EXPERIMENTAL HEPATOLOGY DAYS

April 24-26, 2025

Symposium 240
LYON, FRANCE

12,5 CME CREDITS

12,5 credit hours (CME) have been awarded by the European Union of Medical Specialists (UEMS).

PREFACE

Dear colleagues,

we cordially welcome you to the second symposium of the Falk Foundation Experimental Hepatology Days in Lyon. After the big success of our first Symposium 2023 in Zurich, we are very pleased to bring together again leading scientists and clinician-scientists from around the world who are united in one major aspect: Their interest in innovative aspects of basic and translational hepatology. The structure of the symposium will remain the same: Part A will focus on hepatic injury, inflammation and immunology, while Part B will be about regeneration, fibrosis and carcinogenesis. Both parts will include keynote lectures, panel discussions, poster pitches sessions and talks ranging from novel methodologies and basic mechanisms to clinical applications, thus providing plenty of opportunities for lively and stimulating discussions.

We are confident that building on the tradition of the Falk Foundation Basel Liver days and the success of the first Falk Foundation Experimental Hepatology Days, our symposium will provide the perfect format to discuss and address the emerging open questions in the dynamic area of experimental hepatology. We are pleased that many young scientists and clinician-scientists participate and present their research projects!

Matías Ávila Frank Tacke Robert Thimme Fabien Zoulim
Jessica Zucman-Rossi

EXPERIMENTAL HEPATOLOGY DAYS

April 24-26, 2025

Scientific Organization:
Matías Ávila, Pamplona
Frank Tacke, Berlin
Robert Thimme, Freiburg
Fabien Zoulim, Lyon
Jessica Zucman-Rossi, Paris

Congress Venue:
Marriott Lyon Cité International
70 Quai Charles de Gaulles
69006 Lyon
France

For admission to scientific events your name badge should be clearly visible.

Accompanying persons are not permitted during the conference at any time.

Start of Registration:
Thursday, April 24, 2025
13:00-18:30 h
at the congress office

Thursday, April 24, 2025

13:00	**Networking Lunch**
14:00	Welcome *Fabien Zoulim, Lyon*

PART A: HEPATOCYTE INJURY, INFLAMMATION & IMMUNOLOGY

SESSION I

Hepatocyte injury, metabolism and inflammation

Chairs:	*José C. Fernández-Checa, Barcelona; Sophie Lotersztajn, Paris*
14:05	Keynote lecture I: Metabolism and disease progression in the liver *Eyal Gottlieb, Houston*
14:35	Impact of intestinal neoglucogenesis on liver metabolism and steatosis *Gilles Mithieux, Lyon*
14:55	Liver resident T cells *Mala Maini, London*
15:15	Role of autophagy in liver inflammation/chronic disease progression *Sophie Lotersztajn, Paris*
15:35	Oral poster presentation - Inflammation-educated macrophages drive exacerbated re-injury patterns via innate immune memory *Yuting Wang, Berlin*
15:50	**Coffee break with ePoster session**

SESSION II

Poster pitches session

Chairs:	*Verena Keitel-Anselmino, Magdeburg; Mala Maini, London*
16:20	6 poster pitches (each 5’ pitch plus 2’ discussion)

SESSION III

Translation from discovery to the clinics

Chairs:	*Barbara Testoni, Lyon; Robert Thimme, Freiburg*
17:15	Cell therapies / CAR T cell therapies *Ulrike Protzer, München*
17:30	Therapeutic vaccines *Eleanor Barnes, Oxford*
17:45	iRNA-based therapeutics for metabolic liver diseases *Eliane Sardh, Stockholm*
18:00	From a clinical observation to a start-up *Rajiv Jalan, London*
18:15	**Panel discussion**
18:45	**Welcome Evening**

Friday, April 25, 2025

SESSION IV

Immunology

Chairs: *Eleanor Barnes; Oxford; Mirjam Zeisel, Lyon*

09:00	Keynote lecture II: Immunobiology of HBV infection *Matteo Iannacone, Milan*
09:30	Metabolic programming of innate-like MAIT cells in MASLD *Katrin Böttcher, Munich*
09:50	T cells and viral hepatitis: Lessons from HCV *Georg M. Lauer, Boston*
10:10	T cell immunity in HEV infection *Tobias Böttler, Freiburg*
10:30	Oral poster presentation - Precision-cut liver slices as a pre-clinical model for the evaluation of host-targeting agents against hepatitis B virus and hepatitis delta virus infection *Armando Andres Roca Suarez, Lyon*
10:50	**Coffee break with ePoster session**

SESSION V

Multidimensional analysis of the liver

Chairs: *Matteo Iannacone, Milan; Massimo Levrero, Lyon*

11:20	Novel personalized strategies in times of big data *Carolin Schneider, Aachen*
11:40	Multiparametric analysis of the liver disease *Valérie Paradis, Clichy*
12:00	Human precision-cut liver slices as a platform to study liver diseases *Elena Palma, London*

12:20	Strategies for using AI in diagnosis and prognosis of liver disease *Julien Calderaro, Créteil*
12:40	**Panel discussion**
13:00	**Lunch break with ePoster session**

PART B: REGENERATION, FIBROSIS AND CARCINOGENESIS

SESSION I

Regeneration and fibrosis

Chairs: *Jordi Gracia-Sancho, Barcelona; Frank Tacke, Berlin*

14:00	Keynote lecture III: Gut-liver axis as a modulator for regeneration and fibrosis *Bernd Schnabl, Ja Jolla*
14:30	Oral poster presentation - Inhibition of Adipocyte triglyceride lipase (ATGL) reduces fibrosis and portal hypertension via a CD8+ T-cell dependant manner in murine CCl4 liver fibrosis *Henriette Horstmeier, Vienna*
14:50	Molecular mechanisms of liver fibrosis: Lessons learned from single cell studies *Neil Henderson, Edinburgh*
15:10	Role of polyploidy in transition from NASH to HCC *Chantal Desdouets, Paris*
15:30	Morphogens, metabolism and metabolic dysfunction-associated liver disease *Anna Mae Diehl, Durham*
15:50	**Coffee break with ePoster session**

Friday, April 25, 2025

SESSION II

Poster pitches session

Chairs: *Andreas Kremer, Zurich; Jennifer Rieusset, Lyon*

16:20	6 poster pitches (each 5' pitch plus 2' discussion)

SESSION III

Liver cell plasticity

Chairs: *Annalisa Berzigotti, Bern; Neil Henderson, Edinburgh*

17:15	Cell plasticity in acute-on-chronic liver failure *Schalk Van Der Merwe, Leuven*
17:35	Liver organoids for the study of liver disease and plasticity *Ludovic Vallier, Berlin*
17:55	Liver zonation *Shalev Itzkovitz, Rehovot*
18:15	Dynamic cell heterogeneity in homeostasis and chronic liver disease *Jan Tchorz, Basel*
18:35	**Presentation of ePoster Awards**
19:00	**Networking with light refreshments**

Saturday, April 26, 2025

SESSION IV

Carcinogenesis

Chairs: *Matías Ávila, Pamplona; Jessica Zucman-Rossi, Paris*

09:00	Keynote lecture IV: Immunology and immunotherapy of cholangiocarcinoma *Tim F. Greten, Bethesda*
09:30	Role of liver microenvironment in HCC *Daniela Sia, New York*
09:50	Cholangiocarcinoma-on-a-chip *Ana Lleo, Rozzano*
10:10	Role of immune responses in the development of MASLD-associated liver cancer *Matthias Heikenwälder, Heidelberg*
10:30	Metabolic reprogramming in liver cancer *Renaud Dentin, Paris*
10:50	**Coffee break with ePoster session**

Saturday, April 26, 2025

SESSION V

Opportunities and risks of emerging therapies

Chairs:	*Francesco P. Russo, Padova; Heiner Wedemeyer, Hannover*
11:20	AAV vectorisation for gene therapy *Federico Mingozzi, Philadelphia*
11:40	Gene therapy in liver disease: New approaches *Pasquale Piccolo, Pozzuoli*
12:00	mRNA based therapy for liver metabolic diseases *Sonam Gurung, London*
12:20	New targets for HCC: From genomics to the clinics *Jean-Charles Nault, Bobigny*
12:40	Closing remarks *Jessica Zucman-Rossi, Paris*
12:45	**Closing discussion with lunch**

LIST OF SPEAKERS, MODERATORS AND SCIENTIFIC ORGANIZERS

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REGISTRATION

You can register for the event via our homepage:
www.falkfoundation.org
Registration is only possible online.

CONGRESS FEES

Scientific Program of Symposium 240	EUR 300
Students (copy of student ID required)	EUR 150

The congress fees include:
- Refreshments during coffee breaks
- Welcome dinner on Thursday, April 24, 2025
- Lunch on Thursday, April 24, Friday, April 25 & Saturday, April 26, 2025
- Snacks during networking on Friday, April 25, 2025
- A copy of the final program

CONGRESS OFFICE AND REGISTRATION

Opening Hours:

Thursday, April 24, 2025	13:00 - 19:00
Friday, April 25, 2025	08:00 - 19:00
Saturday, April 26, 2025	08:00 - 12:45

The Falk Foundation will take pictures during the meeting. Additionally, parts of the meeting might be recorded. By participating all attendees consent and agree with the recording and the photo shoots.

ARRIVAL

Marriott Lyon Cite International
70 Quai Charles de Gaulles
69006 Lyon
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By plane
From Lyon Airport (LYS) you can get by taxi to Marriott Lyon Cité International, it takes about 30 minutes.

By train
From the train station Lyon Part Dieu you can take the bus C1, direction Cuire. Stop at “Musée d´Art contemporain”, the Hotel is located in front of the bus stop.

By car
After arrival in Lyon follow the signs to “Cité international (Tête d´Or). Arriving at Cité International, you find new red buildings on your right side, where the Marriott Hotel is located.

POSTER ABSTRACTS

1. Antitumoral activity of G9a inhibitors in hepatocellular carcinoma and its potential combination with immune checkpoint inhibitors
   E. Adan Villaesusa, I. Uriarte, B. Castello-Uribe, A. Lopez-Pascual, M. Latasa, E. Santamaria, J. Elurbide, E. Valbuena-Goiricelaya, A. Irigaray-Miramon, B. Sangro, J. Argemi, M. Arechederra, C. Berasain, J. Totman, V. Gibaja, M. Avila, M. Fernandez-Barrena (Pamplona, ES; Cambridge, US)
2. Unraveling the connection between serum alpha-fetoprotein levels and molecular signatures in hepatocellular carcinoma
   C. Aktan, E. Isik, N. Oruc (Balikesir, Izmir, TR)
3. Defective autophagy in CD4 T cells drives liver fibrosis via type 3 inflammation
   R. Al Sayegh, J. Wan, C. Caer, M. Azoulai, M. Gasperment, S. Baweja, M. Chouillard, J. Kandiah, M. Cadoux, M. Mabire, C. Pignolet, T. Thibault-Sogorb, A. Hammoutene, V. Paradis, L. Saveanu, R. Nicolle, H. Gilgenkrantz, E. Weiss, S. Lotersztajn (Paris, Clichy, FR; Delhi, IN)
4. Role of CCL5 in the pathology of hepatitis delta infection
   E. Batbold, O. Komich, A. Roca Saurez, X. Grand, A. Ivanov, F. Zoulim, B. Bartosch (Lyon, FR; Moscow, RU)
5. Molecular inheritance of insulin resistance
   A. Berthier, C. Gheeraert, B. Staels, P. Lefebvre (Lille, FR)
6. Preliminary study of circulating lipid metabolites difference and mitochondrial dysfunction in patients with hepatitis E virus-triggered acute-on-chronic liver failure B. Bartosch, C. Chen, L. Chen, L. Weixia (Shanghai, CN)
7. Protein tyrosine phosphatase non-receptor type 2 controls hepatic function of cytotoxic T cells in metabolic dysfunction-associated steatohepatitis
   M. Determann, L. Linzmeier, M. Schwarzfischer, A. Niechcial, M. Spalinger, Y. Morsy, D. Poehlmann, M. Wilmink, M. Walker, F. Sella, M. Levesque, V. Koelzer, A. Frei, S. Buch, J. Hampe, C. Datz, C. Schafmeyer, M. Heikenwaelder, M. Scharl, S. Bluemel (Zurich, Basel, CH; Dresden, Rostock, Heidelberg, DE; Salzburg, AT)
8. The PNPLA3 I148M variant initiates metabolic reprogramming in macrophages
   E. Dixon (Vienna, AT)
9. Hepatocyte-derived extracellular vesicles promote endothelial capillarization in chronic liver disease through the miR-153-3p – Pyroptosis axis
   A. Fernandez-Iglesias, L. Abad-Jorda, M. Andres-Rozas, A. Martinez-Alcocer, E. Tonina, Y. Fundora, S. Muntet-Guixe, J. Gracia-Sancho (Barcelona, ES)
10. MAIT cell depletion and dysfunction in patients with portal hypertension undergoing TIPSS
    M. Filipowicz Sinnreich, J. Muerle, M. Esposito, M. Kaech, T. Jaeger, C. Zech, M. Hofmann, A. Chancellor (Basel, CH; Freiburg, DE)
11. mTORC2 as a mediator of lipopolysaccharide-driven lipid metabolism alterations on mouse hepatocytes
    B. Franco Leonardi, M. Abe Honda, A. Souza Peixoto, E. Castro, N. Monteiro Pessoa, E. Monteiro Pessoa, L. Silva Junior, A. Brandao Pires, T. Santos Vieira, F. Tacke, W. Lara Festuccia (Berlin, DE; Sao Paulo, BR)

12. The modulation of the gut microbiota in association with low caloric diet in the treatment of the NASH in obese patients
A. Genunche-Dumitrescu, C. Badea, C. Neagoe, R. Surugiu, C. Deliu, A. Badea (Craiova, Bals, Bucharest, RO)

13. Drug-mediated transcriptional readthrough of HBV RNAs triggers antiviral effects in infected hepatocytes
G. Giraud, X. Grand, A. Diederichs, A. Dubois, M. Michelet, F. De Nicola, F. Zoulim, B. Testoni (Lyon, FR; Rome, IT)

