Investigating MRI-CEST tumor pH imaging to assess prostate cancer aggressiveness

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INTRODUCTION: Altered metabolism[1], in addition to increased glucose consumption, acidosis[2] and hypoxia[3] are recognized as key factors in the progression of prostate cancer (PCa), the primary male malignancy worldwide. However, robust imaging methods are still missing in predicting prostate cancer aggressiveness. In this study we exploited MRI-CEST tumor pH imaging to characterize and distinguish two human PCa murine tumors with different aggressiveness.

METHODS: PC3 and DU145 cancer cells were maintained under normoxia or hypoxia (1% O_2) conditions. Metabolic differences were evaluated by WB staining and RT-PCR. Migration capability was assessed by the wound healing assay by measuring the percentage of wound closure after 8h, 24h, 32h and 48h from the scratch. Athymic Nude-Foxn1nu male mice (n=20) were imaged one month after the orthotopic inoculation into prostate gland of $1x10^6$ cells, with a Bruker's Pharmascan 7T scanner. T1-weighted and T2-weighted images were acquired for prostate localization and volume measurements; a multislice CEST pH imaging (B1 power= 3μT, TS= 3s; slices number 8; slices thickness 1.5mm; FOV 30mm; matrix 128 × 128; acquisition time 9m 55s) was performed measuring whole prostate/tumor pH upon Iopamidol i.v. injection (dose 4 g I/kg b.w.)[4]. Metabolites levels were quantified by 1H single voxel MRS with VAPOR water suppression scheme imaging. Then mice were sacrificed and prostate glands and tumors were excised for WB quantification.

RESULTS: Wound healing assay showed DU145 prostate cancer cells being more aggressive, with a slightly higher migration rate than PC3 cells [Fig.1A] in hypoxic conditions. DU145 cells showed higher expression for glycolytic markers LDH-A and LDH-B in both conditions. Extracellular tumor pH maps [Fig.1B] showed a slightly but statistically significant more pronounced extracellular acidosis in DU145 tumors (mean pH=6.9±0.16) compared to PC3 (mean pH=7.14±0.16), whereas the acidity score showed almost significant differences between the two tumor cell lines. Single-voxel MRS showed altered level of several metabolites, such as Choline and Creatine relative to Citrate, in both prostate tumor models [Fig.1C].

DISCUSSION: In cellulo studies confirmed DU145 as the prostate cancer cell line with higher dysregulated metabolism and increased invasiveness in comparison to PC3 ones. MRI-CEST pH imaging showed increased acidity for the more aggressive prostate tumors (DU145) in comparison to the less aggressive ones (PC3).

CONCLUSION: Iopamidol-based MRI-CEST tumor pH imaging can distinguish between more and less aggressive tumors, potentially providing a novel imaging approach for grading prostate tumors.

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REFERENCES: 1. Qin L, *et all*. Cell Rep 2024;43(1):113575; 2. Irrera P, *et al.* Cancers (Basel) 2022;14(19):4916; 3. Tong D, *et al.* Oncogenesis 2016;5(12):e283; 4. Consolino L, *et al*. Front Oncol 2020;18:10:161;

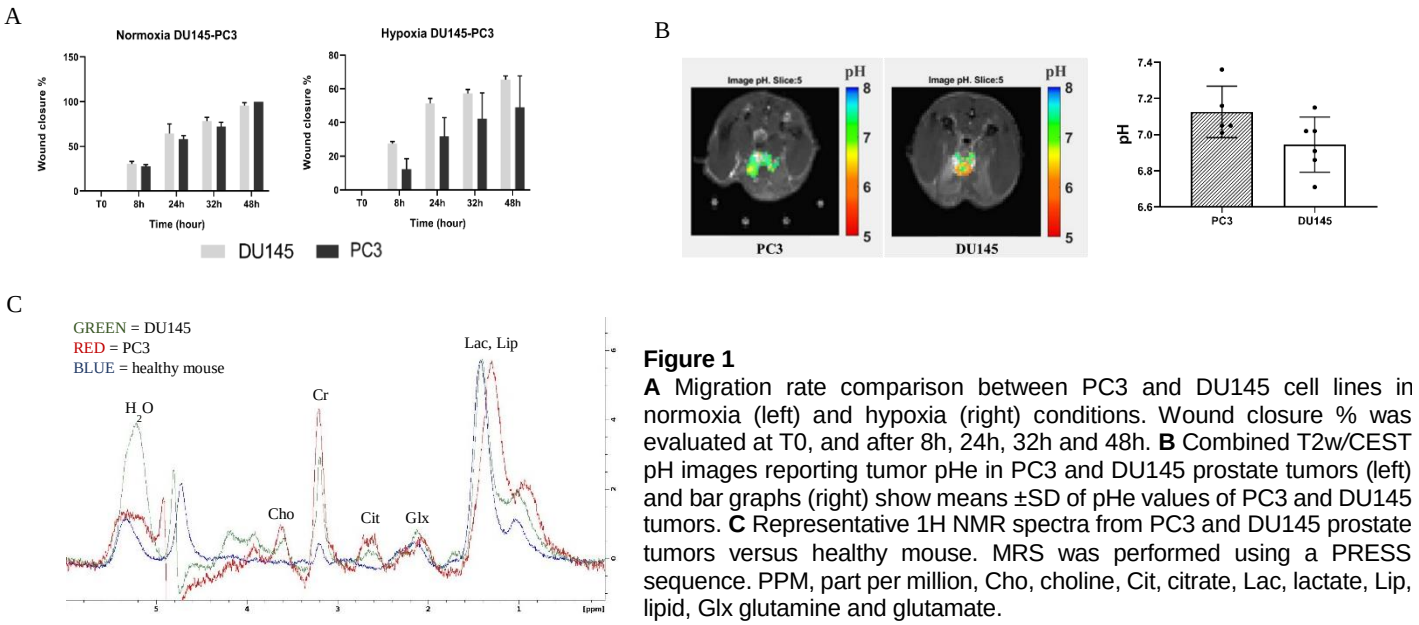

Figure 1
A Migration rate comparison between PC3 and DU145 cell lines in normoxia (left) and hypoxia (right) conditions. Wound closure % was evaluated at T0, and after 8h, 24h, 32h and 48h. **B** Combined T2w/CEST pH images reporting tumor pHe in PC3 and DU145 prostate tumors (left) and bar graphs (right) show means ±SD of pHe values of PC3 and DU145 tumors. **C** Representative 1H NMR spectra from PC3 and DU145 prostate tumors versus healthy mouse. MRS was performed using a PRESS sequence. PPM, part per million, Cho, choline, Cit, citrate, Lac, lactate, Lip, lipid, Glx glutamine and glutamate.