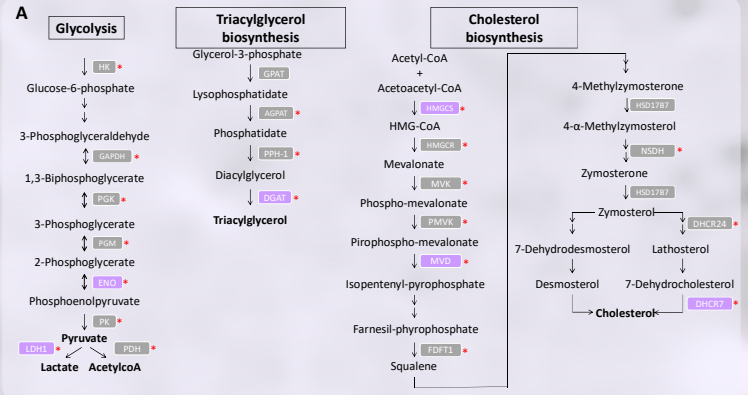
DEFINITION OF METABOLIC SUBTYPES FOR PANCREATIC DUCTAL ADENOCARCINOMA STRATIFICATION

Pilar Espiau-Romera, Beatriz Parejo-Alonso, Alba Royo-García, Sarah Courtois and Patricia Sancho
IIS Aragon, Hospital Universitario Miguel Servet, Zaragoza 50009, Spain

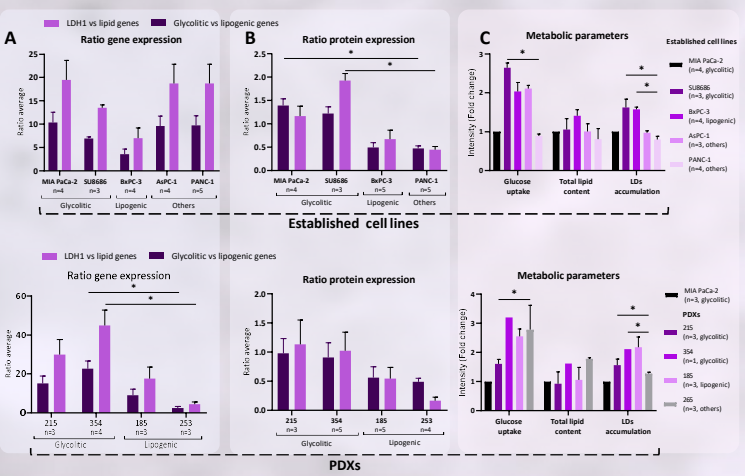
INTRODUCTION: Pancreatic ductal adenocarcinoma (PDAC) is an extremely lethal disease due to late diagnosis, aggressiveness and lack of effective therapies. Considering its intrinsic heterogeneity, patient stratification models based on molecular and metabolic subtypes have been correlated: lipogenic subtype, linked with a classical/progenitor molecular signature, while glycolytic tumors associated with the highly aggressive basal/squamous profile (Espiau-Romera et al. 2020).
MATERIALS: Primary cultures of PDAC patient-derived xenograft models (PDXs), established cell lines and PDXs tumor pieces ex vivo.
METHODS: *Bioinformatic analysis*: Expression and survival data from the GEPIA2 database. *Quantitative PCR (qPCR). Western Blot. Flow Cytometry*: NBDG (glucose uptake), BODIPY 493/503 (total lipid content), LD540 (lipid droplets (LDs) accumulation) and Zombie Violet (cell viability) probes, and human EpCAM antibody. *Statistics:* ANOVA and Kruskal-Wallis-H test.
RESULTS: Through bioinformatic analysis we demonstrated that the representative genes of each metabolic subtype were up-regulated in PDAC samples and predict patient survival. This suggests an association between genetic signature, metabolic profile and aggressiveness of the tumor. Glycolytic versus lipogenic ratios obtained after in vitro RNA and protein expression analysis allowed us to classify both the PDXs and the established cell lines into two metabolic subgroups, one more glycolytic and the other more lipogenic. Moreover, a simplified signature using LDH1 as the unique glycolytic gene showed similar results, thus indicating the strong contribution of this gene to the glycolytic signature. Flow cytometry analysis did not show a clear dependence of subtyping for metabolic requirements. Ex vivo analysis confirmed the results obtained in vitro: analysis of the protein confirmed the two metabolic subtypes defined in vitro, but no differences were detected by flow cytometry with the probes used.

