


3RD CONFERENCE OF THE HUNGARIAN PANCREATIC STUDY GROUP

&

9TH INTERNATIONAL SYMPOSIUM ON ALCOHOLIC LIVER AND PANCREATIC DISEASES AND CIRRHOSIS

Szeged, Hungary
21–23 November, 2014





WELCOME

On behalf of the Organizing Committee, we are warmly welcome you in Szeged to the joint meeting of the 3th Hungarian Pancreatic Study Group (HPSG) and the 9th International Symposium on Alcoholic Liver and Pancreatic Diseases and Cirrhosis (ISALPD/C)"

First of all, let me thank you for attending HPSG and ALPD. We are delighted to have participants from more than 15 countries and are looking forward to a stimulating and interesting conference. During this three days international joint meeting, we will focus on two major organs, which are commonly affected due to chronic alcohol abuse: the liver and the pancreas. Our ultimate goal was to bring together clinicians (Hepatologist and Pancreatologist) and basic scientists from all over the world to present and discuss current topics in clinical research and basic molecular mechanisms. The exchange of the major research advances both in molecular, biochemical and translational aspects may provide future directions in the treatment of alcohol-induced liver and pancreatic diseases. There will be several state-of-the-art lectures delivered by top international experts in order to expanding our knowledge of the clinical implications.

Szeged (our host city), located in the south-eastern part of Hungary, will provide a friendly environment for the meeting. The social programs (welcome reception and folkdance evening) will nicely complement the scientific sessions of the conference.

We hope that you will enjoy both HPSG and ALPD meetings!

On behalf of the Organizing Committee

Péter Hegyi
Chair of HPSG

Viktória Venglovecz
Co-Chair of ALPD

József Maléth
Co-Chair of ALPD

GENERAL INFORMATIONS

ORGANIZING COMMITTEE OF HPSG

Péter Hegyi *(Hungary)*
István Hritz *(Hungary)*
Miklós Sahin-Tóth *(USA)*

ORGANIZING COMMITTEE OF ALPD

Anna Gukovskaya *(USA)*
Péter Hegyi *(Hungary)*
Peter Jansen *(The Netherlands)*
József Maléth *(Hungary)*
Shmuel Muallem *(USA)*
Zoltán Rakonczay Jr. *(Hungary)*
Gyöngyi Szabó *(USA)*
Alexei Tepikin *(UK)*
Hidekazu Tsukamoto *(USA)*
Viktória Venglovecz *(Hungary)*

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FACULTY OF HPSG

Abu-El-Haija, Maisam *(USA)*
Dubravcsik, Zsolt *(Hungary)*
Hegyi, Péter *(Hungary)*
Hritz, István *(Hungary)*
Kahán, Zsuzsanna *(Hungary)*
Lowe, Mark *(USA)*
Párniczky, Andrea *(Hungary)*
Rosendahl, Jonas *(Germany)*
Sahin-Tóth, Miklós *(USA)*
Szűcs, Ákos *(Hungary)*
Uc, Aliye *(USA)*
Witt, Heiko *(Germany)*
Whitcomb, David *(USA)*
Zsoldos, Fanni *(Hungary)*

FACULTY OF ALPD

Apte, Minoti *(Australia)*
Bataller, Ramon *(USA)*
Criddle, David *(UK)*
Fajas, Luis *(France)*
Fernández-Checa, José Carlos *(Spain)*
Gukovskaya, Anna *(USA)*
Hajnóczky, György *(USA)*
Hegyi, Péter *(Hungary)*
Hoek, Joannes *(USA)*
Jansen, Peter *(The Netherlands)*
Kumar, Vipin *(USA)*
Lerch, Marcus *(Germany)*
Maléth, József *(Hungary)*
Martinez-Chantar, Maria Luz *(Spain)*
Muallem, Shmuel *(USA)*
Nathanson, Michael *(USA)*
Parekh, Anant *(UK)*
Pandol, Stephen *(USA)*
Petersen, Ole *(UK)*
Rakonczay, Zoltan *(Hungary)*
Sahin-Tóth, Miklós *(USA)*
Saluja, Ashok *(USA)*
Shah, Yatrik *(USA)*
Singh, Vijay *(USA)*
Szabó, Gyöngyi *(USA)*
Tepikin, Alexei *(UK)*
Torok, Natalie *(USA)*
Tsukamoto, Hidekazu *(USA)*
Varga, Gábor *(Hungary)*
Venglovecz, Viktória *(Hungary)*
Uc, Aliye *(USA)*
Wang, Li *(USA)*
Whitcomb, David *(USA)*
Zakhari, Samir *(USA)*

VENUE:

Hotel Novotel Szeged

Maros utca 1
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Fax (+36)62/562221
E-mail H2996@accor.com
Web http://www.novotel.com/gb/hotel-2996-novotel-szeged/index.shtml

CONFERENCE REGISTRATION:

Registration opening hours:
Thursday, 20 Nov 12:00-20:00 (next to the Hotel reception)
Friday, 21 Nov 08:00-20:00 (next to the Hotel reception)
Saturday, 22 Nov 07:45-18:00 (in front of the Lecture Hall)
Sunday, 23 Nov 07:45-18:00 (in front of the Lecture Hall)

On-site Registration Fees:

HPSG meeting

If registered for ALPD	€50
If not registered for ALPD	€100
Accompanying persons	€20

ALPD meeting

Junior (<30 years, separate room)	€450
Junior (<30 years, shared room with other delegate)	€365
Senior (>30 years, separate room)	€500
Senior (>30 years, shared room with other delegate)	€415
Accompanying person	€125

Registration fees (HPSG) for participants covers the following:

- transport from/to Budapest Ferenc Liszt Airport
- admission to all scientific sessions
- conference materials (including programme leaflet, badge, etc.)
- coffee breaks and lunches
- social events

The registration fee for accompanying persons covers the following: free transport from and to Budapest Ferihegy Airport, lunches and social events.

Registration fees (ALPD) for participants covers the following:

- transport from/to Budapest Ferenc Liszt Airport
- 3 nights (21, 22 and 23 Nov) accommodation in the conference hotel (Hotel Novotel****)
- admission to all scientific sessions
- conference materials (including programme leaflet, badge, etc.)
- coffee breaks and lunches
- social events

The registration fee for accompanying persons covers the following: free transport from and to Budapest Ferihegy Airport, lunches and social events.

NAME BADGES

Participants are requested to wear their name badges at all times.

POWER POINT PRESENTATIONS

All speakers are asked to hand in their presentation (MS Powerpoint 200, 2007, 2010 format) at least before the start of their session.

POSTER EXHIBITIONS

Posters are displayed on Saturday and Sunday in front of the Lecture Hall from 8:00-17:00. The authors of the posters are asked to put up their poster from 8:00 h on Saturday, November 22, 2014 and remove it by 17:00 h on Sunday, November 23, 2014. At least one of the authors must be present during the poster session to answer questions.

TRANSPORT

Bus Transfer from/to Budapest Ferihegy International Airport.

In your return back to the airport you will be picked up in front of the Hotel Novotel. The exact departure time of the shuttles will be listed at the registration desk.

If there are any changes in your departure time please send an email to isalpd2014@gmail.com or call +36-70-560-04-58 as soon as possible.

HEALTH AND SAFETY

Emergency telephone numbers in Hungary
Ambulance: 104
Police: 107
Fire Department: 105
In case of any non-urgent health problems, we can help you out at the ER of the First Department of Medicine, University of Szeged (I. sz. Belgyógyászati Klinika, Szegedi Tudományegyetem). The address is: Korányi fasor 8-10.
Telephone number: +36-62-545-198 or +36-62-545-813

SMOKING

Smoking is prohibited within the Hotel.

INSURANCE/LIABILITY

The Organizers of the HPSG and ALPD do not accept any liability for damages and or losses of any kind which may be incurred by conference participants or accompanying persons. Delegates participate at all events at their own risk.

WIRELESS INTERNET

There is free wireless internet connection for all participants at Hotel Novotel.

DETAILED SCIENTIFIC PROGRAMME

3rd

Conference of the Hungarian Pancreatic Study Group

Focus on:
Pediatric pancreatitis
Pancreas genetics
Results from the Hungarian Pancreas Registry
Prospective Randomized Clinical Trials

21 November, 2014
Szeged, Hungary

Organizers:
Péter Hegyi (Pediatric Pancreatitis)
István Hritz (HPSG)
Miklós Sahin-Tóth (Pancreas Genetics)

8:00 – 9:00 **Breakfast meeting with the International guests**
István Vedres Meeting Room
Moderator: Péter Hegyi (Szeged, Hungary)
Brain storming – by invitation only

9:30 – 10:00 **Opening of the 3rd HPSG meeting**
Lajos Tisza Meeting Room
Chairs: Péter Hegyi (Szeged, Hungary), Miklós Sahin-Tóth (Boston, USA), István Hritz (Budapest, Hungary)
Sponsors: Sager Pharma – Abbott – Berlin Chemie – Szekerke Mária RA

9:30 – 9:40 Welcome Notes by the sponsors

9:40 – 10:00 **Péter Hegyi** (Szeged, Hungary)
HPSG: History and Future perspectives

10:00 – 12:10 **Focus on Pediatric Pancreatitis**
Lajos Tisza Meeting Room
Chairs: Gábor Veres (Budapest, Hungary), Csaba Bereczki (Szeged, Hungary), Natália Lásztity (Budapest, Hungary)
Session introduction by **Péter Hegyi** (Szeged, Hungary)

10:00 – 10:30 **Maisam Abu-El-Haija** (Cincinnati, USA)
AP in Children

10:30 – 10:40 **Aliye Uc** (Iowa, USA)
Introduction of INSPPIRE

10:40 – 11:10 **Aliye Uc** (Iowa, USA)
ARP and CP in Children

11:10 – 11:40 **Mark Lowe** (Pittsburgh, USA)
Treatment of ARP and CP with a focus on TP-IAT

11:40 – 12:10 **Heiko Witt** (Münich, Germany)
Genetic mutations in pediatric CP patients in Germany.

LUNCH

12:50 – 13:20 **Data Administration (lunch session)**
Lajos Tisza Meeting Room
Chair: Andrea Szentesi OPR Director (Szeged, Hungary)

12:50 – 13.20 **Andrea Szentesi OPR Director** (Szeged, Hungary)
Technical guideline for the web based data registry

13:30 – 15:30 **Pancreas Genetic Testing**
Lajos Tisza Meeting Room
Chairs: Márta Széll (Szeged, Hungary), Balázs Németh (Szeged, Hungary)
Session introduction by **Miklós Sahin-Tóth** (Boston, USA)

13:30 – 14:00 **David C. Whitcomb** (Pittsburgh, USA)
Genetics of pancreatitis with a focus on pancreatic ducts

14:00 – 14:30 **Miklós Sahin-Tóth** (Boston, USA)
Genetics of pancreatitis with a focus on trypsin activation

14:30 – 15:00 **Jonas Rosendahl** (Leipzig, Germany)
Genetics of alcoholic and non-alcoholic pancreatitis

15:00 – 15:30 **Federico Canzian** (Heidelberg, Germany)
Genetic susceptibility to pancreatic cancer

COFFEE BREAK

16:00 – 18:00 **HPSG meeting**
Lajos Tisza Meeting Room
Chairs: Áron Vincze (Pécs, Hungary), Balázs Kui (Szeged), Richard Szmola (Budapest, Hungary)
Session introduction by **István Hritz** (Budapest, Hungary)

16:00 – 16:10 **Péter Hegyi** (Szeged, Hungary)
Working rules of HPSG

16:10 – 16:25 **István Hritz** (Budapest, Hungary)
HPSG Cohort: AP

16:25 – 16:40 **Ákos Szűcs** (Budapest, Hungary)
HPSG Cohort: CP

16:40 – 16:55 **Andrea Párniczky** (Budapest, Hungary)
HPSG Cohort: PP

16:55 – 17:10 **Zsuzsanna Kahán** (Szeged, Hungary)
HPSG Cohort PC

17:10 – 18:00 **Prospective studies - Central and Eastern European Working Groups**
Lajos Tisza Meeting Room
Chairs: Ewa Małecka-Panas (Łódź, Poland), Dimitry Bordin (Moscow, Russia), Natalya Gubergrits (Donetsk, Ukraine)

17:10 – 17:30 **Péter Hegyi** (Szeged, Hungary)
Introducing the International Centres, future perspectives

17:30 – 17:45 **István Hritz** (Budapest, Hungary)
Introduction of **EASY** trial

17:45 – 18:00 **Fanni Zsoldos** (Budapest, Hungary)
Introduction of **PINEAPPLE** trial

18:00 – 18:15 **Andrea Párniczky** (Budapest, Hungary)
Introduction of **APPLE** trial

18:15 – 18:30 **Zsolt Dubravcsik** (Kecskemét, Hungary)
Introduction of **PREPAST** trial

11:30 – 15:00 **Paralel meeting for the Hungarian data administrators (in Hungalan) – Párhuzamos előadás magyar adminisztrátorok részére**
Lajos Lechner Meeting Room

11:30 – 12:15 **EBÉD**

12:15 – 12:45 **Szentesi Andrea OPR igazgató** (Szeged, Hungary)
OPR és weboldal újdonságok, induló prospektív vizsgálatok

SZÜNET

13:30 – 15:00 **Szentesi Andrea OPR igazgató** (Szeged, Hungary)
Új űrlapok bemutatása, feltöltéssel kapcsolatos kérdések megbeszélése

20:00 **Joint Dinner of HPSG and the Faculty of ALPD**
FISHER'S RESTAURANT – Szeged H6720 Roosevelt tér 14.

