Australian Experimental Liver Cancer Research Network

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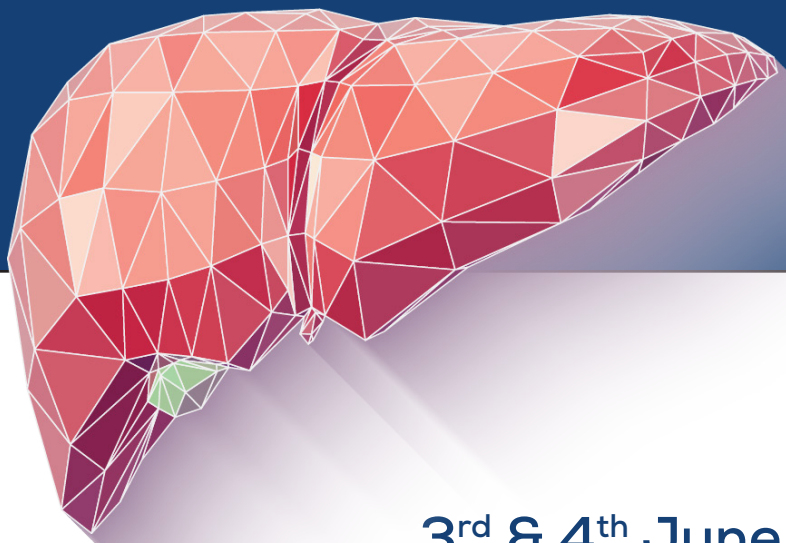

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Prognostic Role of Immune Checkpoint Regulators in Cholangiocarcinoma: A Pilot Study

Lu Cao 1,2,†, Prashanth Prithviraj 3,4,†, Ritu Shrestha 1,2 , Revati Sharma 3,4, Matthew Anaka 5, Kim R. Bridle 1,2, George Kannourakis 3,4, Darrell H.G. Crawford 1,2 and Aparna Jayachandran 1,2,3

1. Gallipoli Medical Research Institute, Greenslopes Private Hospital, Brisbane, QLD 4120, Australia
2. Faculty of Medicine, University of Queensland, Brisbane, QLD 4120, Australia
3. Fiona Elsey Cancer Research Institute, Ballarat, VIC 3350, Australia
4. School of Science, Psychology and Sports, Federation University Australia, Ballarat, VIC 3350, Australia
5. Department of Medical Oncology, University of Alberta, Edmonton, AB T6G 1Z2, Canada
6. † Equal contributors.

Cholangiocarcinoma (CCA) is a hepatobiliary malignancy associated with steadily increasingincidence and poor prognosis. Ongoing clinical trials are assessing the effectiveness and safety of a few immune checkpoint inhibitors (ICIs) in CCA patients. However, these ICI treatments as monotherapies may be effective for a proportion of patients with CCA. The prevalence and distribution of other immune checkpoints (ICs) in CCA remain unclear. In this pilot study, we screened databases of CCA patients for the expression of 19 ICs and assessed the prognostic significance of these ICs in CCA patients. Notably, expression of immune modulator IDO1 and PD-L1 were linked with poor overall survival, while FASLG and NT5E were related to both worse overall survival and progression-free survival. We also identified immune modulators IDO1, FASLG, CD80, HAVCR2, NT5E, CTLA-4, LGALS9, VTCN1 and TNFRSF14 that synergized with PD-L1 and correlated with worse patient outcomes. In vitro studies revealed that the expression of ICs was closely linked with aggressive CCA subpopulations, such as cancer stem cells and cells undergoing TGF-β αnd TNF-α- mediated epithelial-to-mesenchymal transition. These findings suggest that the aforementioned IC molecules may serve as potential prognostic biomarkers and drug targets in CCA patients, leading to lasting and durable treatment outcomes.

YTHDF2 transcriptionally programs effector T cells in tumor immunity

Jiajie Hou, MBBS, PhD, Department of Liver Surgery, State Key Laboratory of Oncology in South China, Collaborative Innovation Center for Cancer Medicine, Sun Yat-sen University Cancer Center, Guangzhou 510060, China, Electronic address: houjj@sysucc.org.cn

Innovative immunotherapy such as immune checkpoint blockade is now defining a paradigm for treating a broad spectrum of cancers, including hepatocellular carcinoma. Tumor-reactive CD8 T cells, which are of paramount importance in endogenous anti-tumor immunity, also form the pillar of successful cancer treatments. While epigenetic changes have been extensively studied in T cell exhaustion, it remains elusive whether and how T cell activation is epigenetically maintained pending cancer treatments. In terms of conserved RNA epigenetics, N6-methyladenosine (m6A) modification is an emerging gene regulatory mechanism that can impact anti-tumor immunity. Despite m6A-bound transcripts have been demonstrated to regulate effector T cell (TEFF) homeostasis, the functional relevance and therapeutic value of m6A modifiers in anti-tumor T cells are unknown.

By enlisting the “writer”, “eraser” and “reader” proteins, dynamic m6A modification prevalently affects post-transcriptional processes in the cytoplasm. Recent reports have shown that m6A can also regulate the chromatin state by integrating nuclear RNA methylation and histone modification through its nuclear reader YTHDC1. This echoes with the notion that RNA-binding proteins are sometimes involved in transcriptional control. However, left unanswered is whether the m6A machinery has an interplay with central transcriptional factors. Unlike other nuclear m6A modifiers, the m6A reader YTHDF2 primarily resides in the cytosol, where it processes mRNA decay. Although it is known that YTHDF2 can enter the nucleus under cell stress, the functionality and molecular basis of YTHDF2 relocation remain obscure. Moreover, one may urge to know whether YTHDF2 and its substrates can affect chromatin accessibility. In the present study, we report a TEFF-specific expression and distribution pattern of YTHDF2, which supports T cell-based tumor immunity and therapy efficacy by incorporating epitranscriptional and transcriptional networks. As such, we not only identify an unprecedented function of YTHDF2, but also uncover a druggable vulnerability for a group of therapy-resistant liver and colorectal cancers. The main findings are as follows:

