The Asian Pacific Association for the Study of the Liver

APASL

Single Topic Conference

Molecular and Cell Biology of the Liver: Recent Evolution to Clinical Application

Program & Abstracts

Term: September 2-3, 2021
City: Osaka, Japan
Venue: Hilton Osaka(Hybrid Meeting)
President: Norifumi Kawada, MD., PhD.

Professor,
Department of Hepatology,
Graduate School of Medicine, Osaka City University

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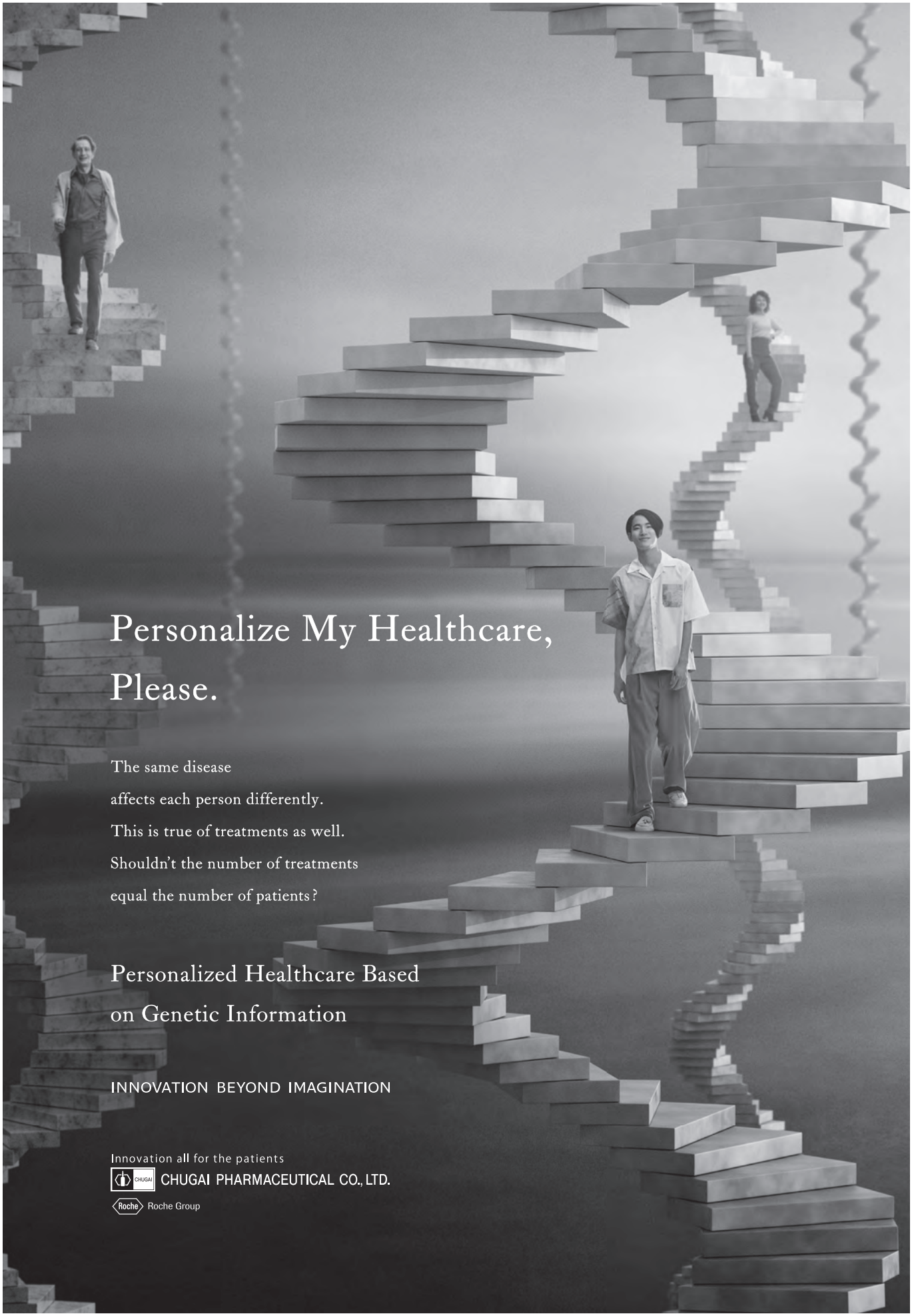
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APASL Single Topic Conference 2021 Osaka

"Molecular and Cell Biology of the Liver: Recent Evolution to Clinical Application"

September 2-3, 2021

Hybrid Meeting (Onsite-Venue: Hilton Osaka)

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Welcome Message

Dear colleagues,

On behalf of the Organizing Committee, it gives us great pleasure to invite you to Asian Pacific Association for the Study of the Liver Single Topic Conference (APASL STC) on the theme of "Molecular and Cell Biology of the Liver: Recent Evolution to Clinical Application", which will be held on September 2-3, 2021 in Osaka, Japan as a Hybrid Meeting (Zoom). (This conference had been originally scheduled in September 2020, however it has been rescheduled because of the pandemic of COVID-19. We ask your understanding and cooperation.)

Chronic inflammation, cellular damage, regeneration and fibrosis are the hallmarks of chronic liver diseases. A recognition of molecular expression patterns in intrahepatic cellular sources in normal and diseased liver provides clues for understanding pathogenic pathways while studies of the structure and function of molecules implicated in liver disease provide insights into their potential as therapeutic targets.

This conference will bring together fundamental researchers and translational scientists to discuss recent successes and highlight challenges across the spectrum of emerging cellular and molecular evolutions. This conference will help to foster interactions and new connections between basic experts focused on molecular-based, cell-based regulation of liver diseases with translational scientists focused on therapeutic approaches currently in development.

The scientific program will consist of invited lectures, plenary sessions, symposia, and free papers which provide the latest information and fresh ideas for hepatologists.

The delegates of experts from all over the world are expected to attend this conference. We are sure that this will provide an excellent opportunity for those of us in the Asian Pacific region to share the latest views, values, experience and practice, and greatly contribute to this field.

We look forward to welcoming you to this exciting event, and hopefully, if the situation allows, we hope you will be able to visit our charming city of Osaka.

With warmest regards,

Norifumi Kawada, MD., PhD.
President of APASL STC 2021 Osaka
Professor, Department of Hepatology, Graduate School of Medicine,
Osaka City University

Invited Guest Speakers / Chairs

Dr. Kazuaki Chayama (Hiroshima University, Japan)
Dr. Massimo Colombo (Liver Center IRCCS San Raffaele Hospital, Italy)
Dr. A Kadir Dokmeci (Ankara University, Turkey)
Dr. Ariel Feldstein (UC San Diego School of Medicine, USA)
Dr. Junji Furuse (Kyorin University, Japan)
Dr. Jacob George (The University of Sydney, Australia)
Dr. Jordi Gracia-Sancho (IDIBAPS Biomedical Research Institute, Spain)
Dr. Eiji Hara (Osaka University, Japan)
Dr. Kiyoshi Hasegawa (The University of Tokyo, Japan)
Dr. Lijian Hui (Shanghai Institute of Biochemistry and Cell Biology, China)
Dr. Yutaka Inagaki (Tokai University School of Medicine, Japan)
Dr. Tatehiro Kagawa (Tokai University School of Medicine, Japan)
Dr. Takanori Kanai (Keio University School of Medicine, Japan)
Dr. Jia-Horng Kao (National Taiwan University, Taiwan)
Dr. Naoya Kato (Chiba University Hospital, Japan)
Dr. Tatiana Kisseleva (UCSD, USA)
Dr. Masatoshi Kudo (Kindai University, Japan)
Dr. George Lau (Humanity and Health Medical Center, Hong Kong)
Dr. Derek A Mann (Newcastle University, UK)
Dr. Giuseppe Mazza (Institute for Liver and Digestive Health, University College London, UK)
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Dr. Hidewaki Nakagawa (RIKEN Center for Integrative Medical Sciences, Japan)
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Dr. Hiroshi Ohno (RIKEN Center for Integrative Medical Sciences, Japan)
Dr. Masao Omata (The University of Tokyo, Japan)
Dr. Diana Payawal (Fatima University Medical Center, Philippines)
Dr. Xiaolong Qi (The First Hospital of Lanzhou University, China)
Dr. Shiv K Sarin (Institute of Liver and Biliary Sciences, India)
Dr. Robert F Schwabe (Columbia University, USA)
Dr. Ekihiro Seki (Cedars-Sinai Medical Center, USA)
Dr. Tatsuhiro Shibata (National Cancer Center, Japan)
Dr. Gyongi Szabo (Harvard Medical School, USA)
Dr. Toshifumi Tada (Japanese Red Cross Society Himeji Hospital, Japan)
Dr. Tetsuo Takehara (Osaka University, Japan)
Dr. Yasuhito Tanaka (Kumamoto University, Japan)
Dr. Hideki Taniguchi (The University of Tokyo, Japan)
Dr. Tawesak Tanwandee (Mahidol University, Thailand)
Dr. Hidenori Toyoda (Ogaki Municipal Hospital, Japan)
Dr. Kaoru Tsuchiya (Musashino Red Cross Hospital, Japan)
Dr. Vincent Wong (The Chinese University of Hong Kong, Hong Kong)
Dr. Jin Mo Yang (The Catholic University of Korea, Korea)
Dr. Hitoshi Yoshiji (Nara Medical University, Japan)
Dr. Man-Fung Yuen (The University of Hong Kong, Hong Kong)

In alphabetical order

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Local Organizing Committee

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Treasurer: Dr. Soichi Kojima
Vice-Treasurer: Dr. Yutaka Yata
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Scientific Secretariat: Dr. Le Thi Thanh Thuy

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	Dr. Yoshiyuki Ueno (Japan)

Conference Information

Registration Fee and Category

	Pre-Registration until August 31, 2021	On Site
APASL Member*	JPY 20,000	JPY 25,000
	Online: JPY 15,000	Online: JPY 20,000
Non-Member	JPY 25,000	JPY 30,000
	Online: JPY 20,000	Online: JPY 25,000
Trainee / Resident**	JPY 15,000	JPY 20,000
	Online: JPY 10,000	Online: JPY 15,000
Accepted Abstract Submitter	JPY 20,000	JPY 25,000
	Online: JPY 15,000	Online: JPY 20,000
Accompanying Person	JPY 5,000	JPY 5,000

JPY=Japanese Yen
*APASL Members who have paid 2021 Membership Fee can apply for discounted registration fee.
*Proof of status is required.
*Registration for viewing of On-demand Presentation is available until September 30th 2021.

Online Participation (Style: Zoom Webinar)

- The conference program will be presented as a hybrid style meeting.
- Attendants are able to enter the webinar through Zoom https://zoom.us/join with the ID and Password of which they have been informed by the conference secretariat. * For Speakers/Chairs, the secretariat sends an individual invitation link to enter the webinar.
- The lectures will be delivered live or by recorded video. After the presentation, the discussion (Q&A) time will be held according to the moderator's instructions. Online viewers are able to send textual questions to the Q&A column, and the onsite participants may ask questions using the microphone in the conference room. We anticipate your active discussions.
- After the conference term, the recorded lectures and discussion will be distributed on-demand from the presentation page of APASL STC Osaka Website http://www. apaslstc-osaka2021.org/index.html
  The viewing period of the on-demand presentation is scheduled to be from September 6 through September 30, 2021. The secretariat will receive the questions by e-mail during the on-demand delivery period and will forward them to each speaker.

[Precautions]

- The organizer cannot handle problems such as computer operation, internet connection, video connection, and audio connection. Please solve such problems by yourself. We recommend the following environment.
  -We would appreciate it if you could use a PC with as much memory as possible (CPU i5 or more, memory 8 Giga or more).
  -Please connect to the Internet via a wired LAN line as much as possible.
- To transfer or share the ID and password, recording of screens and images is strictly prohibited.
- The internet fee at this online conference will be borne by each attendant.

