Characterisation of precision-cut tumour slices, a patient-derived model of cholangiocarcinoma

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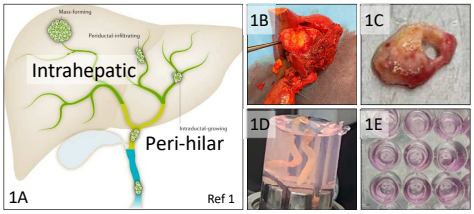
Introduction

- Cholangiocarcinoma is an aggressive malignancy with increasing incidence and persistently poor prognosis.
- The majority of patients present at an advanced stage.
- Current treatment options for cholangiocarcinoma are limited and chemoresistance is common.
- New chemotherapeutic strategies and a greater understanding of the mechanisms related to treatment response/resistance are needed (1).
- Precision-cut tumour slices are patient-derived 3D tissue explants that can be cultured *ex vivo*. They recapitulate critical aspects of cancer biology and crucially retain the tumour microenvironment.
- Here we determine the *ex vivo* viability and phenotype of precision-cut tumour slices and assess their utility as a complex 3D model of cholangiocarcinoma.

Methods

Generation of tissue slices



Cholangiocarcinoma is a cancer of the bile duct, with different types based on their location within the duct. Intrahepatic tumours form within the body of the liver whereas peri-hilar tumours form between the liver and the gall bladder (Fig 1A).

A wedge of tumour (Fig 1C) was excised from resected liver (Fig 1B) from 3 patients with intra-hepatic and 3 patients with peri-hilar cholangiocarcinoma. Cores with a diameter of 5mm were generated. These were embedded in agarose (Fig 1D) and slices of 250µm were generated using a Krumdieck tissue microtome. The slices were cultured in microplates within Millipore well inserts (Fig 1E) (2, 3).

Generation of a cholangiocarcinoma spectral library

A pool of representative tumour samples were lysed in 6M urea/1M ammonium bicarbonate/0.5% deoxycholate by homogenisation followed by sonication. An aliquot of 1.5mg extracted protein was reduced, alkylated and digested in a two-step process with Trypsin/LysC. Following strong cation exchange chromatography, 40 fractions were subjected to nano-LC data-dependent acquisition (DDA) on a Triple TOF 6600.

Acquisition of SWATH data

Individual cultured slices (3 intrahepatic, 3 peri-hilar, 4 time points, 50µg each) lysed in urea buffer were processed using SP3 reversed phase magnetic beads and subjected to nano-LC SWATH/data-independent acquisition (DIA) on a Triple TOF 6600 (4).

MS data analysis

DDA data were processed using ProteinPilot 5 to create the spectral library. SWATH data were aligned to the library in DIA-NN (5) with match between runs, unrelated runs and double pass selected. The output was analysed using Partek Genomics Suite and Ingenuity Pathway Analysis.

Histological characterisation

The histological architecture of the tumour slices in culture was assessed. Standard haematoxylin and eosin staining was performed alongside a immunohistochemical detection of a cell specific marker for cholangiocarcinoma (cytokeratin 19). Cell morphology and viability were assessed by a consultant histopathologist. The cellular architecture of the tissue slices was assessed using QuPath™ software for digital pathology (6).

Assessment of viability

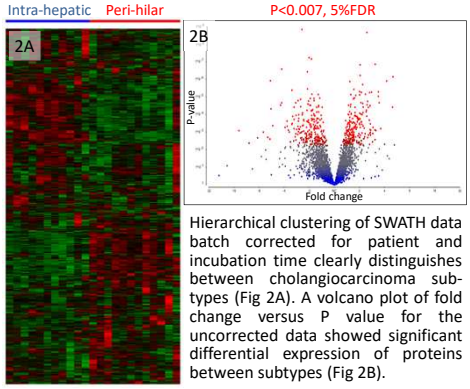
Tissue slice viability was determined at days 0, 3, 7 and 15 in culture by CellTiter 96® AQueous One Solution MTS assay and by histological markers of proliferation (Ki-67) and apoptosis (cleaved-caspase 3) (data not shown).