- YTHDF2 expression increases in response to T cell activation or reinvigoration; loss of YTHDF2 in T cells dampens endogenous or therapy-induced tumor immunity
- Cytoplasmic YTHDF2 destabilizes mitochondrion-related genes as well as its cognate mRNA
- Nuclear YTHDF2 outcompetes the transcriptional repressor IKZF1 and IKZF3 to safeguard T cell activation; the fragility of YTHDF2-deficient T cells could be reversed by lenalidomide (a clinical myeloma drug)
- YTHDF2 relocation to the nucleus of TEFF avoids its self-degradation in the cytoplasm, which relies on T cell activation and m6A deposition
- Conclusively, our work has profound implications for understanding the crosstalk between m6A modification and chromatin organization within anti-tumor T cells, which may inspire novel paths for combinatorial immunotherapy.

Novel miRNA-based drug CD5-2 reduces liver tumour growth in diethylnitrosamine (DEN)-treated mice by normalising tumour vasculature and altering immune infiltrate

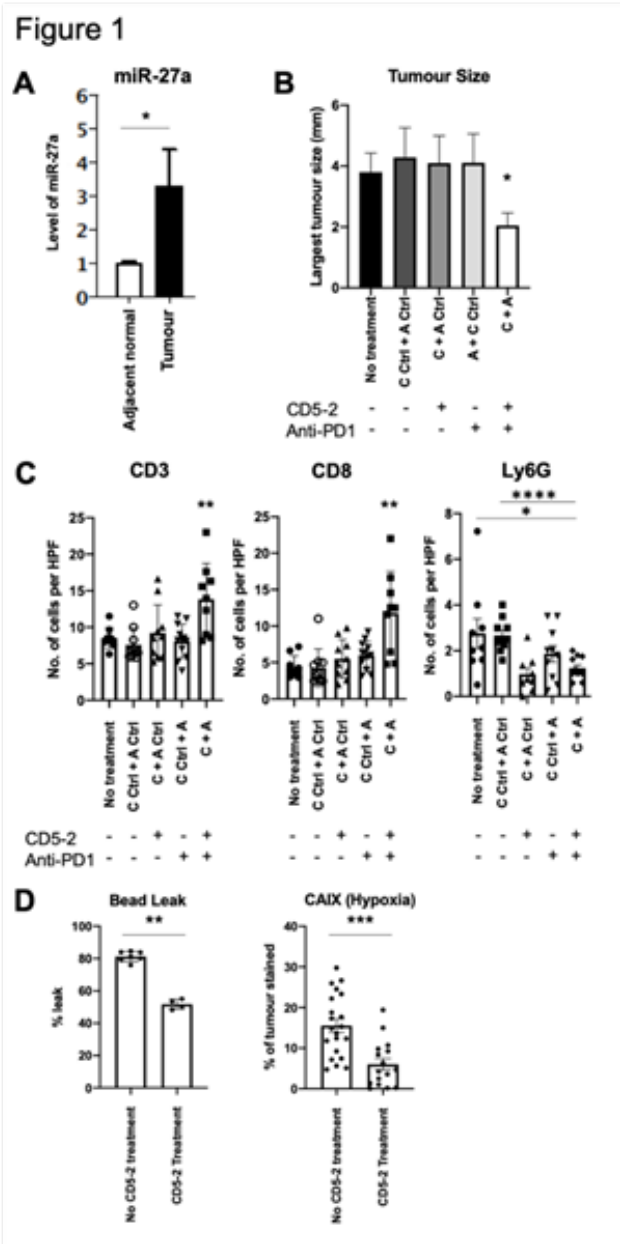
Dr. Ken Liu

Objectives: Normalisation of leaky tumour vasculature is an emerging approach to treat hepatocellular carcinoma (HCC). Blockmir CD5-2 is an oligonucleotide-based inhibitor of the miR-27a interaction with VE-Cadherin, the endothelial specific cadherin. We studied the effect of CD5-2 combined with checkpoint inhibition in the DEN-induced liver tumour mouse model.

Materials and Methods: DEN was given (25mg/kg intraperitoneally) to male C57BL/6 mice at postnatal day 14. CD5-2 (30mg/kg intravenously fortnightly) and/or anti-PD1 antibody (250µg intraperitoneally every 4 days) with their respective controls (4 groups) were given to the mice from age 7-months until harvest at age 9-months.

Results: Human HCC data from The Cancer Genome Atlas showed high miR-27a and low VE-Cadherin were both associated with poorer survival (Log-Rank P=0.02 and P=0.01, respectively). In untreated mice, miR-27a expression was significantly increased in tumours compared to adjacent normal tissue. Mice treated with CD5-2 + anti-PD1 antibody had significantly smaller tumours (50% reduction) compared to mice treated with either agent alone, controls, or untreated mice. Histologically, tumours in the CD5-2 + anti-PD1 antibody group exhibited a more favourable immune infiltrate (significantly higher CD3+ and CD8+ T cells and lower Ly6G+ neutrophils) compared to tumours from other groups. Tumours in CD5-2-treated mice had less leaky vasculature (extravasation of Dextran beads) and tumour hypoxia (carbonic anhydrase IX staining) compared to non-CD5-2-treated mice.

Conclusion: In the DEN mouse model, CD5-2 normalised liver tumour vasculature and reduced tumour hypoxia. CD5-2 plus anti-PD1 antibody reduced tumour size possibly by altering the immune infiltrate to being immunosupportive.