We cordially solicit your understanding and cooperation.

Onsite Registration/PC Pre-view Hours

September 2 (Thursday)	8:00-18:30 (JST)
September 3 (Friday)	7:30-18:30 (JST)

Onsite-Venue

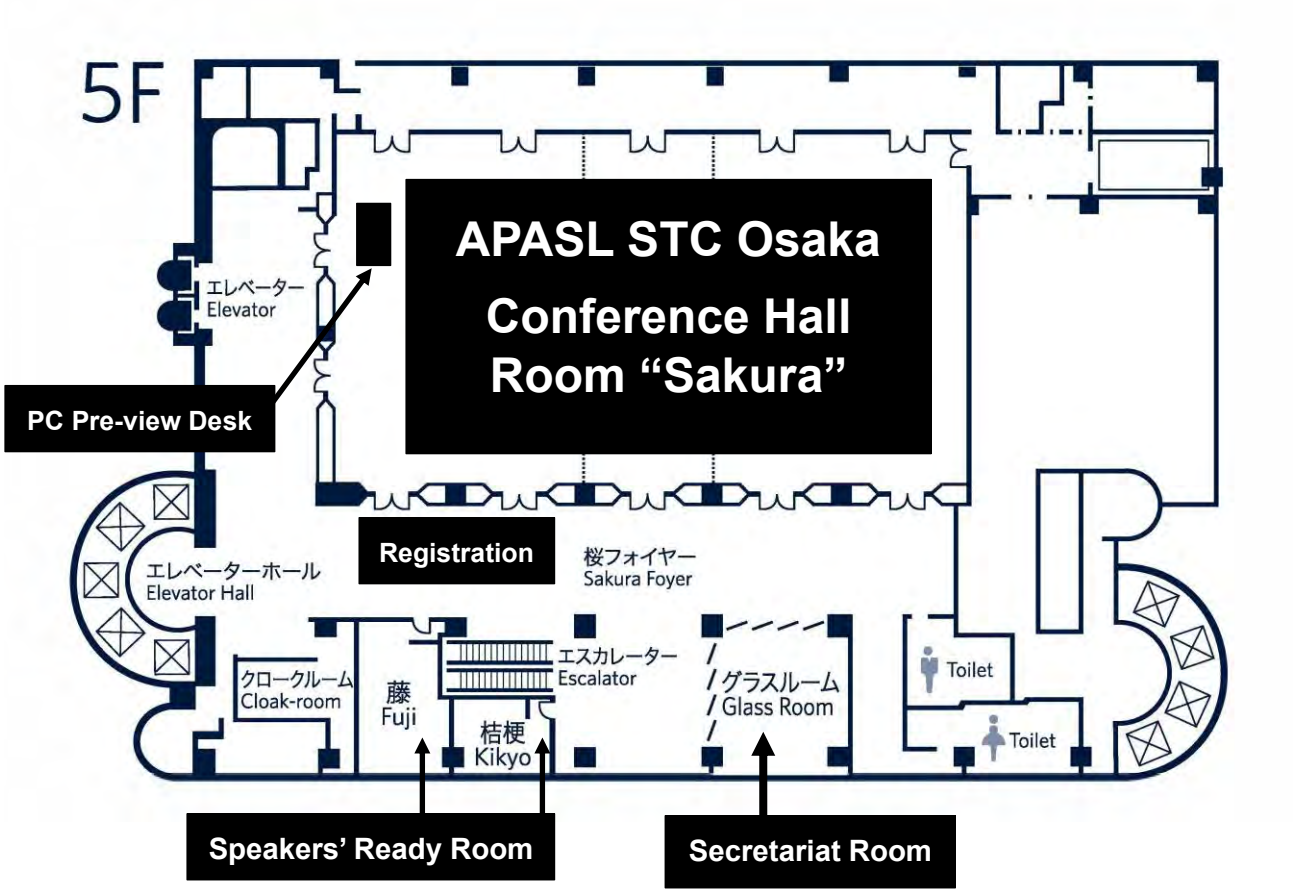
Hilton Osaka

Address:	8-8, Umeda 1-chome, Kita-ku, Osaka, 530-0001, Japan
Tel:	+81-6-6347-7111
Fax:	+81-6-6347-7001
Location:	2 min. walk from JR Osaka Station 25 min. from Osaka Itami Airport by Limousine Bus 65 min. from Kansai Airport by Limousine Bus

URL: https://www3.hilton.com/en/hotels/japan/hilton-osaka-OSAHITW/maps-directions/index.html

Conference Room: “Sakura”, 5th Floor
Registration, PC Preview Desk: In front of “Sakura”, 5th Floor
Secretariat Room: “Glass Room”, 5th Floor
Cloak: Foyer, 5th Floor
Speakers/Chairs Ready Room: “Fuji” “Kikyo”, 5th Floor

Floor Plan Hilton Osaka



Instruction for Oral Presentation

- An invitation email containing information about the login will be sent to the presenters/moderators through the Zoom system. The login URL will be included in the invitation email.
- We would appreciate it if you could conduct a test connection ahead of the conference.
- The general sessions' presentation time is 20 minutes. The selected Free Paper is 5 minutes.
- After presentation, the discussion time (a question-and-answer session) will be held according to the moderator's instructions.

[For those who will participate at the onsite venue]

- Please be seated at the "next speaker's seat" at least 30 minutes before the presentation.
- The slides which you have sent in advance for the presentation are prepared on the computer of the podium. Please operate the slides by yourself. Please note that the presenter tool is not available.

[For those who will attend online]

- Please join Zoom at least 30 minutes before your session begins.
- Please turn on the microphone and the camera only when you are speaking.
- The moderator will introduce the presenter at the beginning of each presentation.
- Then, the secretariat will start the presentation video. (In principle, you do not have to share your presentation by yourself.)
- After finishing the presentation, online viewers will send textual questions to the Q & A column, so please follow the moderator's instructions and answer those questions.
- The following environment is recommended.
  -Create the image resolution in XGA (1024 x 768).
  -Microsoft PowerPoint (2010-2016) can be used as the application software.
  -The fonts that come standard with Microsoft PowerPoint, Times, Arial are recommended.

[About on-demand presentation]

- The secretariat will send you questions received by e-mail and Q & A during the on-demand delivery period, so please answer them if any.

[Precautions]

- Do not post, modify, distribute or reproduce copyrighted material, trademarks, portrait rights or other property rights in any way without the prior written consent of the owners of these property rights.
- Regarding citations, please specify the source of the citation.
- Please exert caution regarding the protection of personal information such as name, age, surgery date, etc. This could lead to the identification of an individual.
- Please pay particular attention to images and videos that may be considered problematic when viewed by the public at this conference.

Instruction for Chairs

[About presentation style]
The conference program will be presented as a hybrid style meeting.
An invitation email that contains information about the login will be sent to the presenters/moderators through the Zoom system. The login URL will be included in the invitation email.
At the real time webinar, the recorded lecture will be presented, and speakers/chairs are requested to join the discussion time. The presentation and Q & A session will be delivered live.
The general oral presentation time is 20 minutes. The selected Free Paper is 5 minutes.
After presentation, the discussion time (a question-and-answer session) will be held according to the moderator's instructions. The online viewer will send questions in the Q & A column. The onsite participants will ask questions using the microphone at the conference venue.
After the conference, the recorded video will be posted on the on-demand presentation page.

[For Chairs who will participate at the onsite conference venue]
Please be seated at the "next chair's seat" at least 30 minutes before the presentation. The seat will be on the left side, closest to the stage.

[For Chairs who will attend online]
Please join Zoom at least 30 minutes before your session begins.
On your entry, the secretariat will check your microphone and camera. At that time, please turn on the microphone and camera.
After checking the microphone and camera, please turn off the microphone (mute) and the camera.
Please turn on the microphone and the camera only when you are speaking. Please mute the microphone otherwise.
Please introduce the presenter at the beginning of each presentation.
Then, the secretariat will start the presentation video.
After finishing the presentation, turn on the microphone and camera again. Online viewers will send textual questions to the Q&A column, and the onsite participants will ask questions using the microphone in the conference room. So please convey those questions and moderate the discussion.
The organizer cannot handle problems such as computer operation, internet connection, video connection, and audio connection. Please solve such problems by yourself. We recommend the following environment.
-We would appreciate it if you could use a PC with as much memory as possible (CPU i5 or more, memory 8 Giga or more).
-Please connect to the Internet via a wired LAN line as much as possible.

Instruction for Poster Presentation

[About presentation style]

・ The conference program will be presented as a hybrid style meeting.

All the speakers are requested to submit PowerPoint presentation data beforehand.

・ After the conference, the presentation will be posted on the on-demand presentation page.

・ Please send your presentation (PowerPoint File 4:3) in advance to the congress secretariat as follows.

・ Microsoft PowerPoint (2010-2016) can be used as the application software

・ File size is limited to 20 MB

・ The number of PPT slides is limited to 15 slides

・ Requested PowerPoint size is 4: 3

・

[Precautions]

・ Do not post, modify, distribute, or reproduce copyrighted material, trademarks, portrait rights, or other property rights in any way without the prior written consent of the owners of these property rights.

・ Regarding citations, please specify the source of the citation.

・ Please exert caution regarding the protection of personal information such as name, age, surgery date, etc. This could lead to the identification of an individual.

・ Please pay particular attention to images that may be considered problematic when viewed by the public at this conference.

If you have any questions, please contact the secretariat below.

Contact: APASL STC 2021 in Osaka Congress Secretariat
Email: info@apaslstc-osaka2021.org
Tel: +81-3-6380-0102 Fax: +81-3-6380-0103
URL http://www.apaslstc-osaka2021.org/index.html

Awards

Excellent papers will be awarded as “Young Investigator Award”.
Awarding Ceremony will be held at the Closing Remark at 19:40 (JST) on September 3rd.

Young Investigator Award (Under 40 years old)

The purpose of the “APASL STC Osaka Young Investigator Award” is to praise outstanding examples of excellence amongst those involved in research training in the early stages of their career.

