Abstract #266 | Oral or Poster presentation

Hyperpolarized [1-^{13}C]pyruvate Magnetic Resonance Spectroscopic Imaging (MRSI) identifies pancreatic cancer subtypes in mice

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Introduction

Pancreatic ductal adenocarcinoma (PDAC) is a heterogeneous disease with poor prognosis in need of non-invasive subtype biomarkers. Two main transcriptional subtypes have been identified: Classical and quasi-mesenchymal (QM)[1]. The QM subtype is associated with increased glucose metabolism[2, 3], high expression of monocarboxylate transporter 4 (MCT4)[4, 5] and can possibly be detected by metabolic imaging. We here present hyperpolarized (HP)-[1-^{13}C]pyruvate chemical-shift imaging (CSI) in combination with Diffusion-Weighted Imaging (DWI) for QM subtype prediction in endogenous murine (m)PDAC.

Methods

Subjects: *24 Ptf1a$^{Cre/wt}$;LSL-KRAS$^{G12D/wt}$;Tp53$^{fl/fl}$* tumor mice, 29 distinct tumor nodules.
MR System: 7T MRI (Bruker/Agilent) with a dual-tuned ^{1}H/^{13}C 31mm volume coil.
Proton Imaging: T_2-weighted anatomical RARE, DWI (0.25x0.25x1mm^3; 12 b-values 12-1500 s/mm²).
^{13}C Imaging: multi-frame single-slice axial 2D phase encoded CSI (2x2x3mm, TR=5s) with hyperpolarized [1-^{13}C]pyruvate (80mM).
Histology: Tumors were removed, formalin fixed, paraffin embedded, cut aligned to the axial imaging plane and histologically processed (H&E, MCT4).
Cytology: Four cell lines were isolated from distinct tumor nodules, cultured under standard conditions (DMEM, 5mM glucose), processed and stained for Vimentin, MCT4, DAPI (Leica Confocal Microscope).
Analysis: Data was analyzed using MATLAB and GraphPad Prism 7.0.

Results/Discussion

We observed high heterogeneity in the *in vivo* metabolic signal of complex endogenous mPDAC compared to orthotopically implanted mPDAC tumors derived from established murine cell lines[5]. Ratios of the area under the curves of lactate to pyruvate spectral peak time-courses (AUC_l/AUC_p) correlated well with the corresponding MCT4 staining (Fig. 1A-C). *In vivo* heterogeneity of AUC_l/AUC_p was independent of tumor

cellularity (Fig. 1D-F). Primary cells derived from AUC_l/AUC_p^{high} tumors (Fig. 1E, F, red) showed an elongated QM-like appearance (Fig. 2A), high Vimentin and MCT4 expression (Fig. 2B-D) and increased lactate dehydrogenase (LDH) activity *in vitro* (Fig. 2E), as compared to cuboid shaped AUC_l/AUC_p^{low} cells (Fig. 1E, F, blue and Fig. 2, blue). Multiparametric MRSI and DWI with HP-pyruvate may be a promising method for non-invasive detection of metabolic phenotypes and corresponding molecular subtypes in PDAC.

Conclusions

We confirmed the link of the QM subtype and MCT4 tissue expression in murine PDAC. We further show that the QM subtype can be detected by multiparametric MRSI/DWI with HP-pyruvate. Considering the observed metabolic heterogeneity in PDAC and its association with known molecular subtypes, implementing metabolic phenotyping in clinical routine might facilitate future patient stratification and treatment monitoring.

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Disclosure

I or one of my co-authors have no financial interest or relationship to disclose regarding the subject matter of this presentation.