9th

International Symposium on Alcoholic Liver and Pancreatic Diseases and Cirrhosis

Focus on:
Cellular metabolism in alcohol-induced pancreatic and liver diseases

22-23 November, 2014
Szeged, Hungary

Organizers:
Anna Gukovskaya *(USA)*
Péter Hegyi *(Hungary)*
Peter Jansen *(The Netherlands)*
József Maléth *(Hungary)*
Shmuel Muallem *(USA)*
Zoltán Rakonczay Jr. *(Hungary)*
Gyöngyi Szabó *(USA)*
Alexei Tepikin *(UK)*
Hidekazu Tsukamoto *(USA)*
Viktória Venglovecz *(Hungary)*

22 NOVEMBER 2014 (Saturday) ALPD symposium

8:00 – 8:15	Opening of the 9th ALPD meeting and Press conference Lajos Tisza Meeting Room
8:15 – 8:30	Break
SESSION 1 :	**State of the Art Lectures " Overview on alcohol-induced pancreatic and liver diseases"** **Chairs:** Viktória Venglovecz (Hungary), József Maléth (Hungary)
8:30 – 8:45	**Alcoholic Liver Disease: Do Genes or Environment Matter More?** **Samir Zakhari** *(USA)*
8:45 – 9:10	**Mechanisms by which alcohol predisposes to pancreatitis: an overview** **Anna Gukovskaya** *(USA)*
9:10 – 9:35	**Morphogens, metabolic reprogramming, and alcoholic liver disease** **Hidekazu Tsukamoto** *(USA)*
SESSION 2 :	**"Selected oral presentations (1-3)"** **Chairs:** Zsuzsanna Helyes (Hungary) and Péter Várnai (Hungary)
9:35 – 9:45	**The genesis of CD133+ progenitor cells in experimental alcoholic hepatitis in mice** **Raymond Wu** *(USA)*
9:45 – 9:55	**TLR2 and TLR9 promote neutrophil-driven alcoholic liver injury through induction of CXCL1** **Yoon Seok Roh** *(USA)*
9:55 – 10:05	**Alcohol intoxication poteniates post burn hepatic damage and interleukin-6 production through increased p38 MAPK signaling in kupffer cells** **Michael M. Chen** *(USA)*
10:05 – 10:20	**COFFEE BREAK**

SESSION 3 : **"Pancreatic and liver cancer development and therapy"**
Chairs: Zsuzsanna Kahán (Hungary) and Richárd Szmola (Hungary)

10:20 – 10:45 **Pancreatic cancer – the microenvironment needs attention too**
Minoti Apte *(Australia)*

10:45 – 11:10 **Cell cycle regulators are key factors in liver pathology**
Luis Fajas *(France)*

11:10 – 11:35 **Neddylation and liver cancer: new therapeutical approach**
Maria Luz Martinez-Chantar *(Spain)*

11:35 – 12:00 **Ca^{2+}, cAMP and migration of pancreatic cancer cells**
Alexei Tepikin *(UK)*

12:00 – 12:25 **Novel therapeutics for pancreatic and liver cancer**
Ashok Saluja *(USA)*

12:25 – 13:30 

LUNCH AND POSTER VIEWING

SESSION 4: **"The effects of alcohol on Ca2+ signalling and on cellular homeostasis"**
Chairs: László Hunyadi (Hungary) and Péter Enyedi (Hungary)

13:30 – 13:55 **Translocation between $PI(4,5)P_2$ microdomains determines STIM1 conformation and gating of Orai1**
Shmuel Muallem *(USA)*

13:55 – 14:20 **NFAT isoform activation by sub-cellular calcium signals**
Anant Parekh *(UK)*

14:20 – 14:45 **Pathological Ca^{2+} signals, their effects and how to prevent them**
Ole Petersen *(UK)*

14:45 – 15:10 **Acid sphingomyelinase in steatohepatitis**
José Carlos Fernández-Checa *(Spain)*

SESSION 5: **"Selected oral presentations (4-6)"**
Chairs: Márta Széll (Hungary) and Gábor Veres (Hungary)

15:10 – 15:20 **MicroRNAs regulates glyicine-n-methyl tranferase in liver cirrhosis**
Fernandez-Tussy Pablo *(Spain)*

15:20 – 15:30 **Implications of neddylation in liver cirrhosis**
Imanol Zubiete Franco *(Spain)*

15:30 – 15:40 **Liver fibrogenesis is controlled by innate immune activation pathways in hepatocyte apoptosis**
Arvin Iracheta-Vellve *(USA)*

15:40 – 16:00 **COFFEE BREAK**

SESSION 6: **"Epithelial physiology and the role of epithelial cells in alcohol induced tissue damage"**
Chairs: Ákos Zsembery (Hungary) and Tamás Bíró (Hungary)

16:00 – 16:25 **Alcohol, calcium signaling, and secretion in cholangiocytes**
Michael Nathanson *(USA)*

16:25 – 16:50 **The role of pancreatic ducts in the pathogenezis of acute pancreatitis**
Zoltán Rakonczay Jr. *(Hungary)*

16:50 – 17:15 **Function and repair of dental enamel – potential role of epithelial transport processes of ameloblasts**
Gábor Varga *(Hungary)*

19:30 – 22:30 **Welcome reception and dinner**
HOTEL TISZA – Szeged H6720 Széchenyi tér 3

23 NOVEMBER 2014 (Sunday)

SESSION 7: **"Genetics of alcohol-related pancreatic diseases"**
Chairs: Jonas Rosendhal (Germany) and Heiko Witt (Germany)

8:00 – 8:25 **How does alcohol contribute to pancreatic diseases?**
David Whitcomb *(USA)*

8:25 – 8:50 **Genetic clues towards a mechanism of alcoholic chronic pancreatitis**
Miklós Sahin-Tóth *(USA)*

8:50 – 9:15 **Genetic susceptibility factors for alcohol-induced chronic pancreatitis**
Marcus Lerch *(Germany)*

9:15 – 9:40 **Genetic models of pancreatitis: how CF animal models can help us understand the pathogenesis?**
Aliye Uc *(USA)*

SESSION 8: **"Selected oral presentations (7-10)"**
Chairs: Attila Mócsai (Hungary) and Ákos Szűcs (Hungary)

9:40 – 9:50 **Ethanol and cigarette smoke extract inhibits CFTR activity in pancreatic ductal cells**
Eleonóra Gál *(Hungary)*

9:50 – 10:00 **Exploring the mechanisms behind cigarette smoke-induced internalization of CFTR**
Mike Gray *(UK)*

10:00 – 10:10 **Atg4B mediates autophagy inhibition in alcoholic pancreatitis**
Olga Mareninova *(USA)*

10:10 – 10:20 **Functional analysis of pancreatitis associated PRSS1, MORC4, and CLDN2 mutations**
Mario Krehan *(Germany)*

10:20 – 10:35 **COFFEE BREAK**

SESSION 9: **"Alcohol-induced inflammation and immune response - Liver"**
Chairs: Ákos Pap (Hungary) and István Hritz (Hungary)

10:35 – 11:00 **Metabolic and microbial danger signals in alcoholic liver disease**
Gyöngyi Szabó *(USA)*

11:00 – 11:25 **Opposing role of type I vs. type II NKT cells in alcoholic liver disease—implications for novel intervention**
Vipin Kumar *(USA)*

11:25 – 11:50 **Cellular and molecular drivers in alcoholic hepatitis**
Ramon Bataller *(USA)*

11:50 – 12:15 **Circadian Clock Control of Alcoholic Liver Disease by Nuclear Receptor Signaling**
Li Wang *(USA)*

12:15 – 13:30 

LUNCH AND POSTER VIEWING

SESSION 10: **"Alcohol-induced inflammation and immune response - Pancreas"**
Chairs: László Czakó (Hungary) and Mark Lowe (USA)

13:30 – 13:55 **The unfolded protein response in protection from alcoholic pancreatitis**
Stephen Pandol *(USA)*

13:55 – 14:20 **Involvement of Alcohol and Fat in Acute Pancreatitis: a Dangerous Liaison**
David Criddle *(UK)*

14:20 – 14:45 **Fat in pancreatitis**
Vijay Singh *(USA)*

SESSION 11: **"Alcohol-related mitochondrial dysfunction"**
Chairs: Georg Beyer (Germany) and Tamás Csont (Hungary)

14:45 – 15:10 **Mitochondrial fusion dynamics is a potential target and mediator of the alcohol-induced tissue injury**
György Hajnóczky *(USA)*

15:10 – 15:35	**Calcium signaling and mitochondrial (dys)function in alcoholic liver disease** **Joannes Hoek** *(USA)*
15:35 – 16:00	**The crucial role of mitochondrial damage and consequent breakdown of bioenergetics in acute pancreatitis** **Peter Hegyi** *(Hungary)*
16:00 – 16:15	**COFFEE BREAK**
SESSION 12:	**"Non-alcoholic and alcoholic fatty liver diseases"** **Chairs:** Pár Gabriella (Hungary) and Pár Alajos (Hungary)
16:15 – 16:40	**Bile acid signalling and non-alcoholic fatty liver disease** **Peter Jansen** *(The Netherlands)*
16:40 – 17:05	**The role of alcohol-induced liver hypoxia** **Yatrik Shah** *(USA)*
17:05 – 17:30	**NADPH oxidases in alcoholic liver disease** **Natalie Torok** *(USA)*
17:30 – 17:35	**Closure of the meeting**
20:00 – 23:00	**Gala dinner, award presentations** **SZEGED THEATRE** – Szeged H6720 Horváth Mihály u. 3.

End of Programme

*Funding for this conference was made possible (in part) by Grant **R13AA20691** from the National Institute on Alcohol Abuse and Alcoholism (NIAAA). The views expressed in written conference materials or publications and by speakers and moderators do not necessarily reflect the official policies of the Department of Health and Human Services; nor does mention by trade names, commercial practices or organizations imply endorsement by the U.S. Government.*

ABSTRACTS

Invited speakers

Alcoholic Liver Disease: Do Genes or Environment Matter More?

Samir Zakhari

DISCUS, Washington, DC, USA

Chronic heavy alcohol consumption results in a group of ailments described as alcoholic liver disease (ALD) which encompasses steatosis, steatohepatitis, fibrosis, cirrhosis and acute alcoholic hepatitis. Although alcoholic cirrhosis (ALC) remains a major cause of morbidity and mortality, it is unknown why only a minority of chronic heavy alcohol abusers develop ALC. A weak relationship exist between the amount of alcohol consumed and ALC development; some develop severe liver disease with less than heavy levels of alcohol use, whereas others with very high levels of consumption only progress to mild liver Injury. A few factors have been identified that contribute to the development of ALC. Although evidence from twin studies supports a genetic component in ALC, recently there has been considerable interest in the role of patatin-like phospholipase domain-containing 3 gene (PNPLA3), the only genetic polymorphism that has been replicated as a risk factor for ALC. Studies involving candidate gene have been too small to yield definitive results, and specific polymorphisms selected were often arbitrarily chosen without ample knowledge of their post-transcription effects. No linkage studies on ALC have yet been described, but allelic variation in candidate genes have been reported to be associated with differences in risk of ALC. Single nucleotide polymorphisms (SNPs) identified by genome-wide search provide an ideal opportunity to Identify genes responsible for this polygenic disorder.

Because of the involvement of the gut flora-liver axis in ALD, there is mounting interest in the role of endotoxin-mediated cytokine release in the pathogenesis of ALD, and in the genes influencing endotoxin response and TLR4. In addition, a convincing genetic association has been reported between ALD and an interleukin-10 (IL-10) promoter-region. Furthermore, genetic factors that influence alcohol-induced hepatocellular carcinoma include polymorphisms in the C677T SNP in the methylenetetrahydrofolate reductase (MTHFR), superoxide dismutase, myeloperoxidase, and glutathione peroxidase.

Needless to say, ALD involves complex disease traits where the ultimate disease phenotype and progression is determined by the interaction between genetic factors and environmental influences. Major environmental factors that interact with chronic heavy alcohol consumption include diet, smoking, and obesity.

Mechanisms by which alcohol predisposes to pancreatitis: an overview

Anna Gukovskaya

Veterans Affairs Greater Los Angeles Healthcare System, University of California at Los Angeles, and Southern California Research Center for ALPD and Cirrhosis, Los Angeles, CA, USA.

Key pathologic responses of pancreatitis are disordering of acinar cell functions, inflammation, cell death, and fibrosis. Alcohol abuse is a major risk factor for pancreatitis; however, both epidemiologic data and experiments with animal models indicate that alcohol alone is not sufficient to initiate pancreatitis but sensitizes pancreas to disease development. Although the mechanisms of ethanol toxicity to pancreas are not fully understood, our knowledge on how ethanol promotes and exacerbates pancreatitis has significantly advanced during the past decade. Several pathogenic pathways have been elucidated, such as activation of the pro-inflammatory transcription factor NF-κB, mediated by novel PKC isoforms; activation of stellate cells, the principal fibrogenic cells in pancreas; and ATP decrease leading to acinar cell necrosis. Toxic effects of ethanol have been shown on acinar cells; many of them are mediated by ethanol's oxidative and non-oxidative metabolites. However, the mechanisms initiating pancreatitis, and how they are affected by ethanol, remain largely unknown. Recent findings indicate that disordering of interrelated cellular organelles (ER, lysosomes, mitochondria), resulting in impaired protein processing, trafficking and degradation, is critical to disease initiation. Further, the data indicate that ethanol and its metabolites cause disordering of acinar cell organelles, manifest by induction of ER stress, loss of the mitochondrial membrane potential, and autophagy impairment. In this overview, I will discuss mechanisms mediating ethanol's toxicity to pancreas, recent findings on ethanol-induced acinar cell organelle dysfunction, and future research directions.