Contact

APASL STC 2021 Osaka Scientific Secretariat
Department of Hepatology, Graduate School of Medicine, Osaka City University
Email: thuylt@med.osaka-cu.ac.jp
Tel: +81-6-6645-3905

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c/o Academia Support Japan
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Asian Pacific Association for the Study of the Liver [APASL]
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Tel: +81-3-5312-7686 Fax: +81-3-5312-7687

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Osaka Convention & Visitors Bureau
The Osaka Medical Research Foundation for Intractable Diseases
The Uehara Memorial Foundation

In alphabetical order

Program at a Glance

Day 1: September 2nd (Thursday) 2021

Day	Day 1 **Thursday September 2nd 2021**		
Room	Conference Hall （Onsite Venue: Room “Sakura” 5F, Hilton Osaka） (Virtual Venue: Zoom Webinar)	**Format**	**Poster**
AM	8:00- Registration (Onsite Venue) 8:30- Webinar Access Open (Virtual Venue)		Poster Free Papers Presentation Through Website
	8:55-9:00 Opening Remark	Live (Zoom)	
	9:00-10:40 Session 1 **Immunology, Inflammation and Regeneration in Hepato-Biliary Diseases** 9:00-10:10 Presentation Time	Recorded Lectures	
	10:10-10:40 Discussion Time	Live (Zoom)	
	10:50-12:30 Session 2 **Molecular Basis of Hepatitis B/C Virus Infection and Therapy** 10:50-12:00 Presentation Time	Recorded Lectures	
	12:00-12:30 Discussion Time	Live (Zoom)	
Noon	12:40-13:40 Luncheon Seminar 1 Sponsored by Eisai Co. Ltd. / MSD K.K.	Live (Zoom)	
PM	13:50-14:20 Meet the Expert 1 **Acute on Chronic Liver Failure**	Recorded Lecture	
	14:30-15:00 Meet the Expert 2 **COVID-19 Vaccination in Chronic Liver Diseases**	Recorded Lecture	
	15:10-16:50 Session 3 **Genetic and Epigenetic Regulation of Liver Diseases** 15:10-16:20 Presentation Time	Recorded Lectures	
	16:20-16:50 Discussion Time	Live (Zoom)	
	17:00-18:40 Session 4 **Molecular Basis of Liver Fibrosis and Cirrhosis Therapy** 17:00-18:10 Presentation Time	Recorded Lectures	
	18:10-18:40 Discussion Time	Live (Zoom)	
	18:50-19:50 Evening Seminar 1 Sponsored by Chugai Pharmaceutical Co., Ltd.	Live (Zoom)	

Program at a Glance

Day 2: September 3rd (Friday) 2021

Day	Day 2 **Friday September 3rd 2021**		
Room	Conference Hall （Onsite Venue: Room “Sakura” 5F, Hilton Osaka） (Virtual Venue: Zoom Webinar)	**Format**	**Poster**
AM	7:30- Registration (Onsite Venue) 7:30- Webinar Access Open (Virtual Venue)		Poster Free Papers Presentation Through Website
	8:00-9:00 Morning Seminar Sponsored by Otsuka Pharmaceutical Co., Ltd.	Live (Zoom)	
	9:10-11:10 Session 5 **Molecular Basis of Alcoholic and Nonalcoholic Steatohepatitis and Therapy** 9:10-10:40 Presentation Time	Recorded Lectures	
	10:40-11:10 Discussion Time	Live (Zoom)	
	11:20-13:00 Session 6 **Use of Pluripotent Stem Cells and Reprogrammed Cells for Therapy** 11:20-12:30 Presentation Time	Recorded Lectures	
	12:30-13:00 Discussion Time	Live (Zoom)	
Noon	13:10-14:10 Luncheon Seminar 2 Sponsored by AbbVie GK	Live (Zoom)	
PM	14:20-16:20 Session 7 **Gut-Liver-Brain Axis in Hepatic Pathophysiology and Clinical Medicine** 14:20-15:50 Presentation Time	Recorded Lecture	
	15:50-16:20 Discussion Time	Live (Zoom)	
	16:30-18:30 Session 8 **Diagnosis and Therapy on Hepatic and Biliary Cancer** 16:30-18:00 Presentation Time	Recorded Lecture	
	18:00-18:30 Discussion Time	Live (Zoom)	
	18:40-19:40 Evening Seminar 2 Sponsored by Gilead Sciences K.K.	Live (Zoom)	
	19:40-19:50 Closing Remark	Live (Zoom)	

Scientific Program

Day 1: September 2nd (Thursday) 2021

8:55-9:00 Opening Remark

Dr. Norifumi Kawada (President of APASL STC Osaka)

9:00-10:40 Session 1: Immunology, Inflammation and Regeneration in Hepato-Biliary Diseases

Chairs: Dr. Robert F. Schwabe (USA) / Dr. Tawesak Tanwandee (Thailand)

09:00-09:20 S1-1 **"Role of Hepatic Stellate Cells in Liver Cancer"**
Dr. Robert F Schwabe (USA)

09:20-09:40 S1-2 **"Multiomics Profiling Identifies the Pro-tumoral Immune Networks in the Steatotic Tumor Microenvironment in Non-viral Hepatocellular Carcinoma"**
Dr. Tetsuo Takehara (Japan)

09:40-10:00 S1-3 **"Inflammasome Modulation to Treat NASH & Liver Fibrosis"**
Dr. Ariel Feldstein (USA)

10:00-10:05 S1-4 **"Toll-like Receptor (TLR)-3 and TLR-9 Genes Polymorphism with Hepatitis C Virus Specific Cell Immunity Outcomes in Egyptian Health-care Workers"**
Dr. Mohamed Abdel-Samiee (Egypt) Selected Paper #10061

10:05-10:10 S1-5 **"The New Immunological Strategy of HCC Prevention to Suppress MICA Shedding"** Dr. Jun Arai (Japan) Selected Paper #10060

10:10-10:40 Discussion Time

10:50-12:30 Session 2: Molecular Basis of Hepatitis B/C Virus Infection and Therapy

Chairs: Dr. Jin Mo Yang (Korea) / Dr. A. Kadir Dokmeci (Turkey)

10:50-11:10 S2-1 **"Being Involved in Molecular Biology for 40 Years; COVID-19 and HCC"**
Dr. Masao Omata (Japan)

11:10-11:30 S2-2 **"Viral Quasispecies Diversity and the Outcomes of Chronic Hepatitis B"**
Dr. Jia-Horng Kao (Taiwan)

11:30-11:50 S2-3 **"Development of New Therapies for CHB-2021 and Beyond"**
Dr. George Lau (Hong Kong)

11:50-11:55 S2-4 **"A Robust Cell Culture System for Anti-hepatitis B Virus Drug Study via Epigenetic Reprogramming"**
Dr. Luc Gailhouste (Japan) Selected Paper #10022

11:55-12:00 S2-5 **"Zinc Chloride Enhances dsRNA-induced beta-interferon Promoter Activity through the Inhibition of Mitogen-Activated Protein Kinase Kinase 3 Expression"**
Dr. Tatsuo Kanda (Japan) Selected Paper #10064

12:00-12:30 Discussion Time

12:40-13:40 Luncheon Seminar 1 (Sponsored by Eisai Co. Ltd. / MSD K.K.)

Chair: Junji Furuse (Japan)

12:40-13:10 **"Consideration of the Decision Process of Treatment in Patients with Hepatocellular Carcinoma"**
Dr. Sadahisa Ogasawara (Japan)

13:10-13:40 **"Clinical Outcome of Lenvatinib Therapy in Japanese Patients with Unresectable HCC"**
Dr. Kaoru Tsuchiya (Japan)

13:50-14:20 Meet the Expert 1

"Acute on Chronic Liver Failure"
Dr. Shiv K Sarin (India)

14:30-15:00 Meet the Expert 2

"COVID-19 Vaccination in Chronic Liver Diseases"
Dr. Xiaolong Qi (China)

15:10-16:50 Session 3: Genetic and Epigenetic Regulation of Liver Diseases

Chairs: Dr. Tetsuo Takehara (Japan) / Dr. Derek A. Mann (UK)

15:10-15:30 S3-1 **"Genomic Landscape of Hepatocarcinogenesis"**
Dr. Tatsuhiro Shibata (Japan)

15:30-15:50 S3-2 **"Epigenetic Changes Caused by Hepatitis C Virus Infection Persist Even after Viral Eradication and Pose a Risk of Hepatocellular Carcinoma"**
Dr. Kazuaki Chayama (Japan)

15:50-16:10 S3-3 **"Epigenetic Control of Hepatic Stellate Cell Activation and Liver Fibrosis"**
Dr. Derek A Mann (UK)

16:10-16:15 S3-4 **"COLCA1 and COLCA2, the Effector Genes Driven by rs1944919 in Primary Biliary Cholangitis (PBC) Susceptibility Locus Chromosome 11q23.1 in the Japanese Population"**
Dr. Yuki Hitomi (Japan) Selected Paper #10002

16:15-16:20 S3-5 **"Palmitate Disrupts the Circadian Rhythm of Mitochondrial Sirtuins in Non-neoplastic Hepatocytes (PH5CH8), While it Restores the Rhythm in Hepatocellular Carcinoma Cell Line (HepG2)"**
Dr. Savera Aggarwal (India) Selected Paper #10062

16:20-16:50 Discussion Time

17:00-18:40 Session 4: Molecular Basis of Liver Fibrosis and Cirrhosis Therapy

Chairs: Dr. Jordi Gracia-Sancho (Spain) / Dr. Xiaolong Qi (China) / Dr. Kazuo Ikeda (Japan)

17:00-17:20 S4-1 **"Treatment Strategy for Liver Fibrosis Based on Deactivation of Fibrogenic Hepatic Stellate Cells"**
Dr. Yutaka Inagaki (Japan)

17:20-17:40 S4-2 **"Molecular Basis of Portal Hypertension Therapy"**
Dr. Jordi Gracia-Sancho (Spain)

17:40-18:00 S4-3 **"The Role of Human Extracellular Matrix in Driving Tissue Fibrosis and Cirrhosis"**
Dr. Giuseppe Mazza (UK)

18:00-18:05 S4-4 **"Behavior of Reactive Cholangiocytes in the Tissue Repair Stage from Chronic Liver Injury"**
Dr. Yasuhiro Nakano (Japan) Selected Paper #10029

18:05-18:10 S4-5 **"Capacity of Extracellular Globins in Suppression of Collagen Production from Activated Hepatic Stellate Cells via Scavenging ROS and Promoting MMP-1 Secretion"**
Dr. Vu Ngoc Hieu (Japan) Selected Paper #10037

18:10-18:40 Discussion Time

18:50-19:50 Evening Seminar 1: Sponsored by Chugai Pharmaceutical Co., Ltd.

Chair: Dr. Kiyoshi Hasegawa (Japan)

"A New Era of Cancer Immunotherapy in Hepatocellular Carcinoma"
Dr. Masatoshi Kudo (Japan)

Scientific Program

Day 2: September 3rd (Friday) 2021

8:00-9:00　Morning Seminar: Sponsored by Otsuka Pharmaceutical Co., Ltd.

Chair: Dr. Hitoshi Yoshiji (Japan)

"Management of Liver Cirrhosis: Summary of JSGE & JSH Evidence-based Clinical Practice Guidelines for Liver Cirrhosis 2020"
Dr. Naoya kato (Japan)

9:10-11:10　Session 5: Molecular Basis of Alcoholic and Nonalcoholic Steatohepatitis and Therapy

Chairs: Dr. Gyongi Szabo (USA) / Dr. Ekihiro Seki (USA) / Dr. Kenichi Ikejima (Japan)

9:10-9:30　S5-1 **"Inflammasome Activation and microRNAs are Molecular Drivers and Therapeutic Targets in Steatohepatitis"**
Dr. Gyongi Szabo (USA)

9:30-9:50　S5-2 **"Treatment Targets for Nonalcoholic Steatohepatitis"**
Dr. Vincent Wong (Hong Kong)

9:50-10:10　S5-3 **"New Therapeutic Strategy for Alcoholic Liver Disease: Role of TLR7 and IL-22"**
Dr. Ekihiro Seki (USA)

10:10-10:30　S5-4 **"Molecular Basis of Alcoholic and Metabolic Steatohepatitis and Therapy"**
Dr. Jacob George (Australia)

10:30-10:35　S5-5 **"Possible Repurposing of the Analgesic Neurotropin for NAFLD/NASH Treatment"**
Dr. Takashi Tsuchiya (USA)　Selected Paper #10014

10:35-10:40　S5-6 **"Gut-liver Axis-mediated Mechanism of NASH-associated Hepatocellular Carcinoma Progression"**
Dr. Ryota Yamagishi (Japan)　Selected Paper #10042

10:40-11:10　Discussion Time

11:20-13:00　Session 6: Use of Pluripotent Stem Cells and Reprogrammed Cells for Therapy

Chairs: Dr. Atsushi Miyajima (Japan) / Dr. Diana A. Payawal (Philippines)

11:20-11:40　S6-1 **"Generation of Quiescent Hepatic Stellate Cells from Human iPSCs and Development of Anti-fibrotic Drugs"**
Dr. Atsushi Miyajima (Japan)