14. Reactive cholangiocyte-derived ORM2 drives a pathogenic modulation of the injured biliary niche through macrophage reprogramming
A. Guillot, H. Liu, G. Yin, T. Lan, B. Franco Leonardi, Y. Ait Ahmed, H. Berger, M. Kohlhepp, N. Amiridze, N. Martagon Calderon, C. Frau, L. Vallier, M. Rezvani, F. Tacke (Berlin, DE)

15. Routine screening of hepatitis C in an antenatal setting – Single-center experience in the United Kingdom
A. Gupta, S. Moreea, R. Simpson, A. Turner, D. Bolton, J. Anderon (Bradford, GB)

16. Evaluation of fatigue and insomnia in patients with primary biliary cholangitis treated with ursodeoxycholic acid
M. Hamzaoui, I. Keskes, G. Gharbi, A. Ben Mohamed, M. Yaakoubi, M. Mahmoudi, A. Khsiba, M. Medhioub (Nabeul, TN)

17. Neutrophil-to-lymphocyte ratio (NLR) as a predictive factor for response to ursodeoxycholic acid in primary biliary cholangitis: A retrospective study
M. Hamzaoui, I. Keskes, A. Ben Mohamed, M. Yaakoubi, G. Gharbi, M. Mahmoudi, A. Khsiba, M. Medhioub (Nabeul, TN)

18. Stem-like TCF-1+ subsets shape the T cell response in chronic viral hepatitis
M. Heuschkel, N. Meier, M. Reinscheid, T. Boettler, B. Bengsch, R. Thimme, M. Hofmann (Freiburg, DE)

19. Inhibition of adipocyte triglyceride lipase (ATGL) reduces fibrosis and portal hypertension via a CD8+ T-cell dependant manner in murine CCl4 liver fibrosis
H. Horstmeier, K. Bonitz, T. Chakma, V. Taru, T. Sorz-Nechay, N. Michalitsch, R. Brettner, C. Fuchs-Steiner, T. Reiberger, P. Schwabl, M. Trauner (Vienna, AT)

20. Conversion of CD4+ T cells to functioning and epigenetically stable induced regulatory T cells in patients with primary biliary cholangitis
K. Kayani, V. Ronca, S. Davies, M. Arai, Y. Nakamura, N. Okamoto, N. Mikami, N. Ohkura, N. Richardson, P. Invernizzi, S. Sakaguchi, Y. Oo (Birmingham, GB; Osaka, JP; Milan, IT)

21. Setting-up a biliary-niche-on-a-chip multicellular model by establishing a cholangiocyte organoid library for the study of liver ductular reaction
M. Kohlhepp, G. Yin, T. Lan, H. Liu, B. Franco Leonardi, N. Martagon Calderon, M. Rezvani, F. Tacke, A. Guillot (Berlin, DE)

22. Taurine reprograms hepatocyte metabolism and its plasma levels are inversely associated with the progression of MASLD
P. Kumar, J. Wang, H. Cosgun, M. Demir, F. Tacke, C. Engelmann (Berlin, DE)

23. HIF-1α/LTBP2 axis activate HSCs to promote liver fibrosis by interacting with LOXL1 via the ERK pathway
C. Liang, C. Liang, L. Mengxin, T. Shuai, Z. Conglin, H. Yuxian (Shanghai, CN)

24. Performance of prognostic scores for biliary cholangitis: A comparative study
M. Medhioub, M. Yacoubi, A. Ben Mohamed, M. Mahmoudi, G. Gharbi, A. Khsiba, L. Hamzaoui (Nabeul, TN)

25. Paneth cells regulate intestinal lymphangiogenesis and lipid metabolism in metabolic dysfunction-associated steatotic liver disease
S. Moghadamrad (Bellinzona, CH)

26. Transcriptomic profiling of vascular invasion in hepatocellular carcinoma: A bioinformatics approach
N. Oruc, E. Isik, C. Aktan (Izmir, Balikesir, TR)

27. The hepatitis C virus (HCV) affects ribosomal RNA methylation and depends on the methyltransferase activity of fibrillarin for translation
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1. Antitumoral activity of G9a inhibitors in hepatocellular carcinoma and its potential combination with immune checkpoint inhibitors

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Introduction: Treatment of advanced Hepatocellular Carcinoma (HCC) has significantly improved due to the advent of immune-checkpoint inhibitors (ICIs). However, many patients still show innate or acquired resistance to ICI-based therapies. Hence, new therapeutic strategies to combat resistance and enhance effectiveness are needed. Epigenetic alterations play a crucial role in liver cancer development. These alterations can suppress the production of certain proteins that aid in immune response against tumors such as chemokines and/or the antigen presentation machinery. Our research has identified a promising target for liver cancer treatment, the histone-methyltransferase G9a. We conducted experiments to evaluate the effectiveness of specific inhibitors of G9a (EZM8266 and CM272), both in vitro and in vivo and their potential combination with ICIs.

Methods: RNAseq analyses of murine HCC cells treated with CM272 alone or combined with IFNɣ were performed. Validation of the data was conducted by using a second G9a inhibitor, EZM8266 and specific siRNAs. Cytokine release was measured by ELISA. MHC was evaluated by flow cytometry analysis. The effects of both G9a inhibitors, CM272 and EZM8266, on the growth of PM299L cells orthotopically implanted in immunocompetent mice, alone and in combination with αPD1 antibodies, were also determined. Multiplex immunofluorescence of tissues was performed to evaluate changes in immune cell populations.

Results: Inhibition of G9a, either genetically or pharmacologically in HCC cells led to a significant stimulation of immune response-related genes. The release of chemokines CXCL9 and CXCL10 was validated by ELISA, and increased MHC-Class I membrane exposure was confirmed by cytometric analysis following G9a inhibition in HCC cells. Mechanistically, transcriptomic analysis showed a substantial upregulation of genes reported as a primary regulators of MHC presentation-related molecules. These results were confirmed through real-time PCR in all tested cell lines. In vivo, both G9a inhibitorsCM272 inhibited the growth of orthotopically implanted tumors and enhanced the effects of α-PD1, resulting in remarkable antitumor activity. These responses were accompanied by increased CD8, CD4, CD11b, and FOXP3 positive cells infiltration. No signs of systemic or liver toxicity were observed.

Discussion/Conclusion: G9a is confirmed as an effective druggable target in HCC. Pharmacological inhibition of G9a increases the expression of immune response-related genes and enhances the efficacy of ICIs. Our findings provide strong support for the combination immunotherapy with epigenetic drugs such as CM272 and EZM8266 for HCC treatment.

2. Unraveling the connection between serum alpha-fetoprotein levels and molecular signatures in hepatocellular carcinoma

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Introduction: Hepatocellular carcinoma (HCC) is a common and aggressive liver cancer, associated with high morbidity and mortality rates. Alpha-fetoprotein (AFP) is a well-known biomarker for diagnosing and monitoring HCC. Elevated AFP levels are often observed, but the relationship between AFP levels and molecular changes in tumor remains complex.

Methods: In this retrospective study, we examined patient records from the Liver Hepatocellular Carcinoma (LIHC) dataset of The Cancer Genome Atlas (TCGA) to identify genetic alterations associated with serum AFP levels

Results: In this study, patients initial serum AFP levels determined at the diagnosis of HCC were categorized into quartiles: Low (n = 58), intermediate (n = 149), and high (n = 69). In the patients with low serum AFP level group, CTNNB1 mutations were found in 42.1% of patients; in the intermediate group, TP53 and CTNNB1 mutations were observed in 32.4% and 27.7%, respectively; and in the high serum AFP group, TP53 and TTN mutations were found in 33.3% and 29.0%, respectively. Survival analysis revealed a significant difference in overall survival between the low and intermediate groups (p-value = 5.482 x 10-3), but no significant difference in disease-free survival (p-value = 0.0726). No significant survival differences were found between the low & high and intermediate & high groups. Molecular analysis identified significantly altered mRNAs (log2FC < -2 or > 2 and q-value < 0.05) across all comparisons. Between the low and intermediate groups, 9 mRNAs were altered, with 8 upregulated in the low group and one in the intermediate group. Between the low and high groups, 294 mRNAs were altered, with 156 upregulated in the high group and 138 in the low group. Similarly, between the intermediate and high groups, 149 mRNAs were altered, with 74 upregulated in the intermediate group and 75 in the high group. The interactions between these genes were mapped, and the signaling pathways they are involved in were identified.

Discussion/Conclusion: These findings suggest that AFP levels, alongside specific mRNA alterations, may serve as prognostic markers and reflect distinct molecular subtypes of HCC.

3. Defective autophagy in CD4 T cells drives liver fibrosis via type 3 inflammation

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Introduction: Sustained inflammation is a driving force of chronic liver disease progression to fibrosis and end-stage cirrhosis. A major source of inflammatory and fibrogenic mediators originates from conventional CD4 T cells, in particular the Th17 cell subset. Dysregulation of autophagy in immune cells is associated with an uncontrolled inflammatory response during various inflammatory diseases. Here, we combined studies in human samples and mice models, to evaluate the role of T cell autophagy in liver fibrosis progression.

Methods: Autophagy was evaluated in the liver and blood T cells isolated from patients with extended fibrosis or controls. Liver fibrosis was induced in mice specifically lacking the

activating (ATG5) or inhibitory (Rubicon) autophagy proteins in T cells, by repeated carbon tetrachloride (CCl4) injections or bile duct ligation (BDL). Genes differentially regulated between CD4 T cells from ATG5Tlymph-/- CCl4-mice and control group were evaluated by RNA sequencing.

Results: Sc-RNA sequencing and functional studies indicated that intrahepatic and circulating CD4 T cells from patients with extended fibrosis show defects in the autophagy machinery. Fibrotic mice deficient for ATG5 in T cells displayed reduced CD4 T cell frequency, a shift toward an activated glycolytic Th17 phenotype, and enhanced type 3 (IL-17A, GM-CSF) cytokine release. These mice were more susceptible to liver inflammation and fibrosis. Co-culture experiments showed that ATG5-deficient CD4 T cells shift hepatic myofibroblasts, hepatocytes and macrophages toward a pro-inflammatory phenotype, associated with enhanced cytokine/chemokine release. As a proof of concept, pharmacological autophagy activation decreased the release of IL-17A and GM-CSF by CD4 T cells from patients with extended fibrosis; in addition, limited fibrosis was observed in mice in which activation of autophagy in T cells occurred following specific T cell deletion of Rubicon.

Discussion/Conclusion: Our findings unravel autophagy in CD4 T cells as a key therapeutic target to control inflammation-driven fibrosis during chronic liver injury.

4. Role of CCL5 in the pathology of hepatitis delta infection

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Introduction: Liver cancer is the sixth most common cancer and the third most common cause of cancer-related deaths worldwide. It is predicted that the number of new cases of liver cancer per year will increase by 55% by 2040. The main causes of liver cancer are hepatitis viruses. Among them, HBV and HCV are considered oncogenic viruses, but the role of HDV in hepatocarcinogenesis is still unknown. HDV is known to accelerate liver disease progression and increase HCC incidence. Thus, it is crucial to understand how HDV is oncogenic and the mechanisms underlying HDV-induced malignant hepatocellular transformation. One of our interested genes is related to HDV is CCL5, expressed in a wide variety of myeloid and lymphoid populations within the liver microenvironment, and increased levels of CCL5 have been implicated in the progression of chronic liver disease towards HCC.

Methods: Differentiated HepaRG and PHH are infected with mono-HDV and HBV/HDV. Infection and cellular response were monitored by RTqPCR, single cell sequencing and western blotting.

Results: In single-cell RNAseq, mono-HDV or HBV/HDV co-infected HepaRG cells are clearly separated from non-infected and HBV-infected cells. Pathway analysis in these populations revealed these differences in the strong inflammatory response associated with HDV infection, particularly IFN signaling. Additionally, the alteration of molecular and metabolic is in hepatocytes by HDV is mostly induced by IFN signaling, not by the virus itself, according to our bulk RNA-seq data. But interestingly, the mono-HDV infected sub-cluster showed a strong upregulation of CCL5, which is induced by HDV infection independently of IFN signaling. Furthermore, CCL5 produced in response to HDV infection was detected in the supernatant of HepaRG cells and PHH. We showed that conditioned medium from HBV/HDV co-infected hepatocyte induced CCL5 and activated LX-2 cells. We have shown that HDV infection, but not S/L Ag expression, induced CCL5, suggesting that RNA sensing mechanisms are involved. Thus, we are exploring the mechanisms in vitro (CRISPR/Cas9) currently. Furthermore, we will validate these data in biopsies and investigate CCL5 cytokine and receptor expression patterns by IHC in resections.

Discussion/Conclusion: Our study reveals a yet unknown upregulation and secretion of chemokine CCL5 induced by HDV mono-infection, and it could, may explain the excessive inflammation phenotype of HDV infected liver and accelerated progression of HDV related fibrosis and HCC.

5. Molecular inheritance of insulin resistance

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Introduction: Metabolic flexibility (MetF) is an organism's ability to adjust to changing metabolic supplies and energy demands. Insulin plays a central role in coordinating MetF through molecular mechanisms such as signaling pathways, transcriptional responses, and circadian regulation. Insulin resistance (IR) can impair MetF, contributing to type 2 diabetes and obesity, often stemming from continuous challenges such as sedentary lifestyles, poor diets, and circadian disruptions. Transient IR episodes, like gestational diabetes or stress-induced hyperglycemia, also heighten the risk of later diabetes development. Yet, the molecular processes post-transient IR remain poorly understood despite their health significance.

Methods: A multi-omic characterization of the hepatic response to a high fat diet challenge in mice previously exposed to a transient IR episode were conducted in this study. We integrated transcriptomic, epigenomic, lipidomic, and molecular clock assessments to provide a molecular basis for the observed dysregulations.

Results: Our study shows that temporarily blocking the insulin receptor in young mice leads to later-life liver issues hindering PPARα-mediated adaptation to a high-fat diet. This is linked to decreased histone active marks at PPARα sites and reduced endogenous PPARα ligands. Transient insulin receptor blockade also altered the liver's molecular clock, particularly affecting PPARα transcriptional responsiveness.