ROS mediated hepatic stellate cell activation via PI3K pathway during chemotherapy in liver cancer

Qi Ruan1,2, Lu Cao2, Haotian Yang1, Leslie J Burke2, Darrell Hg Crawford2, Xiaowen Liang1,2

1. University of Queensland Diamantina Institute, The University of Queensland, Brisbane, Australia
2. Gallipoli Medical Research Institute, Greenslopes Private Hospital, Brisbane, Australia

Objectives: One of the standard treatments of unresectable hepatocellular carcinoma (HCC) is transarterial chemoembolization (TACE), which uses chemotherapeutic drugs such as cisplatin and doxorubicin. However, chemotherapy is considered a "double-edged sword" through renewing tumor microenvironments and activating hepatic stellate cells (HSCs) which can promote tumor growth and chemoresistance. Hence, a better understanding of the molecular mechanism mediated HSC activation in response to chemotherapy could reveal novel potential targets for the treatment of liver cancer.

Materials and Methods: The effect of chemotherapeutic drugs on HSC activation was examined in three in vitro models. Human HSC cell line LX2 cells were co-cultured with cisplatin-treated human HCC cell line Huh7 cells in a transwell system and 3D mixed-cell spheroids. LX2 cells and human primary HSCs were cultured in the conditioned medium (CM) collected from cisplatin/doxorubicin-treated or untreated Huh7 cells. The expression of activation markers was measured. The results were further validated in mouse orthotopic models. Finally, an HSP FRET biosensor was employed to detect reactive oxygen species (ROS) in LX2 cells cultured in Huh7-CM with or without treatment.

Results: HSCs can be activated by cisplatin-pretreated Huh7 cells via three in vitro models with a significant increase in the expression of activation markers. Cisplatin-induced HSC activation was further confirmed in vivo, evidenced by an obvious increase in the activation markers of tumor tissues. Cisplatin-pretreated cancer cells increased intracellular ROS of LX2 cells reflected by an increased ratio of CFP/YFP fluorescent intensity. LX2 cells treated with H2O2 showed an elevated expression level of activation markers, indicating ROS generation can activate HSCs. In addition, the RNAseq result of primary HSCs cultured in CM indicated that PI3K pathway may involve in HSC activation induced by ROS.

Conclusion: HSCs can be activated through a ROS-mediated pathway during chemotherapy in liver cancer. Understanding the HSC activation mechanism induced by chemotherapy could provide potential therapeutic targets as a complement to chemotherapy against liver cancer.

Therapeutic potential of macrophage colony-stimulating factor (CSF1) in chronic liver disease

Sahar Keshvari1, Berit Genz1,2, Ngari Teakle1, Melanie Caruso1, Michelle F. Cestari1, Omkar L. Patkar1, Brian WC Tse3, Kamil A Sokolowski3, Hilmar Ebersbach4, Julia Jascur4, Kelli P. A. MacDonald2, Gregory Miller5, Grant A. Ramm2,6, Allison R. Pettit1, Andrew D. Clouston5,6, Elizabeth E. Powell6,7, David A. Hume1, Katharine M. Irvine1.

1. Mater Research Institute-The University of Queensland, Translational Research Institute, Brisbane, Queensland, Australia
2. QIMR Berghofer Medical Research Institute, Brisbane, Queensland, Australia
3. Preclinical Imaging Facility, Translational Research Institute, Brisbane, Queensland, Australia
4. Novartis Institutes for Biomedical Research (NIBR), Fabrikstrasse 2, Novartis Campus, CH-4056 Basel, Switzerland
5. Envoi Specialist Pathologists, Brisbane, Qld, Australia
6. Faculty of Medicine, The University of Queensland, Brisbane, Australia
7. Department of Gastroenterology and Hepatology, Princess Alexandra Hospital, Brisbane, Queensland, Australia

Resident and recruited macrophages control the development and proliferation of the liver. We showed previously in multiple species that treatment with a macrophage colony stimulating factor (CSF1)-Fc fusion protein initiated hepatocyte proliferation and promoted repair in models of acute hepatic injury in mice. Here we investigated the impact of CSF1-Fc on resolution of advanced fibrosis and liver regeneration, utilizing a non-resolving toxin-induced model of chronic liver injury and fibrosis in C57BL/6J mice. Co-administration of CSF1-Fc with exposure to thioacetamide (TAA) exacerbated inflammation consistent with monocyte contributions to initiation of pathology. After removal of TAA, either acute or chronic CSF1-Fc treatment promoted liver growth, prevented progression and promoted resolution of fibrosis. Acute CSF1-Fc treatment was also anti-fibrotic and pro-regenerative in a model of partial hepatectomy in mice with established fibrosis. The beneficial impacts of CSF1-Fc treatment were associated with monocyte-macrophage recruitment and increased expression of remodeling enzymes and growth factors. These studies indicate that CSF1-dependent macrophages contribute to both initiation and resolution of fibrotic injury and that CSF1-Fc has therapeutic potential in human liver disease.

Matrix optimisation of precision bio-printed HCC organoids for drug screening

Gayatri D. Shirolkar1,2, Johnathan Tibballs2, Michael Wallace1,3, Louise Winteringham1,4, Peter Leedman1,4, Janina E.E. Tirnitz-Parker1,2,4, Benjamin J. Dwyer1,2

1. Liver Cancer Collaborative, livercancerwa.org.au;
2. Curtin Medical School and Curtin Health Innovation Research Institute, Curtin University, Perth WA;
3. Sir Charles Gairdner Hospital, Perth, WA;
4. Harry Perkins Institute of Medical Research, Perth WA

Background and aim: Liver cancer is the third-leading cause of cancer-related deaths worldwide. Hepatocellular carcinoma (HCC) accounts for ~80% of liver cancers. Currently systemic treatments are not effective, and more targeted, effective therapy options are required to ease the burden of disease. Lack of appropriate cell culture models is a major obstacle in high-throughput drug development. Patient-derived organoid (PDO) technology may overcome some limitations of conventional in vitro models. Generation of PDOs require correct matrix support and the reported rate of HCC-PDO generation is ~30% in non-defined matrices, which do not accurately model the tumour microenvironment. Here, we aimed to optimise liver cancer PDO organoid generation and drug screening using fully defined matrices developed by Inventia Life Science.