11:40-12:00　S6-2 **"Cell Identity Conversion and Liver Regeneration"**
Dr. Lijian Hui (China)

12:00-12:20　S6-3 **"Generation of Human Liver using iPS Cells for Regenerative Therapies"**
Dr. Hideki Taniguchi (Japan)

12:20-12:25　S6-4 **"The Therapeutic Effect of Human Placenta Mesenchymal Stem Cell-Derived Exosomes on Primary Sclerosing Cholangitis"**
Dr. Wenyi Chen (China)　Selected Paper #10063

12:25-12:30　S6-5 **"Kruppel-like Factor 15 Induces the Development of Mature-type Hepatocytes from Progenitor Cells"**
Dr. Kota Tsuruya (Japan)　Selected Paper #10044

12:30-13:00　Discussion Time

13:10-14:10　Luncheon Seminar 2: Sponsored by AbbVie GK

Chair: Tatehiro Kagawa (Japan)

13:10-13:40　"**Clinical Outcome of Antiviral Therapy in Patients with Hepatitis C Virus Infection"**
Dr. Toshifumi Tada (Japan)

13:40-14:10　"**Hepatocellular Carcinoma Developing after the Eradication of Hepatitis C Virus: Surveillance, Characteristics, and Prognosis"**
Dr. Hidenori Toyoda (Japan)

14:20-16:20　Session 7: Gut-Liver-Brain Axis in Hepatic Pathophysiology and Clinical Medicine

Chairs: Dr. Shiv K Sarin (India) / Dr. Tatiana Kisseleva (USA)

14:20-14:40　S7-1 **"IL-17 Signaling in Steatotic Hepatocytes Promotes Alcoholic Liver Disease-induced Hepatocellular Carcinoma"**
Dr. Tatiana Kisseleva (USA)

14:40-15:00 S7-2 **"Gut Microbiota and Autoimmune Diseases – Type 1 Diabetes and Multiple Sclerosis –"**
Dr. Hiroshi Ohno (Japan)

15:00-15:20 S7-3 **"The Liver-brain-gut Neural Reflex Maintains the Niche of Gut Regulatory T Cells"**
Dr. Takanori Kanai (Japan)

15:20-15:40 S7-4 **"High-throughput Screening of New Compounds that Remove Senescent Cell Toward Prevention of HCC Development"**
Dr. Eiji Hara (Japan)

15:40-15:45 S7-5 **"Nimbolide Restores Gut Dysbiosis and Prevents Bacterial Translocation by Improving Tight Junction Proteins Expression in an Experimental Hepatocarcinogenesis"**
Dr. Balasubramaniyan Vairappan (India) Selected Paper #10050

15:45-15:50 S7-6 **"Dysbiosis of the Gut Microbiota Associated with Plasma Levels of IFN-&gamma and Viral Load in Patients with Acute Hepatitis E Infection"**
Dr. Jian Wu (China) Selected Paper #10054

15:50-16:20 Discussion Time

16:30-18:30 Session 8: Diagnosis and Therapy on Hepatic and Biliary Cancer

Chairs: Dr. Masao Omata (Japan) / Dr. Jia-Horng Kao (Taiwan)

16:30-16:50 S8-1 **"Whole Genomics and Precision Oncology for Hepato-Biliary Cancer"**
Dr. Hidewaki Nakagawa (Japan)

16:50-17:10 S8-2 **"Biomarkers in the Treatment of Liver Cancer"**
Dr. Naoya Kato (Japan)

17:10-17:30 S8-3 **"Nobel Treatment Strategy for Intermediate-stage Hepatocellular Carcinoma"**
Dr. Masatoshi Kudo (Japan)

17:30-17:50 S8-4 **"HCC Surveillance in Patients with Pharmacologically Treated Viral Hepatitis"**
Dr. Massimo Colombo (Italy)

17:50-17:55 S8-5 **"Analysis of Tumor Microenvironment in Patients with Advanced Hepatocellular Carcinoma Eligible for Systemic Therapy"**
Dr. Hiroaki Kanzaki (Japan) Selected Paper #10008

17:55-18:00 S8-6 **"Soluble PD-1 as a Potential Biomarker for Predicting Response to the Immune Checkpoint Inhibitor in HCC Patients"**
Dr. Yutaka Yasui (Japan) Selected Paper #10027

18:00-18:30 Discussion Time

18:40-19:40 Evening Seminar 2: Sponsored by Gilead Sciences K.K.

Chair: Dr. Yasuhito Tanaka (Japan)

"Novel Agents for HBV Therapy"
Dr. Man-Fung Yuen (Hong Kong, China)

19:40-19:50 Closing Remark
Dr. Norifumi Kawada (President of APASL STC Osaka)

Poster Session Program

P-01 #10028 "**Combination Therapy of Juzentaihoto and Mesenchymal Stem Cells Attenuates Liver Damage and Regresses Fibrosis in Mice"** Dr. Takahiro Iwasawa (Japan)

P-02 #10057 "**The Anti-fibrotic Effect of *ex vivo* Expanded CD34$^+$Cell Transplantation in a Mouse Model of NASH"** Dr. Atsutaka Masuda (Japan)

P-03 #10056 "**Cell Membrane-mediated Direct Crosstalks between Hepatocytes and HSCs"** Dr. Kirara Inoue (Japan)

P-04 #10009 "**Bioinformatics Study of Genes E1 and E2 from Hepatitis C Virus (HCV) with Genotypes 1, 2, 3 and 6 as Vaccine Candidates for Virus-like Particles (VLPs)"** Dr. Rifaldy Fajar (Indonesia)

P-05 #10052 "**Improvement of Liver Fibrosis in Patients Achieving a Sustained Virological Response to DAA Treatment for Hepatitis C"** Dr. Ayumi Sugiura (Japan)

P-07 #10048 "**microRNA-6126 Reduces the Stability of NTCP Messenger RNA and Suppresses its Expression in Hepatocytes"** Dr. Koji Fujita (Japan)

P-08 #10017 "**Molecular Characterization of Hepatitis B Virus in Young People of Vietnam"** Dr. Evgennia V. Lichnaia (Russia)

P-09 #10043 "**Emergence of Novel Resistance-associated Substitutions against Pibrentasvir in Genotype 1b HCV-Infected Mice"** Dr. Takuro Uchida (Japan)

P-10 #10023 "**B-cell Activating Factor Promotes Lipid Synthesis in Murine Hepatocytes"** Dr. Masanori Abe (Japan)

P-11 #10026 "**Development of Human Hepatic Stellate Cell Activation/Deactivation Culture Condition Aiming the Liver Fibrosis Evaluation"** Dr. Seiichi Ishida (Japan)

P-12 #10025 "**Ezetimibe Suppresses the Progression of NASH and NASH-associated HCC"** Dr. Kouichi Miura (Japan)

P-13 #10038 "**Analysis of Liver Fibrosis Progression, Lipid Profile, and Atherosclerosis in NAFLD"** Dr. Shun-ichi Wakabayashi (Japan)

P-14 #10030 "**Glycine Ameliorates Steatohepatitis via Reduction of Oxidative Stress in Hepatocyte-specific PTEN-deficient Mice"** Dr. Kazuyoshi Kon (Japan)

P-15 #10015 "**Synergistic Regulation of Hepatic Fsp27b Expression by HNF4α and CREBH"** Dr. Ichiro C. Kasano-Camones (Japan)

P-16 #10040 "**A Novel Isolation Method of Hepatic Stellate Cells from Tumor Tissue of Obesity-associated Hepatocellular Carcinoma"** Dr. Yi Cheng (China)

P-17 #10036 "**Type IV Collagen 7S is the Most Accurate test for Identifying Advanced Fibrosis in Non-Alcoholic Fatty Liver Disease with Type 2 Diabetes"** Dr. Hiroshi Ishiba (Japan)

P-18 #10055 "**Systemic Inflammation Predict 30-day Bacterial Infection Post-liver Transplantation"** Dr. Jiong Yu (China)

P-19 #10021 "**Elevated Alpha Fetoprotein in the Absence of Hepatic Malignancy in a Patient with Acute Hepatitis"** Dr. Jacklyn M So-Cabahug (Philippines)

P-20 #10005 "**Nanoparticle-mediated Delivery of 2-deoxy-D-glucose Induces Antitumor Immunity and Cytotoxicity in Liver Tumors in Mice"** Dr. Sohji Nishina (Japan)

P-21 #10033 "**The Complication Rate and Clinical Significance of Extrahepatic Autoimmune Diseases (EHAIDs) in Primary Biliary Cholangitis (PBC)"**
Dr. Yuki Yamashita (Japan)

P-22 #10058 "**Role of CD40 in Hepatocellular Carcinoma"**
Dr. Vinh Hanh Ngo (Japan)

P-23 #10049 "**Changes of Soluble Immune Checkpoint Proteins following Antiviral Treatment in Chronic Hepatitis C Patients and the Roles of the CD27-CD70 Pathway in Hepatocellular Carcinoma Development"** Dr. Minh Phuong Dong (Japan)

P-24 #10012 "**Case Report of Overlap Syndrome: Autoimmune Hepatitis and Primary Biliary Cholangitis, a Vietnamese Patient Diagnosing by Pathology"**
Dr. Lieu Quang Dau (Viet Nam)

P-25 #10051 "**Functional Analysis of Liver Cirrhosis-associated Gut Microbiota Uncovers a Unique Mechanism of Hyperammonemia in Hepatic Encephalopathy"**
Dr. Tomonori Kamiya (Japan)

P-26 #10035 "**Role of the FibroScan-aspartate Aminotransferase Score in Risk Stratification for a Japanese Cohort with Fatty Liver Diseases"** Dr. Hideki Fujii (Japan)

P-27 #10039 "**Possible Involvement of ZNF641 with the Prognosis of Hepatocellular Carcinoma"**
Dr. Hirayuki Enomoto (Japan)

P-28 #10004 "**The Long Non-coding RNA of RMRP is Repressed by PERK and Induces Apoptosis in Hepatocellular Carcinoma"** Dr. Atsushi Yukimoto (Japan)

P-29 #10011 "**Identifying UGT1A1 Gene Mutations in Vietnamese Patients with Gilbert Syndrome"**
Dr. Lieu Quang Dau (Viet Nam)

P-30 #10020 "**Validation of Platelets - Albumin - Bilirubin (PALBI) Score for Predicting Overall Survival of Hepatocellular Carcinoma at Hanoi Medical University Hospital, Vietnam"**
Dr. Bich Hang Doan (Viet Nam)

P-31 #10016 "**Validation of ABCR and ART for Predicting Overall Survival in Patient with Hepato-cellular Carcinoma Treated with Transarterial Chemoembolism in Hanoi Medical University Hospital, Vietnam"** Dr. Duc Minh Pham (Viet Nam)

P-32 #10024 "**A Novel Anticancer Therapy with Deferoxamine and Drugs Targeting Iron-chelation-Modulated Metabolism"** Dr. Koichi Fujisawa (Japan)

P-33 #10059 "**Identification of Key Modules and Hub Genes Involved in Cholangiocarcinoma Progression and Prognosis"** Dr. Qigu Yao (China)

P-34 #10031 "**PD-L1 Promotes Cell Proliferation in Liver Cancer Cells"**
Dr. Toshimitsu Tanaka (Japan)

P-35 #10003 "**Efficacy of Zinc Acetate in HCC Cell Lines via the Induction of Apoptosis"**
Dr. Takashi Himoto (Japan)

P-36 #10007 "**Liver Piopsy Technique in the Era of Cancer Genomic Therapies: A Single Center Retrospective Analysis"** Dr. Naoya Kanogawa (Japan)