Metabolic Reprogramming and Cell Fate Regulation in Alcoholic Liver Disease

Hidekazu Tsukamoto

Southern California Research Center ALPD and Cirrhosis and Department of Pathology, Keck School of Medicine of the University of Southern California, Los Angeles, California, USA

Alcoholic liver disease (ALD) should be defined as a life-style metabolic disease. Its pathogenesis is driven by altered cell fate of both parenchymal and non-parenchymal liver cell types, contributing to different pathologic spectra. A critical turning point in progression of ALD is chronic alcoholic steatohepatitis (ASH) or acute alcoholic neutrophilic hepatitis (AH), which markedly predisposes patients to medically most devastating ALD sequela, cirrhosis and liver cancer. Our research identifies the pivotal roles of unique metabolic reprogramming in M1 activation of hepatic macrophages (HM) and myofibroblastic activation (MF) of hepatic stellate cells (HSC) in the genesis of inflammation and fibrosis, the two key histological features of ASH and AH. For M1 HM activation, heightened proinflammatory iron redox signaling in endosomes or caveosomes results from altered iron metabolism and storage, promoting IKK/NF-kB activation via interactive activation of p21ras, TAK1, and PI3K. For MF cell fate regulation of HSC, activation of the morphogen Wnt pathway caused by the nuclear protein NECDIN or the single-pass trans-membrane protein DLK1, reprograms lipid metabolism to establish a positive regulatory loop for MeCP2-mediated epigenetic repression of the key HSC quiescence gene *Ppar-γ*. Conclusions from these studies re-enforce the importance of the morphogen-metabolic reprogramming-cell fate regulation link in the pathogenesis of ASH. (Supported by NIAAA grants and Medical Research Service of Department of Veterans Affairs)

Pancreatic Cancer: The Microenvironment Needs Attention Too!

Minoti Apte

University of New South Wales and Ingham Institutefor Applied Medical Research, Australia

The abundant stromal/desmoplastic reaction, a characteristic feature of a majority of pancreatic adenocarcinomas (PDAC), has only recently been receiving some attention regarding its possible role in the pathobiology of pancreatic cancer. It is now well established that the cells predominantly responsible for producing the collagenous stroma are pancreatic stellate cells (PSCs). In addition to extracellular matrix proteins, the stroma also exhibits cellular elements including immune cells, endothelial cells and neural cells. Evidence is accumulating to indicate the presence of significant interactions between PSCs and cancer cells as well as between PSCs and other cell types in the stroma. The majority of research reports to date, using in vitro and in vivo approaches, suggest that these interactions facilitate local growth as well as distant metastasis of pancreatic cancer, although a recent study using conditional of myofibroblasts in mouse pancreas has raised some questions regarding the central role of myofibroblasts in cancer progression. Nonetheless, novel therapeutic strategies have been assessed, mainly in the pre-clinical setting, in a bid to interrupt stromal-tumour interactions and inhibit disease progression. The next important challenge is for the translation of such pre-clinical strategies to the clinical situation so as to improve the outcome of patients with pancreatic cancer.

Cell cycle regulators are key factors in liver pathology

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Our laboratory has been focusing the last years on the implication of cell cycle regulatory proteins as modulators of metabolic processes. Analysis of genetically engineered mice deficient for cell cycle regulators, including E2F1, cdk4, or, pRB showed that the major phenotypes are metabolic perturbations. We proved that these key cell cycle regulators contribute to lipid synthesis, glucose production, insulin secretion, and oxidative metabolism and how deregulation of those pathways can lead to metabolic perturbations. These examples illustrate the growing notion that cell cycle regulatory proteins can also modulate metabolic processes. We show indeed, that the cdk4-E2F1 network is a sensor of the nutritional and energetic status of the cell. Interestingly we prove that these cell cycle regulators are activated by insulin and glucose. Most importantly we show that these cell cycle regulators trigger the adaptive metabolic switch that normal and cancer cells require in order to proliferate. We prove that cdk4 modulates the AMPK pathway through direct phosphorylation in order to blunt oxidative metabolism in cancer cells. Concomitantly, cdk4 interacts with and activates key proteins in the glycolytic pathway, including PDK4, G6Pisomerase, or phosphoglucomutase. Similarly, we show that E2F1 transcription factor regulates the expression of genes involved in lipid synthesis in cancer cells. Inhibition of the activity of these genes abrogates the ability of E2F1 to transform the cells. In summary, our results show that these factors are essential regulators of anabolic, biosynthetic processes, blocking at the same time oxidative and catabolic pathways in cancer cells.

Neddylation and liver cancer: new therapeutical approach

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BACKGROUND & AIMS: The current view of cancer progression supports the notion that cancer cells must undergo through a post-translational modification regulation and a metabolic switch or reprogramming in order to progress in an unfriendly environment. In an effort to define factors relevant for the acquisition of these abilities we have investigated the effect of NEDDylation in the signaling and metabolism of liver cancer and its potential as a new therapeutic approach.

METHODS: NEDDylation levels were determined on liver tumor from 66 patients. Moreover, assessment of NEDDylation target proteins was evaluated in human hepatoma cells and in Prohibitin 1-KO Hepatocellular Carcinoma animal model where NEDDylation inhibition was achieved through silencing NEDD8 and NAE1 (NEDD8 activating enzyme E1 regulatory subunit) genes or with the experimental drug MLN4924. The bioenergetic alterations of the human hepatoma cells under lack of NEDDylation was detected conveniently and simultaneously using an XFe Analyzer through the oxygen consumption rate (OCR), and extracellular acidification rate (ECAR) as an indicator of glycolysis. The effect of NEDDylation activity by Mass spectrometry-based metabolomic analyses was assessed in the mouse liver cancer model Prohibitin 1-KO.

RESULTS: NEDDylation is specifically enriched in human liver cancers and provide a selective advantage to the tumors. Importantly, a variety of cancer proteins linked to signaling as Akt, LKB1 and Alpha-Fetoprotein, hallmarks of proliferative metabolism in tumoral cells are targets of this post-translational modification. Concomitant, the lack of NEDDylation resulted in a metabolic reprogramming rendering a decrease in the oxidative phosphorylation, an enhanced glycolysis and ending in acute lactic acidosis concordant with a regression of liver cancer. Moreover, loss of NEDDylation caused perturbations in sphingomyelin, diacylglycerol and triacylglycerol metabolism and changes in phosphatidylcholine species produced via the PEMT pathway.

CONCLUSION: The results implicate NEDDylation/signaling/metabolism in the development of liver cancer and pave the way for novel therapeutical approaches.

Novel therapeutics for pancreatic and liver cancer

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Pancreatic cancer, affecting almost 250,000 people worldwide, has very poor prognosis and dismal survival statistics. The current standard of care, Gemcitabine, fails to extend survival. Aggressive biology, high tumor recurrence, and chemoresistance make pancreatic cancer a formidable disease. Additionally, Hepatocellular carcinoma (HCC) affects almost 750,000 people worldwide. Our studies show that Minnelide, a water soluble pro-drug of triptolide (active compound from a Chinese herb) is effective against both pancreatic and liver cancers.

Minnelide was efficacious in multiple *in vivo* models of pancreatic cancer: an orthotopic model of pancreatic cancer using human pancreatic cancer cell, a xenograft model of human pancreatic tumors, and a spontaneous pancreatic cancer mouse model ($KRas^{G12D}$;$Trp53^{R172H}$;Pdx-1Cre). In these models, Minnelide caused tumor regression and inhibited metastasis, improving survival.

In HCC, Minnelide was used in combination with Sorafenib (10 mg/kg), the standard of care. This combination was superior to single drug treatment: increasing cell death *in vitro* and inhibiting tumor growth *in vivo*. Further, combination treatment allowed for treatment with less than 10% of the standard Sorafenib dose.

Together, our results suggest that Minnelide shows promise as a potent chemotherapeutic agent against pancreatic and liver cancers, and is currently being evaluated in a Phase 1 clinical trial.

Ca^{2+}, cAMP and migration of pancreatic cancer cells

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Cell migration and invasion are essential for the formation of metastases. Our laboratory recently developed interest in the role of classical signalling cascades (cAMP and Ca^{2+} signalling) in the migration and invasion of pancreatic ductal adenocarcinoma (PDAC) cells. In particular, we observed that cAMP increase results in the significant inhibition of PDAC cell migration (PANC-1, SUIT-2, CAPAN-2, BxPC3 and MiaPaca-2 cell lines)[1]. The invasion of PDAC cells was also inhibited. Furthermore, we found that the inhibition of migration can be induced by activation of adenylyl cyclases or by inhibition of phosphodiesterases. Using FRET-based sensors we observed clear correlation between the rise of cAMP, trafficking of paxillin from focal adhesion and depolymerisation of actin at the leading edge of PDAC cells. Importantly, we found that the inhibition of migration was triggered by the activation of protein kinase A, whilst another cAMP sensor EPAC actually potentiated migration[1]. Considering the reported close link between the cAMP production and store operated Ca^{2+} entry (SOCE)[2,3] we then focused our investigation on the positioning of the junctions between the endoplasmic reticulum (ER) and the plasma membrane (PM), which serve as platforms for SOCE. We found that ER-PM junctions decorate the leading edge of the migrating PDAC cells, providing signalling domains for interacting Ca^{2+} and cAMP cascades in the immediate proximity to the essential components of the migratory apparatus[4]. We hope that the detailed understanding of the cAMP and Ca^{2+} signalling at the leading edge of migrating PDAC cells will be beneficial for the development of treatment against this type of cancer.

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Translocation Between PI(4,5)P_2 Microdomains Determines STIM1 Conformation and Gating of Orai1

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Receptor-stimulated Ca^{2+} influx is mediated in part by the Ca^{2+} selective channel Orai1. Orai1 is activated by STIM1 in response to Ca^{2+} release from the ER, which results in co-clustering of STIM1-Orai1 in plasma membrane/endoplasmic reticulum (ER/PM) microdomains. Orai1 then undergoes fast and slow Ca^{2+}-dependent inactivation (SCDI). SCDI (and likely FCDI) is mediated by binding of the STIM1 inhibitor SARAF to STIM1. We use SCDI by SARAF as a reporter of the STIM1 conformation and to read the microdomain localization of the Orai1-STIM1 complex. We found that interaction of STIM1 with the C terminus of Orai1 and the STIM1 polylysine-rich domain (K-domain) are required for interaction of SARAF with STIM1 and to cause SCDI of Orai1. Interaction of SARAF with STIM1 required the presence of the STIM1-Orai1 complex in a caveolea PM/ER microdomain that is tethered by Elongated Synaptotagmine 1 (E-Syt1), it is stabilized by Septin4 and is enriched in PI(4,5)P_2. We were able to selectively target STIM1 to a PI(4,5)P_2-rich or to PI(4,5)P_2-poor microdomain using targeting motifs. Significantly, targeting of STIM1 to PI(4,5)P_2-rich or to PI(4,5)P_2-poor microdomains revealed that SCDI by SARAF is observed only when the STIM1-Orai1 complex is within the PI(4,5)P_2-rich microdomain. Notably, measuring the dynamics of STIM1-Orai1 complex localization in live cells using PI(4,5)P_2-rich or PI(4,5)P_2-poor microdomain probes revealed that store depletion is followed by transient STIM1-Orai1 complex formation in the PI(4,5)P_2-poor microdomain where the channel is fully active, which then translocates to the PI(4,5)P_2-rich domain to recruit SARAF and initiates SCDI. These findings reveal a role of PM/ER tethers in the regulation of Orai1 function and Ca^{2+} influx and describe a new mode of regulation by PI(4,5)P_2 involving translocation between PI(4,5)P_2 microdomains.

NFAT isoform activation by sub-cellular calcium signals

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Protein isoforms are widely expressed in biological systems. How isoforms that co- exist within the same sub-cellular domain are differentially activated remains unknown. Here we compare the regulatory mechanism of two closely related transcription factor isoforms, NFAT1 and NFAT4, that are widely expressed in mammalian tissues including the immune system and the pancreas. NFATs migrate from the cytoplasm to the nucleus following the increase in intracellular Ca2+ that accompanies opening of store-operated Orai1/CRAC channels. Here, I describe how NFAT1 has a private line of communication with Orai1, activating in response to Ca2+ microdomains near the open channels. By contrast NFAT4 stimulation requires both local Ca2+ entry and a rise in global Ca2+. We mapped differences in nuclear location to amino acids within the NFAT regulatory domain. The different Ca2+-dependencies enable agonist to recruit different isoform combinations as stimulus strength increases.

Pathological Ca^{2+} signals, their effects and how to prevent them

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In pancreatic acinar cells, physiological secretagogues evoke release of Ca^{2+} from internal stores, which in turn triggers opening of store-operated Ca^{2+} entry channels in the plasma membrane. The resulting repetitive short-lasting elevations of the cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) then activate fluid and enzyme secretion (1). Pathological stimulation of pancreatic acinar cells with, for example, combinations of alcohol and fat - generating fatty acid ethyl esters intracellularly (2) - induce massive intracellular release of Ca^{2+} followed by excessive Ca^{2+} entry through the store-operated channels, resulting in a sustained elevation of $[Ca^{2+}]_i$. This causes impairment of mitochondrial function, intracellular trypsin activation and necrotic cell death (1-3). There are several components of store-operated Ca^{2+} entry, but the most important pathway is through classical Ca^{2+} release-activated Ca^{2+} (CRAC) channels, which are extremely Ca^{2+}-selective (3). Specific blockade of CRAC channels might therefore be used as a therapy against severe acute pancreatitis. The relatively selective CRAC channel blocker GSK7975A dramatically inhibited store-operated Ca^{2+} entry and therefore prevented the sustained elevation of $[Ca^{2+}]_i$ that normally follows emptying of intracellular stores. Most importantly, the CRAC channel blocker markedly inhibited the intracellular protease activation evoked by palmitoleic acid ethyl ester as well as the resulting necrosis (3). These data provide proof in principle that specific CRAC channel blockade could be the basis of a drug-based therapy of acute pancreatitis (1,3).