Methods: 19 primary liver cancer biopsy samples have been received (n=17 HCC, n=2 cholangiocarcinoma). Among HCC cohort, aetiology was varied ranging from non-cirrhotic, cirrhotic (HBV, NASH), alcohol and fatty liver. Ultrasound-guided core biopsy samples were obtained by interventional radiologist, enzymatically digested and plated in Cultrex Basement Membrane Extract (BME2) to generate organoids in defined medium. Samples were observed for 2-3 weeks to confirm initial organoid growth. Five samples were plated in three defined matrices (Inventia) to optimise organoid derivation. Organoid cultures were stained for alpha-fetoprotein, cytokeratin 19 and albumin to confirm tumour characteristics. Successful lines were subjected to precision-bioprinting using the RASTRUMTM 3D bioprinting platform (Inventia). Three novel matrices of 1.1kPa and 3kPa stiffnesses were used for bioprinting to define a matrix supporting optimal HCC-PDO growth.

Results: Eight organoid lines have been successfully generated (~42% success). Two out of five samples showed promising growth on Inventia matrices at the early stage. Currently, characterisation of successful lines is being carried out. Additionally, three PDO cultures have been successfully optimised for precision-bioprinting, which will be used for future drug screening.

Conclusions: This study underlines the importance of using defined matrices in derivation and bio-printing of liver cancer PDOs.

Impact of vessels that encapsulate tumour clusters vascular pattern on hepatocellular carcinoma recurrence following liver transplantation – a 10-year Australian study

Dr. Ken Liu

Background and Aim: Vessels that encapsulate tumour clusters (VETC) is a novel vascular pattern seen on histological examination of hepatocellular carcinoma (HCC) tissue which has been shown to independently predict tumour recurrence and survival after surgical resection. Its prognostic value in patients receiving liver transplantation (LT) for HCC is unknown.

Methods: We retrospectively studied consecutive adults who underwent deceased-donor LT for the indication of HCC between January 2010 and December 2019. Formalin-fixed, paraffin-embedded tissue of liver explants were serially sectioned and stained for haematoxylin and eosin and CD34. Sections with viable tumours were then assessed for their VETC count (average number of VETC per 100x field in the 5 most vascularised areas as previously described by Fang et al. Hepatology 2015). Recipient clinical and outcomes data were collected from a prospective LT database and electronic medical records. The primary endpoint was recurrence-free survival.

Results: During the study period, 158 patients received a LT for the indication of HCC (81% male, median age 58 years [IQR 65-61]). Main aetiologies for underlying liver disease were hepatitis C (56%), hepatitis B (17%) non-alcoholic fatty liver disease (11%), and alcohol (10%). The median model for end-stage liver disease score at time of LT was 15 (IQR 10-20) and patients' Child-Pugh categories were: A 29%, B 25%, C 22% and non-cirrhotic 23%. Most patients (85%) had HCC treatment prior to LT: transarterial chemoembolisation (81%), thermal ablation (17%), resection (11%), ethanol ablation (7%), stereotactic radiotherapy (1%), radioembolisation (1%). The median time from HCC diagnosis to LT was 18 months (IQR 10-32) and the median time spent on the waitlist was 6 months (IQR 3-13). The median pre-LT alpha-foetoprotein level was 7 kIU/L (IQR 3-21). Based on pre-LT multiphase imaging, 4%, 3% and 1% of patients were outside Milan, University of California San Francisco (UCSF) and Metroticket 2.0 criteria, respectively. On examination of liver explants, the median number of viable tumour nodules was 2 (IQR 1-3) with a maximum diameter of 20mm (IQR 14-27). Based on this, 27%, 19% and 13% of patients were outside Milan, UCSF and Metroticket 2.0 criteria, respectively. Microvascular invasion was present in 25% and perineural invasion in 1%. VETC was seen in 75% of explants. The median VETC count was 1.5 (IQR 0.02-10.7) per patient and 1.0 (IQR 0.02-6.3) per viable tumour on explant. Patients with VETC pattern had slightly larger tumours on explant compared to those who did not (20mm [IQR 15-30] vs. 18mm [IQR 10-25], P=0.011). Otherwise VETC was not significantly associated with any baseline variables, being inside/outside of any HCC LT criteria or having microvascular invasion. After a median follow-up of 56 months (IQR 37-79), 13/158 (8%) patients developed HCC recurrence after a median of 21 months (IQR 14-23) post-LT. On Cox regression neither the presence of VETC (P=0.634) nor VETC count (P=0.651) were associated with HCC recurrence. Conversely, patients with vs. without HCC recurrence had similar median VETC counts (1.5 [IQR 0.3-10.8] vs. (1.0 [IQR 0-8.3], P=0.826). Significant univariable predictors of HCC recurrence post-LT are shown in Table 1. Multivariable analysis was no possible due to significant multicollinearity between these variables.

Conclusion: In this single centre study, the rate of HCC recurrence is low (8%). Unlike in HCC resection, VETC pattern does not predict for HCC recurrence post-LT. Traditional predictors including Milan and UCSF criteria, number of tumours and presence of microvascular invasion seen on explant remain most useful.