P-37 #10041 "**Intracellular Gaps Formation of Liver Sinusoidal Endothelial Cells Facilitates Cancer Cell Engraftment in Liver"** Dr. Truong Huu Hoang (Japan)

P-38 #10045 "**Role of SKI in the Suppression of Cholangiocarcinoma Cell Proliferation by Inducing G1-phase Arrest: Validation of Results from Clinical Specimens"**
Dr. Etsushi Kawamura (Japan)

P-39 #10034 "**The Association between Plasma Free Amino Acids and Sarcopenia in the Course of Hepatocellular Carcinoma Recurrence"** Dr. Shunichi Tsuge (Japan)

P-40 #10010 "**A Survey on Approaches towards Patients with Elevated Liver Enzymes before Surgery in Different Vietnamese Hospitals"** Dr. Hang Dao (Viet Nam)

APASL Single Topic Conference 2021 Osaka

"Molecular and Cell Biology of the Liver: Recent Evolution to Clinical Application"

Abstracts

Meet the Experts

Meet the Experts-1

Dr. Shiv Kumar Sarin
Senior Professor, Department of Hepatology
Institute of Liver and Biliary Sciences (ILBS)
New Delhi, India

Acute and acute-on-chronic liver failure

ACLF is a serious medical ailment, with high short-term mortality. Amongst its many definitions; two are most often used. According to the APASL, ACLF It has been variously defined is an acute hepatic insult manifesting as jaundice (serum bilirubin ≥5 mg/dl [≥85 ųmol/l]) and coagulopathy (INR ≥1.5 or prothrombin activity <40%) complicated within 4 weeks by clinical ascites and/or encephalopathy in a patient with previously diagnosed or undiagnosed chronic liver disease or cirrhosis, and is associated with a high 28-day mortality. The APASL ACLF Research Consortium (AARC) established in 2012, has more than 56 collaborative centers and have collected nearly 7,250 patients in a short span.

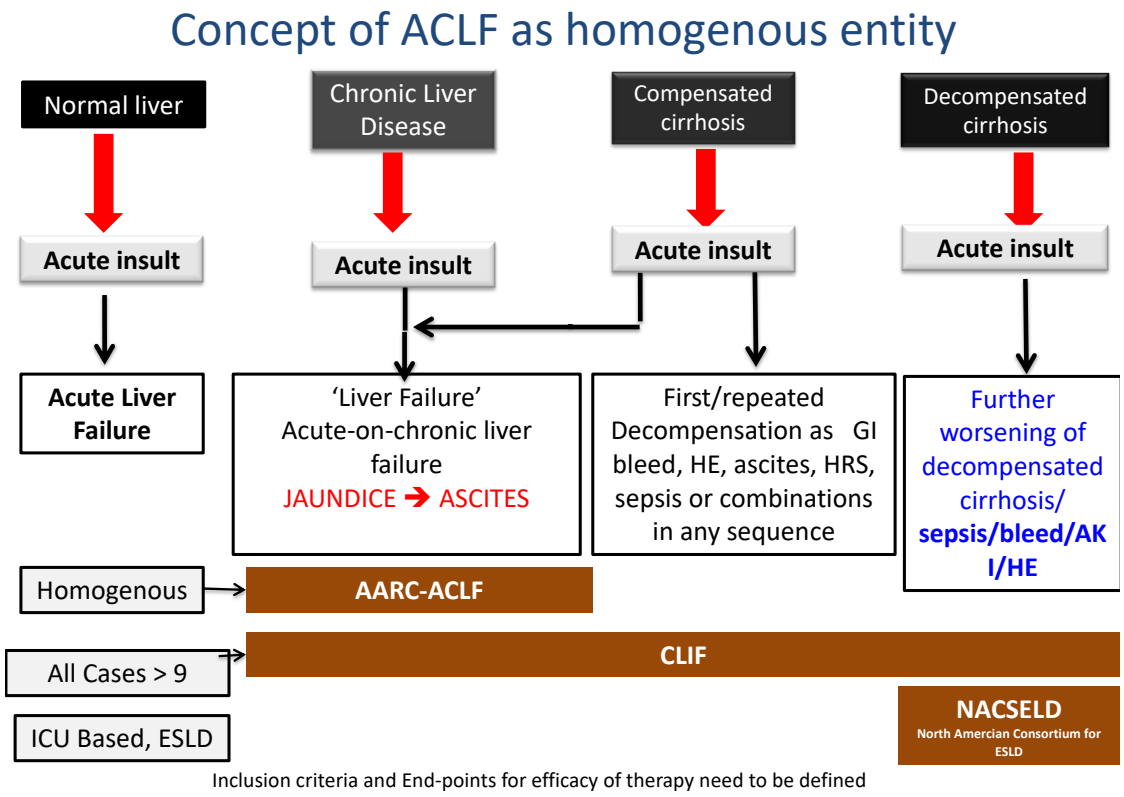
On the other hand the Western (CLIF SOFA) definition involves an acute deterioration of pre-existing cirrhosis, usually related to a precipitating event and is associated with an extrahepatic organ failure and increased mortality at 3 months. Sepsis and extra-hepatic organ failure are integral part of the Western definition of ACLF. This makes diagnosis of patient late. Further, CLIF definition includes already decompensated cirrhosis patients, which make the group very heterogenous (Fig.1).

Acute insults include alcohol, hepatotropic viruses and drugs whereas the underlying chronic liver disease is generally cirrhosis due to alcohol, hepatitis B or C, or NASH. The pathophysiology of ACLF relates to persistent inflammation, immune dysregulation with initial wide-spread immune activation, a state of systematic inflammatory response syndrome and subsequent sepsis due to immune paralysis. A short 'golden window' of 7 days precedes sepsis development and organ(s) failure, and provides opportunity for immunomodulation with GCSF and other interventions; extrahepatic organ failure indicates severity of illness, prognosis and helps guide management. Artificial liver support systems such as Liver dialysis using MARS or Prometheus and plasma exchange, help remove toxins, PAMPS, DAMPS and toxic metabolites and serve as a bridge to liver transplantation. These have been tried in patients with ACLF and work on the principle of albumin dialysis. These studies showed an improvement of biological cholestasis and hepatic encephalopathy with albumin dialysis but without survival benefit. In a recent retrospective study, however, survival benefit has been reported. Therapeutic Plasma-exchange (TPE) is associated with an improvement in the systemic inflammatory response syndrome, reduced damage-associated molecular patterns (DAMPs), and bacterial endotoxin proinflammatory cytokines, endothelial function, and monocyte phagocytic function in patients with ACLF. Combining TPE with continuous renal replacement therapy may be a better strategy that needs to be explored.

Clinical recovery is expected with the use of nucleoside analogues for hepatitis B, and steroids for severe alcoholic hepatitis and severe autoimmune hepatitis. Artificial liver support systems (Liver dialysis using MARS or Prometheus and Plasma exchange) help remove toxins and metabolites and serve as a bridge to liver transplantation. Hepatic regeneration during ongoing liver failure, although challenging, is possible through the use of growth factors such as GCSF. Liver transplantation is the definitive treatment and a good outcome is achieved with early transplantation in carefully selected patients. The severity of ACLF is reliably assessed by the APASL ACLF Research Consortium (AARC) score and a score above 11, or Grade III liver failure, portends, high mortality and need for liver transplant in 7 days (Fig.2). Patients with a MELD of ≥30 and a delta MELD of >4 points in 7 days should also be considered for early liver transplantation. The results of liver transplant are good with nearly 85% one year survival.

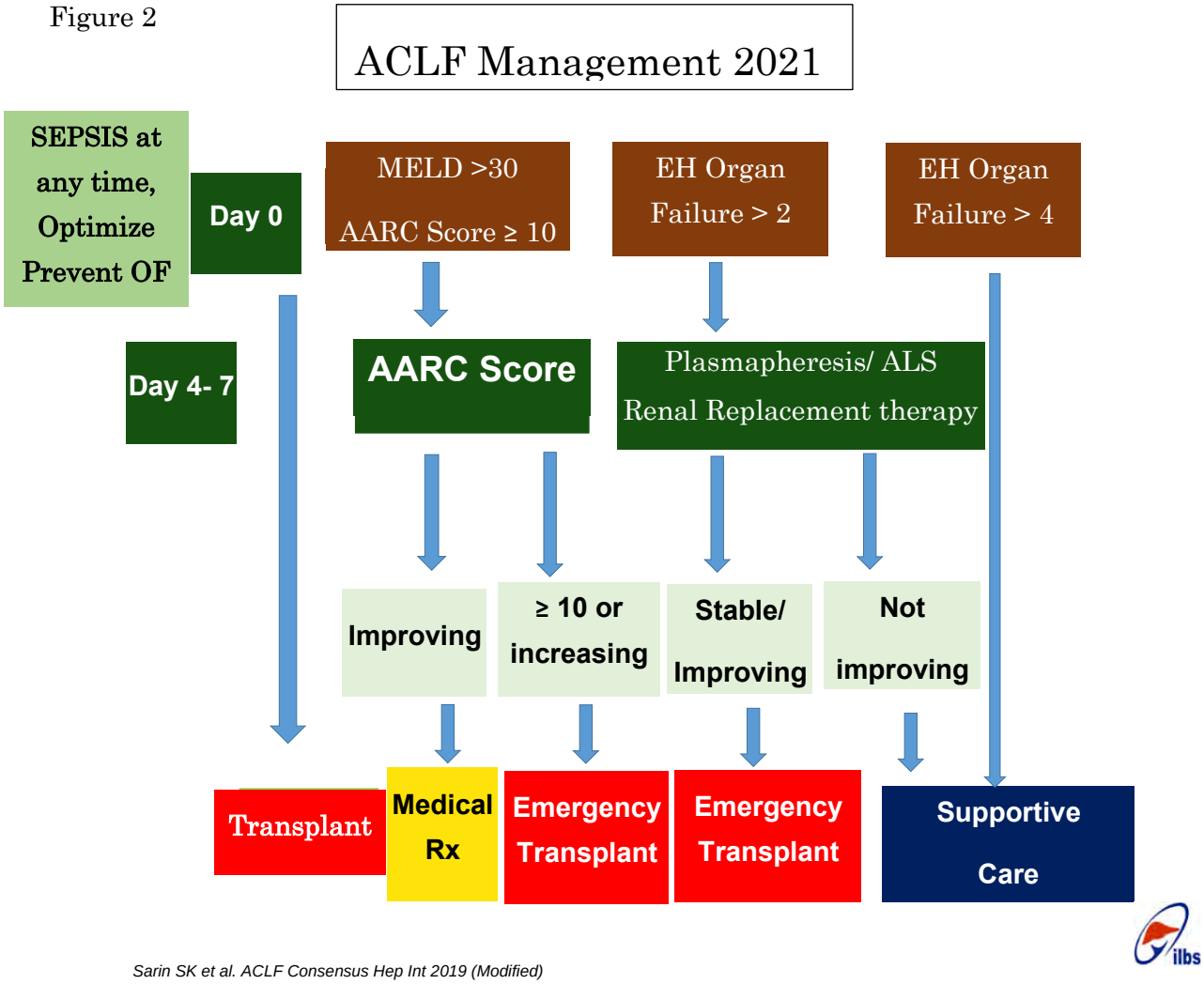
Additionally, hepatic regeneration during ongoing liver failure, although challenging, is possible through the use of growth factors such as GCSF and EPO. Heterologous human adult liver-derived progenitor cells (HepaStem) is also being evaluated, although safety issues remain to be solved. There have been attempts to develop bio-artificial liver using a decellularization approach or by developing small bio-reactors. These are however, still in experimental stages.