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Acid sphingomyelinase in steatohepatitis

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Steatohepatitis (SH) is an intermediate stage of fatty liver disease and one of the most common causes of chronic liver disease worldwide that may progress to cirrhosis and liver cancer. SH encompasses alcoholic (ASH) and nonalcoholic steatohepatitis (NASH), the latter being of particular concern due to its association with obesity and insulin resistance and a major cause of liver transplantation. ASH is a major health concern of alcohol abuse and a leading cause of liver-related morbidity and mortality. The molecular mechanisms governing the transition from steatosis to SH are not fully understood. Recent evidence in ASH and NASH has indicated in that acid sphingomyelinase (ASMase), a specific mechanism of ceramide generation, is required for the activation of key pathways that regulate steatosis, fibrosis, insulin resistance and lipotoxicity, including endoplasmic reticulum (ER) stress, autophagy and lysosomal membrane permeabilization. The alcohol-induced ASMase-mediated ER stress was independent of the disruption of methionine metabolism and hyperhomocysteinemia caused by alcohol. Pharmacological inhibition of ASMase prevented alcohol-mediated ER stress, steatosis, and sensitization to LPS-mediated liver injury. These findings indicate that ASMase regulates multiple pathways and that its inhibtion may be of potential relevance in SH.

Alcohol, calcium signaling, and secretion in cholangiocytes

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Alcoholic hepatitis is a life-threatening complication of alcoholic liver disease, thought to result largely from hepatocellular inflammation. However, evolving evidence suggests cholestatic liver injury also occurs in alcoholic hepatitis. Cholestasis typically involves cholangiocytes, which condition bile secreted by hepatocytes and are particularly important for biliary bicarbonate secretion. The best described signaling pathway that regulates cholangiocyte secretion links secretin receptors via cyclic AMP to activation of CFTR, resulting in chloride secretion and chloride/bicarbonate exchange. However, CFTR activation also results in luminal secretion of ATP, which then stimulates apical P2Y receptors, leading to autocrine calcium signaling and calcium-mediated chloride and bicarbonate secretion. Calcium signals in cholangiocytes are mediated by inositol 1,4,5-trisphosphate (InsP3). The type III InsP3 receptor (InsP3R3) is the predominant isoform in cholangiocytes. It is concentrated in the apical region, where calcium signals originate. Knockdown of InsP3R3 impairs calcium signaling and bicarbonate secretion, so this autocrine pathway may be required for normal secretion. Furthermore, InsP3R3 expression is lost in cholangiocytes from patients with a range of cholestatic diseases, including primary biliary cirrhosis, sclerosing cholangitis, biliary atresia, and bile duct obstruction. Therefore loss of InsP3R3 may be a final common element in cholestasis. We now have found that >70% of patients with alcoholic hepatitis have an elevated serum alkaline phosphatase, suggestive of cholangiocyte damage. Moreover, in each of seven liver biopsies from patients with alcoholic hepatitis, InsP3R3 expression in cholangiocytes was either reduced or mis-localized. These findings suggest that cholangiocyte damage may occur frequently in alcoholic hepatitis, resulting in impaired calcium signaling and bile secretion.

The role of pancreatic ducts in the pathogenesis of acute pancreatitis

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Pancreatic ducts secrete 2.5 l of alkaline, bicarbonate-rich fluid daily which greatly contributes to the homeostasis of the pancreas. The ducts are also important in the pathophysiology of the pancreas, the alteration of ductal function can lead to severe diseases such as cystic fibrosis and chronic pancreatitis. The role of pancreatic ducts in the development of acute pancreatitis has only been uncovered recently. Pancreatitis inducing agents like bile acids and ethanol dose-dependently affect pancreatic ductal secretion; low concentrations inhibit, whereas high concentrations stimulate secretion. The majority of the presentation will focus on the central role of cystic fibrosis transmembrane conductance regulator (CFTR), a critical protein in the regulation of ductal secretion, in the pathogenesis of acute pancreatitis which is highlighted by numerous studies. Downregulation of CFTR expression results in increased severity of acute pancreatitis in mice. Furthermore, human genetic studies have demonstrated statistically significant association of *CFTR* mutations with acute recurrent pancreatitis. Overall, the data suggest that the stimulation of pancreatic ductal secretion may serve as a new therapeutic target in the treatment of acute pancreatitis.

Function and repair of dental enamel - potential role of epithelial transport processes of ameloblasts

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The hardest mammalian tissue, dental enamel is produced by ameloblasts, which are electrolyte-transporting epithelial cells. Although the end product is very different, they show many similarities to transporting epithelia of the pancreas, salivary glands and kidney. Enamel is produced in a multi-step epithelial secretory process. First, "secretory" ameloblasts form the entire thickness of the enamel layer, but with low mineral content. Then they differentiate into "maturation" ameloblasts, which remove organic matrix from the enamel and in turn further build up hydroxyapatite crystals. The protons generated by hydroxyapatite formation need to be buffered, otherwise enamel will not attain full mineralization. Buffering requires a tight pH regulation and secretion of bicarbonate by ameloblasts. The whole process has been the focus of many immunohistochemical and gene knock-out studies, but, perhaps surprisingly, up till now, no functional data existed for mineral ion transport by ameloblasts. However, most recent studies including ours provided evidence for the nature of molecular mechanism of mineral transport, as well as for the bicarbonate secretory processes in enamel formation. The secretory regulation is not completely known as yet, but its significance is crucial. Impairing regulation retards or prevents completion of enamel mineralization and results in the development of hypomineralized enamel that easily erodes after dental eruption. Factors that impair this function are fluoride and disruption of pH regulators. Revealing these factors may eventually lead to the treatment of enamel hypomineralization related to genetic or environmentally induced malformation.

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Genetics of alcohol-related pancreatic diseases in North America

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Alcohol is clearly associated with chronic pancreatitis, but the exact mechanism is unknown. Until recently, up to 90% of chronic pancreatitis (CP) cases were thought to be from alcoholism. However, more recent studies suggest that, in many populations alcohol-related pancreatitis is less then 50%. In addition, only about 3% of heavy alcoholics develop clinically recognized chronic pancreatitis, suggesting that alcohol alone does not cause pancreatitis.

The North America Pancreatitis Study II (NAPS2) is a multicenter, cross-sectional cohort study designed to complete a detailed clinical, demographic, risk factor, and genetic study of recurrent acute pancreatitis (RAP) and chronic pancreatitis (CP); 33 centers recruited and fully phenotyped 1851 cases (RAP, n=590; CP n=1261) and 1111 controls.

Key findings related to alcohol include a threshold effect of 5 or more drinks a day to increase risk of pancreatitis from alcohol. Smoking was found to be an independent, dose related risk factor, with a synergistic risk with combined alcohol and smoking. Neither alcohol or smoking appeared to be a major risk factor for acute pancreatitis (AP), but patients with recurrent AP (RAP) had rapid transition to CP (fibrosis) with alcohol – as previously demonstrated in our animal models. Alcohol increased the risk of RAP, and risk of progression to CP was increased in patients with a high-risk variant at the *CLDN2* locus, located on the X chromosome. Since men only have one X chromosome, there is a higher likelihood that drinking men will have a high-risk genotype then drinking women. The risk of progressing from AP to CP is also increased in patients with *CTRC* mutations, and this risk appears to be highest in smokers.

Conclusion: Chronic alcoholic pancreatitis is a complex gene x environment disorder that includes an initial episode of AP, RAP, smoking, *CLDN2* and *CTRC* gene variants.

Genetic clues towards a mechanism of alcoholic chronic pancreatitis

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Idiopathic (non-alcoholic) chronic pancreatitis with or without a family history is a genetically determined disorder. Risk for disease development may be determined by a single strong genetic risk factor (e.g. some *PRSS1* and *CPA1* variants) or a combination of multiple, weaker genetic risk factors (e.g. *SPINK1*, *CTRC* and *CFTR* variants). Alcoholic chronic pancreatitis, on the other hand, has been regarded as a disease determined largely by environmental risk factors (alcohol and smoking) with a smaller genetic component. Here we review the role of various genetic risk factors in alcoholic chronic pancreatitis and argue that this disease entity is largely determined by the same set of susceptibility genes as non-alcoholic pancreatitis with the notable difference that genetic variants that confer smaller risk (e.g. *PRSS1* promoter variants or *CLDN2* variants) are more often observed than in idiopathic disease. In fact, an inverse relationship seems to exist between genetic effect size and frequency in the patient population as a function of age. Finally, an important corollary to our argument is that mechanistically alcoholic chronic pancreatitis is similar to idiopathic disease and both are driven by a trypsin-dependent pathological pathway and/or the more recently emerging misfolding-dependent pathway.

Genetic susceptibility factors for alcohol-induced chronic pancreatitis

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The last decade has seen enormous progress in understanding the genetic susceptibility factors for chronic pancreatitis – most prominently for the hereditary variety of the disease. When pancreatitis is passed on in families in an autosomal dominant manner, the associated gene mutations most commonly affect the cationic trypsinogen (PRSS1) gene. For chronic pancreatitis associated with immoderate alcohol consumption the situation was, until recently, quite different and no genetic susceptibility factors had been identified. Smaller studies in ethnically defined populations have found that not only polymorphism affecting proteins involved in the metabolism of ethanol, such as Alcohol Dehydrogenase and Aldehyde Dehydrogenase, can confer a risk for developing chronic pancreatitis but also mutations that had previously been reported in association with idiopathic pancreatitis, such as SPINK1 mutations. In a much broader approach employing genome wide search strategies the NAPS study has found that polymorphisms in Trypsin (PRSS1 rs10273639), and Claudin 2 (CLDN2 rs7057398 and rs12688220) confer an increased risk of developing alcohol-induced pancreatitis. These results from North America have now been confirmed by a European consortium. In another genome wide approach polymorphisms in the genes encoding Fucosyltransferase 2 (FUT2) non-secretor status and blood group B were found to more than double the risk for developing alcohol-associated chronic pancreatitis. These novel genetic associations now allow to investigate the pathophysiological and biochemical basis of alcohol-induced chronic pancreatitis on a cellular level.

Metabolic and microbial danger signals in alcoholic liver disease

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Inflammation defines the progression of alcoholic liver disease (ALD) from reversible to advanced stages. The translocation of bacterial lipopolysaccharide (LPS) to the liver from the gut is thought to be necessary for inflammation in ALD. In this study we discovered that liver inflammation in ALD is dependent on endogenous danger signals released from damaged hepatocytes and that these signals drive inflammation in synergism with gut-derived LPS. We administered Lieber-DeCarli ethanol diet or intragastric ethanol to WT, IRF3- or ATP receptor 2x7 (P2rx7)-KO mice, or to mice overexpressing uricase or treated with probenecid or allopurinol to assess the role of hepatocyte death, ATP and uric acid in liver inflammation. We found that hepatocytes damaged by ethanol released endogenous danger signals, uric acid and ATP, with subsequent activation of the inflammasome and development of liver inflammation prevent liver inflammation in ALD. Absence of hepatocyte death (in IRF3-KOs), depletion of uric acid or ATP, or lack of ATP signaling prevented activation of inflammasome and its major downstream cytokine, interleukin (IL)-1β. Uric acid and ATP mediated paracrine inflammatory cross-talk between damaged hepatocytes and liver immune cells, and pharmacological depletion of uric acid with allopurinol or probenecid provided significant protection from alcohol-induced inflammation, steatosis and liver damage. Our data suggest that inflammation in ALD results from a synergism between host-derived molecules released from damaged hepatocytes and microbial components such as LPS translocated from the gut. Inhibition of signaling triggered by uric acid and ATP may have therapeutic implications in ALD.

Type I NKT cells mediate while type II NKT cell activation attenuates alcoholic liver disease

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Innate immune mechanisms leading to liver injury following chronic alcohol ingestion are poorly understood. Natural killer T (NKT) cells, enriched in the liver and comprised of at least two distinct subsets, type I and type II, recognize different lipid antigens presented by CD1d molecules. We have investigated whether differential activation of NKT cell subsets and their interactions with other innate immune cells are decisive in orchestrating inflammatory events leading to alcoholic liver disease (ALD). We found that following chronic plus binge feeding of Lieber-DeCarli liquid diet in male C57BL/6 mice, type I but not type II NKT cells are activated leading to recruitment of inflammatory cells, including neutrophils into liver. Thus liver injury is significantly inhibited in Ja18-/- mice deficient in type I NKT cells as well as following their inactivation by sulfatide-mediated activation of type II NKT cells. Furthermore we have identified a novel pathway involving all-trans retinoic acid (ATRA) receptor gamma signaling that inhibits type I NKT cells and consequently ALD. We have used quantitative PCR to examine hepatic gene expression of several key molecules and signaling pathways shared in human disease and found that the upregulation of a number of cytokine and chemokine genes in ALD is dependent upon differential activation of NKT cell subsets. These studies provide a better understanding of the involvement of key innate immune mechanisms centered on activation of CD1d-retricted NKT cells following ethanol ingestion and should allow identification of novel immune targets for potential therapeutic intervention in ALD.

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CELLULAR AND MOLECULAR DRIVERS IN ALCOHOLIC HEPATITIS

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Patients with underlying alcoholic liver disease and continuous heavy alcohol intake can develop abrupt jaundice and liver-related complications, a clinical condition known as alcoholic hepatitis (AH). The pathogenesis of AH is incompletely understood, in part due to the lack of animal models that reproduce the main clinical and histological features. Traditionally, it was felt to be an inflammatory reaction to the toxic effects of ethanol in the liver, mainly mediated by TNFα. Consequently, TNFα-blocking agents were tested in patients with severe AH, with disappointing results. More recent translational studies including human samples from patients with AH showed that CXCL chemokines, rather than TNFα, are markedly overexpressed in AH and correlated with survival. Other inflammatory mediators such as Fn14, CCL20 and osteopontin are potential targets for therapy in AH. Moreover, inflammatory molecules in AH can derived from a leaky gut that contains an unusual content of gram-negative bacteria. Gut-derived bacterial products represent pathogen-associated molecular pattern (PAMPs) circulate through the portal system and induce inflammatory actions in resident liver cells (mainly stellate cells and Kupffer cells) through TLR4. Another type of antigens that can stimulate the innate immune system is the so-called damage-associated molecular pattern (DAMPs). DAMPs are intracellular molecules that are released by dying cells and can stimulate sterile inflammation. Among DAMPs, high mobility group box-1 (HMGB1) has been implicated in the pathogenesis of alcoholic steatohepatitis. Besides inflammation, recent studies indicate that bilirubinostasis and severe fibrosis are major histological components of AH and are associated with a poor prognosis. Interestingly, the presence of PMN cells is associated with a better prognosis, probably reflecting that livers with active wound healing are more prone to regenerate upon cessation of alcoholic intake. Moreover, a detailed analysis of liver explants from patients with AH that underwent a liver transplantation showed that impaired regeneration is a hallmark finding in patients with severe AH. The mechanisms leading to inefficient liver regeneration in AH are unknown. We found that AH is characterized by a massive proliferation of progenitor cells, which do not yield mature hepatocytes. Promoting maturation of hepatic progenitor cells is an appealing strategy to treat AH.

