy suggested previously [1].

References

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