Decoding and targeting metabolic heterogeneity in pancreatic neuroendocrine tumors: MCT1 and MCT4 in the crosshair for precision therapy

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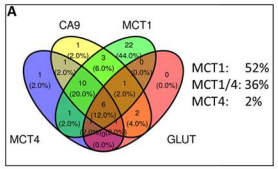
Background:

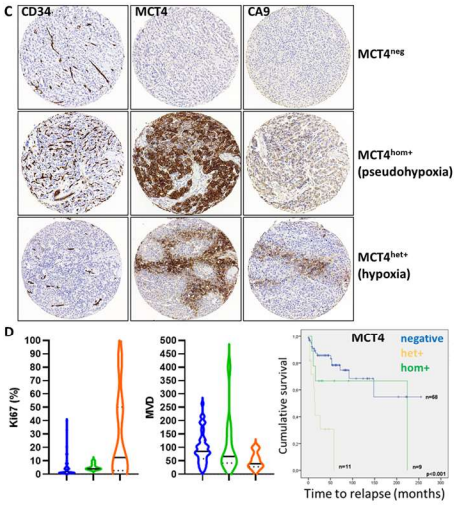
Over half of patients with pancreatic neuroendocrine tumours (PanNETs) present with metastasis at diagnosis or experience relapse post-surgery. Predictive biomarkers for disease progression are missing, including factors driving the development of aggressive and metastatic PanNET. Our transcriptome and epigenome studies indicate a stepwise progression towards aggressive behaviour, marked by increased proliferation, dedifferentiation, and hypoxia-related metabolic reprogramming. Given our limited understanding of the metabolic landscape of PanNET, the aim of this study is to identify metabolic subtypes of PanNET and investigate metabolic vulnerabilities for therapeutic purposes.

(1) Expression of MCT1, MCT4 and CA9 in PanNET defines metabolic subtypes associated with hypoxia and aggressive disease progression



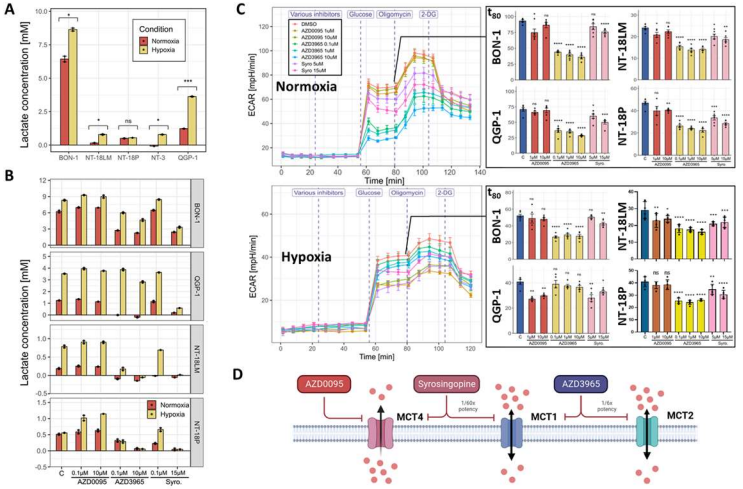
B

	MCT4	CA9	MCT1
CA9	0,000		
MCT1	0,010	0,000	
Age	0,137	0,324	0,092
Sex	0,694	0,185	0,263
Grade	0,000	0,000	0,232
DAXX/ATRX	0,152	0,115	0,979
Methylation groups	0,630	0,630	0,522
CNA groups	0,669	0,241	0,045
T-stage	0,001	0,002	0,516
N-stage	0,02	0,002	0,332
M-stage	0,171	0,103	0,080
Hormones	0,09	0,003	0,544
ARX	0,572	0,279	0,228
PDX1	0,002	0,003	0,386
SOX9	0,038	0,106	0,092
Relapse	0,002	0,002	0,119
Tumorspecific Death	0,933	0,744	0,124



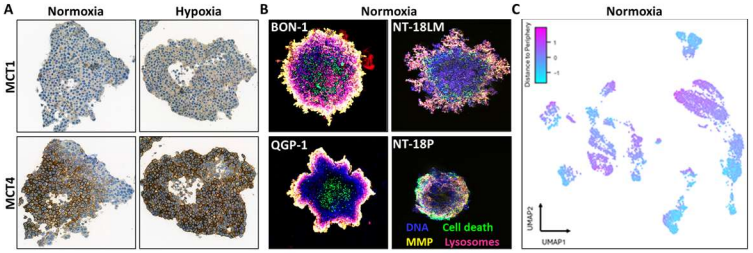
(A) Immunohistochemistry (IHC) of monocarboxylate transporters MCT1/4 and hypoxia marker CA9 in tissue microarrays of two independent patient cohorts (n=109 and n=102 cases) revealed frequent co-expression. (B) Correlation analysis of MCT1/4 and CA9 expression with clinico-pathological parameters of aggressive disease (p values, chi-square test). (C) Examples of MCT4 subtypes based on negative, homogeneous positive or heterogeneous positive staining of MCT4 and CA9. High microvessel densitiy (CD34) in MCT4^{hom+} PanNETs suggests pseudohypoxia, whereas MCT4 and CA9 expression distant from microvessels in MCT4^{het+} PanNETs suggests regional hypoxia and metabolic heterogeneity. (D) MCT4^{hom+} (green) and MCT4^{het+} PanNET (orange) showed higher Ki67 staining, reduction in microvessel density (MVD) and significantly reduced time to relapse when compared to MCT4^{neg} tumours.