Circadian Clock Control of Alcoholic Liver Disease by Nuclear Receptor Signaling

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Our research has been focused on elucidating hepatic function and metabolic diseases regulated by an orphan nuclear receptor small heterodimer partner (Shp, Nrob2). Our studies revealed the pleotropic function of Shp in fatty liver disease, liver cancer, and microRNA regulation. Circadian rhythms play a fundamental role in regulating metabolic diseases including non-alcoholic and alcoholic fatty liver. Using transcriptomics (RNA-seq) and metabolomics (GC/MS) analyses, we uncovered a global impact of *Shp*-deletion on the circadian rhythmic expression of liver clock and metabolic genes, as well as the oscillation of intermediate metabolites in various metabolic pathways. The ER stress signaling and homocysteine homeostasis were modulated by the liver clock machinery, which was markedly altered by ethanol-binge in both SHP-dependent and SHP-independent fashion. The results demonstrate that alcoholic liver disease is tightly controlled by the circadian clock that is integrated by nuclear receptor signaling.

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The Unfolded Protein Response in Protection from Alcoholic Pancreatitis

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The acinar cell of the pancreas is highly specialized for the synthesis and transport of large quantities of digestive enzymes and consequently, has the most highly developed endoplasmic reticulum (ER) and protein processing and trafficking organelles of any mammalian cell. Spliced X-box binding protein-1 (sXBP1) is uniquely responsible for the development of the protein secretory apparatus of the pancreatic acinar cell and its protein synthesis, processing and trafficking properties. In acinar cells, the high rate of protein synthesis is associated with a baseline ER stress, which is manifest by constitutive expression of sXBP1 which is also an ER stress response factor. In this regard, we have found that sXBP1 increases in the pancreas of animals fed alcohol and that genetic inhibition of sXBP1 results in marked deterioration of the acinar cell organellar structure associated with the development of pancreatitis. From these findings we have hypothesized that not only is sXBP1 necessary for exocrine pancreatic development and maintenance of protein synthesis but necessary for adaptation and prevention of pancreatic damage with exogenous pancreatic stressors.

We find that when sXBP1 expression is inhibited that there is activation of another ER originating pathway that is associated with inflammation and cell death- the PKR-like endoplasmic reticulum kinase (PERK) and C/EBP homologous protein (CHOP) pathway.

These findings lead us to an overall hypothesis that key ER stress pathways are deployed for adaptation and protection from potentially injurious stimuli. However, when these adaptive and protective pathways are inhibited or otherwise breached, pathologic ER originating pathways ensue and lead to pancreatitis.

The Role Of Fat And Alcohol In Acute Pancreatitis: A Dangerous Liaison

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Excessive alcohol consumption is a major trigger for severe acute pancreatitis which may lead to multi-organ dysfunction and premature death of the individual. Hyperlipidaemia is a risk factor for both acute and chronic pancreatitis and the role of fat has received increasing attention in recent years. In the pancreas ethanol is metabolised by both oxidative and non-oxidative pathways, the latter generating fatty acid ethyl esters (FAEEs) from fatty acid (FA) substrates via the action of diverse enzymes (FAEE synthases) including carboxylester lipase (CEL). Inhibition of the oxidative pathway promotes formation of FAEEs which induce sustained elevations of cytosolic calcium leading to inhibition of mitochondrial function, loss of ATP and necrosis of isolated pancreatic acinar cells[1,2]. Furthermore, FAEEs undergo hydrolysis in mitochondria releasing free FAs that exert toxic effects. Our recent work has shown that pharmacological inhibition of CEL by 3-BCP ameliorated detrimental effects of non-oxidative ethanol metabolism in isolated pancreatic acinar cells *in vitro* and in a new *in vivo* experimental model of alcoholic acute pancreatitis, revealing a specific enzyme target for ethanol-induced injury[3].

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Fatty acid ethyl esters are less toxic than their parent fatty acids and may play a protective role in pancreatitis

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Background: Fat is predominantly stored as triglycerides, forming >80% of adipocyte mass, the amount of which is increased in obesity. Fatty acid ethyl esters (FAEEs) were first noted to be present in the pancreas, adipose tissue of dying alcoholics [*Science 231(4737):497-9 (1986*)], and since then have sometimes been thought to play a deleterious role in alcoholic AP. Interestingly, the alcoholics in whose tissues FAEEs were noted to be elevated did not have pancreatitis and had died from unrelated causes such as a motor vehicle accident. The safety of FAEEs is also supported by their use to treat hypertriglyceridemia. Fatty acids (FAs) are elevated in the sera and pancreatic necrosis of patients with severe acute pancreatitis (SAP) [*Sci. Transl. Med. 3, 107ra110 (2011*)], and cause pancreatic necrosis along with organ failure. We therefore used pancreatic acini and *in vivo* models of SAP to compare the relative toxicities of FAEEs and their parent FAs, alone, or those generated by triglyceride lipolysis. **Methods:** Fatty acids were measured in the pancreatic necrosis debridement of patients with SAP or in a non-inflammatory group comprising pancreatic cystic neoplasms. Mice pancreatic acini were exposed to FAs (linoleic; LA, oleic; OA, palmitic; PA) and equimolar amounts of the corresponding FAEEs (i.e. LAEE, OAEE, PAEE) at concentrations relevant to human pancreatic debridement fluids. Necrotic cell injury was assessed by LDH leakage, propidium-iodide (PI) uptake and cytochrome c leakage, ATP levels, and cytosolic calcium, mitochondrial depolarization using JC-1 were also measured. The triglyceride Glyceryl trilinoleate (GTL) alone or with the lipase inhibitor orlistat (GTLO), Glyceryl-trioleate (GTO), or the ethyl ester of OA i.e. OAEE were injected intraductally in rats to simulate models of biliary and obesity associated SAP. Serum amylase, lipase, pancreatic necrosis, organ failure (blood urea nitrogen; BUN, Lung MPOs, TUNELS), cytokines and mortality were studied. Values are reported as mean±SEM. **Results:** LA and OA caused higher LDH leakage, PI uptake, ATP depletion, cytochrome c leakage, mitochondrial depolarization and cytosolic calcium increase than LAEE, OAEE. PA or PAEE did not affect these parameters. Serum amylase and lipase, pancreatic necrosis, mortality, serum oleic acid, IL-1b, IL-6, IL-18 and KC/GRO, BUN along with lung injury were higher in GTO infuse compared to OAEE infused rats. GTL, similar to GTO caused SAP, with extensive necrosis, renal, lung injury and 100% mortality which were completely prevented in the GTLO infused group. **Conclusions:** Unsaturated FAs generated from lipolysis of visceral triglyceride cause worse acinar necrosis, inflammation, organ failure and mortality than the corresponding FAEEs noted to be elevated in the viscera of alcoholics. Therefore the accumulation of FAEEs alcoholics is likely to be protective by reducing FA toxicity.

Mitochondrial fusion dynamics is a potential target and mediator of the alcohol-induced tissue injury

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The central role of mitochondria in alcohol metabolism and alcohol-induced tissue injury was established long ago. In the past 20 years, a burst of discovery in mitochondrial research has revealed fundamental roles for mitochondria in cell survival and signaling and dynamics. Recent studies have indicated that these aspects of mitochondrial function are also highly relevant for alcohol-linked disorders. In the context of mitochondrial dynamics, our recent work has demonstrated that (1) chronic EtOH exposure interferes with mitochondrial fusion dynamics in skeletal muscle fibers, cardiomyocytes and hepatocytes, and (2) at least in the skeletal muscle, the relevant target of the EtOH effect is mitofusin 1 (Mfn1), and (3) impairment of fusion/mitochondrial quality control produces myopathy phenotype. These results will be overviewed in the presentation.

Calcium Signaling and Mitochondrial (Dys) function in Alcoholic Liver Disease

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Calcium serves as a critical signaling mediator in the transmission of hormonal and stress signals in the liver, integrating metabolic, genetic and morphological information to optimize cell and tissue function as well as driving cell survival and cell death decisions. Mitochondria participate in the events that regulate hepatocyte calcium homeostasis both in shaping the patterns of cytosolic calcium responses and as targets of the calcium signals to drive metabolic integration and the response to stress and death signals. Both acute and chronic ethanol treatment profoundly affect the metabolic environment in the liver and alter the mitochondrial energy and redox balance. Ethanol treatment also impacts on the spatiotemporal organization of calcium signals in the liver. These changes are accompanied by an increased susceptibility of the mitochondria to pro-apoptotic signals. We will consider how the changes in calcium homeostasis can deregulate cellular stress response capacity and contribute to mitochondrial dysfunction that impacts on the susceptibility to alcoholic liver disease.

The crucial role of mitochondrial damage and consequent breakdown of bioenergetics in acute pancreatitis

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Acute pancreatitis is an inflammatory disease with no specific treatment. One of the main reasons behind the lack of specific therapy is that the pathogenesis of acute pancreatitis is poorly understood. During the development of acute pancreatitis, the disease-inducing factors can damage both cell types of the exocrine pancreas, namely the acinar and ductal cells. Because damage of either of the cell types can contribute to the inflammation, it is crucial to find common intracellular mechanisms that can be targeted by pharmacological therapies. Despite the many differences, recent studies revealed that the most common factors that induce pancreatitis cause (1) uncontrolled Ca2+release leading to intracellular Ca2+ overload and toxicity and (2) mitochondrial damage with the consequent breakdown of bioenergetics, that is, ATP depletion in both cell types. This presentation summarizes the variety of Ca2+ signals and the mitochondrial function and damage within both pancreatic acinar and ductal cells. We also suggest that colloidal ATP delivery systems for pancreatic energy supply may be able to protect acinar and ductal cells from cellular damage in the early phase of the disease. An effective energy delivery system combined with the prevention of further mitochondrial damage may, for the first time, open up the possibility of pharmacological therapy for acute pancreatitis, leading to reduced disease severity and mortality.

Bile acid signaling in non-alcoholic and alcoholic fatty liver disease

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Non-alcoholic liver disease is the most prevalent liver disease in western society. Like alcoholic liver disease, the histopathology of NASH is characterized by steatosis, inflammation, fibrosis, apoptosis and ballooning of hepatocytes. Thus from a pathology point of view NASH and alcoholic liver disease are very similar. In fact the 'non-alcoholic' in NASH is based on the arbitrary definition of less than 3 alcoholic drinks per day in men and less than 2 in women. There are more similarities between NASH and alcoholic liver disease. Overweight, insulin resistance and type 2 diabetes mellitus are prevalent both in NASH and alcoholic liver disease.

Therapy of NASH is based on changes of life style with emphasis on exercise and reduced calorie intake. However, life style changes are difficult to maintain, especially in this population. Therefore effective drug therapy would be an important contributor to any successful treatment program for NASH and this also applies for alcoholic liver disease.

With the discovery of nuclear hormone receptors about twenty years ago came the recognition that bile acid signaling plays an important role in regulation of metabolism, in particular regulation related to the fed/fasting cycle (1). The farnesoid X-nuclear receptor (FXR), the peroxisome proliferator-activated receptors PPARα and PPARγ and the trans-membrane bile acid receptor TGR5 are major players in bile acid- and fatty acid-mediated regulations of metabolism. Various bile acid metabolites and fatty acids are natural ligands for these receptors but drugs such as 6-ethyl-chenodeoxycholic acid (INT-747, Obeticholic acid) have 100 times higher affinity for FXR that the natural ligands. Drugs addressing TGR5 and the PPARs are being developed. The first results from phase 3 RCTs with Obeticholic acid in NASH are promising (2). Trials in alcoholic liver disease with this drug are planned.

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The role of alcohol-induced liver hypoxia

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Alcohol consumption is a major risk factor hepatocellular carcinoma. However, the molecular basis for the alcoholic progression of liver cancer is still unclear. Using our hypoxia reporter mouse line we show that mice treated with alcohol have a rapid and robust activation of the hypoxic signaling pathway. Oxygen dynamics in the liver is a central signaling mediator controlling hepatic homeostasis, and dysregulation of cellular oxygen dynamics is associated with liver injury. Our recent data demonstrates that the transcription factor relaying changes in cellular oxygen levels, hypoxia-inducible factor (HIF), is critical in liver metabolism. To help define the HIF signal transduction pathway, an animal model of HIF overexpression was generated and characterized. In this model, overexpression was achieved by Von Hippel-Lindau (*Vhl*) disruption in a liver-specific temporal fashion. Acute disruption of *Vhl* induced hepatic lipid accumulation in a HIF-2α-dependent manner. In addition, HIF-2α activation rapidly increased liver inflammation and fibrosis demonstrating that steatosis and inflammation are primary responses of the liver to hypoxia. Sustained HIF overexpression in hepatocytes led to hepatocellular tumors. The progression from steatosis to staetohepatitis and then fibrosis and liver tumors resembles sequelae of alcoholic liver disease (ALD). To demonstrate that HIF-2α is a critical link between alcohol and liver cancer we generated an inducible disruption of HIF-2α specifically in hepatocytes and we tested these mice in a novel alcohol-induced liver cancer model. Preliminary data demonstrates that HIF-2α is essential in alcoholic liver tumor promotion. To identify downstream effectors, a global microarray expression analysis was performed a revealing a time-dependent effect of HIF-2α on gene expression. A rapid increase in pro-inflammatory cytokines, iron regulatory proteins, and fibrogenic gene expression was observed. In vivo chromatin immunoprecipitation assays revealed novel direct targets of HIF-2α signaling that may contribute to hypoxia-mediated inflammation and tumorigenesis.