Figure 1. Genes from the Glycolytic and lipogenic pathways are significantly overexpressed in tumoral *versus* normal tissues and predict PDAC prognosis



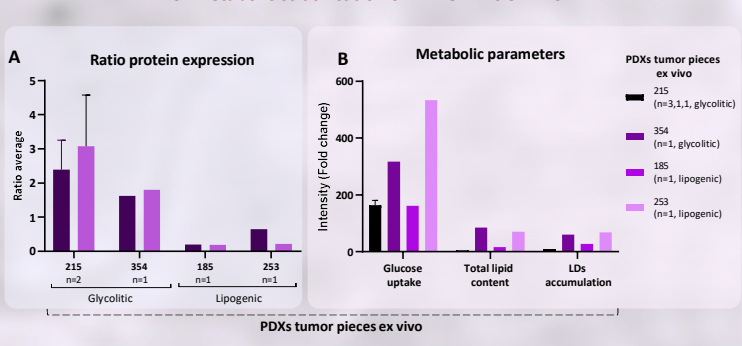
*A. Genes involved in glycolysis and triacylglycerol and cholesterol biosynthesis. The genes selected for the study are shown in violet. Other genes present in the pathways are shown in grey. Significant differences in gene expression between cancerous (n=179) and non-cancerous (n=171) tissues was calculated by ANOVA test and shown with an asterisk (*p ≤0.05). B. Disease-free survival analysis based on the expression of the genes selected for the study. Top: LDH1 and ENO2; Middle: MVD, HMGCS1, DHCRT7 and DGAT1; Bottom: Ratio LDH1/lipid signature (MVD, HMGCS1, DHCR7 and DGAT1). Comparison of upper and lower population quartiles is displayed. Log-rank test and Hazard Ratio (HR) were calculated, based on the COX model (significance when p(HR)≤0.05). The significance and survival data was obtained from the GEPIA2 webtool, using public gene expression information collected in the TCGA and GTEx databases.*

Figure 2. The expression ratio of selected glycolytic and lipogenic enzymes allows for metabolic stratification of PDAC cell lines and PDXs *in vitro*



*A. Ratio between glycolytic (ENO2 and LDH1) and lipogenic (MVD, HMGCS1, DHCR7 and DGAT1) average gene expression. B. Ratio between glycolytic (ENO2 and LDH1) and lipogenic (MVD and DGAT1) average protein expression. C. Flow cytometry analysis of glucose uptake, total lipid content and LDs content (relative to MIA PaCa-2). Top: Established cell lines (MIA Paca-2, SU8686, BxPC-3, AsPC-1, PANC-1) Bottom: PDXs (215, 354, 185 and 253). Data is represented as mean +SEM. Comparison of quantitative data was done by Kruskal-Wallis non-parametric analysis (*p value ≤0.05). Graph obtained by GraphPad Prism 8.0.2 (GraphPad Software, La Jolla, CA, USA).*

Figure 3. The expression ratio of selected glycolytic and lipogenic enzymes allows for metabolic stratification of PDAC PDXs *ex vivo*



*A. Ratio between glycolytic (ENO2 and LDH1) and lipogenic average protein expression (MVD and DGAT1). B. Flow cytometry analysis of glucose uptake, total lipid content and LDs content (relative to cells without probe staining intensity). PDXs tumor pieces ex vivo (215, 354, 185 and 253). Data are represented as mean +SEM. Comparison of quantitative data was done by Kruskal-Wallis non-parametric analysis (*p value ≤0.05). Graph obtained by GraphPad Prism 8.0.2 (GraphPad Software, La Jolla, CA, USA).*

CONCLUSION: Although further work is needed, our results experimentally support previous molecular and transcriptional analyses suggesting the existence of different metabolic subtypes in PDAC.

Unión Europea
FONDO EUROPEO DE DESARROLLO REGIONAL
FONDO SOCIAL EUROPEO
worldwide cancer research
aecc Contra el Cáncer

REFERENCES
1. Daemen, Anneleen, David Peterson, Nisebita Sahu, Ron McCord, Xiangnan Du, Bonnie Liu, Katarzyna Kowanetz, et al. 2015. «Metabolite profiling stratifies pancreatic ductal adenocarcinomas into subtypes with distinct sensitivities to metabolic inhibitors». Proceedings of the National Academy of Sciences of the United States of America 112 (32): E4410-17.
2. Espiau-Romera, Pilar, Sarah Courtois, Beatriz Parejo-Alonso, y Patricia Sancho. 2020. «Molecular and Metabolic Subtypes Correspondence for Pancreatic Ductal Adenocarcinoma Classification». Journal of Clinical Medicine 9 (12).
3. Sancho, Patricia, Emma Burgos-Ramos, Alejandra Tavera, Tony Bou Kheir, Petra Jagust, Matthieu Schoenhals, David Barneda, et al. 2015. «MYC/PGC-1α Balance Determines the Metabolic Phenotype and Plasticity of Pancreatic Cancer Stem Cells». Cell Metabolism 22 (4): 590-605.