Quantitative proteomics for therapeutic biomarker discovery in pancreatic ductal adenocarcinoma

Claire Chew [1,2], Esther Cheow [1,2], Lee Yi Fang [1,2], Edward Chow [3], Glenn Bonney [4]

1. Surgical Proteomics Laboratory, iHealthTech 2. Department of Surgery, National University Singapore

3. Cancer Science Institute, National University Singapore 4. Department of Surgery, National University Hospital

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is an aggressive cancer with a dismal prognosis. Unfortunately, there are at present few clinically relevant biomarkers for therapeutic selection. Organoids models have been applied to PDAC research and are a promising platform for precision oncology. In this study, we aim to use quantitative proteomic profiling of patients derived pancreatic cancer organoids (PDOs) to identify biomarkers of therapeutic sensitivity to standard-of-care chemotherapy agents.

Methodology

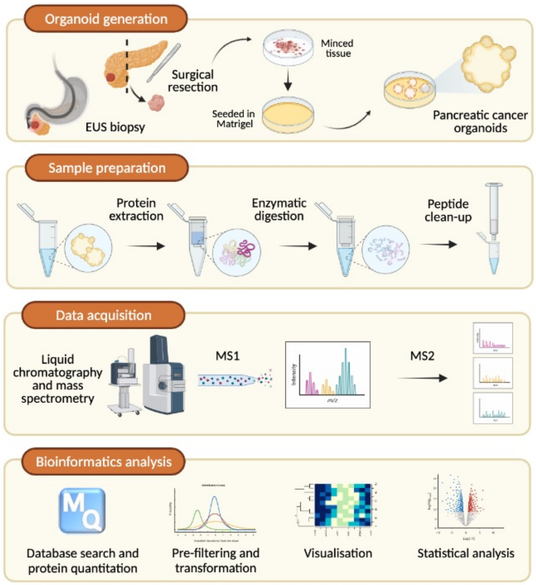


Fig. 1. Illustration of overall workflow for quantitative proteomics. (Created with BioRender)

All patient samples were collected with informed patient consent from the National University Hospital, Singapore under IRB approval.

Organoids were according to a modified published method by Hans Clevers (STAR Protoc. 2020 Dec 4;1(3):100192.). Briefly, EUS tissue biopsies or surgically resected specimens are minced, plated in Matrigel (Corning) and cultured in complete media containing multiple growth factors. Growth medium is changed every 4 days. Organoids are propagated by digesting with TrypLE enzyme (Gibco) to obtain single cells, before subsequent plating in Matrigel.

Label-free quantification (LFQ) and isobaric labeling quantification (ILQ) are among the most popular protein quantification workflows in discovery proteomics. We utilised both techniques in this study (TMT 10-plex workflow and label free data dependent workflow). By comparing both datasets we were able to more reliably identify deregulated proteins by limiting identification to only proteins that were identified in both data sets. We analysed the PDOs from 4 patients, with 2 biological replicates (different passages) included from each patient.

Results

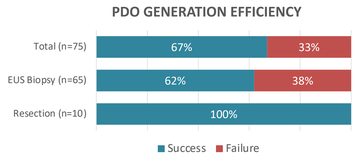


Fig 1. Generation of established PDAC organoids after 5 passages (P5).

Lower rates of generation efficiency in EUS biopsies compared to surgically resected specimens is attributable to insufficient cellular material obtained. Overall generation efficiency of 67% is comparable to published studies. Tumour PDOs can be passaged indefinitely and are considered established once they can be propagated beyond 5 passages.

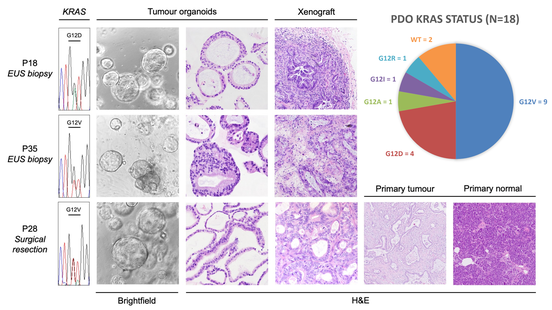
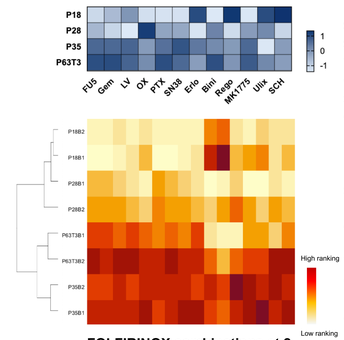


Fig 2. (Left to right) Brightfield images of PDOs, H&E stain of PDOs and xenografted tumours, comparison with primary. Tumour, KRAS status determined by Sanger sequencing.