NADPH oxidases in alcoholic liver disease

Natalie Torok

Alcoholic liver disease (ALD) is a major cause of morbidity and mortality worldwide, and currently there are no successful therapeutic approaches available. The spectrum of liver disease includes simple steatosis, alcoholic hepatitis, and chronic liver disease with progressive fibrosis leading to cirrhosis. The factors implicated in the initiation of injury are poorly understood. Oxidative stress is an early event in liver injury leading to hepatocyte stress signaling and cell death, macrophage recruitment and activation and activation of the hepatic stellate cells leading to progressive fibrosis. Some important sources of oxidative radicals in alcoholic liver injury e.g. CYP2E1 system have been described, but potential targets for therapeutics are still lacking.

Superoxide and H_2O_2 (hydrogen peroxide) have been recognized as the main intrinsic signaling molecules and NADPH oxidases (NOXs) as major sources of ROS. These membrane bound-enzymes catalyze the reduction of molecular oxygen to superoxide using NADPH as an electron donor. There are seven known NOX homologues, and in the liver NOX1, 2 and 4 were shown to be expressed. NOXs are induced and expressed differentially, produce different ROS (superoxide *vs.* hydrogen peroxide); and the dynamics and the regulation of their activity are distinct and depend on the stimulus and co-activators present. In macrophages and neutrophils the phagocytic NADPH oxidase, NOX2 is the main source of ROS whereas in hepatocytes NOX4 induction dominates upon alcoholic injury. Stellate cells express NOX1, 2 and 4 upon transdifferentiation to myofibroblasts. Strategies that reduce redox injury thus should result in the improvement of liver injury and fibrosis. Multiple attempts however, using various anti-oxidants in the past have been disappointing owing to the differences in animal models and human disease; and the stage and cell-specific regulation of oxidant and anti-oxidant pathways. In this talk we will summarize the current knowledge of NOX-mediated alcoholic redox injury in the liver and potential of NOX-targeting approaches.

Submitted abstracts

PROHIBITIN-1 DEFICIENCY PROMOTES FIBROGENESIS IN THE LIVER THROUGH HDAC4

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Introduction. PHB1 is a ubiquitously expressed protein that participates in diverse processes depending on its subcellular localization. It is known for its chaperone-like function in mitochondria but it can exert its transcriptional activity in the nucleus. Given the implication of PHB1 in many vital functions we generated liver-specific *Phb1* KO mice, which develop spontaneously liver fibrosis and HCC.

Objectives. Identify the protective mechanism of PHB1 in the liver.

Materials and methods. We isolated primary hepatocytes from WT and *Phb1* KO mice and treated them with bile acids and performed bile duct ligation in animals. PHB1, OPA1 and HDAC4 silencing and parthenolide administration were carried out before BDL and BA treatment. Protein expression was evaluated by IHC and WB and gene expression by qPCR. Liver injury was evaluated by histopathology, serumaminotransferases and caspase 3 activation.

Results. PHB1 expression is markedly reduced in cholestatic diseases like PBC, biliary atresia and Alagille syndrome. Importantly, the lack of PHB1 sensitizes the liver to injury through a mitochondrial-independent pathway. In addition, HDAC4 is highly expressed and more nuclear localized in the *Phb1* KO hepatocytes, deregulating the expression of genes related to bile acid metabolism and cell survival. Downregulating the levels of HDAC4 we could restore the expression of those genes and attenuate liver damage in the *Phb1* KO animals.

Conclusions. We identify PHB1 as a mediator of cholestatic liver injury regulating the activity of HDAC4. These results identify potential strategies to treat liver injury and fibrosis, particularly as a consequence of chronis cholestasis.

CORRECTION OF HEMOCOAGULATION DISORDERS IN THE COMPLEX TREATMENT OF SEVERE ACUTE PANCREATITIS

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Introduction: In acute pancreatitis (AP), coagulation disorders abnormalities range from localized intravascular thrombosis to disseminated intravascular coagulation (DIC). Results from experimental animal studies suggest that modulation of hemostasis may provide a therapeutic target for treatment of AP.

Objectives: The study was dedicated to improve the results of the complex treatment of severe acute pancreatitis (SAP) by prediction and correction of hemocoagulation disorders.

Materials and methods: This study was based on the results of clinical examination and treatment of 112 patients with AP.Fibrinogen, prothrombin time, thrombin time, partial thromboplastin time,D-dimer and antithrombin-III (AT-III) levelswereassessedonday1, 3, 7 and14ofadmission.

Results: Hemocoagulation disorders during severe SAP develop and related to disease severity. SAP is characterized by the development of DIC, which is accompanied by excessive activation of hemostasis that leads to secondary activation of fibrinolysis and as a consequence, the development of multiple organ dysfunction syndrome (MODS). The determination of the level of AT-III ≤ 68% and D-dimer levels ≥ 693 ng/ml allows early diagnosis hemocoagulation disorders ofSAP.In order to correct hemocoagulation disorders in patients with SAP treatment algorithmwas developed, including the plazmapheresis, transfusion of fresh frozen plasma, and the introduction of low molecular weight heparins.

Conclusion: Correction of hemocoagulation disorders may predict andimprove outcomes in patients with SAP:reduce the incidence of pancreatic infection by 6.6%, 19.3% MODS and mortality of 13.2%.

ALCOHOL INTOXICATION POTENIATES POST BURN HEPATIC DAMAGE AND INTERLEUKIN-6 PRODUCTION THROUGH INCREASED P38 MAPK SIGNALINGINKUPFFER CELLS

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Introduction: Alcohol intoxication precedes nearly half of all adult burn injuries in the USA and leads to worsened clinical outcomes.The major site of alcohol metabolism and toxicity, the liver is also critical to the post-burn response. We recently reported systemic inflammation after intoxication and burn is partly caused by increased hepatic damage and interleukin-6 (IL-6) production though the cellular source and mechanisms remain unknown.

Objective: Determine the mechanism of alcohol potentiation of post-burn hepatic damage and IL-6 production.

Methods: C57/BL6 mice were given ethanol (1.2g/kg) by oral gavage 30min prior to a 15% total body surface area burn.Antecedent depletion of Kupffer cells (KCs) was achieved with clodronate liposomes (0.5mg/kg).KCs were isolated and analyzed for p38 MAPK with and without LPS stimulation (100ng/ml)and p38 inhibition (SB203580, 20μM).

Results: The absence of KCsattenuated hepatic damage as measured by a reduction of 53% in serum ALT ($p<0.05$), 44% in serum AST ($p<0.05$), a 37% in hepatic triglycerides ($p<0.05$), as well as 77% in hepatic IL-6 mRNA expression ($p<0.05$) compared to intoxicated burned mice receiving control liposomes. KC's isolated from intoxicated burned mice demonstrated a >2-fold ($p<0.05$)and 3-fold ($p<0.05$) elevation of baseline and LPS-stimulated p38activation, respectively. This corresponded to a 2-fold ($p<0.05$) increase in IL-6 production which was decreased by 80% ($p<0.05$) when p38 was inhibited before LPS stimulation. Intoxication alone did not alter baseline p38 phosphorylation but potentiated LPS signaling.

Conclusions: Alcohol exacerbatespost-burn hepatic damage and IL-6 production through p38 MAPK signaling in Kupffer cells.

HIV, HEPATITIS C, AND ABSTINENCE FROM ALCOHOL IN A LARGE SAMPLE OF INJECTION AND NON-INJECTION DRUG USERS

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Introduction: Individuals who use illicit drugs such as opiates, cocaine, and/or amphetamines are at heightened risk for (a) heavy drinking and (b) infection with HIV and/or Hepatitis C Virus (HCV). Despite the medical consequences of drinking with HIV and/or HCV, whether drug users with either or both of these conditions are more likely to abstain from alcohol remains unclear.

Objective:: We propose to examine the mainand interactive effects of HIV and HCV on abstinence from alcohol in a largesample of drug users.

Materials and Methods: Ina sample of 4672 injection and non-injection drug users who participated in alarge, cross-sectional study of risk factors for HIV, we investigate the mainand interactive effects of HIV and HCV on abstinence from alcohol. We examine models with and without control for demographic characteristics and injection drug use status.

Results: HIV and HCV interacted in predicting abstinence in all models. HCV was associated with increased probability of abstinence from alcohol for drug users with HIV(Odds Ratio [OR] = 3.20) and withoutHIV (OR=1.97), but effects for those with HIV were stronger and more robust to control for covariates.

Conclusions: Drug users with HCV are more likely to abstain from alcohol than those without HCV, especially in the HIV-infected population. Findingssuggest that HCV-infected patients (especially HIV/HCV co-infected patients) are aware of alcohol-related risk. However, the majority of drug users in all diagnostic groups did drink, indicating need for increased alcohol intervention among drug users with HIV and/or HCV.

microRNAs REGULATES GLYICINE-N-METHYL TRANFERASE IN LIVER CIRRHOSIS

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Introduction: The enzyme GNMT is known to have an essential role in the liver, maintaining SAMe levels and regulating the amount of methionine. GNMT expression is also known to be regulated during liver processes as proliferation, regeneration and tumoral transformation (low levels are found) or differentiation (increasing levels). Uncovering the mechanisms underlying this regulation would help to better understand the above mentioned process and the liver pathologies characterized by low GNMT.

Objectives: Study the regulation of GNMT expression by microRNAs (miRNAs) evaluating its possible modulation under different pathophysiological conditions in the liver

Material and Methods: Potential miRNA were selected according to different databases. Specific rtPCR and qPCR to study the expression of candidates miRNAs were performed. *In vivo* (bile duct ligation) and *in vitro* (primary hepatocytes) models were developed to confirm candidates miRNAs/GNMT correlated modulation. Specific siRNA to inhibit or overexpress selected miRNAs were used in these models.

Results: GNMT expression is regulated by the miRNA A under different pathophysiological conditions. In the studied cases, the blockage of the miA results in the maintenance of GNMT levels, contributing to reduce the induced damage in the liver and preserving the differentiated state of the hepatocyte.

Conclusions: GNMT levels are, in part, regulated by miRNA. As demonstrated in this work, this regulation can be modulated to maintain normal levels and the grade of differentiation of the hepatocyte, attenuating malignant signaling through the regulation of the miRNA A levels and sustaining the GNMT expression constant.

ETHANOL AND CIGARETTE SMOKE EXTRACT INHIBITS CFTR ACTIVITY IN PANCREATIC DUCTAL CELLS

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Introduction & Aim: Smoking represents an independent risk factor for the development of chronic pancreatitis, however, the pathomechanism remains unknown. Secretion of fluid and bicarbonate plays a crucial role in maintaining the integrity of the gland, therefore, the aim of this study was to investigate the effects of cigarette smoke extract (CSE) on pancreatic ductal fluid secretion and on cystic fibrosis transmembrane conductance regulator (CFTR) Cl^- channel activity.

Methods: Intra/interlobular pancreatic ducts were isolated from guinea pig pancreas. Basal and forskolin stimulated fluid secretion were measured by videomicroscopy, whereas, CFTR currents were detected by whole cell configuration of the patch clamp technique. CSE was prepared by smoking of 3 cigarettes into 40ml distilled water by a smoking machine and 10x (21µg/ml), 40x (5.25µg/ml) and 400x (0.5µg/ml) dilution of the extract were studied.

Results: Administration of 5µM forskolin activated CFTR currents by 10-15-fold in magnitude. 15 min administration of 0.5, 5.25 and 21 µg/ml CSE inhibited the currents by 44%, 64.6% and 79.4%, respectively (n=2-4). Concerning the fluid secretion, the basal volume of isolated intact pancreatic ducts in bicarbonate-free solution was considered to be 1.0. Administration of 25mM bicarbonate increased the relative luminal volume up to 1.57±0.02 (n=7). Administration of 5 mM forskolin further increased the luminal volume to 1.87±0.1 (n=16). Simultaneous administration of 21µg/ml CSE decreased fluid secretion by 24% (1.42±0.06; n=12).

Conclusion: CSE inhibits pancreatic ductal fluid secretion and the activity of the CFTR which may play role in the smoke-induced pancreatic damage.

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LIVER FIBROGENESIS IS CONTROLLED BY INNATE IMMUNE ACTIVATION PATHWAYS IN HEPATOCYTE APOPTOSIS

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Introduction: While liver inflammation contributes to liver fibrogenesis, it is not known whether other events, such as hepatocyte death, also promote liver fibrogenesis. Both liver inflammation and hepatocyte death are controlled by the Interferon regulatory factor 3 (IRF3). In hepatocytes, IRF3 is activated via the Stimulator of Interferon Genes (STING).

Objective: To investigate whether hepatocyte death is an independent factor in liver fibrogenesis.

Methods: Hepatocyte injury was assessed in mouse models of acute or chronic carbon-tetrachloride (CCl_4) injury, in WT, STING- and IRF3-deficient mice.

Results: Acute CCl_4 administration to WT mice resulted in early activation of IRF3 and Type I IFNs, and was followed by hepatocyte death via activation of caspase-3 in the liver. These events were accompanied by significant liver fibrosis upon repeated administration of CCl_4, as measured by increased α-SMA. Activated IRF3 was associated with STING and triggered the mitochondrial pathway of hepatocyte apoptosis via Bax. Remarkably, mice deficient in IRF3 or STING showed no hepatocyte death or fibrosis after acute or chronic CCl_4 administration. Deficiency in TRAM or TRIF, two canonical activators of IRF3, provided no protection from liver injury, suggesting the causal involvement of STING and IRF3.

Conclusions: Our novel findings demonstrate that IRF3-driven hepatocyte death is a pre-requisite for liver fibrosis. The CCl_4-induced death of hepatocytes is mediated by the interaction between STING and IRF3, resulting in activation of the mitochondrial pathway of hepatocyte apoptosis. Thus, liver damage and hepatocyte death likely represent a permissive initial event for liver fibrogenesis.