Table 1. Univariable predictors of HCC recurrence post-liver transplantation

Variable	Hazard Ratio	95% Confidence Interval	P
Time spent on waitlist (per month increase)	1.026	1.000-1.052	0.048
Outside Milan criteria on pre-LT imaging (vs. inside Milan)	4.526	1.002-20.445	0.049
Outside UCSF criteria on pre-LT imaging (vs. inside UCSF)	5.637	1.247-25.449	0.025
Number of viable tumours on explant (per tumour increase)	1.271	1.028-1.573	0.027
Presence of microvascular invasion (vs. absence)	3.741	1.257-11.136	0.018

c-Myc driven dedifferentiation and growth require GTP biosynthesis.

Talhah M. Salmi1,2, Malcolm J. McConville3, and Andrew G. Cox1,2,3

1. Peter MacCallum Cancer Centre, Parkville, Victoria, Australia.
2. Sir Peter MacCallum Department of Oncology, The University of Melbourne, Parkville, Victoria, Australia.
3. Department of Biochemistry and Pharmacology, The University of Melbourne, Parkville, Victoria, Australia

Liver cancer remains one of the most lethal cancers worldwide. Although liver cancer is a heterogeneous disease at the genetic level, one of the unifying features is the deregulation of transcription factors. c-Myc is a frequently amplified oncogenic transcription factor known to regulate cell fate, proliferation and metabolism. In this study, we took advantage of an inducible zebrafish model of liver cancer, in which c-Myc is overexpressed specifically in hepatocytes upon exposure to doxycycline (TO-Myc). Using this model, we demonstrated that c-Myc induces hepatomegaly in larvae and liver cancer in adults. At the cellular level, c-Myc overexpression caused hepatocytes to dedifferentiate into hepatoblast-like progenitors with an expansion of the nucleolus, consistent with enhanced ribosomal biogenesis. RNA-seq analysis confirmed that c-Myc altered cell fate and induced genes associated with ribosomal biogenesis. Interestingly, amongst the c-Myc upregulated genes, we observed an enrichment of genes involved in de novo purine biosynthesis. Pharmacological inhibition of IMPDH, a rate-limiting step in GTP biosynthesis, suppressed nucleolar expansion and hyperplasia in TO-Myc larvae whilst having no impact on normal liver growth. Notably, the effects of IMPDH inhibition were rescued by re-supplementation with guanosine. Together, these studies highlight that GTP biosynthesis is a therapeutically actionable metabolic vulnerability in c-Myc driven cancer.

The Characterisation of Tumour Self-Seeded Cells in Liver Cancer

Haotian Yang1, Darrell HG Crawford2, Xiaowen Liang1,2

1. University of Queensland Diamantina Institute, The University of Queensland, Brisbane, Australia
2. Gallipoli Medical Research Institute, Greenslopes Private Hospital, Brisbane, Australia

Background: Recent studies indicated a multi-directional seeding of circulating tumour cells (CTCs). Apart from seeding to distant tissues (metastases), CTCs can also reinfiltrate and colonise already established tumours. This process of “tumour self-seeding” provides new insights into the dynamics of tumour progression, and has been indicated to promote tumour growth, angiogenesis and invasion. However, tumour self-seeded cells (TSCs) have not been well identified and characterised due to unsuitable animal models. Therefore, this study aims to develop a novel animal model to recapitulate the process of tumour self-seeding and characterise the transcriptional and functional profiles of TSCs in liver cancer.

Methods: Kikume Green-Red (KikGR) is an irreversibly photoconvertible protein that changes fluorescence from green (KikGreen) to red (KikRed) upon violet light irradiation. Human tumour cell lines were knocked-in with KikGR plasmid and injected into mouse liver. After tumour formation, KikGreen+ CTCs in the blood vessels were photoconverted to KikRed+ CTCs. KikRed+ CTCs that recolonised the primary tumour were identified as KikRed+ TSCs, which could be distinguished from non-photoconverted KikGreen+ primary tumour cells (PCs). KikRed+ TSCs and KikGreen+ PCs were isolated by fluorescence-activated cell sorting (FACS) and analysed by RNA sequencing and functional assays. Potential TSC population in cancer patients was investigated using single-cell RNA sequencing data.

Results: A novel animal model was successfully developed to fully recapitulate the process of tumour self-seeding. 114 genes were significantly and differentially expressed between TSCs and PCs, with potential TSC markers identified (TM4SF1, EMP1, SH3BGRL3). Further analysis showed TSCs were enriched with gene ontology relating to metastases and cell proliferation. Functional assays demonstrated that TSCs were more invasive and tumorigenic than PCs. Moreover, potential TSC population was identified in cancer patients.

Conclusions: TSCs present as a cell subpopulation within the primary tumour with enhanced invasiveness and tumorigenesis, which may provide novel cell targets with diagnostic, prognostic or therapeutic potential.

The HGF/c-MET signalling pathway is a key regulator of liver progenitor cell function and maintenance of epithelial cell characteristics

Sara Pasic1, Pieter J. Eichhorn1, Grant A. Ramm2, Janina E.E. Tirnitz-Parker1

1. Curtin Medical School and Curtin Health Innovation Research Institute, Curtin University.
2. QIMR Berghofer Medical Research Institute

Background: During chronic liver injury, bipotential liver progenitor cells (LPCs) become activated, proliferate, and migrate to the injury location to replace lost epithelial cells. Hepatocyte growth factor (HGF)/cellular mesenchymal-epithelial transition (c-MET) signalling has been identified as a key pro-regenerative and anti-fibrotic pathway in chronic liver disease.a Since LPCs express c-MET and proliferate in fibrotic liver conditions, this study focussed on the role of HGF/c-MET signalling in LPCs in vitro.