FOLFIRINOX combinations at 2 dose concentrations each drug

Fig. 3. High-throughput drug screening in PDOs reveals sensitivities to a range of therapeutic agents. (Top) 12 compounds tested in 24 PDOs. The z-scores of obtained IC50 values are depicted in the heatmap. High values (indicating resistance) in dark blue and low values (indicating sensitivity) in light blue. (Bottom) FOLFIRINOX, a standard of care 4-drug combination, is tested by varying concentrations of each drug using the Quadratic Phenotypic Optimisation Platform (Sci Transl Med. 2018 Aug 8;10(453). Combinations are ranked against all possible combinations of 12 compounds (>500,000 combinations). PDOs classified into responders (P18, P28) and non-responders (P35, P63) to FOLFIRINOX.

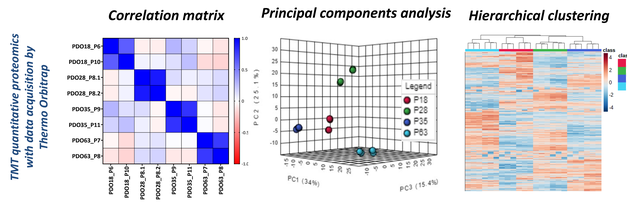
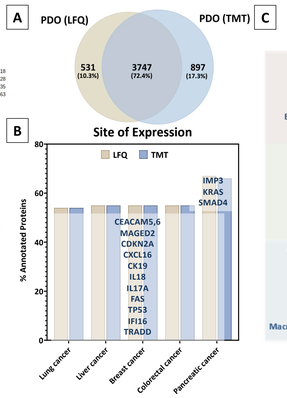


Fig 3. Quantitative evaluation of clonal variation between passages of PDOs

- (Left) High degree of correlation of quantified proteins between clonal passages
- Unsupervised 3D-PCA plot (middle) and hierarchical cluster heatmap (left) show clustering of PDOs of different passages together.
- Clear inter-patient protein diversity demonstrated by cluster heatmaps, verifying the known heterogeneity of pancreatic cancer.
- Robust TMT and LFQ (data not shown) proteomics workflow with minimal clonal protein evolution between propagation



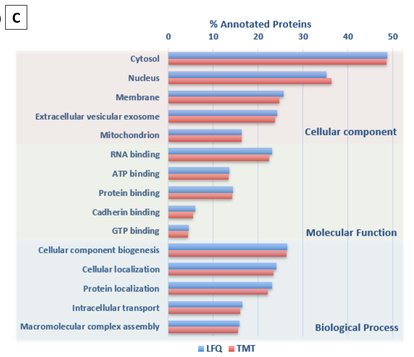
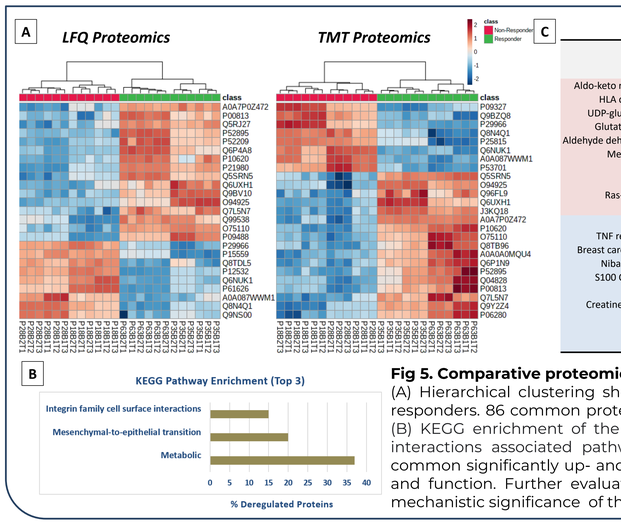