Discussion/Conclusion: Seemingly reversible and unnoticed metabolic challenges in early adulthood may predispose the liver to exacerbated metabolic dysfunctions when confronted with chronic challenges later in life.

6. Preliminary study of circulating lipid metabolites difference and mitochondrial dysfunction in patients with hepatitis E virus-triggered acute-on-chronic liver failure

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Introduction: Several previous data suggested a correlation between circulating lipid metabolites and disease of hepatitis E virus-triggered acute-on-chronic liver failure (short for HEV-T-ACLF). The mitochondrion, as the center of cellular energy metabolism in the body, also has a very important impact on lipid metabolism in organs and the circulation.

Methods: Liquid chromatography-mass spectrometry (LC-MS) was used to detect circulating lipid metabolites. Peripheral blood samples were collected from HEV-T-ACLF patients, and PBMCs were isolated for transcriptome sequencing to explore the differential mitochondrial gene expression among groups. qPCR was used to externally verify the differentially expressed genes. In addition, XFe-24 hippocampal apparatus was used to detect the changes in mitochondrial function of PBMCs.

Results: There were differences in the overall levels of circulating lipid metabolites among the healthy controls (HCs), HEV-T-ACLF survivors and HEV-T-ACLF non-survivors. The functional level analysis of circulating differential lipid metabolites showed that the differential

lipid expression was correlated with immune and inflammation-related genes (antiviral response, cytokine/cytokine receptor, etc.) in PBMCs, which revealed the complex relationship between multiple omics in HEV-T-ACLF. The comparison of PBMC high-throughput sequencing data showed that there were different degrees of gene up-regulation and down-regulation between the pairwise comparison groups. The enrichment of differentially expressed genes showed that there were differences in mitochondria-related metabolic pathways among the three groups ($p < 0.05$). The qPCR results showed that there were significant differences in the expression of genes related to mitochondrial β-oxidation, aerobic glycolysis, mitochondrial dynamic balance and bioenergetic function between the groups. Subsequently, the mitochondrial function test in PBMCs showed that basal oxidative phosphorylation, maximal oxidative phosphorylation and reserve oxidative phosphorylation were significantly decreased in patients with HEV-T-ACLF (both survivors and non-survivors) compared with HCs ($p < 0.05$). In addition, compared with survivors, the basal oxidative phosphorylation in non-survivors was significantly decreased ($p < 0.05$). Compared with HCs, the basal glycolytic capacity, maximal glycolytic capacity and reserve glycolytic capacity in HEV-T-ACLF patients (both survivors and non-survivors) were significantly decreased ($p < 0.05$). Furthermore, compared with survivors, the basal glycolytic capacity, maximum glycolytic capacity and reserve glycolytic capacity in non-survivors were significantly decreased ($p < 0.05$).

Discussion/Conclusion: The disease progression of HEV-T-ACLF may involve complex correlations among multiple omics, such as metabolomics, transcriptomics and immune inflammation. Abnormal circulating lipid metabolites in serum and mitochondrial dysfunction in PBMCs is associated with poor clinical prognosis, suggesting that energy metabolism may play an important role in the progression of HEV-T-ACLF.

7. Protein tyrosine phosphatase non-receptor type 2 controls hepatic function of cytotoxic T cells in metabolic dysfunction-associated steatohepatitis

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Introduction: Obesity affects 39% adults globally and is linked to metabolic dysfunction-associated steatohepatitis (MASH), which impacts up to 25% of the population. MASH is characterized by liver damage and fibrosis driven by cytotoxic CD8+ T cells. Protein tyrosine phosphatase non-receptor type 2 (PTPN2) modulates inflammatory CD8+ T cell responses. This study investigates the role of PTPN2 in MASH pathogenesis and its effect on CD8+ T cell function within the liver.

Methods: PTPN2 mRNA and protein expression levels were measured in human liver samples using Sequence Read Archive (SRA) (n = 106) and multicolor immunofluorescence (IF) staining (n = 8–12). The single nucleotide polymorphism (SNP) rs2542151, which leads to PTPN2 dysfunction, was evaluated in German and Austrian Metabolic Dysfunction-associated Steatotic Liver Disease (MASLD) patients (n = 742–997). Murine models, including T cell-specific, hepatocyte-specific, and myeloid cell-specific PTPN2 knockout (KO) mice, along with wild-type littermate controls, were fed a western-style fast food diet for 28 weeks to clarify the contribution of the different hepatic cell populations to MASH pathogenesis.

Results: Human MASH livers had elevated PTPN2 mRNA and protein levels compared to healthy and MASLD controls with increased PTPN2+CD8+ cells in MASH patients. The SNP rs2542151 was associated with reduced risk of severe liver fibrosis and cirrhosis in MASH patients. T cell-specific PTPN2 KO mice were protected from MASH and the development of liver fibrosis, despite exhibiting an increase in hepatic exhausted CD8+ T cells. In contrast, other tested PTPN2 KO models did not display distinct phenotypes, emphasizing the critical role of T cell-PTPN2 in MASH progression.

Discussion/Conclusion: Our results suggest that targeting PTPN2 may help reduce CD8+ T cell-mediated liver damage in MASH. Future research into PTPN2-focused immunomodulatory therapies could improve clinical outcomes for MASH patients.

8. The PNPLA3 I148M variant initiates metabolic reprogramming in macrophages

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Introduction: The impact of the patatin-like phospholipase domain-containing protein 3 (PNPLA3) I148M variant on MASLD has been studied extensively in hepatocytes and stellate cells. However, its role in macrophages, as critical players in disease progression, has not been addressed yet. Therefore, we explored the impact of the PNPLA3 I148M variant on the metabolic state, mitochondrial function, and proinflammatory phenotype of macrophages.

Methods: We established a stable THP-1 cell line overexpressing the PNPLA3 wildtype (WToe) and I148M variant isoforms. We conducted metabolomics, gene expression analysis, extracellular flux analysis, immunofluorescent imaging by confocal microscopy, and flow cytometry. These in vitro studies were complemented by spatial transcriptomics of liver biopsies of metabolic dysfunction-associated steatohepatitis (MASH) patients carrying the PNPLA3 WT and I148M variants.

Results: In I148Moe M1 macrophages GLUT1, HK1, LDHA, and HIF-alpha were significantly upregulated compared to the WToe, consistent with the increase of both the basal (+20%) and maximum (+15%) glycolytic activity. Conversely, reduced CPT1-alpha (-63%) in the I148M variant macrophage was in line with reduced mitochondrial respiration (-48%). Furthermore, I148Moe macrophages had an impaired mitochondrial electron transport chain (ETC) system as reflected by significantly reduced cofactors, FAD and NAD metabolites, as well as genes linked to the ETC complexes, MT-ND1, MT-SHDA, MT-CYTB, MT-CO1, MT-CO2, and ATP6 in both MASH patients and macrophages carrying the PNPLA3 I148M variants, accompanied by reduced mitochondrial ROS production (-15%). Liver biopsies from MASH patients and M1 macrophage with the PNPLA3 I148M variant revealed reduced PGC1-alpha, NRF1/2, mt-ND1, and 16sRNA mRNA transcripts compared to wildtype. In line, immunofluorescence showed a pronounced staining for COXIV protein in WToe macrophage. Spatial transcriptomics data also revealed differentially expressed COXIV, MT-CO3, and MT-ND6 in the livers of MASH patients carrying the PNPLA3 I148M variant. Since phagocytosis involves mitochondrial function, we explored the impact of the PNPLA3 I148M on phagocytosis. Interestingly, phagocytic components like MAFB (-57%), FCR2B (-30%), components of the phagolysosome NOS2 (61%), NOX2 (55%), and the overall phagocytic effect were reduced in the I148Moe macrophage.

Discussion/Conclusion: The PNPLA3 I148Moe reprograms macrophage metabolism because of reduced mitochondria number and/or mitochondrial dysfunction, thus affecting their phagocytotic capacity.

9. Hepatocyte-derived extracellular vesicles promote endothelial capillarization in chronic liver disease through the miR-153-3p – Pyroptosis axis

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Introduction: Liver cells communicate with each other through the release of extracellular vesicles (EVs). The aim of this study was to investigate the role of microRNAs (miRNAs) enclosed in hepatocyte-derived EVs (hepEVs) in sinusoidal endothelial dedifferentiation during chronic liver disease (CLD).

Methods: EVs were obtained from healthy (CT) or cirrhotic (CH) hepatocytes from human and rat livers. Healthy rats received fluorescent hepEVs-CT or hepEVs-CH (200 µg/day, 3 days) or vehicle (n = 10) to assess their biodistribution and the phenotype of LSECs through RNAseq, IF, and WB. Dysregulated miRNAs in human and rat hepEVs-CH were analyzed, and the common miRNAs were overexpressed in CT-LSECs for RNAseq. The most dysregulated pathway was studied in CH-LSECs, in cirrhotic human and rat livers, and in CT-LSECs treated with hepEVs overexpressing a specific miRNA. Cirrhotic rats were treated with the caspase-1 (casp1) inhibitor VX-765 (15 mg/kg, 2 weeks) or vehicle (n = 11) to evaluate hepatic hemodynamic, liver function, and endothelial phenotype.

Results: In vitro treatment with hepEVs-CH increased the expression of pro-inflammatory, fibrosis, angiogenesis and cell death related genes in CT-LSECs. In vivo administered hepEVs-CH accumulated in hepatic endothelium, deregulating profibrogenic and inflammatory genes, together with vWF up-regulation and nitric oxide synthase decrease, suggesting a direct effect on endothelial dedifferentiation. Human hepEVs-CH showed 37 miRNAs significantly deregulated, validating miR-200a-3p and miR-153-3p in rat hepEVs-CH. Transfection of CT-LSECs with miR-153-3p deregulated 771 genes involved in inflammation and pyroptosis, with 51% homologous to CH-LSECs transcriptome. Cirrhotic LSECs and livers, and hepEVs-miR-153-3p treated CT-LSECs showed activation of pyroptosis. Finally, CH rats treated with VX-765 exhibited reduced hepatic pyroptosis (-60% active casp1), resulting in a significant improvement in portal hypertension (-16.5%), fibrosis (-11.4%), and endothelial phenotype (-42% vWF).

Discussion/Conclusion: miRNAs embedded in hepEVs actively contribute to hepatic endothelial dedifferentiation in CLD through the activation of the miR-153-3p-pyroptosis pathway, suggesting casp1 inhibition as a new therapeutic strategy for endothelial dysfunction in cirrhosis.

10. MAIT cell depletion and dysfunction in patients with portal hypertension undergoing TIPSS

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Introduction: Mucosal-associated invariant T (MAIT) cells represent the most abundant T cell type in human liver. They respond to bacterial metabolites derived from riboflavin synthesis that are presented by the MHC-like molecule, MR1. The most potent stimulatory MAIT cell antigen (Ag) is called 5-OP-RU. MAIT cells are known to participate in immune-regulatory, an-

ti-bacterial as well as profibrogenic responses. We recently identified increased levels of MAIT cell stimulatory ligands in the sera of patients with portal hypertension. Whether these ligands affect portal blood MAIT cell phenotype and pathogenesis in these patients is unknown.

Methods: We examined the phenotype of unstimulated and Ag/cytokine-stimulated MAIT cells in the portal and peripheral blood of patients undergoing transjugular intrahepatic porto-systemic shunt (TIPSS) placement and in the peripheral blood of sex- and age-matched healthy subjects using a spectral flow cytometry panel including markers for T cell activation/exhaustion, degranulation, cytokine/chemokine receptors, as well as intracellular cytokines. Data were analyzed and visualized using hierarchical clustering and UMAP dimension reduction.

Results: Our analyses reveal heterogenous MAIT cell activation states. We observe that MAIT cells are strongly depleted in patients with portal hypertension in both peripheral and portal blood compared to healthy subjects. The remaining MAIT cells show an activated phenotype and impaired responsiveness to 5-OP-RU/IL-12/IL-18-stimulation with reduced expression of activation markers and lower cytokine production. These findings underscore significant alterations in MAIT cell frequency, activation, and functionality in the context of portal hypertension.

Discussion/Conclusion: Our analysis highlights the unique characteristics of MAIT cells in advanced liver disease complicated by portal hypertension and their potential contribution to disease progression. Severe depletion and functional impairment of MAIT cells in both peripheral and portal blood reflect a state of compromised immunity which may also exacerbate complications associated with portal hypertension, such as increased susceptibility to infection.

11. mTORC2 as a mediator of lipopolysaccharide-driven lipid metabolism alterations on mouse hepatocytes

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Introduction: Metabolic dysfunction-associated steatotic liver disease (MASLD) is a highly prevalent chronic liver condition with varying stages ranging from simple steatosis to more severe cirrhosis. Robust evidence indicates that intestinal dysbiosis and gut-derived lipopolysaccharides (LPS) play critical roles in MASLD development and progression, exacerbating lipid deposition, inflammation and liver injury. Nonetheless, the exact cellular and molecular actors remain to be identified. The canonical mechanistic target of rapamycin complex 2 (mTORC2)-protein kinase B (PKB/Akt) signaling pathway, a major activator of liver de novo lipogenesis (DNL) and lipid deposition, was shown to be activated by LPS in macrophages. However, it is unknown whether mTORC2-Akt signaling is activated and mediates LPS effects on hepatocytes. Thus, this study aims to investigate the potential implications of hepatocyte mTORC2 as a mediator of pathogenic LPS effects in the liver.

Methods: Animal procedures were conducted in compliance with ethical regulations for animal testing approved by CEUA-ICB/USP (607118112). Male C57BL6/J mice (n = 4–5 from each group) bearing (L-RicKO) or not (L-RicWT) hepatocyte-specific deficiency of Rictor, an essential mTORC2 component in hepatocytes, were injected intraperitoneally with LPS (0.1 or 1 mg/kg) or vehicle (PBS) for 7 days and evaluated for body and tissue weight, serum parameters and hepatic intracellular signaling.