Figure 1



Sarin S K et al. ACLF Consensus – update Hep Int 2019, Chaudhary et al Hepatology2019

Figure 2



Sarin SK et al. ACLF Consensus Hep Int 2019 (Modified)

Dr. Xiaolong Qi
Institute of Portal Hypertension,
The First Hospital of Lanzhou University Lanzhou, China

COVID-19 Vaccination in Chronic Liver Diseases

Background and Aims: Concerns have been raised recently about SARS-CoV-2 vaccine responses in the large population of patients with chronic liver diseases (CLD). The study aimed to explore the safety and immunogenicity of COVID-19 vaccination in patients with CLD in a large Chinese cohort.

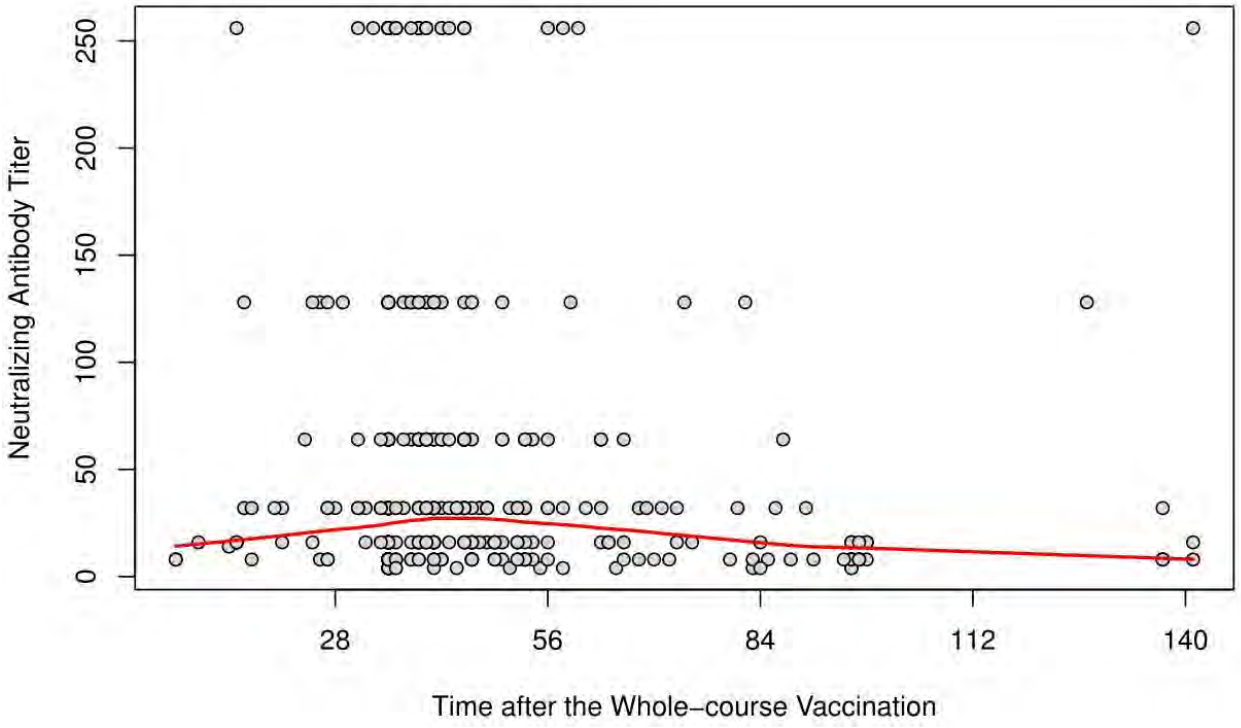
Method: This multicenter study included CLD patients without a history of SARS-CoV-2 infection, from 11 designated centers in China. The primary safety outcome was the incidence of adverse reactions within 7 days after each injection and the overall incidence of adverse reactions within 28 days, and the primary immunogenicity outcome was neutralizing antibody response at least 14 days after the whole-course vaccination.

Results: A total of 390 patients with pre-existing CLD were included in the analysis. The median age was 39.0 years (IQR, 33.0-48.3 years) and 185 (47.4%) were male. Of these, 381 (97.7%) patients had non-alcoholic fatty liver disease. The number of 7-days adverse reactions after each injection and adverse reactions within 28 days totaled 98 (25.1%) and 115 (29.5%) of patients, respectively. The most common adverse reactions were 71 (18.2%) injection site pain, followed by 23 (5.9%) muscle pain, and 21 (5.4%) headaches. All adverse reactions were mild and self-limiting, and no grade 3 adverse reactions were recorded. Notably, neutralizing antibodies were detected in 372 (95.4%) patients. According to the locally weighted scatterplot smoothing, the neutralizing antibody titers were maintained over the time since whole-course vaccination (Figure 1).

Conclusion: The inactivated COVID-19 vaccine appears to be safe with good immunogenicity in patients with CLD.

Figure 1. Correlation between the time since whole-course vaccination and the neutralizing antibody titer.

General trend line is shown in red and was calculated using the locally weighted scatterplot smoothing algorithm.



APASL Single Topic Conference 2021 Osaka

"Molecular and Cell Biology of the Liver: Recent Evolution to Clinical Application"

Abstracts

General Sessions

Dr. Robert F Schwabe
Division of Digestive and Liver Diseases,
Department of Medicine, Columbia University, USA

Role of Hepatic Stellate Cells in Liver Cancer

Hepatic stellate cells (HSC) are the main fibrogenic cell type of the liver and contribute to the development of liver fibrosis. HSC also become activated in many forms of liver cancer. Here we will discuss the role of HSC in two forms of desmoplastic liver tumor: Cholangiocarcinoma and liver metastasis. Genetic HSC depletion shows a tumor-promoting role for HSC in desmoplastic but not in non-desmoplastic liver tumors. Single cell RNA-sequencing clustered HSC into different subtypes and revealed candidate mediators. Genetic deletion of candidate mediators revealed tumor-promoting roles for HSC-derived HGF and Has2. Surprisingly, despite activation of mechanosensitive signals, type I collagen restricted tumor growth in models of desmoplastic liver metastasis in vitro and in vivo. Together, our results demonstrate the activation of tumor-promoting and tumor-restraining pathways by HSC, opening up novel therapeutic opportunities for cholangiocarcinoma and liver metastasis.

Dr. Tetsuo Takehara
Department of Gastroenterology and Hepatology,
Graduate School of Medicine, Osaka University, Japan

Multiomics Profiling Identifies the Pro-tumoral Immune Networks in the Steatotic Tumor Microenvironment in Non-viral Hepatocellular Carcinoma

Background & Aims: Incidence of non-viral hepatocellular carcinoma (HCC) has increased rapidly but its molecular and immunological features have not been fully characterized. We profiled non-viral HCCs by omics approach.

Methods: We performed RNA-sequence of tumor tissues in 113 non-viral HCC patients who underwent curative surgical resection. For 55 tumors, we further sequenced cancer genomes using gene panels focusing on 68 genes in which recurrent genetic alterations were reported in HCC. Intratumoral abundances of immune cell types were estimated by CIBERSORT analysis of transcriptomic data. We used the 10x Genomics Visium platform to define the spatial topography of gene expression in tumor tissues of 2 HCC patients.

Results: Unsupervised hierarchical clustering of tumor transcriptomes classified non-viral HCCs into 3 molecular classes (Class I, II, III), which were not associated with etiology of background liver disease but stratified patient prognosis. Class I with the poorest prognosis had significantly higher rate of TP53 mutations, whereas class III with the most favorable prognosis showed the immune-excluded phenotype with frequent CTNNB1 mutations. The multivariate Cox proportional hazard analysis identified heavy drinking, M2 macrophage infiltration and class I as independent predictive factors of poor prognosis in non-viral HCC patients. Among class II, we identified a pro-tumor immune subclass (k1) characterized by T cell exhaustion signature, M2 macrophage and stromal infiltration, high PD-L1 expression, and TGF-β signaling activation. Pathologically, k1 subclass was characterized by intratumoral steatosis and lipidomics-based intratumor free fatty acid profiling showed the increase in palmitic acid (PA) in this subclass. Lipid accumulation by PA supplementation in HCC cells upregulated PD-L1 and TGF-β expression *in vitro*. Furthermore, lipid-accumulated HCC cells promoted M2 polarization of co-cultured macrophages and upregutated TGF-β expression of co-cultured fibroblasts. Spatial transcriptomics of steatotic HCCs in k1 subclass showed that $CD163^+$ M2 macrophages and VIM^+THY1^+ cancer-associated fibroblats may be in close proximity to $NR4A1^+CD8^+$ exhausted T cells, suggesting that pro-tumoral immune networks were furnished in the steatotic tumor microenvironment.

Conclusions: Multiomics profiling identified the pro-tumor immune subclass in non-viral HCCs and suggested the link between intratumor steatosis and pro-tumor immune microenvironment.

Session 1: Immunology, Inflammation and Regeneration in Hepato-Biliary Diseases

Dr. Ariel Feldstein
Department of Pediatrics,
University of California San Diego,
USA

Inflammasome Modulation to Treat NASH & Liver Fibrosis

The immune system plays an essential role in protecting the host against infections and to accomplish this task has evolved mechanisms to recognize microbes and destroy them. In addition, it monitors the health of cells and responds to ones that are under stress, in the process of dying or death, even if this occurs under sterile conditions. In the liver, this process is often initiated when damage hepatocytes that are undergoing cell death expose intracellular molecules that can be recognized by cells of the innate immune system. As a consequence of this recognition, dendritic cells are activated in ways that help to promote T-cell responses to antigens associated with the dying cells. Additionally, resident macrophages or Kupffer cells are stimulated to produce the cytokine interleukin-1 that may then act on hepatocytes and non-parenchymal cells in the liver in ways that drive a robust inflammatory response. In addition to dead cells, a number of other sterile particles and altered physiological states can similarly stimulate an inflammatory response and do so through common pathways involving the inflammasome and interleukin-1. These pathways are evolving as key players in a number of acute and chronic liver diseases. In this symposium, I will discuss new insights into specific triggers of sterile inflammation, the role of inflammasomes, and downstream signals that contribute to liver damage and fibrosis, as well as the implications for the development of anti-fibrotic strategies.

Session 2: Molecular Basis of Hepatitis B/C Virus Infection and Therapy

Dr. Masao Omata
Department of Gastroenterology,
Yamanashi Central & Kita Hospitals,
University of Tokyo, Japan

Being Involved in Molecular Biology for 40 Years; COVID-19 and HCC

1982 was the first time we started to be involved in molecular biological study of HBV. Since then, we have been studying the viruses particularly related to hepatitis. However, since February 11th, last year when we had two American passengers from Diamond Princess, we have been deeply involved in the prevention and treatment of COVID-19 in our hospital.

In fact, the two American passengers from Diamond Princess were among 437 from their country and among 10, who required intensive care unit cares (Ref 1). Luckily, they returned to Florida after several months care by our team (Ref 2).

In this lecture, in addition to ordinary caring on all the patients suffering from liver diseases, hepatocellular carcinoma in particular, I may report about how we conducted the biological studies on the occurrence of COVD-19 and how we have been able to maintain the “routines” without a single day cessation of the daily clinical practice in this Single Topic Conference.