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STEATOSIS INDUCES HEPATIC PROGENITOR CELL PROLIFERATION BY AUGMENTING THE WNT/β-CATENIN PATHWAY DURING LIVER CANCER DEVELOPMENT

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Liver cancer is an aggressive and deadly disease with poor outcome. Approximately half of hepatocellular carcinoma (HCC) presents progenitor cell phenotype. Our lab established a mouse model specifically lacking PTEN (phosphatase and Tensin homologueon Chromosome 10), a negative regulator of PI3K/AKT, in liver hepatocytes. We have shown that loss of PTEN induces fatty liver mediated hepatic injury followed by progenitor cell expansion and upregulation of hepatic progenitor cell markers. Additionally, factors governing progenitor niche includingWnt ligands, receptorandβ-catenin are upregulated. Wnt/β-catenin has been shown to promote self-renewal and proliferation of progenitor cells. Furthermore, interrupting Wnt/β-catenin pathway using shRNA and small molecule inhibitor attenuated progenitor cell proliferation. The **objective** of this study is to unveil how hepatic steatosis stimulates progenitor cell activation and liver cancer development through the Wnt/β-catenin pathway. **Methods:**In this study, we investigate the role of hepatic steatosis in progenitor cell mediated liver cancer development using high fat diet and calorie restriction. Our **results** demonstrate lipotoxic liver injury induced by high fat diet induces β-catenin whereas calorie restriction not only blocks lipid accumulation but also tumorogenesis. **Conclusion:**This study addresses how metabolic changes dictate fatty liver formation,Wnt/β-catenin activation and progenitor cell expansion during liver tumerogenesis. Given that both obesity and liver cancer epidemic is on the rise in the US, understanding how fatty liver contributes to hepatic tumorigenesis will not only delineate the interaction between lipid metabolism and cancer development, but also hold promise for developing effective treatment and eventually eradicating liver cancer.

FUNCTIONAL ANALYSIS OF PANCREATITIS ASSOCIATED PRSS1, MORC4, AND CLDN2 MUTATIONS

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Introduction: Pancreatitis is an inflammatory disease in which the pancreas digests itself. Alcohol abuse is long known to be a major risk factor. But in the last two decades genetic analysis showed that several mutations also contribute to pancreatitis development.

Only recently, polymorphisms in the promoter region of *PRSS1* and *MORC4* as well as an mutation in the intron region of *CLDN2* were shown to be associated with alcoholic and non-alcoholic chronic pancreatitis.

Aims: As little is known about the promoter structure related to the pathogenic phenotype we intend to analyse the promoter with respect to these mutations.

Materials & methods: Promoter variants are cloned into a luciferase reporter vector and promoter activity is quantified by light emission. Different cell lines (HeLa, 3T3, pancreatic cancer cell lines) will be utilised.

Results: Preliminary data in HeLa cells point to an enhanced promoter activity by *PRSS1* and *MORC4* mutations. The *CLDN2* mutation has decreased promoter activity.

Conclusion: Results have to be verified with other pancreatic cell lines.

ALCOHOL-INDUCED SMALL BOWEL INFLAMMATION IS PREVENTED BY MICRO-RNA-155 DEFICIENCY

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Introduction: The effects of alcohol ingestion on gut are partially understood. Inflammatory cytokine production is induced and gut barrier function is impaired in alcoholism. Inflammatory modulator, micro-RNA-155 (miR-155) stabilizes pro-inflammatory cytokine, tumor necrosis factor-α (TNFα) mRNA.

Objectives: Our goal was to investigate the effects of acute binge and chronic alcohol feeding and the role of miR-155 in murine small bowel (SB).

Materials and methods: In acute alcohol binge, wild-type (WT) mice received 3 days oral gavage of 5 g/kg 50% alcohol/d or equal amount of water. WT and miR-155-deficient (miR-155-knockout [KO]) mice received ethanol containing Lieber-DeCarli or isocaloric control diet for 5 weeks. MiR-155, regenerating islet-derived 3-beta (Reg3b), occludin, Src homology 2-containing inositol phosphatase-1 (SHIP1), interleukin-17 (IL17), TNFα, and nuclear factor-κB (NF-κB) were measured in SB. Endotoxin was measured in serum.

Results: Acute alcohol binge increased, whereas chronic alcohol feeding decreased Reg3b in SB. Both acute binge and chronic alcohol feeding increased serum endotoxin levels, intestinal NF-κB activation, TNFα and IL17 mRNA levels. Acute alcohol decreased, however there was no change in occludin levels in chronic alcohol-fed mice. TNFα protein and miR-155 were increased after chronic alcohol feeding in SB. MiR-155-KO mice were protected from chronic alcohol-induced increase in serum endotoxin, intestinal TNFα, IL17 and NF-κB activation. Alcohol-fed miR-155-KO mice had no attenuation of Reg3b or SHIP1 levels.

Conclusion: Our results show that both acute binge and chronic alcohol administration result in increased serum-endotoxin levels. We demonstrate a novel role of miR-155 in chronic alcohol-induced intestinal inflammation and barrier dysfunction.

ALCOHOLIC PANCREATITIS- EPIDEMIOLOGY FEATURES

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Introduction: Acute pancreatitis is a disease with an increased incidence in the last years. **Objectives:** The aim of this study is to determine the incidence of alcoholic pancreatitis in our area and to analyse epidemiological factors in patients with acute pancreatitis admitted in our department.

Materials and methods: We performed a retrospective study in a series of patients who were admitted in the Gastroenterology Department of Targu Mures County Clinical Emergency Hospital between 01.08.2013-31.07.2014. We included only patients with acute pancreatitis. We exclude patients with chronic pancreatitis. Patients were, according to etiology, divided into four groups: alcoholic, biliary, metabolic, and other. After severity pancreatitis was stratified in mild, moderate and severe.

Results: We included 66 patients admitted with acute pancreatitis, with a median age of 58,6 years. There was a strong predominance of males 66.66% (44) with a male/female ratio of 2/1. Alcoholic etiology was found in 27 patients (40,9%), biliary in 15 (22.72%), metabolic in 14 (21.21%) and other in 10 patients (15.15%). The patients with the alcoholic etiology of the disease were younger than the other groups of patients (median age 49.09) and all the patients were males In the biliary etiology we found a predominance of female gender. We found 4 (6.06%) severe pancreatitis, 20 (30.30%) moderate and 42 (63.63%) mild pancreatitis. The most prevalent etiology in the severe pancreatitis was alcoholic. Mortality rate was zero in our series.

Conclusions: Our data showed that alcoholic etiology is the most frequent etiology in our area especially in young male patients.

Keywords: pancreatitis, epidemiology

ATG4B MEDIATES AUTOPHAGY INHIBITION IN ALCOHOLIC PANCREATITIS

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Background & Aims: Impaired autophagy mediates non-alcoholic pancreatitis; however, the effects on autophagy of alcohol, a major risk factor for pancreatitis, remain elusive. Here we show that ethanol inhibits pancreatic autophagy through up-regulation of Atg4B, a protease that inactivates (delipidates) LC3-II, a key mediator of autophagosome formation.

Methods: Mice were pair fed Lieber-DeCarli diet followed by low-dose cerulein. In ex-vivo model, acinar cells were transduced with mock or Atg4B-shRNA adenovirus and incubated with 50-100mM ethanol followed by 100pM CCK-8. Immunoblotting, immunofluorescence, electron microscopy, and enzymatic assays were used to measure autophagy and the effects of manipulating Atg4B on acinar cell death.

Results: Ethanol inhibited autophagosome formation in both in vivo and ex-vivo pancreatitis models, shown by EM and LC3-II decrease. Ethanol treatment up-regulated protein level and activity of Atg4B in tissue and cells (the latter measured with an Atg4B active-site probe). Atg4B activity correlated with changes in its level. Ethanol plus cerulein/CCK caused impaired autophagy, manifest by accumulation of large vacuoles and deficient clearance of p62 protein aggregates in acinar cells. Atg4B shRNA knockdown restored LC3-II and autophagosome formation, induced autophagic flux, and protected ethanol-treated acinar cells from necrosis and trypsinogen activation.

Conclusions: Atg4B plays a key role in pancreatic autophagy. Ethanol stimulates Atg4B-mediated LC3-II delipidation, thus down-regulating autophagy in acinar cells. shRNA knockdown of Atg4B induces autophagic flux and alleviates alcoholic pancreatitis. The results indicate that this novel mechanism is important in alcohol's predisposing effect to pancreatitis.

EXPLORING THE MECHANISMS BEHIND CIGARETTE SMOKE-INDUCED INTERNALIZATION OF CFTR

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Introduction: Cigarette smoke (CS) results in the rapid internalization and aggregation of CFTR in airway epithelial cells, and a consequent decrease in CFTR-mediated anion secretion. Since CFTR has a clear role in the development of acute and chronic forms of pancreatitis, and that people who smoke have a higher risk of developing pancreatitis, it is therefore possible that the reason for the increased risk may involve a CS-induced dysfunction in CFTR activity in the exocrine pancreas.

Objectives: To establish the mechanism behind the CS-induced internalization of CFTR.

Methods: To determine the time course of internalization, HEK293T cells were transiently transfected with GFP-tagged wt-CFTR and exposed to 10 puffs of freshly prepared CS or air control before incubating at 37°C for timed intervals over 60 min before fixation with 4% PFA.

Results: No change in fluorescence intensity was seen over 60 min in air exposed cells. However, after CS exposure, CFTR membrane fluorescence exponentially decreased with a half-life of 10.2 min, and intracellular CFTR appeared with similar kinetics. FRET-based experiments using GFP- and RFP-tagged constructs showed that aggregation of CFTR after CS exposure occurred intracellularly, rather than at the membrane. Removal of either the PDZ-binding motif (L1254X CFTR) and/or NBD2 of CFTR (K1174X CFTR) had no effect on the CS-induced CFTR intracellular accumulation.

Conclusion: Despite the C-terminus of CFTR having an established role in normal endocytosis of CFTR, the PDZ domain and NBD2 are not involved in the CS-induced internalization of CFTR. Funded by NIH R01 HL108927 and MRC (UK).

ELECTRON MICROSCOPIC CHANGES IN THE LIVER TISSUE OF PATIENTS WITH CHRONIC ALCOHOLISM

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Introduction: High prevalence of alcoholism among Ukrainian young adults is a serious medical and social problem. Visualizing electron-microscopic changes in liver tissuesof patients with alcoholic liver disease (ALD), including sub-microscopic evidence of progressive fibrosis is necessary.

Objectives: To determine changes in liver tissue by electron microscopy (EM) with alcoholic liver disease (ALD).

Materials and methods: Histological investigation of liver tissues of 12 patients with chronic alcoholism aged 29 to 47 years (11 males and 1 female). Criteria for inclusion was confirmed ALD on ICD-10 (K.70). Liver biopsy was performed with ultrasonography under local anesthesia.

Results:

1. ALD and low histological activity: moderate deposition of lipid granules (LG) in centrolobular zone, high degradation of LG incentro-lobular and periportal areas, accompanied by increased beta-oxidation of fatty acids inmitochondria, increased LG inbiliary capillaries from space of Disse.

2. ALD and high histological activity: decreased degradation of LG, significant morphological abnormalities in mitochondria, significant decrease in glycogen content incytoplasm, reduced area of granular endoplasmic mesh activated Kupffer cells and cell transformation processes in fibroblasts, collagen fibers mainly in centrolobular zone.

Conclusion: Electron microscopic examination in ALD allow objective assessment of severity of hepatocyte injury, severity of profibrogenic factors, degree of fibrotic changes useful in determining treatment policy and prognosis of disease.

MINNELIDE DECREASES PANCREATIC CANCER INVASION THROUGH NF-KB

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Introduction: Triptolide, a compound derived from the traditional Chinese herb, Tripterygium wilfordii Hook F, has been demonstrated to effectively induce cell death within pancreatic cancer cell lines and several murine models of pancreatic cancer and effectively inhibited metastasis.

Objectives: In this study, we aimed to determine the effect of Minnelide (a pro-drug derived from triptolide) on the Epithelial-Mesenchymal Transition (EMT) and cellular invasiveness and to elucidate the mechanism by which triptolide limits pancreatic cancer invasiveness.

Materials and Methods: Human pancreatic tumor samples propagated in SCID mice and pancreatic tumors derived from KPC (LSL-$Kras^{G12D/+}$;LSL-$Trp53^{R172H/+}$;Pdx-1-Cre) mice were utilized for these experiments. Several established human pancreatic cancer cell lines were used for *in vitro* experiments. Boyden chamber invasion inserts were used to determine invasion. Quantitative PCR and western blot were used to determine expression of markers involved in EMT and invasion. Triptolide and Minnelide were used for *in vitro* and *in vivo* treatment, respectively.

Results: *In vitro* invasion decreased with triptolide treatment within several pancreatic cancer cell lines. EMT transcription factors and mesenchymal markers were downregulated both *in vitro* and *in vivo* upon treatment with triptolide and Minnelide, respectively. This effect was mediated through modulation of NF-kB activity. Inhibition of NF-kB through pharmacological or IKK inhibition resulted in downregulation of EMT markers and attenuation of *in vitro* invasion.

Conclusion: Minnelide downregulates EMT transcription factors and mesenchymal marker expression, resulting in decreased invasion *in vitro* and metastasis *in vivo*. EMT induction and invasion was mediated by NF-kB activation, inhibited by Minnelide treatment.

THE ROLE OF PLASMA MEMBRANE CA^{2+}-ATPASE IN PANCREATIC DUCTAL CA^{2+} HOMEOSTASIS

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Introduction: The plasma membrane Ca^{2+}-ATPase (PMCA) is an ATP-driven pump that is critical for maintaining a low resting cytosolic Ca^{2+} concentration in all eukaryotic cells. Impaired Ca^{2+} metabolism and cytosolic Ca^{2+} overload in pancreatic acinar cells have been implicated as the cardinal pathological events common to most forms of pancreatitis. The regulation of PMCA in pancreatic acinar cell is relatively well characterized but in pancreatic ductal epithelial cells has not yet been investigated.