Results: When compared to controls, chronic administration of LPS increased liver mTORC2 activity as evidenced by increased hepatic content of Akt phosphorylated (p) at Ser473 in L-RicWT. Besides, LPS increased serum triacylglycerol levels, liver mass and hepatic contents of interleukin (IL)-1β and DNL-related enzymes acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS) and stearoyl-CoA desaturase-1 (SCD1) in L-RicWT. Hepatocyte mTORC2 deficiency (L-RicKO) attenuated all LPS effects mentioned above.

Discussion/Conclusion: Taken together, our findings indicate that hepatocyte mTORC2-Akt signaling is an important mediator of LPS-driven disease progression. Funding: FAPESP (2020/04159-8; 2021/14419-0; 2024/063448)

12. The modulation of the gut microbiota in association with low caloric diet in the treatment of the NASH in obese patients

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Introduction: This study assessed the efficacy of association between usual therapies, probiotic and low caloric diet in modifying of the liver function and effects on hepatic steatofibrosis.

Methods: We studied 42 patients with NASH and obesity. We excluded patients with viral or autoimmune hepatitis, genetic liver disease, diabetes mellitus or drug abuse. Group A was composed of 18 cases which received UDCA (13–15 mg/kg/day) in association with probiotics (which contain Lactobacillus acidophilus, Bifidobacterium infantis and Enterococcus faecium, 20 billion colony forming unit/day). The B group consist of 24 cases, treated with UDCA 13–15 mg/kg/day or combined therapy with UDCA and vitamin E (400 IU twice a day). We evaluated the liver function tests, serum lipids, TNF-alpha, serum ferritin and BMI at baseline, after 2, 4 and 12 months. We evaluated the steatosis and fibrosis stages with non-invasive testing, but in a few cases liver biopsy was necessary. FAST Score and FIB-4 Index were calculated.

Results: In whole group, 37 patients had high level of serum aminotransferase and the lipid profile was: 13 cases with hypercholesterolemia, 7 cases with hypertriglyceridemia and 11 with both. In group A, mean value of serum ALT decreased from 89.19 + 22.7 U/l at baseline, to 52.12 + 16.8 U/l at 2 months. In B group, serum ALT was moderately reduced (in mean with 19.3 + 7.2 U/l) after 2 months and the cholesterol level was significantly improved only in 5 cases (41.67%). In the A group the mean values of cholesterol, HDL and TNF-alpha were more decreased comparative with B group. After four months the normalisation rates of ALT was 88.89% in the A group and 73.33% in B group. The steatosis grade improved in 83.34% of patients in the A group and in 70.84% in B group. Also, the fibrosis score was more reduced in the A group. Multivariate analysis showed that the BMI > 32 kg/m2 and high values of serum ALT were associated with the steatosis grade. High levels of TNF-alpha and the ferritin values was associated with fibrosis score. Patients which associated UDCA therapy with probiotics and low caloric diet, had a better and quickly response.

Discussion/Conclusion: The combination of UDCA and probiotics significantly improves aminotransferase levels, HDL and steatosis grade. TNF-alpha level was correlated with the severity of NASH.

13. Drug-mediated transcriptional readthrough of HBV RNAs triggers antiviral effects in infected hepatocytes

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Introduction: Current treatments against chronic hepatitis B (CHB) infection, albeit efficient in maintaining the infection under control, fail to completely eradicate hepatitis B virus (HBV). As a consequence, treated CHB patients remain at high risk of developing severe liver diseases, including hepatocellular carcinoma. There is thus a compelling need to design more efficient novel therapeutic strategies to achieve a functional cure. HBV persistence is due to its minichromosome, the so-called covalently closed circular (ccc)DNA, which associates with host and viral factors to adopt a stable episomal structure in the nucleus of infected hepatocytes. cccDNA serves as the template for the transcription by the host RNA polymerase II of the six main viral transcripts. These include the pregenomic RNA, which is reverse-transcribed by the viral polymerase while being encapsidated to generate new infectious particles. As host transcripts, a series of co-transcriptional processing participate in HBV RNAs maturation and contribute to HBV replication. These include polyadenylation of their 3' end extremity, which is initiated by the recognition of a polyadenylation signal (cPAS) that is common to all viral transcripts and that is linked to transcription termination. We previously identified a heterogeneity of the 3' end extremity of HBV RNAs with a significant proportion of transcripts showing a transcriptional readthrough and stopping at a distal PAS. We demonstrated that this transcriptional readthrough destabilises HBV RNAs and is associated with a lower replication rate (Chapus, Giraud, et al. J. Hepatol. 2024). This study thus functionally linked HBV transcription termination and replication opening the door to the usage of novel compounds to trigger this transcriptional readthrough. Here, we target HBV RNA transcriptional termination via the termination factor CPSF3, which favours the recognition of proximal PAS. JTE-607, which was previously described as an anti-inflammatory drug, inhibits this factor and showed anti-proliferative effects in cancer cell lines by inducing transcriptional readthrough on CPSF3-regulated genes. This prompted us to determine whether JTE-607 treatment of HBV-infected HepG2-NTCP cells also induced HBV transcriptional readthrough and to investigate its effect on HBV replication

Methods: We performed 3'RACE-PCR combined with single molecule sequencing using the Oxford Nanopore Technology to appreciate the ability of JTE-607 treatment of HBV-infected HepG2-NTCP cells to induce HBV transcriptional readthrough. HBV RNAs were quantified by RT-qPCR and viral protein levels were assessed by Western blot and ELISA experiments. Finally, viral replication was assessed by the quantification of HBV viremia in the supernatant of HepG2-NTCP cells.

Results: First, we performed 3'RACE-PCR assays combined with Oxford Nanopore single molecule sequencing to demonstrate that, indeed, as for host transcripts, JTE-607 treatment induced HBV transcriptional readthrough. Furthermore, the quantification of viral parameters demonstrated that JTE-607 treatment significantly decreased the levels of secreted antigens, HBV viremia and intracellular HBsAg and HBc proteins. As expected, these changes were associated with the significantly decreased HBV RNA levels due to their destabilisation. These results were confirmed in primary human hepatocytes. Finally, transfection assays of HepG2-NTCP cells with HBx constructs carrying the 3'UTR with either wild-type or mutated cPAS showed that the effect of JTE-607 treatment on HBV RNAs was strictly dependent on its ability to induce HBV transcriptional readthrough.

Discussion/Conclusion: Altogether, these data demonstrated that HBV transcriptional termination might represent a new target for emerging antiviral strategies.

14. Reactive cholangiocyte-derived ORM2 drives a pathogenic modulation of the injured biliary niche through macrophage reprogramming

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Introduction: Injured or reactive biliary epithelial cells participate in most chronic liver injuries in a process referred to as ductular reaction, which involves multicellular interactions with marked local infiltration of macrophages and fibrogenic cell activation. The direct roles of biliary epithelial cells in shaping their cellular niche remain unknown. We aimed at investigating the effects of biliary epithelial cell-derived acute phase response protein orosomucoid 2 (ORM2) in shaping monocyte/macrophage response to liver injury.

Methods: Transcriptome datasets from human and mouse livers were used, results were confirmed with multiplex immunofluorescence. A multicellular biliary-niche-on-a-chip derived from primary liver and blood cells (wild-type, Mdr2-/- mice) was established to model ductular reaction. Human blood cells collected from healthy donors and intrahepatic cholangiocyte organoids derived from normal and cirrhotic liver patients were used.

Results: Our transcriptome dataset and multiplex immunofluorescence analyses indicated a previously unrecognized involvement of the acute phase response protein ORM2 in ductular reactions in both human and mouse livers. ORM2 gene expression was increased in biliatresone-, bile acid- and acetaminophen-challenged cholangiocytes. Cholangiocyte-derived ORM2 induced unique transcriptome changes and functional adaptation of liver macrophages. ORM2-activated macrophages exacerbated cholangiocyte cell stress and Orm2 expression, but also promoted fibrogenic hepatic stellate cell activation. Mechanistically, ORM2 effects were mediated by an inositol 1,4,5-trisphosphate receptor type 2 (ITPR2)-dependent calcium pathway.

Discussion/Conclusion: This study reveals a paracrine communication circuit during ductular reaction, in which reactive cholangiocyte-derived ORM2 reprograms liver macrophages, participating in a pathogenic remodeling of the immune biliary niche.

15. Routine screening of hepatitis C in an antenatal setting – Single-center experience in the United Kingdom

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Introduction: Hepatitis C virus (HCV) remains a major health concern globally accounting for approximately 290,000 deaths and 1.5 million new infections annually. The United Kingdom (UK) has committed to the World Health Organization's viral hepatitis elimination targets which aim to support the elimination of viral hepatitis as a public health threat by the year 2030. New case finding strategies, such as antenatal testing, may be needed to achieve the target. Universal opt-out HCV testing is not currently recommended as part of antenatal screening in the UK because of low risk of mother to child transmission, lack of trial evidence on the efficacy of treatment for HCV in pregnancy, and treatment not being recommended for the babies. Few areas in United Kingdom being an area with high HCV prevalence, we rolled out a screening program for HCV testing in the antenatal clinic to evaluate the feasibility of this case finding strategy.

Methods: After discussion with the antenatal team, we added HCV antibody test to the antenatal screening panel which included HIV, Hepatitis B and syphilis serology. This panel can be ordered on our electronic system as a 'one touch' request. We agreed with the laboratory that any positive HCV Ab tests would be reflexly tested for HCV PCR. We interrogated our electronic database to obtain the following: number of pregnancies, demographics, uptake of the new antenatal screening panel, number of positive HCV Ab/PCR tests and outcome of treatment of PCR positive cases.

Results: Between October 2022 to March 2024 (17 months), there were 9383 pregnancies registered with the antenatal service (5031 [53.6%] Asians, 3051 [32.5%] Caucasians, 605 [6.4%] Black ethnicity,188 [2%] mixed population and 494 [5.2%] others). 9066 pregnancies (96.6%) were screened for HCV antibody. 57 (0.62%) tested positive for HCV antibody (39 [68.4%] Asians, 13 [22%] Caucasians, 5 [8.7%] others). 7/57 (12.2%) were HCV PCR positive (5 [71.4%] Asians; 2 [28.6%] Caucasians). 50/57 were HCV antibody positive but PCR negative, 11 (22%) of whom having received treatment for HCV prior to their pregnancies and 39 (78%) having spontaneously cleared HCV. 2/7 of the HCV PCR positive cases have been started on treatment, the remaining 5 awaiting to be treated post-partum

Discussion/Conclusion: Screening for HCV was very successful in the antenatal setting, 97% taking up the test. The prevalence of HCV Ab (0.62%) in our population is reflective of the UK prevalence. The spontaneous rate of HCV clearance (78%) was higher than expected. Based on these figures, we have shown that antenatal case finding is feasible and will help towards HCV elimination in the UK, hopefully before the WHO deadline of 2030. We would recommend national roll out of case finding in the antenatal setting.

16. Evaluation of fatigue and insomnia in patients with primary biliary cholangitis treated with ursodeoxycholic acid

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Introduction: Chronic fatigue and insomnia are common symptoms observed in patients with primary biliary cholangitis (PBC) throughout the course of their disease. Ursodeoxycholic acid (UDCA), the first-line treatment for PBC, slows the progression to cirrhosis and its related complications. However, its impact on associated symptoms such as fatigue and insomnia remains incompletely characterized. The aim of this study was to evaluate the effect of UDCA on improving fatigue and insomnia in patients with PBC.

Methods: A retrospective study was conducted among patients diagnosed with PBC from January 2001 to December 2023. The biochemical response to UDCA was assessed according to the Paris II criteria. The patients were contacted by phone to complete the symptoms questionnaires. Fatigue was evaluated using the 11-question Fatigue domain of the PBC-40 scale, with each item scoring from 1 to 5 (total range 11 to 55). Sleep disturbances were assessed using the Insomnia Severity Index (ISI), comprising five items scored from 0 to 4; scores of 15 to 21 were considered moderate insomnia and scores ≥ 22 were considered severe insomnia. Patients with comorbidities capable of influencing fatigue (chronic respiratory disease, cardiac disease, severe anemia) were excluded. The associations between variables were evaluated using Pearson's chi-square test, with statistical significance defined as $p < 0.05$.

Results: Fifty-seven patients with PBC were initially included; five were excluded due to severe comorbidities and 11 could not be reached, leaving 41 patients in the final analysis. Of these, 33 patients (80.5%) reported chronic fatigue and 26 (63.4%) also had insomnia. The

mean age at diagnosis among patients with chronic fatigue was 60 years. Only one patient was male. All patients received UDCA at a dose of 13–15 mg/kg/day. A biochemical response was achieved in 20 patients (60.6%). The mean fatigue and ISI scores were 19.2 and 7.2 in responders, compared to 36.2 and 17.8 in non-responders. A significant negative correlation was observed between the biochemical response and both fatigue ($p = 0.01$) and insomnia ($p < 0.001$). Among the responders, cirrhosis ($p = 0.039$) and female sex ($p = 0.01$) were associated with persistent fatigue, while cirrhosis alone ($p = 0.02$) was associated with persistent insomnia. Multivariate analysis revealed that age, menopausal status, seronegative PBC, delay in treatment initiation, and duration of treatment were not significant predictors of persistent fatigue or insomnia in patients who achieved a biochemical response.

Discussion/Conclusion: In this study, the UDCA response was associated with a reduction in fatigue and insomnia among patients with PBC. The severity of liver disease and female sex were significantly associated with persistent fatigue, while only cirrhosis was associated with persistent insomnia. These findings underscore the importance of identifying and addressing the factors that contribute to the persistence of symptoms despite adequate biochemical control.

17. Neutrophil-to-lymphocyte ratio (NLR) as a predictive factor for response to ursodeoxycholic acid in primary biliary cholangitis: A retrospective study

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Introduction: Primary biliary cholangitis (PBC) is a chronic and progressive autoimmune liver disease characterized by inflammation and destruction of the intrahepatic bile ducts. Ursodeoxycholic acid (UDCA) is the first-line treatment for PBC, demonstrating its efficacy in improving liver tests and preventing disease progression. However, the response to treatment varies between patients and the underlying mechanisms of this variability remain unclear. The neutrophil-lymphocyte ratio (NLR), a simple and inexpensive biomarker, has recently emerged as a potential indicator of systemic inflammation in various diseases. This study aims to investigate the association between baseline NLR and response to UDCA treatment in patients with PBC.