(2) MCT1/4 cooperatively direct lactate efflux in normoxia and hypoxia



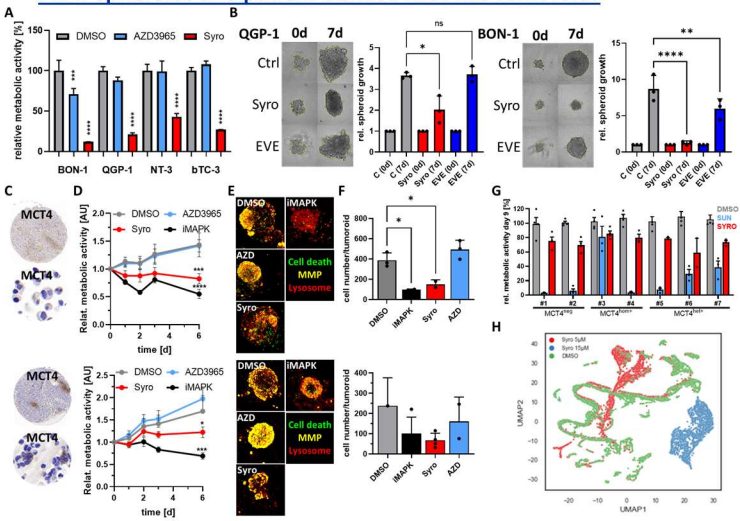
(A) Lactate secretion of PanNET cells in normoxia and hypoxia (1.5% O_2, 72h) and (B) in the presence of MCT1/4 inhibitors. (D) Seahorse metabolic flux assay in normoxia and hypoxia to measure extracellular acidification (ECAR) of PanNET cells treated with MCT1/2 inhibitors. Bar chart inserts show ECAR at t=80 min. (E) Schematic of MCT1/2/4 function, lactate transport direction and inhibitor specificity.

(3) PanNET 3D models capture regional hypoxia and spatial metabolic heterogeneity



(A) IHC of MCT1 (top panel) and MCT4 expression (bottom panel) in QGP1 spheroids grown in normoxic and hypoxic conditions (1.5% O_2 for 72h). (B) Confocal fluorescence microscopy (CFM) of fluorescent reporters of cell death, mitochondrial membrane potential (MMP) and lysosomal activity demonstrates spatial metabolic heterogeneity in PanNET spheroids cultured in normoxic conditions. (C) Single-cell dimensionality reduction analysis of spatially resolved morphometric and fluorometric data by UMAP demonstrates multiple distinctive cell clusters based on the fluorescent sensor probes shown in C.

(4) Dual inhibition of MCT1/4 reduces metabolic activity and growth of PNET spheroids and patient-derived islet-like tumoroids



(A) Metabolic activity and (B) representative micrographs of time lapse microscopy to measure growth of PanNET spheroids treated for 7 days with MCT1/4 inhibitors. (C) Representative IHC images of two tumour punches and single cell suspension for patient-derived tumoroid (PDT) generation from a primary (top) and metastatic MCT4^{het+} PNET (bottom) after MCT4 staining. (D) Real time analysis of metabolic activity of the same two MCT4^{het+} PDTs treated for six days followed by (E) Confocal fluorescence microscopy to measure cell death, mitochondrial membrane potential (MMP), lysosomal activity and (F) cell number per PDT. (G) Analysis as in D at endpoint (6d). (H) Single-cell dimensionality reduction analysis of spatially resolved morphometric and fluorometric data by UMAP demonstrates reduction of metabolic heterogeneity after MCT4 inhibition when compared to vehicle (DMSO).

(5) Conclusions

- PanNETs display intra-tumoral heterogeneity, regional hypoxia and pseudohypoxia.
- MCT1/4/CA9 expression defines metabolic subtypes which are associated with more aggressive disease.
- MCT1/4 cooperatively direct lactate efflux in PanNET cells.
- 3D PNET models capture metabolic heterogeneity and regional hypoxia.
- MCT4 inhibition reduces PanNET tumoroid growth and metabolic heterogeneity.

Affiliations & Acknowledgements

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