Response to chemotherapy

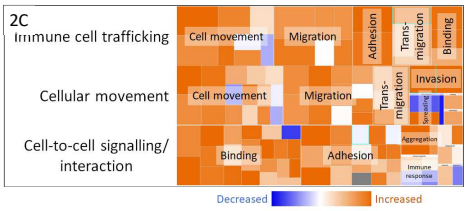
Tissue slices were generated as previously described and allowed to recover in culture for 24 hours. The slices underwent an MTS assay to assess baseline viability and then received 72hours of treatment with gemcitabine. Another MTS assay was performed to assess changes in viability.

Results

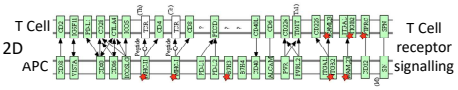
Intra-hepatic and peri-hilar tumours are different



Hierarchical clustering of SWATH data batch corrected for patient and incubation time clearly distinguishes between cholangiocarcinoma sub-types (Fig 2A). A volcano plot of fold change versus P value for the uncorrected data showed significant differential expression of proteins between subtypes (Fig 2B).

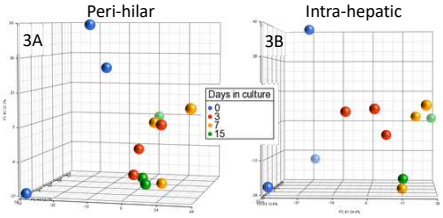


Ingenuity Pathway Analysis revealed that peri-hilar tumours are associated with increased immune cell infiltration and cell adhesion compared to intra-hepatic tumours (Fig 2C).

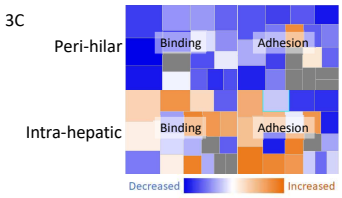


Functional Enrichment Analysis (DAVID) revealed that T cells may be amongst the infiltrating immune cells (Fig 2D).

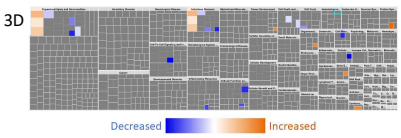
Tumour subtype phenotype is retained in culture



Principal Component Analysis indicated that the proteome of both subtypes of tumour changes between day 0 and day 3 in culture, but stabilises thereafter (Fig 3A and B).

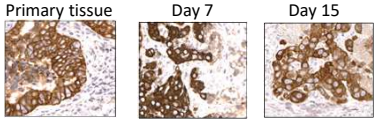


Ingenuity Pathway Analysis revealed that, after 3 days in culture, peri-hilar slices display reduced whereas intra-hepatic slices display increased cell adhesion (Fig 3C).

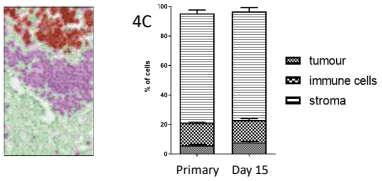


Comparison of cell function between day 3 and day 15 revealed that few further changes take place on prolonged *ex vivo* culture (intra-hepatic as example, Fig 3D).

Tumour histology is retained in culture

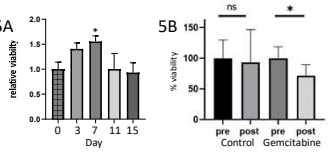


Sections of tissue slices after 7 and 15 days in culture were stained for cytokeratin 19 and compared to that observed in the primary tissue from which they were derived (Fig 4A). This suggested that the histological phenotype of the tumour is also maintained in culture.



QuPath™ software showed that the tumour micro-environment remains stable in the slices after 15 days in culture (Fig 4B and C).

Viability and drug response are retained in culture



There is no loss in the viability of the tumour slices after 15 days in culture, as assessed by MTS assay (Fig 5A). Treatment of the slices with gemcitabine for 72h resulted in a decrease in cell viability consistent with the expected tumour response to chemotherapy (Fig 5B).

Conclusion

- Precision-cut tumour slices from resected cholangiocarcinoma specimens can be generated with good viability.
- Tumours derived from intra-hepatic and peri-hilar bile ducts are distinct, suggesting that treatment regimes may be tailored for each subtype.
- Following a recovery period, the proteomic, histological and chemotherapeutic response phenotypes of the slices are retained after prolonged culture *ex vivo*.
- These experiments reveal the potential of precision-cut tumour slices as a patient-derived model to assess response/resistance to treatment and as a potential platform for novel drug discovery.

References

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