Aims: Our aim was to characterize the role of PMCA in pancreatic ductal Ca^{2+} homeostasis.

Materials & methods*: Inter- and intralobular ducts were isolated from guinea pig pancreas. To determine, whether changes in extracellular* Ca^{2+} and Na^{+} concentrations influence the $[Ca^{2+}]_i$, ion withdrawal techniques were used. $[Ca^{2+}]_i$ was measured by microflourometry. The mRNA expression of different PMCA isoforms in PDEC was investigated by RT-PCR.

Results: *Removal of* Na^{+} from the extracellular solution caused an increase in $[Ca^{2+}]_i$, which suggests a Na^{+}-dependent Ca^{2+} transport mechanism in guinea pig PDEC. *Withdrawal of extracellular* Ca^{2+} and both Na^{+} and Ca^{2+} decreased the $[Ca^{2+}]_i$, meaning that a Na^{+}-independent transport mechanism plays role in Ca^{2+} efflux. The expression of *PMCA1 and 4 mRNA isoforms was detected in guinea pig PDEC.*

Conclusion: *Our results showed that the* Ca^{2+} efflux in guinea pig PDEC after Ca^{2+} removal from the extracellular solution is a Na^{+} independent process. Further investigations are needed to determine whether PMCA is the key Ca^{2+} exit pathway in guinea pig PDEC.

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TLR2 AND TLR9 PROMOTENEUTROPHIL-DRIVEN ALCOHOLIC LIVER INJURY THROUGH INDUCTION OF CXCL1

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Introduction: Neutrophil infiltration is a hallmark of alcoholic hepatitis (AH) and correlates with the severity of alcoholic liver disease (ALD). Activation of Toll-like receptor (TLR) signaling induces the production of cytokines and chemokines through MyD88. However, the cellular and molecular links between TLRs and neutrophil-dependent alcohol-mediated liver injury have not been elucidated.

Materials & methods*:* Alcohol-induced liver injury model was developed by feeding Lieber-DeCarli diet containing 6.3% (vol/vol) ethanol plus binge drink (5g/kg BW) in wild-type (WT), TLR2-deficient and TLR9-deficient mice.

Results: TLR2 and TLR9-deficient mice exhibited reduced alcohol-induced liver injury compared with WT mice. Induction of neutrophil-recruiting chemokines, including *Cxcl1*, *Cxcl2* and *Cxcl5*, and hepatic neutrophil infiltration were prevented in TLR2 and TLR9-deficient mice compared with WT mice fed an alcohol-containing diet. Interestingly, *in vivo* depletion of Kupffer cells (KCs) reduced the expression of proinflammatory cytokines (*Il1b*, *Il6* and *Tnf*), but not the expression of *Cxcl1*, *Cxcl2* and *Cxcl5* and neutrophil infiltration. Hepatocytes and hepatic stellate cells (HSCs) isolated from ethanol-fed mice had increased expression of *Cxcl1*. Notably, the treatment with TLR2 and TLR9 ligands synergistically induced CXCL1 production in hepatocytes. Chemical inhibitors of CXCR2, a receptor for CXCL1, and MyD88 attenuated alcohol-induced neutrophil infiltration and liver injury. Consistent with the above findings, hepatic CXCL1 expression was found to be highly upregulated in patients with alcoholic hepatitis.

Conclusion: The TLR2 and TLR9-dependent MyD88-dependent pathway mediates CXCL1 production in hepatocytes and HSCs, which promotes neutrophil infiltration into the liver via CXCR2, resulting in the development of ALD.

THE EPIDEMIOLOGY OF ALCOHOLIC LIVER CIRRHOSIS IN OUR CASUISTRY

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The **object** of our work is a one- year evaluation of the epidemiology of alcoholic liver cirrhosis at patients hospitalized in the Department of Gastroenterology, Emergency Clinical County Hospital of Tîrgu Mureș.

Material and methods: We retrospectively studied 1221 patients, admitted for longer time that one day, in our department, during the period of 01.08.2013 – 31.07.2014. We followed-up the presence of liver cirrhosis, alcoholic and non-alcoholic etiology and the age and sex of the patients.

Results: We found 364 cases of liver cirrhosis (29.81%), of which 196 cases alcoholic cirrhosis (53.85%) and 168 cases non-alcoholic liver cirrhosis (46.15%). We found 84,7% men with alcoholic liver cirrhosis, and 52,97% women with non-alcoholic liver cirrhosis. Observing the age of the patients, at men under 60 years old, we found the alcoholic liver cirrhosis more frequent (71,08%) than the non-alcoholic liver cirrhosis (58.75%).

Conclusions: The prevalence of alcoholic liver cirrhosis in our study was 16.05%, predominantly in males, under 60 years old. The non-alcoholic liver cirrhosis was predominantly at women and has an age-related increasing until 70 years of age at both sexes.

Keywords: alcoholic liver cirrhosis, epidemiology

METHIONINE AND S-ADENOSYLMETHIONINE LEVELS ARE CRITICAL REGULATORS OF PP2A ACTIVITY MODULATING LIPOPHAGY DURING STEATOSIS

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Introduction: S-Adenosylmethionine (SAMe) is synthetized from methionine by a reaction catalyzed by the methionine adenosyltransferase. Several conditions that lead to abnormally elevated seric levels of methionine and SAMe, including Glycine N-methyltransferase (*Gnmt*) deficiency or ingestion of relative large amounts of methionine, are associated with liver steatosis. Recent studies have shown that alterations in autophagy could lead to liver steatosis.

Objectives: We want to study if elevated levels of methionine and its metabolite SAMe, both well known inactivators of autophagy, could contribute to hepatic steatosis development by blocking autophagy-mediated lipid catabolism.

Materials and methods: We have used hepatocytes cultured with methionine and SAMe, and hepatocytes and livers from *GNMT*-KO mice to study the effect of high levels of both metabolites in autophagy functionality.

Results: We detected an increase in methionine levels in a cohort of 358 serum samples from NAFLD patients. Lipophagy was impaired in livers and hepatocytes in the presence of high methionine and SAMe. Elevated levels of methionine and SAMe activates PP2A by methylation inducing a chronic inactivation of MTOR, a well-known inactivator of autophagy. However, despite this inactivation of MTOR, SAMe and methionine inhibited autophagy by inducing PP2A/cAMP/PKA functionality and leading to lysosomal peripheral localization. In addition, SAMe and methionine contributed to this lysosomal peripheral localization by preventing the intracellular pH basification induced during starvation. **Conclusion:** Elevated levels of methionine and SAMe lead to a malfunction in autophagic clearance of lipids contributing to liver steatosis.

ROLE OF CYCLOPHILIN-D IN ARGININE-INDUCED PANCREATITIS

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Introduction & Objectives: We previously showed that mitochondrial dysfunction mediates pancreatitis induced by L-arginine (Arg) in rodents. The mechanisms linking mitochondrial damage to pathologic responses of pancreatitis remain poorly understood. Here we investigate the role of mitochondrial dysfunction in mediating autophagy, ER stress, inflammatory and cell death responses of pancreatitis. We focus on the role in pancreatitis responses of cyclophilin D, a key regulator of mitochondrial permeability transition pore and ATP-synthase.

Materials & Methods: Pancreatitis in wild type and cyclophilin D knockout (CypD KO) mice was induced by 3 *i.p.* injections of 3 g/kg Arg. Changes in mitochondrial dynamics, autophagy, ER stress responses, inflammatory infiltration, cell death were measured.

Results: Arginine causes inhibition of mitochondrial dynamics manifest by a decrease in the markers of mitochondrial fusion OPA-1 and Mfn-1 and fission DLP-1 and Fis-1. This was associated with autophagy impairment measured by accumulation of p62/sequestosome. Inhibition of both mitochondrial dynamics and autophagy was prevented in pancreatitis on mice deficient in CypD. We found that one of the early manifestations of Arg-pancreatitis is release in cytoplasm of nuclear protein HMGB1, a key **damage-associated molecular pattern** molecule mediating necrosis and inflammation. CypD genetic ablation prevented HMGB1 release into cytosol followed by reduction in inflammation and necrosis cell death. Our data also indicate that CypD knockdown attenuates markers of ER stress response of Arg-pancreatitis.

Conclusion: The results show the role of CypD in linking mitochondrial damage to pancreatitis responses; they indicate CypD inactivation as a promising approach for pancreatitis treatment.

THE GENESIS OFCD133+ PROGENITOR CELLS IN EXPERIMENTAL ALCOHOLIC HEPATITIS IN MICE

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Alcoholic hepatitis (AH) is a distinct spectrum of alcoholic liver disease (ALD) with intense neutrophilic inflammation and high mortality.We recently reported that the histological and clinical features of AHare produced by giving weekly alcohol bingeto mice developing alcoholic steatohepatitis (ASH) characterized by steatosis, mononuclear cell infiltration, and pericellular and perisinusoidal liver fibrosis.To produce ASH, male mice were given 40% calories derived from *ad libitum* consumption of the Western diet high in cholesterol and saturated fat and the rest from intragastric feeding (*iG*) of alcohol liquid diet to sustain alcohol intoxication. Weekly alcohol binge causes a drastic histological shift to AH from chronic ASH in 57% of mice with clinical symptoms such as hypoalbuminemia, bilirubinemia, peritoneal effusion and splenomegaly. Besidesupregulation of inflammatory chemokines and neutrophil-related genes, a microarray analysisrevealed induction of cancer- or progenitor cell-related genes (*Cd133,Cd24, Epcam, Anilin, Sox 4, Spp1*)in AH livers compared to ASH livers, recapitulating a key feature of AH patients. Immunofluorescent microscopy analysis revealed cells positive for TLR4 and NANOG, TLR4 and AFP or CD133 and CD49f in the inflamed parenchyma of AH livers, suggesting the genesis of TLR4+ NANOG+ or CD133+CD49f+ liver tumor-initiating cells recently characterized by us (J Clin Invest. 2013; 123:2832-49). As a step towardcharacterizing these cells, we succeeded in isolation of the three distinct populations of CD133+CD49f+CD45-, CD133+CD49f-CD45- and CD133+CD49f+CD45+ cellsfrom AH livers using the fluorescent activated cell sorting. Currently, RNA-seq analysis is being performed on these cells. To this end, our new mouse model provides an opportunity to study molecular mechanisms underlying the appearance of progenitor cells in livers during the genesis of AHinduced by alcohol abuse and Western diet.

US-GUIDED PERCUTANEOUS CATHETER DRAINAGE IN MANAGEMENT OF ACUTE NECROTIZING PANCREATITIS

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Introduction: Necrotizing pancreatitis is associated with a high rate of complications and death.

Aim: To define the appropriate time of US-guided percutaneous drainage.

Materials and methods: We reviewed the data of 81 patients with acute pancreatic fluid collections from 2012 to 2014. Patients were divided in 3 groups according to the terms of percutaneous drainage: 1-7 days after onset of acute pancreatitis (n=32, group A), 8-15 days (n=33, group B), after 15 days (n=16, group C). The CT severityindex, Ranson scores, and maximum diameter of abdominal fluid collections were calculated for all patients.

Results: The CT severity index and acute Ranson scores were not different between the 3 groups ($p>0,05$). The duration of hospitalization did not differ significantly in groups A and B with a mean length of hospital stay of 26,2 days and 32,7 days, respectively ($p=0.16$), but was longer in group C 41,3 days ($p=0,013$). The mortaility was 3 (9,5%) in group A, 6 (18,1%) in group B, 3 (18,5%) in group C ($p>0,05$). Surgical conversions occurred significantly more often in groups B and C in 14 (42,4%) and 10 (62,5%) respectively in comparison to group A – 8 (25%) ($p=0,021$). Aspirates were primary culture-positive in 9/32 cases in group A, 15/33 in group B ($p=0,05$), 11/16 in group C ($p<0,01$). Aspirates turned culture-positive in 22 of 46 patients (47,8%). Prolonged catheter drainage was successful as final treatment in 45 (55,6%) patients.

Conclusion: Early US-guided percutaneous drainage (up to 7 days after onset of acute pancreatitis) proved to be effective in decrease of septic complications. Time of US-guided percutaneous drainage didn't affect mortaility rate.

IMPLICATIONS OF NEDDYLATION IN LIVER CIRRHOSIS

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Introduction: Cirrhosisis a consequence of chronic liver disease, characterized by replacement of liver tissue with scartissue, impairing normal activity. Cirrhosis is also a predisposing factor for hepatocellular carcinoma. Evidence is starting to appear pointing to a dysregulation of protein neddylation in many diseases such as cancer. Nedd8 is a small ubiquitin like protein who sema in substrate are cullins.

Objectives: To determine the effect of neddylationon cirrhosis progression.

Materials and Methods: To study neddylation in cirrhosis we used MLN4924, an experimetnal Nedd8 activating enzyme inhibitor, on *in vivo* bile duct ligation and CCL_4 treated mice models. We studied changes in protein expression and mRNA levels of oncogenic or profibrogenic targets. Liver metabolism changes were also studied using UPLC/MS. *In vitro* experiments testing the effect of Nedd8 inhibition on human stellate cell activation after TGFβ were also done. We studied cell viability after MLN4924 treatment and levels of TGFβ reponse proteins as well as mRNA levels of TGFβ target genes.

Results: Treatment of injured mice lead to an improved outcome of cirrhosis seen by reduced collagen levels and inflammation markers. Treated mice had changes in their metabolism compared to control or cirrhotic mice. Inhibition of Nedd8 in stellate cells led to a reduction in their activation seen by lower mRNA levels of TGF beta target proteins and an increased cell death.

Conclusions: In conclusion, neddylation levels of cirrhotic liver can facilitate, either directly or indirectly, TGFβ signaling and can stabilize pro-oncogenic proteins increasing the probability of disease progression and malignant transformation.

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