Methods: The clonal LPC line BMOL-TATa (c-MET wildtype, WT) was utilised to establish baseline cellular characteristics and HGF/c-MET signalling pathway status, prior to molecular manipulation. CRISPR/Cas9 technology was used to construct a clonal c-MET knockout (KO) BMOL-TAT cell line. BMOL-TATc-MET-WT and BMOL-TATc-MET-KO were then assessed by MTS proliferation assay, scratch wound migration assay, and comparatively evaluated for expression of HGF/c-MET and TGFβ signalling components through western blotting and immunofluorescence. Additionally, morphological changes were investigated by cellular adherence assay and confocal evaluation of epithelial-to-mesenchymal transition markers (E-cadherin, N-cadherin, vimentin).

Results: BMOL-TATc-MET-KO cells showed significantly decreased proliferation and migration, compared to their BMOL-TATc-MET-WT counterpart. CRISPR KO of c-MET signalling led to an increase in pro-fibrotic TGFβ signalling in BMOL-TATc-MET-KO cells, while they also switched from high expression of the epithelial cell marker E-cadherin to a mesenchymal phenotype with strong expression of N-cadherin and vimentin. Interestingly, the cells were significantly more adherent, consistent with their decreased migratory potential, compared to BMOL-TATc-MET-WT cells.

Conclusion: These data suggests that the HGF/c-MET signalling pathway is vital for LPC biology and their functional role during liver regeneration, where they proliferate and migrate to sites of injury to regenerate the lost parenchyma. Additionally, the morphological changes in BMOL-TATc-MET-KO cells from an epithelial to a more mesenchymal nature highlight the importance of c-MET signalling in the maintenance of the epithelial LPC phenotype. Considering that HGF/c-MET signalling has been identified as a promising therapeutic target to inhibit hepatocellular carcinoma formation but signalling ablation in LPCs resulted in a pro-fibrotic mesenchymal LPC phenotype, c-MET-targeting approaches will need to be carefully considered to avoid inhibition of regenerative LPCs.

a) Ishikawa T et al. Hepatocyte growth factor/c-Met signaling is required for stem-cell-mediated liver regeneration in mice. Hepatology (2012).

b) Tirnitz-Parker JEE et al. Isolation, culture and immortalisation of hepatic oval cells from adult mice fed a choline-deficient, ethionine-supplemented diet. IJBCB (2007).

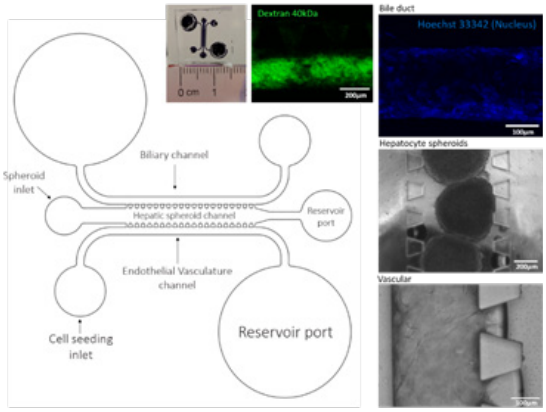
A functional MSC-derived liver on a chip for modelling liver diseases

Anastasia Brooks1, Darrell HG Crawford2, Xiaowen Liang1,2, Haolu Wang1,2

1. University of Queensland Diamantina Institute, The University of Queensland, Brisbane, Australia
2. Gallipoli Medical Research Institute, Greenslopes Private Hospital, Brisbane, Australia

Objectives: Current study of liver diseases and drug development is hampered by the lack of an appropriate model. Most current liver models are unable to replicate the complex liver structure and function due to a lack of optimal co-culture systems which can include all the parenchymal and non-parenchymal cells found in the liver. Microfluidics offers a new potential platform for creating an in vivo like environment to better study and understand disease and drug development. Using patient derived mesenchymal stem cells (MSCs), we aim to derive a liver-on-a-chip which houses a hepatic vasculature, a bile duct, and a hepatic compartment.

Materials and Methods: The microfluidic chip was designed using standard soft lithography procedure. MSCs were cultured and differentiated into hepatocytes, endothelial cells and cholangiocytes. The cells were seeded into three independent but interconnected channels. Cell culture conditions were optimised by modifying channel surface coating, cell seeding density, and cell rotation to yield the idealised bile duct, endovasculature and hepatic spheroids.



Results: MSC-derived functional hepatic cells including hepatocytes, endothelial cells and cholangiocytes have been successfully differentiated and characterised. MSC-derived cholangiocytes and endothelial cells successfully formed a dense tube with a functional lumen. The cholangiocyte tube had low dextran permeability and high rhodamine 123 uptake. MSC-derived hepatocytes cultured into functional spheroids which were successfully loaded into a hepatic compartment.

Conclusion: The microfluidic device designed was capable of creating a functional liver-on-a-chip that could serve as a reliable model replica of a simplified liver in structure and organ-level functions. With this model, personalised drug testing, analysis and disease modelling could be further developed and investigated. The independent channels allow modelling of different areas or nodes of disease. The current chip is modelled for biliary diseases, but can also be used for hepatocellular carcinoma modelling by replacing MSC-hepatocytes with hepatocellular carcinoma cells.