Methods: We conducted a retrospective study that included patients followed for PBC in our center from 2001 to 2023. All patients treated with UDCA (13–15 mg/kg/day) were included. Demographic, clinical, biological, and radiological data were collected from patient records. Response to treatment was evaluated after 12 months based on alkaline phosphatase (ALP) levels, defined as ≤ 1.5 times the upper limit of normal. NLR was calculated at baseline as the ratio of neutrophils to lymphocytes. A multivariate logistic regression analysis was performed to assess the association between baseline NLR and response to UDCA at 12 months.

Results: A total of 57 patients with PBC were included in the study. The mean age of the patients was 57.6 years, ranging from 20 to 83 years. Only three patients were male. Most of the patients (80.8%) were symptomatic at the time of diagnosis, with the most common symptoms: fatigue (33.3%) and pruritus (19.4%). Cirrhosis was present in 31.6% of the patients. Six patients (16.7%) had seronegative PBC. Additionally, autoimmune diseases were associated with PBC in 42.1% of patients. Multivariate regression analysis revealed that a higher baseline NLR was significantly associated with a non-response to UDCA treatment after 12 months, with an odds ratio (OR) of 1.480 (95% CI: 1.066–1.761; $p = 0.002$). These results suggest that elevated NLR at baseline is an independent risk factor for non-response to UDCA.

Discussion/Conclusion: This study suggests that NLR could be a useful biomarker to predict the response to UDCA in PBC. An elevated NLR at baseline is associated with a non-response to treatment, which may have clinical implications for the management of patients with PBC by identifying those who may require closer monitoring or alternative therapeutic strategies.

18. Stem-like TCF-1+ subsets shape the T cell response in chronic viral hepatitis

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Introduction: Virus-specific CD8+ T cells are major effector cells in determining the outcome and pathogenesis in viral hepatitis. In both acute-resolving and chronic viral hepatitis, the virus-specific T cell response is shaped by heterogeneous subsets, including stem-like, effector, and terminally differentiated cells. In chronic infections like hepatitis B (HBV) and C (HCV), distinct memory-like and exhausted T cell subsets emerge. These memory-like T cells, marked by IL-7 receptor alpha-chain (CD127) and TCF-1 expression, exhibit stem-like properties, allowing for sustained immunity and a heterogeneous T cell response. However, identifying stem-like/progenitor cells within these populations and comparing them across chronic infections remains challenging. Based on the hypothesis that stem-like subsets drive virus-specific immunity in chronic viral hepatitis, we deeply profiled antigen-specific TCF1+ CD8+ T cells in various chronic hepatitis infections, notably chronic HBV and HCV infections, in order to identify correlates of T cell stemness.

Methods: Using spectral and conventional flow cytometry and single cell transcriptomics, we identified heterogeneous profiles of HBV- and HCV-specific TCF-1+ CD8+ T cells in chronically infected patients.

Results: In particular, differences were observed in the expression of the transcription factor EOMES, the cell surface glycoprotein CD38, and the immune checkpoint receptor PD-1. These distinct virus-specific TCF-1+ CD8+ T cells were linked to different programs of CD8+ T cell dysfunction in chronic viral hepatitis including T cell exhaustion and beyond.

Discussion/Conclusion: These results highlight the need to explore further the heterogeneous nature of T cell responses in chronic infections and raise questions about the involvement of stem-like cells in immune alterations. Understanding these mechanisms could pave the way for novel therapeutic strategies aimed at redirecting or restoring T cell function in chronic viral hepatitis.

19. Inhibition of adipocyte triglyceride lipase (ATGL) reduces fibrosis and portal hypertension via a CD8+ T-cell dependant manner in murine CCl4 liver fibrosis

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Introduction: Immune cells orchestrate hepatic inflammation and fibrogenesis. Importantly, their intrinsic lipid metabolism is among their key phenotypic determinants in addition to the critical impact of lipotoxicity on hepatocellular injury. We investigated the role of lipolysis in liver fibrosis by studying adipocyte triglyceride lipase (ATGL) that cleaves triacyl glycerides

stored in lipid droplets. Hepatic ATGL deficiency promotes lipid accumulation but in turn, prevents lipotoxicity originating from lipolysis-derived intrahepatic free fatty acids (FFAs). Thus, targeting lipolysis via ATGL may represent a novel therapeutic approach in fibrosis.

Methods: Liver fibrosis was induced in male C57BL/6 mice by administration of carbon tetrachloride (CCl4; 3x/week, 2 ml/kg, oral gavage) for 8 weeks, while healthy control groups received olive oil (OO). Healthy and diseased mice were treated with an ATGL inhibitor (atglistatin, 2 mmol/kg), via oral gavage (2x/day for 4 weeks; 200 µmol/kg, n = 10), and diet supplementation (2 mmol/kg chow diet for 8 weeks, n = 10), whereas the respective control groups (n = 10 each) received olive oil as vehicle control under pair-fed conditions. Hepatic collagen proportionate area (CPA) was assessed histologically and portal pressure measured invasively, while fibrogenic and inflammatory gene expression were quantified by qPCR and bulk tissue RNA sequencing. Hepatic immune cells were profiled using flow cytometry.

Results: ATGL inhibition strongly improved features of liver fibrosis (CPA: 5.07 ± 1.16 vs. 3.09 ± 1.28%; $p < 0.0002$) and portal hypertension (7.94 ± 0.97 vs. 6.87 ± 0.62 mmHg; $p = 0{,}004$). This was accompanied by markedly reduced profibrogenic (Col1a1, Acta2, Tgfb1, Timp1) gene expression and reduced ALP (89.3 ± 11.4 vs. 77.4 ± 14.3; $p = 0.04$) in serum biochemistry. Intrahepatic lipid accumulation was confirmed by histology supporting sufficient hepatic ATGL inhibition by atglistatin. Moreover, atglistatin remarkably increased liver to body weight ratio (4.915 ± 0.48 vs. 5.97 ± 0.49%) and induced a proinflammatory transcriptional signature (Mcp1), suggesting inflammatory hepatic immune cell infiltration. In liver flow cytometry, this was predominantly reflected by a strongly increased abundance of CD3+ T-cells – further identified as CD8+ T-cells (14.32 ± 3.29% vs. 6.76 ± 1.58 of the CD45+ population). The role of hepatic CD8+ T-cells, promoted by atglistatin treatment, will be functionally dissected in vitro.

Discussion/Conclusion: Our data shows a protective role for atglistatin in a model of toxic liver fibrosis. The underlying potential mechanism is a profound alteration of the liver immune cell niche and points towards a novel role for ATGL-driven lipolysis in T cell biology during fibrosis. Ongoing transcriptome sequencing and cytokine profiling will allow deciphering of our findings on lipase-mediated immunoregulation in liver fibrosis.

20. Conversion of CD4+ T cells to functioning and epigenetically stable induced regulatory T cells in patients with primary biliary cholangitis

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Introduction: Primary biliary cholangitis (PBC) is a chronic, autoimmune liver disease. Regulatory T cells (Tregs) are a subset of CD4+ T lymphocytes whose activity is driven by the expression of a key transcription factor, Forkhead box P3 (FOXP3). A reduction in Treg frequency and functionality has been proposed as an underlying pathogenic mechanism of PBC. We aimed to characterise the natural Treg (nTreg) epigenetic profile in PBC patients. Additionally, we set out to induce functional and stable Tregs (SF-iTregs) from PBC-derived effector CD4 cells in vitro, via cyclin-dependent kinase (CDK8/19) inhibition.

Methods: CD4+ T cells were magnetically enriched from PBC patient peripheral blood mononuclear cells (PBMCs). These were activated by CD3+ activator antibodies only (without CD28 co-stimulation) and cultured with IL-2 and a CDK8/19 inhibitor. FoxP3, CTLA4 and Helios expression in SFiTregs was assessed via flow cytometry and bisulphite sequencing

pre- and post-activation in the presence of Th1 polarising cytokines. SFiTreg suppressive function was investigated by measuring CellTrace Violet dye-labelled effector T-cell proliferation when co-cultured with SFiTregs in varying ratios. nTreg and SFiTreg Foxp3 gene locus STAT5 binding, H3K27ac, and chromatin status were characterised by Chromatin immunoprecipitation followed by sequencing (ChIP-seq) and assay for transposase-accessible Chromatin sequencing (ATAC-seq).

Results: Deprivation of CD28 costimulation, with CDK8/19 inhibition in CD4 T cells induces stable, functional Tregs with DNA hypomethylation in Treg signature genes. In two weeks, our protocol generated a 100-fold expanded, > 95% pure FOXP3 expressing population. ATAC-seq and ChIP-seq confirmed Treg specific epigenetic changes in SFiTregs, with features comparable to those of nTreg. To replicate the inflamed PBC hepatic environment, we cultured nTregs and SFiTregs in Th1-conditioning media containing IL-12 and IFN-γ for 6 days and found SFiTregs had superior lineage stability compared to nTregs.

Discussion/Conclusion: We applied a novel methodology to generate abundant stable, functional induced regulatory T cells from PBC patient peripheral blood conventional CD4+T cells. This approach could facilitate the production of stable, functional induced Tregs from antigen-experienced disease-mediating T cells at scale, for application as a GMP cell-therapy in PBC.

21. Setting-up a biliary-niche-on-a-chip multicellular model by establishing a cholangiocyte organoid library for the study of liver ductular reaction

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Introduction: Ductular reaction (DR), characterized by cholangiocyte proliferation with inflammation and liver fibrosis, is a common hallmark of virtually all chronic liver diseases. Diverse cells are involved in DR and, therefore, we aimed at developing a versatile in vitro system for cellular crosstalk investigation. We previously reported on a perfusable, multicellular and primary cell-based liver-on-a-chip model mimicking the liver sinusoid microenvironment.

Methods: Intrahepatic cholangiocytes were isolated from wild-type (WT) and Mdr2-deficient (KO) mice by positive selection and expanded to generate 3-dimensional organoids. Bulk RNA sequencing and immunostaining confirmed cell identity. Mouse primary intrahepatic cholangiocytes, hepatic stellate cells, macrophages and endothelial cells were seeded into the biochip to assemble the biliary niche-on-a-chip (BoC). Fresh blood circulating immune cells were perfused. Immunostaining and RT-qPCR were performed on the cells adherent to the membrane, and the cells remaining in the perfusate were collected for flow cytometry.

Results: Primary cholangiocytes, WT and KO mICOs were successfully isolated and cultured in conventional culture conditions and into the perfusable, primary cell-based biochip to create a BoC. Gene expression analysis and flow cytometry revealed an increased inflammation in the BoC treated with the cholangiotoxin biliatresone. Transient gene expression interference in mICOs or macrophages modulated the BoC inflammatory response.

Discussion/Conclusion: To circumvent the technical limitations of primary cholangiocyte isolation, we generated mouse intrahepatic cholangiocyte organoids (mICOs) from healthy and a DR mouse model and optimized our seeding protocol, to establish a biliary niche-on-a-chip (BoC) model. Mouse ICOs represent a valuable cell source for in vitro DR studies. The multicellular BoC model may be used to study the crosstalk between different cell types involved in DR, allowing to dissect the relevance of cell-type specific molecular pathways for liver biology and pathogenesis ex vivo. Ongoing efforts aim at developing a similar BoC using human cells.

22. Taurine reprograms hepatocyte metabolism and its plasma levels are inversely associated with the progression of MASLD

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Introduction: Liver diseases, particularly those related to metabolic disorders like metabolic dysfunction-associated steatotic liver disease (MASLD), pose a significant global health challenge. Identifying reliable biomarkers for early diagnosis and understanding metabolic changes is essential for effective management. Taurine, a semi-essential micronutrient synthesized in the liver, declines with aging. Hepatocytes, the primary cells for fat accumulation and metabolism in the liver, interact with immune cells, influencing the progression of MASLD. This study investigates taurine as a potential biomarker for MASLD and explores its regulatory role in hepatic metabolism-related to this condition.

Methods: The study examined polar metabolomics in EDTA-plasma from age-matched healthy subjects (n = 5) and MASLD patients at stages F0–F2 (n = 10) and F3–F4 (n = 10). Primary mouse hepatocytes were treated with 1 mM taurine for 24 hours, followed by 0.3 mM free fatty acids (FFA) (1:1, palmitic and oleic acid) for another 24 hours. Neutrophils were isolated using negative selection microbeads, while bone marrow-derived macrophages were obtained with anti-F4/80 microbeads. Cellular bioenergetics were assessed using 10 mM glucose, 2 mM pyruvate, and 1 mM glutamine, with fuel preference evaluated via the XFe Seahorse analyzer.

Results: Levels of taurine decreased 2-fold in MASLD patients at stages F0–F2 ($p < 0.05$), and further decreased by 2.5-fold from F0–F2 to F3–F4 ($p < 0.05$). This decline in taurine levels correlates with the progression of chronic liver disease severity toward fibrosis and cirrhosis, as confirmed in a validation cohort that showed a 50% reduction in taurine between healthy individuals (n = 29) and those with compensated cirrhosis (n = 43, $p < 0.0001$). Since taurine is primarily synthesized in the liver, we aim to examine its role and mechanism in steatotic hepatocytes. Our findings indicate that taurine reduces fatty acid uptake and lipogenesis by downregulating CD36, FATP2, and FATP5, maintaining its effects even with FFA treatment. Taurine supplementation halved lipid accumulation ($p < 0.001$) without increasing fatty acid oxidation, suggesting its benefits arise from reduced FFA uptake and lipogenesis. FFA-exposed steatotic hepatocytes preferred glucose over fatty acids for ATP production, but taurine decreased glucose-dependent oxidative phosphorylation by 2-fold ($p < 0.001$). Additionally, taurine reduced the attraction of neutrophils and bone marrow-derived macrophages (BMDMs) to steatotic hepatocytes by 1.5-fold and 2-fold, respectively ($p < 0.001$), indicating its potential to mitigate liver inflammation.

Discussion/Conclusion: Taurine serves as an early biomarker for liver disease, showing significant decreases in plasma levels at different disease stages. Additionally, taurine supplementation reduces fatty acid uptake and lipid accumulation while modulating inflammatory responses, highlighting its potential therapeutic benefits in managing liver conditions.