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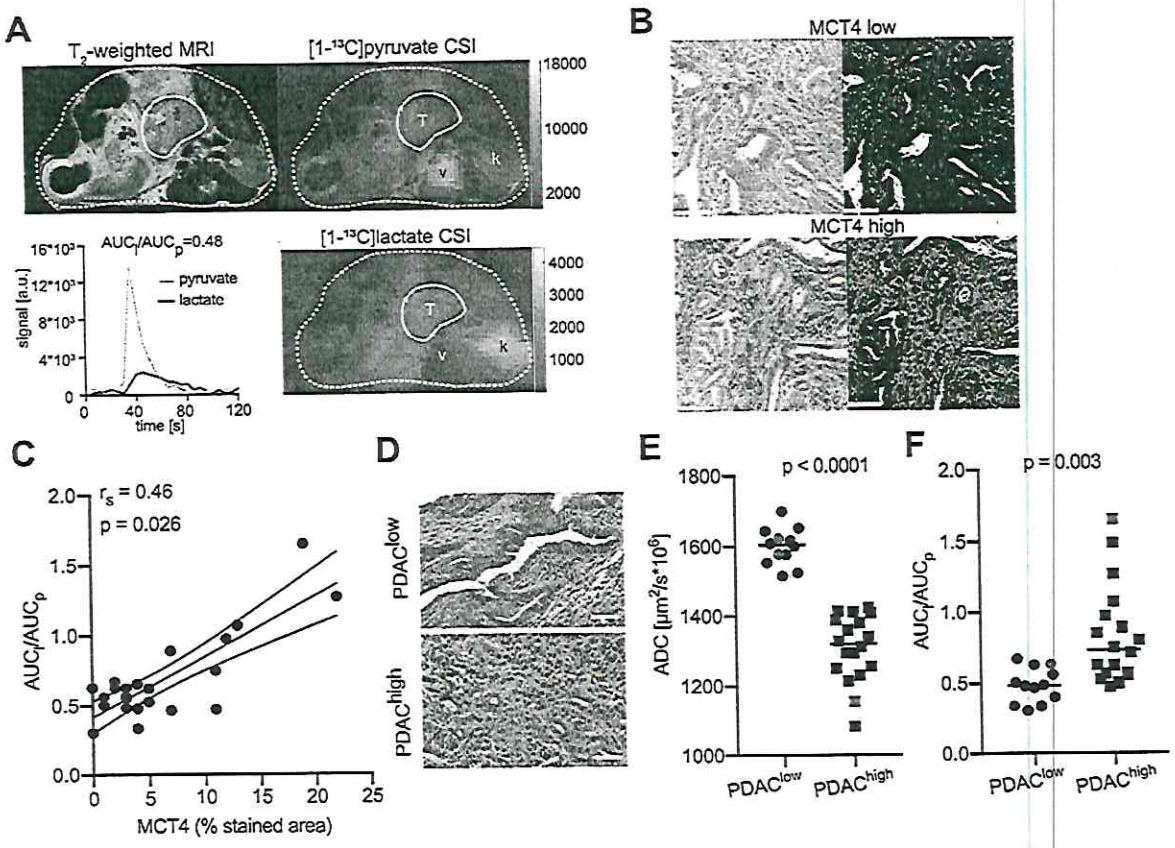
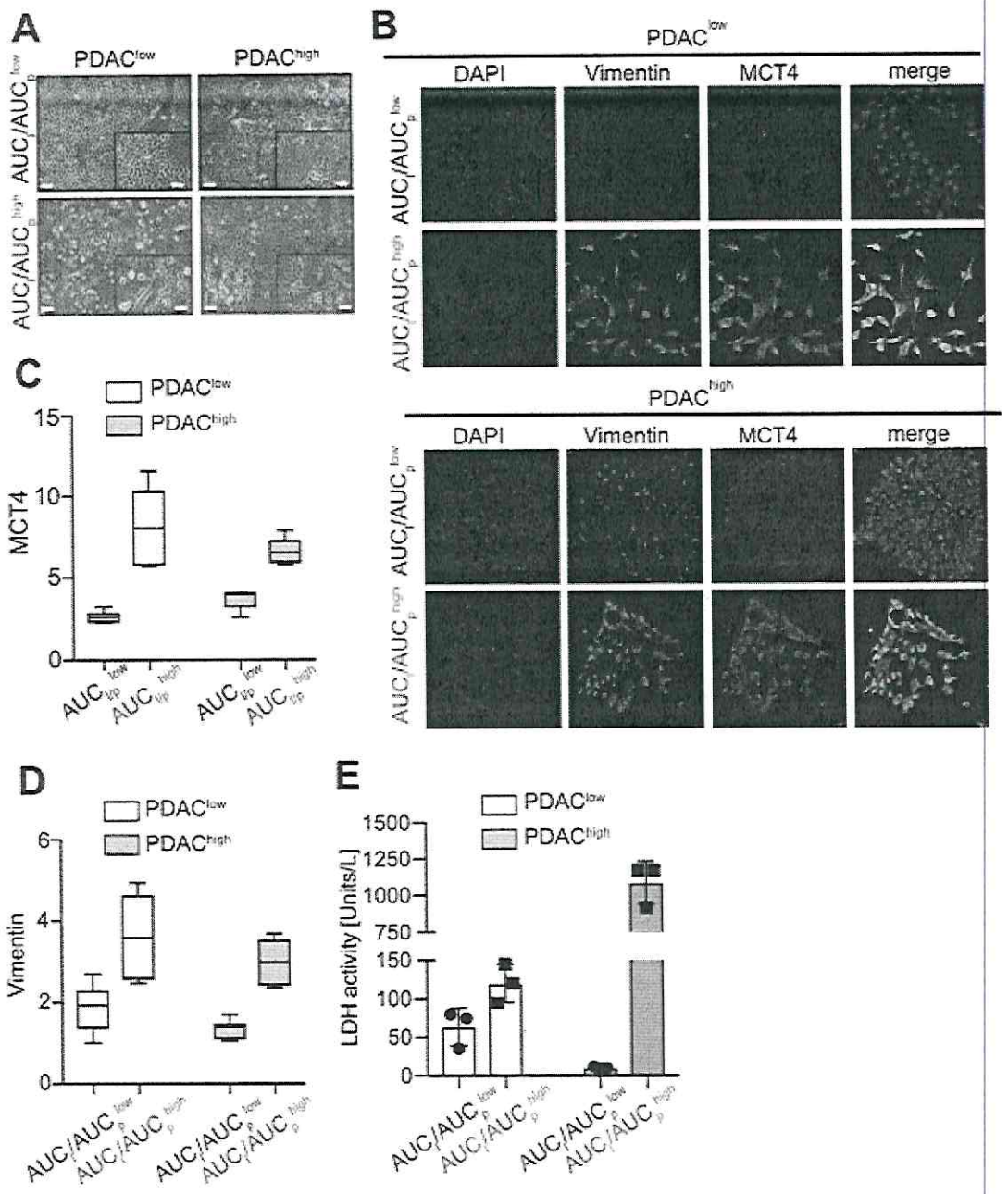