Interrogating the tumour microenvironment in hepatocellular carcinoma

Felix Marsh-Wakefield1,2, Positha Santhakumar1,3, Angela L. Ferguson1,2, Ken Liu1,3, Joo Shin4, Umaimainthan Palendira2, Geoffrey McCaughan1,3

1. Liver Injury & Cancer Program, Centenary Institute, Sydney, Australia
2. Human Immunology Laboratory, The University of Sydney, Sydney, Australia
3. A.W. Morrow Gastroenterology and Liver Centre, Royal Prince Alfred Hospital, The University of Sydney, Sydney, Australia
4. Tissue Pathology and Diagnostic Oncology, Royal Prince Alfred Hospital, Sydney, NSW, Australia

The tumour microenvironment (TME) is made up of cancer, stromal and immune cells, and is associated with various cancer types. In hepatocellular carcinoma (HCC), the TME landscape has been associated with prognostic outcomes, but there is still more to be investigated. Our work aims to provide further insight into the TME landscape within HCC, and how it contributes to prognostic outcomes. Thirty-two treatment naïve patients with HCC undergoing curative resection at a single tertiary institution were identified. Tissue microarrays (TMAs) were created from regions of interest within the tumour, invasive margin, and peri-tumour. An imaging mass cytometry panel consisting of forty immune markers was optimised and stained on the TMA. Spatial analyses were undertaken to interrogate differences between regions and clinical parameters. Our high-dimensional panel allowed the identification of a wide range of cancer, stromal and immune cell subsets. Using principal component analysis and spare partial least squares-discriminant analysis we revealed differences in subset density and interactions between cell subsets in each region. These in turn were associated with various patient aetiologies, including microvascular invasion and viral-associated HCC. Our analyses provide insight on how various regions contribute to clinical outcomes.

Effect of Gut influence on the immune environment in pre-clinical HCC

Sj Shen1, Miriam Jackson1, Saroj Khatiwada1, Anita Raposo1, Abhishek Vijayan2, Emad El-Omar1,3, Jason Behary1,3, and Amany Zekry1,3

1. UNSW Microbiome Research Centre, St George and Sutherland Clinical School, UNSW Sydney, NSW, Australia
2. School of Biotechnology and Biomolecular Sciences, UNSW Sydney, NSW, Australia
3. Department of Gastroenterology and Hepatology, St George Hospital, Kogarah, NSW, Australia

Background: The gut microbiota has been shown to influence several immune and metabolic responses central to hepatocellular carcinoma (HCC) growth and survival. The aim of this study was to assess in preclinical model of HCC whether gut manipulation by faecal microbiota transplantation (FMT) could influence the systemic and local immune responses associated with HCC survival.

Methods: We utilised C57Bl/6J mice and the diethylnitrosamine (DEN) and high fat diet (HFD)-induced model of HCC. We used a refined, validated, and published protocol from our group to achieve gut decontamination by antibiotics, prior to successful engraftment by donor FMT. Mice were administered weekly FMT by oral gavage from HCC patients (HCC FMT) or healthy controls (Healthy FMT) that had their microbiome composition pre-characterised by 16sRNA sequencing. Blood was collected and plasma cytokines were assessed by 28-plex cytokine array (MagPix ®). At 37 weeks (HCC time point), mice were humanely sacrificed. Tumours were separated from adjacent liver tissue and snap frozen for qPCR analysis. OPAL multiplex immunofluorescence (IF) was performed on paraffin embedded tissue sections.

Results: All mice had established HCC at 37 weeks. With HCC FMT, gene expression analysis of tumour regions showed significantly increased expression of Cd4, Cd8, Ifng, and Cxcl1 compared to Healthy FMT ($p<0.05$). Multiplex IF staining confirmed that HCC FMT led to increased infiltration of CD4+/FOXP3+ T cells within tumour regions compared to adjacent liver tissue, whilst Healthy FMT led to enrichment of CD8+ T cells within tumour regions. Correspondingly, HCC FMT led to an increased CD4-predominant milieu in the peripheral circulation with increased plasma levels of IL-2, IL-4, & IL-17 compared to healthy FMT.

Conclusions/significance: The data confirm that gut manipulation influences the tumour and systemic immune environment, with HCC FMT enticing a CD4-predominant milieu and a gene expression profile which favours tumour invasion (Cxcl1). In contrast, Healthy FMT led to increased infiltration of CD8+ T cells into the tumour environment. The data provide insight into the critical role gut microbiota plays in modulating immune responses relevant to HCC progression or regression.

Salivary biomarkers of liver fibrosis: Evidence for a potential role in diagnosis.

Lucas Trevisan França de Lima1,2, Darrell Crawford2,3, Daniel Broszczak4, Xi Zhang5, Kim Bridle2,3, Chamindie Punyadeera4,2,6.

1. The School of Environment and Science, Griffith Institute for Drug Discovery (GRIDD), Griffith University;
2. Gallipoli Medical Research Foundation, Greenslopes Private Hospital, Greenslopes, QLD, Australia;
3. The University of Queensland, Faculty of Medicine;
4. Institute of Health & Biomedical Innovation, School of Biomedical Sciences, Queensland University of Technology;
5. Griffith Institute for Drug Discovery (GRIDD), Griffith University; 6Menzies Health Institute Queensland (MIHQ), Griffith University, Queensland, Australia

Background: Liver fibrosis is a common feature in chronic liver disease, and a common underlying condition in hepatocellular carcinoma (HCC). Liver biopsy, the gold-standard to assess fibrosis, presents several limitations and is not suitable for - screening of at-risk populations. Thus, the use of non-invasive assessment tools such as ultrasound and blood biomarkers have gained attention. In this study, we investigated the clinical utility of salivary biomarkers to detect liver cirrhosis. We developed the Saliva Liver Fibrosis (SALF) score, the first saliva-based algorithm for the screening and/or early diagnosis of liver fibrosis.

Methods: Saliva samples from 135 patients were collected, including: liver cirrhosis (n=41), intermediate stages of liver fibrosis (n=20), liver disease with minimal fibrosis (n=50), and healthy controls (n=41). The stages of liver fibrosis were determined by liver stiffness measurement (LSM) assessed using transient elastography (TE). Individuals with LSM<7.0 kPa were considered to have minimal/nil fibrosis, those with LSM between 8.0 kPa and 12.0 kPa were categorized as intermediate degrees of fibrosis, and liver cirrhosis was classified as LSM>14.0 kPa. Concentration of clinically used serum biomarkers were measured in saliva samples, and then utilized in a logistic regression analysis.