23. HIF-1α/LTBP2 axis activate HSCs to promote liver fibrosis by interacting with LOXL1 via the ERK pathway

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Introduction: LTBP2 is a multi-domain exocrine protein present in the ECM and associated with the fibrosis of numerous organs. Nevertheless, the function of LTBP2 in liver fibrosis remains poorly comprehended. This study aims to investigate the role and mechanism of LTBP2 in liver fibrosis.

Methods: The expression of LTBP2 was assessed via public databases and validated by immunohistochemistry. The migration and proliferation of HSCs were determined through wound healing, CCK-8 and cell cycle assays. Epithelial-mesenchymal transition and MAPK pathway markers were evaluated using Western blotting. Chromatin immunoprecipitation was employed to detect DNA-protein interactions. The interaction between LTBP2 and LOXL1 was discovered through database prediction and molecular docking. RNA sequencing was utilized to identify the pathways of LTBP2.

Results: The expression of LTBP2 is positively correlated with the severity of liver fibrosis and significantly increased in fibrotic liver tissues in both human and mice. More importantly, in vivo LTBP2 inhibition significantly alleviates CCL4-induced liver fibrosis by reducing collagen accumulation and HSCs activation in mice. In vitro, knockdown or overexpression of LTBP2 inhibits or enhances the proliferation, migration and expression of fibrotic genes in LX-2 cells. Then, HIF-1α promoted LTBP2 expression by directly binding to the LTBP2 promoter. Moreover, western blot results showed that LTBP2 promoted HSCs activation and EMT. Mechanically, LTBP2 interacting with LOXL1 via ERK1/2 signaling pathway to promote the EMT and HSCs activation.

Discussion/Conclusion: HIF-1α/LTBP2 axis promoted HSCs activation and EMT by interacting with LOXL1 via ERK signaling and may be a potential target in liver fibrosis.

24. Performance of prognostic scores for biliary cholangitis: A comparative study

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Introduction: Response to ursodeoxycholic acid (AUDC) is a prognostic factor in primary biliary cholangitis (PBC). This response can be assessed after 12 months of treatment using simple qualitative criteria (Barcelona, Paris-1, Paris-2, Rotterdam and Toronto criteria) or quantitative scores (GLOBE score, UK-PBC score). The aim of the study was to compare the performance of qualitative and quantitative scores in assessing the prognosis of a cohort of BPC patients treated with AUDC.

Methods: monocentric, retrospective, cross-sectional, analytical study carried out in the hepato-gastroenterology department, over a 20-year period from March 2003 to March 2023, including all patients with PBC. Simple qualitative (Barcelona, Paris-1, Paris-2, Rotterdam and Toronto criteria) and quantitative (GLOBE score, UK-PBC score) prognostic scores were calculated for all patients at 12 months of treatment. Events were defined as the need for liver transplantation, death or cirrhotic decompensation. Survival was assessed according to Kaplan Meier. The sensitivity and specificity of each score were evaluated using ROC curves.

Results: In our series, 66 patients were colonized, with a mean age of 52 years [31.78 years] and a sex ratio (M/F) of 0.02. Twenty-five patients (36%) had cirrhosis-stage PBC, classified as Child A, B and C in 52%, 36% and 12% of cases respectively. The mean duration of follow-up was 8.7 years [1–29 years]. During this follow-up, about half the patients (n = 33, 45.8%) developed an AE. Median event-free survival was 15 years. Overall event-free survival at 5 years was 88%. Using the Kaplan-Meier method, all prognostic scores showed significantly different survival between responders and non-responders. To identify the score with the best predictive performance for survival, ROC curve analysis showed a better discriminatory ability of the UK-PBC score and the GLOBE score. The ASCROCs for predicting events at 5 years were as follows: UK-PBC score: 0.885; GLOBE score: 0.863; Paris 1: 0.820; Paris-2: 0.762; Barcelona: 0.631; Toronto: 0.797 and Rotterdam: 0.838.

Discussion/Conclusion: In our study, the UK-PBC and GLOBE scores were the best predictors of event-free survival in PBC.

25. Paneth cells regulate intestinal lymphangiogenesis and lipid metabolism in metabolic dysfunction-associated steatotic liver disease

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Introduction: Paneth cells (PC) are involved in the regulation of intestinal microbiota, and their function in intestinal lymph- and angiogenesis has been recently described. Uptake of dietary lipids is largely dependent on intestinal lymphatic vessels, and impairments in their density or function may result in significant depletion of lipids in the liver. In this study, we assessed the effects of PC deficiency in a model of metabolic dysfunction associated steatotic liver disease (MASLD).

Methods: We induced functional inactivation of PC in male and female mice (Sox9lox/loxVil-CreERT2) by intraperitoneal injection of 1 mg/day of tamoxifen for three consecutive days. One week later, all mice, PC-deficient, and control littermates were randomly subjected to a high sucrose/fructose – high fat (60% lard) (HFD) or standard (SD) diet for 16 weeks. Intraepithelial leakage of FITC-albumin was measured in vivo using laser-probe endomicroscopy. Several tissues were harvested for histological examination, molecular biology analyses, and imaging mass cytometry (IMC). Fecal samples were collected at both the start and end of the experiment to assess intestinal dysbiosis.

Results: All mice fed HFD gained weight compared to SD, but the weight gain in PC-deficient-HFD was significantly lower than in HFD-controls. Consistent with these findings, the severity of hepatic steatosis was significantly lower in PC-deficient-HFD compared to HFD-control mice. IMC of liver tissue revealed lower levels of collagen deposition and steatosis associated with fewer macrophages and neutrophils activation/infiltration in PC-deficient-HFD than in control-HFD mice. In vivo, microscopy showed a lower disruption of intestinal vascular barriers in PC-deficient-HFD than in HFD-control mice. The intestinal expression of distinct lymphangiogenic genes (VEGFC, VEGFR3, Prox1) was reduced in the PC-deficient HFD or SD. The alpha diversity of the fecal microbiota showed no significant differences based on gender or PC deficiency in the SD groups. Nevertheless, the beta diversity showed significant differences in PC-deficient fed SD after 16 weeks. Furthermore, we found significant alpha- and -beta diversity associated with decreased Bacteroidota and increased Firmicutes in HFD and PC-deficient mice.

Discussion/Conclusion: PC deficiency attenuated the severity of steatosis and fibrosis in mice, suggesting their regulatory role on intestinal lymphatics contributing to the pathogenesis of MASLD. Thus, Paneth cells may represent a novel target for intervention in MASLD

26. Transcriptomic profiling of vascular invasion in hepatocellular carcinoma: A bioinformatics approach

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Introduction: Hepatocellular carcinoma (HCC) exhibits significant heterogeneity in terms of clinical outcomes and therapeutic responses, posing challenges in predicting disease progression and determining optimal treatment strategies. The prognosis of the disease is typically predicted based on liver tumor size at diagnosis, vascular invasion, and tumor proliferation markers.

Methods: In this retrospective study, we examined patient records from the Liver Hepatocellular Carcinoma (LIHC) dataset of The Cancer Genome Atlas (TCGA) to identify genetic alterations associated with vascular invasion in HCC (n = 377 patients).

Results: Of the cases analyzed, 27.9% (n = 94 patients) exhibited microvascular invasion, while 4.51% (n = 17 patients) showed macrovascular invasion. No statistically significant changes in mRNA expression were found between patients with no vascular invasion and those with microvascular invasion. However, significant differential expression was observed between microvascular invasion and macrovascular invasion cases for the genes ODAM (q-value = 0.0259) and FMO6P (q-value = 0.0259), with higher expression levels in the microvascular invasion group. When comparing patients with no vascular invasion to those with macrovascular invasion, 16 genes were found to be significantly differentially expressed (q-value < 0.05). Among these, NALF2 (q-value = 0.0116), SLC7A1 (q-value = 0.0116), GPD1L (q-value = 0.0432), and CCDC198 (q-value = 0.0456) were significantly more highly expressed in the macrovascular invasion group. DNA methylation analysis revealed that out of 154 proteins analyzed, 26 proteins exhibited significantly higher methylation levels in the macrovascular invasion group. Notably, SLC7A1 and GPD1L displayed lower methylation levels. Functional enrichment analysis revealed that the differentially expressed genes were associated with several pathways, including the Insulin signaling pathway, JAK-STAT signaling pathway, Type II diabetes mellitus, FOXO-mediated transcription of oxidative stress, metabolism, Interleukin-6 family signaling, and regulation of beta-cell development.

Discussion/Conclusion: Our findings contribute to a deeper understanding of the molecular landscape of HCC and emphasize the importance of considering vascular invasion in clinical decision-making and treatment strategies

27. The hepatitis C virus (HCV) affects ribosomal RNA methylation and depends on the methyltransferase activity of fibrillarin for translation

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Introduction: Ribosomes are central to cell translation. Their intrinsic plasticity relies on chemical modifications of ribosomal RNAs (rRNAs), events known to modulate translation. 2'-O-methylation (2'-O-Me) is rRNA's most abundant alteration. 2'-O-Me rates in defined rRNA positions correlate with, or even condition, outcomes in leukemia and breast cancer. RNA viruses may be considered as ribosomal parasites for persistence, as being incapable to encode them. Thus, liver oncogenic viruses such as HCV are relevant models for the study of 2'-O-Me. In this context, we considered the mutual impacts between 2'-O-Me and HCV, which remains a useful tool to study hepatic pathogenesis, and of which replication intensely relies on cellular translation due to its (+)strand RNA status.

Methods: For better clinical relevance, in vitro infections were done with the HCV genotype 1 strain TNcc-HI. 2'-O-Me scores were measured by RiboMeth-seq, a authoritative quantitative approach, in in vitro and patients samples, that were obtained from one local (Lyon U. Hospital) and one national (French Liver Biobank) chronic liver disease (CLD) cohort. These two cohorts consider all main etiologies and fibrosis stages (n = 90 cases) of CLD, and propose normal livers (n = 16) as references. RNAi knock-down of the rRNA 2'-O-methyltransferase fibrillarin (FBL) was achieved to decrease the global rate of 2'-O-Me to study the implication of 2'-O-Me in HCV replication. Results were confirmed by mutating the 2'-O-Me domain of FBL in inducible hepatocytic lines.

Results: In vitro, 2'-O-Me was decreased on two rRNA positions in HCV-infected cells. While the global impairment of 2'-O-Me decreased by ≈50% HCV RNA levels, it triggered a near-disappearance of HCV proteins core and NS5A, suggesting i) the relevance of 2'-O-Me for viral replication, and ii) a potential new and likely impactful layer of regulation for viral translation. Of note with respect to the understanding of chronic liver disease pathogenesis, the virally triggered clinical 2'-O-Me profile persisted after viral clearance. Strikingly, these two HCV-altered rRNA spots in vitro were also decreased in human biopsies obtained from all main etiologies and fibrosis stages. Thus, as demonstrated in other pathologies, the regulation of 2'-O-Me could be impactful in hepatic pathogenesis.

Discussion/Conclusion: These datasets indicate the salient interest of ribosome chemistry regulation in hepatic pathology. They also provide preliminary insight on the identification of processes leading to these molecular alterations in a causal fashion, using e.g. viral infection.

28. Impact of HLA class I restriction on CD8+ T cell responses in HCV infection

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Introduction: Chronic hepatitis C virus infection (HCV) and coinfection with HCV and human immunodeficiency virus (HIV) are still global health problems. CD8+ T cells represent the major antiviral effector cells and are associated with spontaneous viral clearance of HCV infection as well as viral suppression in HIV-infected patients. However, chronic antigen exposure also leads to T cell exhaustion characterized by an altered phenotypic, metabolic, epigenetic and functional program of the exhausted T cell which in turn favors viral persistence. Recognition of viral epitopes by CD8+ T cells needs epitope presentation by HLA class I molecules, and some HLA alleles like HLA-B*2705 or HLA-B*1501 have been associated with spontaneous viral clearance. However, the impact of the HLA restriction on CD8+ T cell differentiation remains largely unknown. In this study we aim to identify phenotypic, functional and transcriptional determinants of CD8+ T cell differentiation in chronic HCV infection and HCV/HIV-coinfection with special interest in the impact of HLA restriction on CD8+ T cell phenotype.

Methods: Using in vitro expansion followed by flow cytometric analysis of cytokine production and peptide-loaded MHC class I tetramer staining, 174 patients positive for at least one of the HLA class I alleles, HLA-A*0101, HLA-A*0201, HLA-A*0301, HLA-B*0702, HLA-B*0801, HLA-B*1501 and HLA-B*5101, with chronic HCV (genotype [GT] 1 or 3) infection were screened for CD8+ T cell responses recognizing previously published or in silico-predicted HCV epitopes. CD8+ T cells specific for epitopes detectable in the screening cohort were further isolated using peptide-loaded MHC class I tetramer-based enrichment strategy and characterized by high-dimensional flow cytometry in patients with chronic HCV infection as well as resolved HCV infection after antiviral therapy or spontaneous clearance. Finally, the impact of HIV coinfection on the phenotype of HCV-specific CD8+ T cells in HCV/HIV-coinfected patients compared to HCV monoinfected patients was analyzed.

Results: Out of the 22 epitopes derived from HCV GT 1a/b or GT3a used for epitope screening, 5 epitopes showed reliable responses in the majority of patients positive for the respective HLA class I allele. CD8+ T cells from chronically HCV-infected patients recognizing these confirmed epitopes (HLA-A*0101 NS31436 GT1a/b, HLA-A*0201 NS5b2594 GT3a, HLA-B*1501 NS5b2540 and E2693 GT1a/b and HLA-B*5101 NS31373 GT1a/b) expressed CD38 as a marker of antigen recognition, pointing towards chronic antigen stimulation. CD38+ CD8+ T cells

from patients with chronic HCV infection specific for the different epitopes differed phenotypically e.g. in the expression of PD-1 with higher expression in CD8+ T cells specific for the HLA-B*1501- and the HLA-B*5101-restricted epitopes compared to the HLA-A*0101-restricted epitope. After therapeutic clearance of the chronic HCV infection, however, most differences observed between CD8+ T cells specific for the different HLA class I-restricted epitopes were diminished. Furthermore, after spontaneous resolution of HCV infection, CD8+ T cell responses restricted to HLA class I alleles HLA-B*1501 and HLA-B*2705 associated with high rates of spontaneous clearance showed higher magnitude and activation compared to CD8+ T cells restricted by HLA-A*0201 even after viral clearance. As CD8+ T cells require additional stimulation from CD4+ T cells in order to gain full functionality, HCV-specific CD8+ T cell responses from patients with HCV/HIV coinfection were studied. Interestingly, we observed no marked differences in all markers analyzed in CD8+ T cells specific for HLA-A*0201-restricted epitopes, indicating a minor impact of HIV coinfection on these HCV-specific CD8+ T cell responses.