Ref 1. MM Plucinski et al. Coronavirus Disease 2019 (COVID-19) in Americans

Aboard the Diamond Princess Cruise Ship. Clin Infect Dis. 2021:72 May 18 e448-57

Ref 2. Miyashita et al. Bronchoscopic drainage with full personal protective equipment (PPE) saved a life of COVID-19 infected patient with ventilator associated pneumonic athelectasis from the Diamond Princess Cruise Ship. Case Report in Internal Medicine. 2020, Vol7;1-4

Dr. Jia-Horng Kao
Hepatitis Research Center,
National Taiwan University Hospital, Taiwan

Viral Quasispecies Diversity and the Outcomes of Chronic Hepatitis B

Chronic hepatitis B virus (HBV) infection remains a challenging global health problem. Although a significant portion of HBV patients may experience HBeAg seroconversion with disease remission, many of chronic hepatitis B (CHB) patients develop adverse clinical outcomes, including cirrhosis, hepatocellular carcinoma (HCC) and liver decompensation, so timely antiviral therapy including interferon (IFN) or nucleos(t)ide analogue (NA) is required for those at risk. HBV replicates through the error-prone reverse transcriptase and exhibits a high mutation rate, leading to heterogeneous viral populations termed “quasispecies” within an infected individual. The selection pressure imposed by the host immune responses or antiviral viral treatment also contributes to the diversity of viral quasispecies. Viral quasispecies diversity has been associated with the outcomes of treatment-naïve CHB patients, such as HBeAg seroconversion in HBeAg-positive CHB patients. However, it remains largely unknown regarding how viral quasispecies evolves before and after NA treatment and its association with the durability of off-therapy responses. By using the next-generation sequencing (NGS) approach, our recent data confirmed the association between viral quasispecies characteristics and evolution of HBV and clinical outcomes of HBV patients. First, we demonstrated that higher HBV quasispecies diversity was associated with spontaneous HBeAg seroconversion and IFN-induce HBeAg seroconversion with low viremia, conferring a favorable clinical outcome. Second, we also showed that higher baseline HBV quasispecies diversity is associated with more durable off-therapy viral suppression in HBeAg-negative CHB patients. In addition, combination of baseline viral nucleotide diversity and HBcrAg at end-of-therapy could identify patients at high risk for virological relapse after stopping NA.

Dr. George Lau
Humanity and Health Clinical Trial Center,
Humanity & Health Medical Group,
Hong Kong SAR China

Development of New Therapies for CHB-2021 and Beyond

Over the past two decades, the landscape of registered treatment for chronic hepatitis B (CHB) infection have embraced two categories of therapy-pegylated interferon and nucleosi(t)e analogues (NUCs). However, with the use of these therapies, a "CURE" with loss of hepatitis B surface antigen (HBsAg) to enable lasting off-treatment disease remission, have been disappointingly low. In real-life, due to the drastic reduction of cost and excellent long-term safety records of NUCs, a lot of CHB patients are currently placed on life long NUCs. With the use of these existing registered therapies, the low "CURE" rate has been attributed to the lack of direct effects on viral covalently closed circular (ccc) DNA in infected hepatocytes and inability to restore a functional antiviral immune response. This has been clearly demonstrated in human studies with adoptive immunity clearance of CHB in the setting of allogenic hemopoietic stem cell therapy. In the past few years, based on the understanding of HBV viral life cycle, direct acting antivirals agents that block HBV hepatocyte entry, silence or deplete the cccDNA pool, inhibit viral core assembly, degrade RNase-H, interfering RNA molecules, and nucleic acid polymers, have been developed. On the other hand, immunomodulatory therapy, such as Toll-like-receptors, Retinoic acid Inducible Gene-1 (RIG-1), stimulator of interferon genes (STING) agonists or checkpoint inhibitors, therapeutic vaccines, vector-based vaccines, or adoptive transfer of genetically-engineered T cells aim towards the restoration of T cell function, are also being investigated. Disappointingly, none of the clinical studies with the use of agents, have provided convincing evidence of "CURE" with sustained off-treatment disease remission. The development of new therapies needs also to consider the additional cost-effective benefit to the public at-large, in the "NUCs" era. To this end, carefully designed clinical studies by experienced clinical investigators with emphasis to understand the impact of these "novel agents" on host immunity and viral markers (with standardized laboratory protocol), should be called upon. Without stringent clinical and translational studies, further development of any "CURE" to CHB will be futile.

Dr. Tatsuhiro Shibata
Laboratory of Molecular Medicine, Human Genome Center,
The Institute of Medical Science, The University of Tokyo Japan

Genomic Landscape of Hepatocarcinogenesis

Hepatocellular carcinoma (HCC) is a global health issue and the fourth leading cause of cancer deaths worldwide. Large-scale HCC genome sequencing analyses have identified core drivers (*TERT, TP53*, and *CTNNB1/AXIN1*) as initial molecular events, and other low-frequent drivers that include therapeutically targetable ones. The recent genetic analysis uncovered a distinctive driver gene landscape in precancerous lesions, arguing a discontinuous process at early HCC development. In advanced tumors, intra-tumoral heterogeneity through clonal evolution processes is common, and it displays clear geographic segregation genetically and epigenetically. The genetic information of individual tumors has been utilized for optimizing treatments, early diagnosis, and monitoring recurrence. It will expand the opportunity for screening and prevention of high-risk populations in the near future.

Dr. Kazuaki Chayama
Collaborative Research Laboratory of Medical Innovation,
Hiroshima University, Japan

Epigenetic Changes Caused by Hepatitis C Virus Infection Persist even after Viral Eradication and Pose a Risk of Hepatocellular Carcinoma

Chronic hepatitis C virus (HCV) infection is an important risk factor for development of liver cirrhosis and hepatocellular carcinoma (HCC). Most patients who received direct-acting antiviral agent therapy against HCV achieve a sustained virologic response (SVR). The eradication of the virus reduces the risk of development of HCC, but the risk is still elevated, especially in patients with advanced fibrosis. Some patients develop HCC more than ten years after achieving SVR. To elucidate the mechanism of such late development of HCC, we performed analysis of epigenetic changes that might be related to HCC development. Enrolling patients from Europe and Japan, we performed genome-wide ChIPmentation-based ChIP-Seq and RNA-seq analyses of liver tissues from 6 patients without HCV infection (controls), 18 patients with chronic HCV infection, 8 patients with chronic HCV infection cured by DAA treatment, 13 patients with chronic HCV infection cured by interferon therapy, 4 patients with chronic hepatitis B virus infection, and 7 patients with nonalcoholic steatohepatitis. HCV-induced epigenetic modifications were mapped by comparative analyses with modifications associated with other liver disease etiologies. We also performed analysis using uPA/SCID mice engrafted with human hepatocytes. Mice were infected with HCV by injection of serum samples obtained from chronic HCV-infected patients. Some of the mice were treated withhara DAAs to eradicate the virus. Pathways associated with HCC risk were identified by integrative pathway analyses and validated using analyses of paired HCC tissues from 8 patients who achieved SVR following DAA treatment for HCV infection. We found that chronic HCV infection induces specific genome-wide changes in H3K27ac that were correlated with changes in expression of mRNAs and proteins. These changes persisted after SVR resulting from DAA- or interferon-based therapies. Integrative pathway analyses of liver tissues from patients and mice with humanized livers demonstrated that HCV-induced epigenetic alterations were associated with liver cancer risk. Computational analysis revealed that increased expression of SPHK1 was associated with higher risk of HCC. We validated these findings in an independent cohort of patients with HCV-related cirrhosis (n = 216), a subset of whom (n = 21) achieved viral clearance. Cumulative incidence of HCC development was significantly higher in patients with high SPHK1 expression. These analyses identified epigenetic and gene expression alterations associated with HCC risk. These alterations might be targeted to prevent liver cancer in patients treated for HCV infection.

Dr. Derek A Mann
Faculty of Medical Sciences,
Newcastle University, UK

Epigenetic Control of Hepatic Stellate Cell Activation and Liver Fibrosis

Hepatic stellate cell (HSC) activation is critical for the development and progression of liver fibrosis. The phenotype of the HSC changes dramatically during activation and requires changes in the expression of hundreds of genes that encode transcripts for protein as well as non-protein products. Underpinning these alterations in gene expression are epigenetic modifications at the level of DNA methylation and histone modifications. In this presentation the basic epigenetic mechanisms by which HSC alter their phenotype in response to liver injury will be reviewed as will evidence that fibrosis in humans is at least in-part determined by epigenetic factors. The role of key epigenetic regulators such as DNA methyltransferases, histone modifying enzymes (e.g. EZH2) and chromatin associating factors such as MeCP2 will be described. The potential for fibrosis susceptibility to be pre-determined by ancestral experiences that are transmitted epigenetically will be discussed as will the opportunities for blood-based epigenetic surrogate markers of fibrosis and therapeutic target of epigenetic regulators for the treatment of fibrosis. Finally, the potential for pre-clinical modelling of epigenetic therapies for fibrosis in human precision cut liver slices will be outlined with examples.

Dr. Yutaka Inagaki
Department of Innovative Medical Sciences,
Tokai University School of Medicine, Japan

Treatment Strategy for Liver Fibrosis Based on Deactivation of Fibrogenic Hepatic Stellate Cells

Hepatic stellate cells (HSCs), a key player in the progression of liver fibrosis, are activated by various inflammatory stimuli and converted to myofibroblast-like cells with excessive collagen production. Despite many attempts to suppress activation of HSCs or inhibit collagen production in activated HSCs, their clinical applications have not been established yet. Recently, the deactivation of HSCs has been reported as a mechanism underlying the reversibility of experimental murine liver fibrosis.

We have identified Tcf21 as a novel deactivation factor of fibrogenic HSCs by screening transcription factors whose expression is up-regulated in parallel to the differentiation of murine fetal HSCs and down-regulated during hepatic fibrogenesis in both mice and humans. This reduced Tcf21 expression observed in the fibrogenic process was recovered during the spontaneous regression of murine liver fibrosis. Tcf21 was also examined for its effects by adeno-associated viruses serotype 6-mediated *Tcf21* gene transfer into cultured activated HSCs and mice with carbon tetrachloride- or methionine-choline deficient diet-induced liver fibrosis. Overexpression of Tcf21 in activated HSCs not only suppressed fibrogenic gene expression, but also restored cells, at least in part, to a quiescent phenotype, both *in vitro* and *in vivo*. These phenotypic changes of HSCs were accompanied by the regression of steatohepatitis and fibrosis, and improved hepatic architecture and function.

Using the *in silico* homology modeling, we clarified the ternary structure of the complex between Tcf21, its counterpart protein, and the target DNA. We also revealed the functionally important region in Tcf21 that mediates its effect on HSC deactivation. Work is in progress in our laboratory to identify small molecules that mimic the relevant region of Tcf21. These studies provide insight into a treatment strategy for the otherwise intractable liver fibrosis.

Session 4: Molecular Basis of Liver Fibrosis and Cirrhosis Therapy

Dr. Jordi Gracia-Sancho
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Molecular Basis of Portal Hypertension Therapy

Portal hypertension represents one of the major clinical consequences of chronic liver disease, having a deep impact in patients' prognosis and survival. Its pathophysiology defines a pathological increase in the intrahepatic vascular resistance as the primary factor in its development, being subsequently aggravated by a paradoxical increase in portal blood inflow. Although extensive pre-clinical and clinical research in the field has been developed in the last decades, no effective treatment promoting its improvement has been defined. It is well known that cirrhosis due to hepatitis C or hepatitis B virus infection is declining, nevertheless excessive alcohol consumption and the increasing incidence of non-alcoholic steatohepatitis are two major etiological factors leading to cirrhosis and portal hypertension. Despite the wide use of liver transplantation, the most successful strategy to treat cirrhosis is removing the etiologic agent. Moderate improvement of portal hypertension may be spontaneously reached after cessation of etiologic factor, and include the following underlying mechanisms: a) cessation of chronic damage; b) shifting the sinusoidal milieu from inflammatory to restorative; c) promoting the deactivation and/or elimination of hepatic stellate cells and myofibroblasts, and d) degradation of extracellular matrix and repopulation by functional hepatocytes. However, even after successful suppression of the etiological factor, some advanced patients keep progressing and die because of the liver disease if transplantation is not possible, thus, pharmacologically driven regression of portal hypertension is an urgent need. Major underlying mechanisms contributing to increase the intrahepatic vascular resistance, and therefore representing therapeutic targets for portal hypertension, are diverse and include the arachidonic acid – cyclooxygenase - thromboxane A2, the endothelin-1, the kruppel-like factor 2 – nitric oxide synthase - nitric oxide-cGMP, and the peroxisome proliferator-activated receptors pathways, among others. More importantly, novel therapeutics targeting the de-regulated phenotype of sinusoidal cells, and not just a specific pathway, may indeed represent a distinctive strategy to ultimately promote portal hypertension improvement.