Results: Salivary concentrations of hyaluronic acid (HA), tissue inhibitor of metalloproteinase 1 (TIMP-1), and α-2-macroglobulin (A2MG) were significantly increased in patients with liver fibrosis/cirrhosis compared to those where fibrosis was absent and healthy individuals. By combining these biomarkers, we developed a novel diagnostic score to detect clinically significant liver fibrosis, the Saliva Liver Fibrosis (SALF) score. In a training cohort, the median SALF scores of patients with liver cirrhosis (0.921±0.09) and fibrosis (0.819±0.285) were significantly higher than the scores of patients with minimal/nil fibrosis (0.061±0.29) and healthy controls (0.034±0.05). The SALF score identified patients with significant liver fibrosis with an AUC of 0.970, with a performance that was similar or superior to clinically validated diagnostic algorithms such as the Fibrosis-4 (FIB-4, AUC: 0.740) and Hepascore (AUC: 0.979). Using the optimal cut-off of 0.51, the validation of the SALF score using an independent cohort of patients showed an AUC of 0.920 for the detection of significant liver fibrosis.

Conclusion: To our knowledge, this is the first demonstration of the utility of saliva to diagnose significant liver fibrosis/ liver cirrhosis. The SALF score is a novel and accurate tool to improve the screening for cirrhosis in asymptomatic populations.

A Novel Mediator of Cholangiocarcinoma that has potential as a Diagnostic and Treatment Target

Dr. L Wickramasuriya
Dr. Y He, Prof. John Hooper, Dr D Crawford, Dr T O'Rourke

1. University of Queensland / Mater Research, AUS

Purpose/Introduction:CDCP1 (CUB-domain containing protein 1) is a cell surface receptor that has been found to be upregulated in a variety of malignancies. This research will involve CDCP1 and its role in the pathogenesis and dissemination of cholangiocarcinoma (CCA). Our hypothesis is that CDCP1 drives CCA pathogenesis and is a rational target for its detection and treatment.

Methodology: To characterise CDCP1 and its function in CCA by utilising a variety of in vitro and in vivo assays using established cell lines and mouse models. Specific cytotoxic Antibody Drug Conjugates (ADCs) to CDCP1 were used in subcutaneous CCA mouse models to determine their efficacy in delivering targeted treatment specifically to tumour tissue. To validate their efficacy in an environment more closely mimicking that of human CCA an attempt will be made to create orthotopic and patient derived xenografts (PDXs) using human CCA specimens collected fresh via various interventions; percutaneous biopsy, endoscopic retrograde cholangiopancreatography (ERCP) and surgical specimens.

Results: Our preliminary results in relation to CDCP1 and its role in CCA show that the ADC 41-2MMAE and Chimera 41-2MMAE have high efficacy and specificity in a subcutaneous CCA xenograft models and dramatically improve the response of CCA to gemcitabine, suggesting significant potential of CDCP1-directed agents in the treatment of this malignancy.

Conclusion/s: CDCP1 appears to be an exciting new prospect as a novel theranostic target in fight against cholangiocarcinoma.

Loss of primary cilia and PTEN in biliary epithelial cells contributes to intrahepatic cholangiocarcinoma formation in TAA-treated adult mice

Jinbiao Chen a,b, Ngan Ching Cheng a, Jade A. Boland a, Ken Liu a,b,c, James G. Kench b,d, D Neil Watkins e,f, SofiaFerreira-Gonzalez g, Stuart J. Forbes g, Geoffrey W. McCaughan a,b,c

1. Liver Injury and Cancer Program, Centenary Institute of Cancer Medicine and Cell Biology, Camperdown, NSW 2050, Australia;
2. Sydney Medical School, Faculty of Medicine and Health, University of Sydney, Camperdown, NSW 2050, Australia;
3. A.W. Morrow Gastroenterology and Liver Centre, Australian Liver Transplant Unit, Royal Prince Alfred Hospital, Camperdown, NSW 2050, Australia;
4. Department of Tissue Pathology & Diagnostic Oncology, NSW Health Pathology, Royal Prince Alfred Hospital, Camperdown, NSW 2050, Australia;
5. Research Institute in Oncology and Hematology, CancerCare Manitoba, Winnipeg, Manitoba, Canada;
6. Department of Internal Medicine, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, Manitoba, Canada
7. Centre for Regenerative Medicine, University of Edinburgh, 5 Little France Drive, EH16 4UU Edinburgh, United Kingdom

Intrahepatic cholangiocarcinoma (iCCA) is the second most common primary liver cancer. It is one of the deadliest cancers. The disease urgently needs more research. We lack an ideal animal model for iCCA, which should develop from the intrahepatic biliary epithelial cell (BEC) in immunocompetent mice with chronic inflammation, have a modifiable microenvironment, and represent iCCA genomic features.

The genomic characterisation of iCCA have been partially revealed over the past decade. Primary cilia (PC) loss, active PI3K-AKT pathway, Kras mutation, p53 mutation, and FGFR fusion are common genomic alterations in iCCA. Therefore, we will test whether loss of both primary cilia and PTEN in biliary epithelial cells mice will lead to iCCA in mice with chronic liver injury.

We found polycystic liver diseases in thioacetamide (TAA)-treated adult mice after primary cilia removal in BECs, but not in PC loss mice without TAA. Furthermore, iCCA was not found in TAA-treated PC-depleted mice. When PTEN was also removed in BECs of PC-loss mice, iCCAs formed 18 weeks after gene knockout. Importantly, these tumours highly expressed common features of human iCCA, including CK19, Sox9, pERK, aSMC, PCNA, CD3, and pFRS2.

Thus, a novel iCCA model was established. It shows that loss of primary cilia and PTEN in biliary epithelial cells leads to the formation of intrahepatic cholangiocarcinoma formation in TAA-treated adult mice. Its further characterisation and application are in progress.