Discussion/Conclusion: In our comprehensive study of CD8+ T cell responses from chronic and spontaneously cleared HCV infection, out data points towards a clear impact of the HLA-restriction and against a dominant effect of HIV coinfection on CD8+ T cell phenotype. The impact of viral mutations/viral escape on CD8+ T cell responses against differently restricted CD8+ T cell epitopes is currently being investigated. Further studies will be needed to clarify e.g. the role of different CD4+ counts on CD8+ T cell responses in HIV/HCV coinfected patients and the exact impact of HLA class I-types on clinical outcome of HCV infection.

29. Precision-cut liver slices as a pre-clinical model for the evaluation of host-targeting agents against hepatitis B virus and hepatitis delta virus infection

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Introduction: The development of new therapeutic strategies against hepatitis B virus (HBV) and hepatitis delta virus (HDV) is of high priority to cure these infections. However, pre-clinical evaluation of host-targeting agents (HTAs) with available in vitro and in vivo models remains challenging. Precision-cut liver slices (PCLS) represent a promising approach, as it preserves the multi-cellular architecture of the human liver, while offering the practicality of an in vitro system. Therefore, we aimed to: 1) assess PCLS permissiveness to HBV/HDV infection, 2) evaluate the antiviral effect of HTAs in co-infected PCLS, and 3) employ this model to characterize the impact of HTAs on the hepatic microenvironment.

Methods: Human PCLS were prepared using resected liver tissue samples from patients without history of chronic liver disease. Slices were mono- or co-infected ex vivo with HBV and HDV for a five-day period. Tissue architecture and viability were evaluated by histological analysis and quantification of intracellular ATP. Viral infection was assessed by the presence of HBV RNA, covalently closed circular (ccc)DNA and HDV RNA by RT-qPCR, as well as hepatitis delta antigen (HDAg) by Western blot. The antiviral impact of HTAs targeting hepatocytes (Bulevirtide, BLV, 1 µM), non-parenchymal immune cells (Selgantolimod, SLGN, 1 µM) or multiple populations (Lonafarnib, LNF, 2 µM) was evaluated by the quantification of viral parameters and the production of inflammatory cytokines. Additionally, we developed a protocol combining PCLS with single-cell (sc)RNA-seq to characterize the response to SLGN.

Results: Our ex vivo PCLS infection protocol allowed the detection of HBV RNA, cccDNA, HDV RNA and HDAg at the end of the five-day observation period. Treatment of PCLS with BLV prior to inoculation led to a marked decrease in viral parameters for both HBV and HDV. LNF treatment post-infection was associated with the intracellular accumulation of HDAg. The TLR8 agonist SLGN induced a significant decrease of HBV RNA levels. The immunomodulatory action of SLGN was confirmed by the production of inflammatory cytokines (e.g. IL-6) in the culture supernatant. Moreover, scRNA-seq analysis showed the activation of TLR8-expressing Kupffer cells, as well the indirect modulation of non-TLR8-expressing populations (e.g. NK cells). PCLS architecture and viability were comparable between the different treatment conditions.

Discussion/Conclusion: Our results represent the first characterization of PCLS as a relevant ex vivo study model of HBV/HDV co-infection. Moreover, the evaluation of HTAs directed against parenchymal and non-parenchymal hepatic cell populations highlights the potential relevance of this system for the pre-clinical study of novel molecules aimed to achieve HBV/HDV cure.

30. Decipher hepatocellular carcinoma (HCC) progression through cell-cell competition between hepatocytes and tumor cells using an interface 3D model

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Introduction: Cell competition is a fitness sensing process essential for tissue homeostasis. During tumorigenesis, cancer cells will gain competition against surrounding non-tumoral cells. Hepatocellular carcinoma (HCC) is a highly heterogeneous cancer that develops over an extended period in a pathological liver. HCC patients are classify according tumor proliferative statue into non-proliferative or proliferative HCC. Few studies have examined the impact of cell-cell competition during HCC progression and it remain unclear how tumor cells gain cell competition in proliferative HCC. In this context, our hypothesis is that the surrounding liver may play a crucial role in HCC progression. The aim of this project is to recreate an HCC 3D model recapitulating the cellular interface between tumor cells and the surrounding pathological liver in order to understand the molecular mechanisms involved in cell-cell completion in proliferative HCC.

Methods: We therefore have a clear need to reproduce the mechanical constraints of the liver parenchyma on a tumor in vitro in new 3D models. In this context, we have established a co-culture 3D model using HepaRG or AML12 and HUH7 HCC tumor cells in different ratios. Moreover, in order to highlight pathway involved in cell-cell competition, we perform proteomic on the 3D coculture model in different conditions, non-proliferative and proliferative HCC tumor.

Results: We recreate the cellular interface in a spheroid organized as tumor surrounded by healthy cells. We perform a characterization of the model by IHC, Incucyte live images, confocal microscopy and functional assays (proliferation, apoptosis). This model maintains a good viability during 2 weeks with no apoptosis or hypoxia in the spheroid core. The proliferation of tumor cells is reduced in the co-culture model compared to Huh7 monoculture spheroid and is inversely correlated with the increase number of normal cells around the Huh7 spheroid. HepaRG pressure and indirect co-culture are not sufficient to reduce HUH7 proliferation compare to the direct co-culture experiment suggesting the importance of cell-cell contact and competition at the molecular level. Proteomic analysis of this 3D model evolution in non-proliferative and proliferative tumor cells conditions will reveal molecular processes controlling tumor progression.

Discussion/Conclusion: In this study, we recreate proliferative and non-proliferative tumor surrounded by normal hepatocytes. To investigate the molecular pathways involved in cell-cell proliferation, proteomic has been performed and will allow us to identify targets involved in cell-cell competition. A silencing or overexpression strategy will be use to modulate the expression of identified targets in the 3D model. Moreover, all the data obtained during these experiments will be compile for numerical simulations of the evolution and proliferation of cancer cells. Additionally, to recapitulate the physiopathology of ALD or MASH liver in the model, we plan to add to treat the model with alcohol or a fatty medium and to add mechanical constraints recapitulating liver stiffness in F1 to F4 fibrosis stages.

31. Inhibition of VEGF signalling may affect human CCC cell line (HuCCT-1) tumour growth

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Introduction: Cholangiocarcinoma (CCC) is a common hepatic malignant tumour with growing incidence and poor prognosis. Multiple studies confirmed that high vascular endothelial growth factor (VEGF) expression in cancer tissues is a significant biomarker for the poor prognosis of patients with intrahepatic CCC. It was found that no significant association was detected between VEGF expression and age, gender, and tumor size; high VEGF expression was significantly correlated with lymph node metastases and advanced TNM stage. Inhibition of VEGF signalling may affect tumour growth via several mechanisms and showed good results in studies of hepatocellular carcinoma. However, studies involving CCC are rare and present various and often disappointing results. Therefore, we hypothesized that VEGF inhibiting may be similarly effective on CCC as on hepatocellular carcinoma.

Methods: A commonly used for research purposes human CCC cell line (HuCCT-1) established from an extrahepatic bile duct carcinoma was cultivated in modified medium with 10% fetal bovine serum seeded onto well plates. VEGF-targeting drug Sorafenib, which inhibits cellular signalling by targeting different receptor tyrosine kinases including receptors for VEGF, 0.05 mg/ml added in study group cultures. Two types of controls were used, the HuCCT-1 cultures without treatment and treated Hep G2/Hep 3B HCC cultures, for comparison of pro-apoptotic effects on different liver tumours. General cellular count and nuclei morphology were visualized via the TUNEL-staining protocol and cells viewed with a fluorescence microscopy (magn. ×400). The number of apoptotic cells calculated in percentage of total nuclei. Apoptosis related cytokines (caspase-9, caspase-3, and caspase-6) were analysed by Western blotting.

Results: In study group HuCCT-1 cell line after 48 hours of treatment with Sorafenib compared to control changes become evident leading to a significant time-dependent reduction of cell numbers of 59.6–82.4% ($p < 0.01$). Cells became sparse, rounded, and detached from the dishes representing morphologic signs of apoptosis. These findings correlated with overexpression of caspase-9, caspase-3, and caspase-6. Obtained changes were similar to changes observed in hepatocellular carcinoma Hep G2/Hep 3B lines used for control with comparable pro-apoptotic effect during same time interval.

Discussion/Conclusion: VEGF-targeted therapy may act through parallel mechanisms that have more or less important role depending on tumour type. In certain malignancies VEGF-targeted therapy has significant activity, whereas in other has no clinical benefit. Our study shows positive pro-apoptotic effect of VEGF-targeting therapy in CCC. However, there is no clear evidence that other CCC cells lines will be similarly sensitive for VEGF-targeting therapy as HuCCT-1.

32. In vitro study of peroxisome proliferator activated receptor-γ activation influence on cholangiocarcinoma cells

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Introduction: Peroxisome proliferator activated receptor gamma – NR1C3 (PPARγ) plays an important role in various biological processes including, but not limited to lipid and glucose metabolism. Activation of PPARs may significantly influence tumour progression in various tissues, including colon and liver. Whereas different PPAR isotypes may either suppress or promote tumour development depending on the specific tissue or ligands, the mechanism remains generally unclear, raising the question of any respective compounds' use for targeted treatment, while PPARs are embrace more interest as possible therapeutic targets for a number of disorders. PPARγ agonists have been used in treatment of different metabolic disorders and MASH decreasing steatosis, inflammation, and fibrosis. In addition, recent studies show its pro-apoptotic and antiproliferative effect. Numerous clinical studies are being conducted on PPARs as possible therapeutic targets for liver cancer. Therefore, the aim of the study was to clarify the perspectives for cholangiocarcinoma (CCC) targeted therapy with thiazolidinediones, compunds known to activate PPARγ in liver.

Methods: A culture of CCC HuCCT-1 cell line was selected for this in vitro study, cultivated in modified with 10% fetal bovine serum medium seeded onto standard well plates. PPARγ agonist pioglitazone 0.5 to 10 mmol/l added in study group cultures. General cells count and nuclei morphology in study wells and intact control were visualized with the TUNEL-staining protocol and cells viewed with a fluorescence microscope (magn. ×400). The number of apoptotic cells calculated in percentage of total nuclei. Apoptosis related cytokines were analyzed by Western blotting.

Results: Activation of PPARγ by pioglitazone caused marked growth inhibition in a time- and dose-dependent manner. Pioglitazone inhibited growth of cholangiocarcinoma cell lines by both inducing apoptosis and by cell cycle regulation, and this was associated with caspase-3, 6 and caspase-9 activation. These changes were similar to changes observed in hepatocellular carcinoma Hep G2/Hep 3B lines used for additional control with comparable pro-apoptotic effect during same time interval but different pioglitazone dosage.

Discussion/Conclusion: This study demonstrates that PPARγ activation may have positive pro-apoptotic and antiproliferative effect on CCC cells as well as other liver tumour types. Therefore, molecular targeting with thiazolidinediones, nuclear receptor ligands, may be a promising strategy for treating cholangiocarcinoma. However, this study has several limitations, among them the experimental character of research and the fact our previous study of PPARγ agonists and hepatocellular carcinoma raised question of individual susceptibility/resistance for this approach in different tumour cell lines

33. Liver resident natural killer T-cells in experimental diet-induced metabolic dysfunction associated liver disease

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Introduction: A set of dietary and lifestyle related factors like obesity, metabolic syndrome, diabetes, insulin resistance are the main factors underlying metabolic dysfunction

associated liver disease (MASLD), which becomes one of the most common liver conditions in civilized world. The expression of adipose tissue inflammation showed directly correlation with the MASLD severity and progression to MASH, cirrhosis, fibrosis and liver cancer. Consumption of higher caloric intake is increasingly emerging as a fuel of metabolic inflammation not only in obesity-related disorders but also MASLD. T-cell receptor bearing lymphocytes are among the most prominent and versatile immune cells. Whereas, most T-cells respond to peptide-antigens display an adaptive immune behaviour, allowing them to establish specific immune memory against their respective cognate antigens and the sources these are derived from, a group of T-cell differ in the way they recognize antigen or function, displaying innate-like features of antigen-independent responses driven by cytokine sensing. Natural killer (NK) T-cells, an innate immune cells, are abundant in the liver, accounting for 20–35% of total hepatic lymphocytes in rodent experimental models and can specifically recognise glycolipid antigens, and produce both Th1 and Th2 cytokines when activated, and sharing characteristics with both T-cells and natural killer cells. However, the role of NK T-cells in hepatic fibrogenesis is poorly understood as well as, while the role of diet in progression of MASLD and MASH is well established, the role of NK T-cells in diet related influence on liver remains unclear. Therefore, we aimed to analyze possible associations of different diets with NK T-cells, and proinflammatory cytokines in MASLD.

Methods: Fifty adult Wistar-line rats underwent 16 weeks of either MCD diet (Methionine-Choline-Deficient Diet, containing high sucrose and fat without methionine and choline, essential for hepatic mitochondrial β-oxidation and for synthesis of VLDL), the classic dietary model for studying MASH, or FFD (Fast-Food diet, containing high fat, high cholesterol, high fructose), which is another dietary tool for MASLD modelling. The study fully conforms to international bioethical standards and approved by respective institutional body. Another similar fifteen animals formed control, receiving normal diet with not more than 10–12% of calories from fat. Liver bioptates were a source for liver histology, NK T-cells, fibrosis, and proinflammatory cytokines determination.