CSI with HP[1-13C]pyruvate detects metabolic heterogeneity of mPDAC in vivo.

__A:__ Axial T_2w abdominal images, HP[1-^{13}C]pyruvate and HP[1-^{13}C]lactate images at peak (25 s, 5 frames) and their metabolite time courses of mPDAC. __B:__ Respective automated analysis mask of MCT4 (IHC) staining divided by median, scale bar 100µm. __C:__ Spearman's correlations of AUC_l/AUC_p ratios and MCT4 expression. __D:__ H&E stains of $PDAC^{low}$ and $PDAC^{high}$ tumors. Scale bar 200µm. __E, F:__ $PDAC^{low}$ and $PDAC^{high}$ subgroup comparison of mean ADC(__D__) and AUC_l/AUC_p(__E__) values. Blue and red dots represent tumors from which AUC_l/AUC_p^{low} (blue) and AUC_l/AUC_p^{high} (red) primary cell lines were derived.



MCT4 expression correlates with classical and QM phenotype in primary tumor cell lines of mPDAC.
***A**: Transmitted light microscopy images of cell lines derived from AUC/AUC$_p^{low}$(blue) and AUC/AUC$_p^{high}$ (red) tumors. Note cuboid and elongated shape of AUC/AUC$_p^{low}$ and AUC/AUC$_p^{high}$ cells respectively. **B**: Representative images of co-stained immunofluorescence of MCT4 and Vimentin in the AUC/AUC$_p^{low}$ and AUC/AUC$_p^{high}$ cells. Scale bar 50µm. **C, D:** Quantification of the fluorescent signal in 6 images/cell line for MCT4 (**C**) and Vimentin (**D**) presented as Box-Whisker-Plot of min to max. **E**: LDH enzyme activity analysis in the AUC/AUC$_p^{low}$ and AUC/AUC$_p^{high}$ cells.*