Fig. 4. Qualitative evaluation of PDAC proteome using Gene Ontology (GO) Functional Enrichment analyses. Only proteins with ≥2 unique peptides and strict FDR of < 1% were assessed. (A) Venn diagram showing that across both datasets, expression levels of 5175 proteins were determined, with overlap of 70%. TMT workflow identified 7% more proteins. (B) Proteins identified were significantly enriched in pancreatic cancer as compared with other major cancer types. Pancreatic lineage proteins e.g. IMP3, CK19 and S100P, along with the top 4 mutated proteins in PDAC (KRAS, SMAD4M,TP53, CDKN2A) were identified in both datasets, strongly indicating that the PDOs are representative of PDAC. (C) Functional GO enrichment analyses were performed to define and compare the key categories (p<0.05) of cellular components, molecular function and biological pathway enriched from both datasets.



Protein Name	LFQ Fold Change	TMT Fold Change	Function
Aldo-keto reductase family 1 member C2	9.908	3.584	Steroid metabolism
HLA class I antigen, A α chain	6.318	2.96	Antigen-specific T cell immune response
UDP-glucuronosyltransferase 1A10	5.678	2.968	Excretion of metabolites
Glutathione S-transferase Mu 1	5.498	3.304	Detoxification of electrophilic compounds
Aldehyde dehydrogenase family 1 member A3	5.218	2.762	Catalyzes formation of retinoic acid
Metalloreductase STEAP3	5.024	1.7218	Role in downstream of p53/TP53
Calcyphosin	4.628	1.7864	Calcium-binding protein. Role in signaling events .
Clusterin	4.48	2.128	Involved in cell death, tumor progression.
Ras-related protein R-Ras2	4.442	1.7922	Activates signal transduction controlling cell proliferation.
Caveolin-1	4.068	2.426	Tumor suppressor, negative regulator of Ras-p42/44 cascade
Mucin-1	-2.238	-2.21	Modulates ERK, SRC and NF-kappa-B signalling
TNF receptor family member 6	-2.92	-1.7628	Regulation of programmed cell death
Breast carcinoma-amplified sequence 1	-4.268	-2.644	Required for myelination
Niban Apoptosis Regulator 1	-5.026	-2.088	Regulation of p53-mediated apoptosis
S100 Calcium Binding Protein P	-5.058	-3.734	Regulation of cellular processes
Villin-1	-5.182	-4.124	Reorganizes microvillar actin filaments
Creatine kinase U-ty, mitochondrial	-5.504	-2.05	Energy transduction
Scinderin	-5.952	-3.052	Ca(2+)-actin filament-severing protein, regulates exocytosis
Claudin-18	-5.988	-2.94	Integral membrane proteins and components of tight junction
Cocaine esterase	-7.808	-2.294	Detoxification of xenobiotics

Fig 5. Comparative proteomics of responders vs non-responders to FOLFIRINOX.
(A) Hierarchical clustering showing deregulated proteins between FFX responders and non-responders. 86 common proteins were significantly (p<0.05) deregulated across both datasets. (B) KEGG enrichment of the modulated proteins showed that metabolic, MET and integrin interactions associated pathways were the top 3 commonly altered functions. (C) Top 10 common significantly up- and down-regulated proteins, alongside their degree of fold-change and function. Further evaluation of the existing literature will be required to elucidate the mechanistic significance of these differences in expression.

Conclusions

- ✓ Successful generation of PDOs generated from EUS biopsies and surgical resections of pancreatic cancer with generation efficiency of ~70%.
- ✓ Application of combinatorial drug screening to identify responders and non-responders to FOLFIRINOX
- ✓ Application of of two robust quantitative proteomics workflows to characterise the proteome of PDAC, demonstrating the PDOs are indeed representative of the molecular landscape of PDAC.
- ✓ Comparatives proteomics to identify potential biomarkers associated with response to FOLFIRINOX
- ✓ Further interrogation of existing literature is needed to elucidate their mechanistic significance