Results: Both MCD and FFD diets significantly impacted body mass during the study period compared to control. FFD increased body mass by 95.36 ± 12.71% ($p < 0.01$), while MCD-related change was opposite – it decreased body mass by 37.14 ± 11.09% ($p < 0.05$), supporting previous studies. Further changes in body mass were insignificant in both groups. FFD fed animals developed mainly perisinusoidal/pericellular histological changes associated with mild to moderate NASH 1 stage fibrosis, whereas MCD fed animals presented with paraacinar macrovesicular steatosis, severe inflammation, hepatocellular ballooning, more advanced stage of fibrosis (mostly 2–3 stages), occurring in a perisinusoidal/pericellular, perivenular or bridging fibrosis patterns, associated with severe MASH. FFD diet animals led to significant decrease of NK T-cells in liver bioptates compared to both control and MCD group ($0.01 < p < 0.05$), while difference between control and MCD group was insignificant ($p = 0.11$). IL-4 demonstrated no valid changes in the FFD fed animals ($p = 0.19$), but was significantly higher in MCD fed animals compared to control ($p < 0.01$).

Discussion/Conclusion: Inflammation and immune response play a key role in the pathogenesis of obesity-associated metabolic diseases, including MASLD. Studies on pathogenic and protective role of NK T-cells in MASLD are controversial: it was shown that lack of NK T-cells may promote steatosis, inflammation, and liver fibrosis in high-fat or choline-deficient diets, whereas other studies showed NK T-cells play a role in promoting liver fibrogenesis and cirrhosis. This study confirms existing associations of dietary habits and development of metabolism associated liver conditions.

34. Time-dependent apoptosis induction of the Hep-3B and Hep-G2 HCC cells by selective inhibition of cyclooxygenase-2

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Introduction: The incidence of hepatocellular carcinoma is growing, especially in the developed world, with 50–75% increase over the last two decades. Previous studies provided significant evidence database that the overexpression of cyclooxygenase-2 (COX-2) is associated with carcinogenesis and COX-2 inhibition or prostaglandin endoperoxide synthase-2 knockout can reduce tumor growth. COX-2 has become a perspective target in cancer therapy for its role in carcinogenesis following different expression patterns in neoplastic and normal tissues. However, most of the studies were limited to pulmonary and colon cancer cells, whereas it is still debatable whether COX-2 sufficiently contributes to the hepatic malignant growth and whether inhibition of COX-2 modifies the malignant potential of liver tumors. Exact mechanisms of how COX-2 inhibiting influences hepatic malignancies remains unclear, too. The aim of the study was to clarify the pro-apoptotic and anti-proliferative effect of selective COX-2 inhibition.

Methods: The study is performed in vitro; Hep-3B, p53-negative mutant, and Hep-G2, p53-wild type HCC cells were cultivated in modified media seeded onto well plates divided into study and control groups. Celecoxib 50 μmol/l a commonly used selective COX-2 inhibitor was added into the study group cultures. Apoptosis-related cytokines were analyzed in Western blotting. Apoptotic nuclei were visualized via the TUNEL-staining protocol and cells viewed in fluorescence microscopy (magn. ×400), with the number of apoptotic cells calculated as percentage of total nuclei.

Results: After 48 hours of treatment, COX-2 inhibition related changes compared to control become evident in Hep G2/Hep 3B cell lines cultures leading to a significant time-dependent reduction of cell numbers of up to 80% ($p < 0.05$). Cells became sparse, rounded, and detached from the dishes representing morphologic signs of apoptosis. This finding correlated with activation of caspase-9, caspase-3, and caspase-6 cytokines. Moreover, exposure of cell cultures to 3 g/ml PgE2 eliminated the COX-2 inhibiting and eliminated pro-apoptotic effect on HCC cells. This fact indicates that the antineoplastic properties of COX-2 inhibiting are dependent on reduced conversion of arachidonic acid to PGE2 attributable to COX-2 inhibition.

Discussion/Conclusion: It is known that COX-2 induces cancer stem cell like activity and promotes resistance to apoptosis, inflammation, neoangiogenesis, aggravates proliferation, invasion, and metastasis of various cancer cells. This study shows that selective inhibition of COX-2 causes marked inhibition of different human liver tumor cells, based on the induction of apoptosis and inhibition of proliferation. The exact mechanism by which COX-2 inhibiting-related apoptosis is realized is still unclear as well as involvement of other factors into antiproliferative effect of COX-2 inhibitors.

35. Exploring the multi-organ inflammatory responses in a mouse toxic acute-on-chronic liver disease model

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Introduction: Acute-on-chronic liver failure (ACLF) is characterized by an excessive systemic inflammatory response, organ failure(s), and high short-term mortality. We aimed to develop and characterize a representative ACLF mouse model.

Methods: Toxic liver cirrhosis was induced in 10 weeks old, C57BL/6J male mice by carbon-tetrachloride (CCl4; p.o. for 12 weeks), while control animals received olive oil (OO). ACLF was induced by a single i.p. injection of increasing doses of lipopolysaccharide (LPS; 5–200 µl/kg) 24 hours prior to direct portal pressure (PP) readings, blood and organ sampling. Respective groups (n = 8/group) were OO; CCl4; CCl4+LPS low; CCl4+LPS mid; CCl4+LPS high. Mice were carefully monitored for 8 hours using the standardized Murine Sepsis Score (MSS). The degrees of hepatic fibrosis, organ inflammation and systemic inflammation were quantified by histologic collagen proportionate area (CPA), RT-qPCR gene expression in liver and kidney, and a multiplex assay for plasma biomarkers, respectively.

Results: Cirrhotic animals displayed a significantly increased liver/bodyweight (BW) and spleen/BW ratio, compared to controls ($p < 0.001$). The latter further increased upon LPS treatment in a dose-dependent manner.

CCl4 treatment induced significant liver fibrosis (CPA%: 1.4 ± 0.09 vs. 4.5 ± 0.3, $p < 0.001$), which remained unaltered in LPS-induced ACLF mice (CPA%: 5.1 ± 0.3). PP was elevated in cirrhotic animals and further increased in the respective LPS groups (OO vs. CCl4 vs. CCl4+LPS high: 4.9 ± 0.2 vs. 6.2 ± 0.3 vs. 7.1 ± 0.4 mmHg, $p = 0.001$). In line, the proinflammatory gene expression was increased in cirrhotic animals and further stimulated by LPS (CCl4 vs. CCl4+LPS low vs. CCl4+LPS mid vs. CCl4+LPS high), both, in liver (Ccl2: $p < 0.001$; Il6: $p < 0.001$; Tnf: $p < 0.001$; Il1b: $p < 0.001$; Cxcl1: $p < 0.001$), kidney (Ccl2: $p < 0.001$; Il6: $p = 0.001$; Tnf: $p < 0.001$; Il1b: $p < 0.001$; Cxcl1: $p = 0.004$) and lung (Ccl2: $p < 0.001$; Il6: $p < 0.001$; Tnf: $p < 0.001$; Il1b: $p < 0.001$; Cxcl1: $p < 0.001$) tissues. Plasma levels of G-CSF and chemokines (CCL2, CCL3, CCL4 and CCL5) increased with LPS dose, whereas cytokines (IL-1beta, IL-6, IL-8, IL-10 and TNF-alpha) were only modestly affected by short-term LPS.

Discussion/Conclusion: This study characterized a murine ACLF model based on toxic liver fibrosis, stimulation with LPS i.p. injection and a subacute endpoint at 24 hours. This mouse model recapitulates key features of human ACLF, including immune cell recruitment, organ dysfunction and inflammation.

36. A rapid-onset MASH model captures non-parenchymal disease determinants that affect myeloid cell positioning, liver fibrosis and cellular regeneration

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Introduction: MAFLD to MASH progression can be explored using different mouse models that partially reflect certain aspects of human liver disease pathophysiology. While models with slow disease onset may mirror human disease evolution over time, rapid onset MASH models may better capture rapid-evolving spatiotemporal immune cell dynamics. Focusing on immediate disease characteristics, we here exploited a rapid-onset MASH diet model and generated a rich single-cell RNA-sequencing (scRNA-seq) atlas reference.

Methods: A choline deficient, ethionine-supplemented (0.05%; CDE) diet was given to male C57BL/6J mice for 3–21 days and liver cells were isolated using two isolation strategies at five different timepoints to generate a scRNA-seq atlas containing 76 k cells. Disease stage was assessed by histologic assessment, liver function tests and multicolor immunofluorescence (IF). FACS and real-time PCR were conducted to confirm tissue-specific shifts in cell

abundance. Pathway enrichment, ligand-receptor interaction analysis and trajectory inference were performed on cell populations of interest.

Results: The CDE scRNA-seq data captured MAFLD/MASH disease progression as objectified by liver function tests, histopathological scoring, FACS analysis and general health assessment. IF visualized intralobular heterogeneous myeloid foci at regions of hepatocyte cell death and increased collagen deposition. scRNA-seq data captured hepatocyte disease progression and myeloid dynamics indicated an intricate heterocellular communication network between Kupffer cells, recruited monocytes and lipid associated macrophages, that may govern macrophage polarization and myeloid cellular positioning.

Outlook: To identify spatial niche domains that may be of importance for macrophage/monocyte subtype positioning and differentiation in MASH, we currently generate high resolution spatial transcriptomics data. At the same time, we explore the role of myeloid subpopulations and their impact in inducing a fibrotic or liver regeneration response.

37.Inflammation-educated macrophages drive exacerbated re-injury patterns via innate immune memory

Yuting Wang (Berlin, DE)

Introduction: Chronic liver injury leads to pronounced immune alterations, but the persistence of these changes and the impact of the immunological reprogramming on the liver's response to re-injury remains uncertain. Patients with chronic liver diseases suffer from phases of high disease activity followed by months of injury regression.

Methods: Here we used a mouse model of chronic toxicity (CCl4) and regression of liver injury and simulated re-injury by administering a single dose of CCl4 after regression.

Results: We found that while liver architecture and damage returned to normal levels during regression, rechallenge injury resulted in significantly more severe liver damage compared to long-term chronic injury and acute injury on an otherwise healthy liver. Through the utilization of fate-mapping tools, intravital imaging, and multiplex flow cytometry, we show that chronic injury resulted in a significant influx of monocytes into the liver, with infiltration of monocyte-derived macrophages, which persisted into the regression phase. These monocyte-derived macrophages displayed a proinflammatory profile and engaged in frequent and prolonged interactions with circulating neutrophils. Furthermore, they were able to rapidly secrete cytokines such as TNF-α and IL-1β upon re-stimulation, indicating an increased pro-inflammatory potential.

Discussion/Conclusion: Our study reveals reprogramming of liver macrophages during the regression of chronic liver injury, resulting in a hyperirritable immune state of the liver and a heightened inflammatory response upon re-injury.

38. Role of mitochondria in the HBV life cycle and associated pathologies

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Introduction: Chronic hepatitis B virus (HBV) infection affects approximately 250 million people worldwide and is a leading cause of cirrhosis and hepatocellular carcinoma. The HBV protein HBx plays a key role in HBV pathogenesis by disrupting mitochondrial function through interactions with mitochondrial proteins. This study investigates how HBx affects mitochondrial apoptosis and its role in HBV-induced mitochondrial dysfunction in hepatocytes.

Methods: We assessed the effect of HBx on mitochondrial apoptosis by cell viability (Neutral Red staining), cytochrome c release assays and caspase activation in an HBx-inducible hepatocyte model system. Immunoprecipitation of HBx from isolated mitochondria was used to determine its mitochondrial interaction partners and their activation was analysed by chemical cross-linking experiments.

Results: HBx expression protected against cytotoxic drugs by reducing cytochrome c release from mitochondria, thereby inhibiting caspase activation and apoptosis. Mechanistically, HBx interacted with BAK, BAX and VDAC1, proteins involved in mitochondrial membrane permeabilisation. This interaction reduced their oligomerisation into higher molecular weight complexes, likely explaining the reduced cytochrome c release.

Discussion/Conclusion: HBx localises to mitochondria, interacts with membrane permeabilising proteins and alters their activation to reduce apoptosis. Future studies will investigate the molecular basis of HBx-induced mitochondrial dysfunction in more physiologically relevant models of HBV infection. This will improve our understanding of HBV-induced liver pathology and identify novel therapeutic targets.

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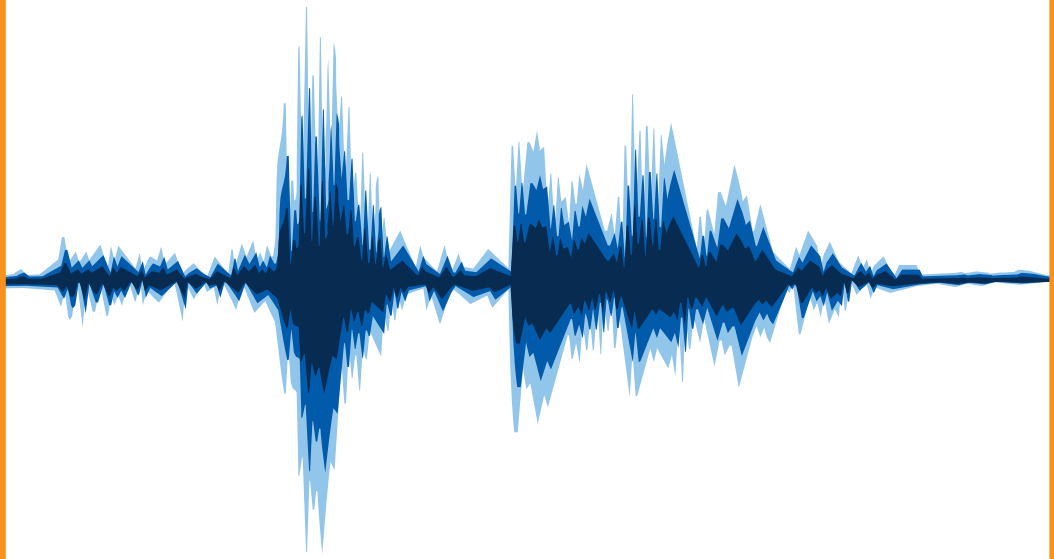

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