This lecture will summarize the current knowledge in portal hypertension treatment, focusing on those therapeutic strategies driven by the disease pathophysiology and the associated underlying cellular mechanisms.

Dr. Giuseppe Mazza
Principal Investigator at UCL
Institute for Liver and Digestive Health
Co-Founder and CEO at Engitix Therapeutics

The Role of Human Extracellular Matrix in Driving Tissue Fibrosis and Cirrhosis

Progressive fibrosis remains one of the most vexing problems in modern medicine. Its seeming intractability does not result from a lack of scientific attention. Instead, it might be possible that the formulation guiding even the best studies may be incomplete. Most experimental work has been guided by inferences from the advanced state of knowledge about fibrosis that follows a discrete injury, not by studies focused on self-sustaining progressive fibrosis as a discrete entity. In parallel with our increased understanding of fibrosis initiation, we have learned that fibrosis progression involves both cell-intrinsic/autonomous and extracellular matrix (ECM)-driven mechanisms. ECM being the non-cellular component present within all tissues and organs provides not only essential physical scaffolding for the cellular constituents, but also initiates crucial biochemical and biomechanical signals. These signals are required for tissue morphogenesis, differentiation, and homeostasis. Indeed, the ECM is a highly dynamic structure that is constantly remodeled by cells that are constantly rebuilding and modifying the ECM through synthesis, degradation, and reassembly of its 3D structure. These processes are complex and need to be tightly regulated to maintain tissue homeostasis, especially in response to injury associated with chronic diseases, as a dysregulated ECM remodeling represents a key feature leading to disease progression.

The idea of mutual interaction between ECM and cells has been always identified as key factor in orchestrating physiological and pathological conditions. However, it remains largely undefined whether extracellular support is to be seen as a passive structure or as a bioactive micro-environment affecting the cross-talk. Early two-dimensional (2D) cell culture technologies could not reproduce this complex 3D microenvironment, thus showing certain limitations when applied to disease modelling and drug discovery. Consequently, technologies developed in recent years attempt to reproduce the tissue microenvironment by using 3D ECM biomaterials developed by the decellularization of healthy and diseased human liver tissues. Tissue-specific and disease-specific human ECM biomaterials may therefore facilitate the understanding of both composition and biology of human ECM with the possibility of identifying novel targets regulating tissue fibrosis and cirrhosis as well as to improve the drug discovery process in liver diseases.

Dr. Gyongi Szabo
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Beth Israel Deaconess Hospital

Inflammasome Activation and microRNAs are Molecular Drivers and Therapeutic Targets in Steatohepatitis

Both alcohol-associated liver disease (ALD) and non-alcoholic fatty liver disease (NAFLD) can progress to steatohepatitis that marks chronic progression to fibrosis and advanced liver disease. Thus, understanding triggers and molecular mechanisms of inflammation is critical in steatohepatitis. Of the numerous mechanisms that were shown to promote immune cell activation and recruitment to the liver in alcoholic hepatitis and NASH, we found that the intracellular inflammasome complex appears to be critical in progression of liver and systemic inflammation. We showed that NLRP3 inflammasome activation is increased in AH and it contributes to interleukin (IL)-1β release. Mechanisms for NLRP3 activation in AH is linked to ATP and uric acid plus LPS primarily in immune cells. We also showed that NLRP3 inflammasome is activated in NASH both in hepatocytes and immune cells through fatty acids and LPS. In AH but not in NASH, NLRP3 inflammasome activation induced the adaptor protein apoptosis-associated speck-like protein containing CARD (ASC) oligomerization into specks for interleukin (IL)-1β release. Extracellular ASC specks derived from alcohol- or LPS-stimulated macrophages or hepatocytes can trigger IL-1β release in alcohol-naïve monocytes, to sustain inflammation. Importantly, MCC950, an NLRP3 inhibitor, prevented liver damage in a mouse model of ALD suggesting that NLRP3 is a potential therapeutic target. Inflammation is also regulated by various microRNAs including miR-155. We found that mice deficient in miR-155 were partially protected from steatosis, inflammation and fibrosis in a mouse model of ALD. In macrophages, alcohol upregulated miR-155 via NF-kB transcriptional activation and increased TNF production. Furthermore, miR-155KO mice showed attenuation of liver damage both in high cholesterol-high fat and high sucrose diet-induced and in MCD diet-induced NASH in mice. Our results suggest that miR-155 inhibition could be a potential target in steatohepatitis.

Dr. Vincent Wong
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Hong Kong, China

Treatment Targets for Nonalcoholic Steatohepatitis

In the past two decades, nonalcoholic steatohepatitis (NASH) has emerged as an important cause of cirrhosis and hepatocellular carcinoma in Western countries, and modelling studies suggest that Asia will follow suit. Compared with other chronic liver diseases such as chronic viral hepatitis, NASH has a more complex pathophysiology through the interaction of genetic, lifestyle and metabolic factors.

Currently, a number of agents have entered phase 2 to 3 clinical development. Many more are being evaluated in preclinical and early phase studies. In general, investigational drugs can be divided into metabolic (upstream) and anti-inflammatory and anti-fibrotic (downstream) drugs. So far, none of the downstream drugs has succeeded in late phase studies. Some of the inflammatory pathways, such as apoptosis signal-regulating kinase 1 and chemokine receptors, have shown great promise in preclinical studies but failed to reverse NASH or improve liver fibrosis in subsequent phase 3 studies despite evidence of target engagement. This is not to say that anti-inflammatory and anti-fibrotic drugs will never have a role in NASH treatment. Nonetheless, it is likely that they have to be at least combined with metabolic treatments to exert their effect.

In contrast, metabolic drugs appear to be more successful. Obeticholic acid is a potent farnesoid X receptor agonist that inhibits bile acid production and modulates glucose and lipid metabolism. In the phase 3 REGENERATE study, it significantly increased the rate of fibrosis improvement without worsening of NASH (23% in the 25 mg group versus 12% in the placebo group) at 18 months. However, pruritus and atherogenic dyslipidaemia are adverse events that will limit its wider application. Inhibitors of acetyl-CoA carboxylase, diacylglycerol O-acyltransferase 2 and fatty acid synthase effectively suppress *de novo* lipogenesis and reduce intrahepatic fat. Whether this will translate into NASH resolution and fibrosis improvement requires further studies. Thyroid hormone receptor-beta agonists alter hepatic metabolism without systemic haemodynamic effects and may be beneficial for NASH. Even more upstream would be drugs targeting insulin resistance such as glucagon-like peptide-1 receptor agonists and various peroxisome proliferator-activated receptor agonists.

Finally, genomic studies in the past 15 years have identified a number of genetic determinants of NASH. How this would influence therapeutic response remains to be seen. To the extreme, genetic based therapies such as targeting the PNPLA3 mutant protein in patients carrying the gene polymorphism are under evaluation. Ultimately, personalised and combination therapy will likely be the key to success.

Dr. Ekihiro Seki
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New Therapeutic Strategy for Alcoholic Liver Disease: Role of TLR7 and IL-22

Alcohol-associated liver disease (ALD) is a significant health concern. However, effective therapies for ALD are limited. Alcoholic hepatitis (AH) is a severe form of ALD that is characterized by chronic liver injury and inflammation. The current treatment strategy for AH involves the use of corticosteroids, despite a lack of evidence supporting their efficacy. Therefore, the discovery of new therapeutic agents for ALD, including AH is greatly warranted. Toll-like receptor 7 (TLR7) is a pattern recognition receptor for single-stranded RNA, and its activation prevents liver fibrosis. Here, we demonstrate that activation of TLR7 signaling protects against liver injury via production of IL-22 in an AH mouse model. First, we determined the role of endogenous TLR7 signaling in ALD development by examining liver and intestinal damage in *Tlr7*$^{-/-}$ mice. In an AH mouse model, hepatic steatosis, injury, and inflammation were induced in wild-type mice. In contrast, *Tlr7* deficiency exacerbated hepatic steatosis, injury, and inflammation induced by ethanol-containing diet feeding. These results prompted us to hypothesize that exogenous TLR7 activation may be an effective strategy for ALD treatment. To test this hypothesis, we used 1Z1, a synthetic TLR7 ligand that does not elicit systemic side effects, in the AH mouse model. Oral administration of 1Z1 was well tolerated and has a significant beneficial effect on the intestine. Oral 1Z1 ingestion preserved intestinal barrier functions and inhibited bacterial translocation, which is associated with the suppression of ethanol-induced liver injury, steatosis, and inflammation. Additionally, 1Z1 treatment increased the expression of anti-microbial peptides, Reg3b and Reg3g, in the intestinal epithelium, along with the modulation of the composition of gut microbiome. We found that 1Z1 treatment decreased *Bacteroides* and increased *Lactobacillus* in the gut. Notably, we discovered that 1Z1 treatment induced the upregulation of intestinal IL-22 expression. We validated that IL-22 deficiency abolished the protective effects of 1Z1 in ethanol-induced liver and intestinal damage. This suggests that the mechanism of action of 1Z1 largely relies on the induction of IL-22 expression in the intestine to suppress the development of AH in mice. Taken together, our results indicate that TLR7 signaling exerts protective effects in the AH mouse model and that a novel TLR7 ligand, 1Z1, has therapeutic potential for the treatment of AH.

Dr. Jacob George

Storr Liver Centre, Department of Gastroenterology and Hepatology, Westmead Hospital, The Westmead Institute of Medical Research and the University of Sydney, Australia

Molecular Basis of Alcoholic and Metabolic Steatohepatitis and Therapy

Metabolic (dysfunction) associated fatty liver disease (MAFLD) affects half the global population and together with alcohol related liver disease (ALD) represents the commonest reasons for end stage liver disease and liver cancer. While the incidence and prevalence of MAFLD is rising across the world, that of ALD overall remains stable. Moderate alcohol consumption occurs in about two thirds of cases with MAFLD. While MAFLD is slowly progressive, its inflammatory form (metabolic steatohepatitis) is the usual harbinger of progressive liver disease and of the associated extrahepatic complications.

The pathogenesis of MAFLD can be considered at 4 levels; effective management of the disease will only occur in the context of effectively targeting each level. At the societal level, MAFLD is a consequence of the many complex factors governing food choices and patterns, as also physical activity and sedentary patterns. At the individual level, MAFLD is the hepatic outcome of cross talk between multiple organ systems including the neuroendocrine axis, adipose, muscle and pancreas, as also the liver and gut interacting with a person's genetic and epigenetic signature. At the hepatic level, the interaction of these factors results in hepatic and peripheral insulin resistance, the accumulation of excess liver lipid and the activation of inflammatory signaling cascades. At the level of the cell, this results in cellular stress, cell death and the activation of the fibrosis cascade that ultimately results in cirrhosis and adverse liver outcomes, including cancer. The pathogenesis of ALD is similarly complex, multifactorial and multi-level.

Lifestyle intervention with calorie restriction for weight loss, improved dietary composition and physical activity are the cornerstones for therapy in MAFLD. While no drug therapies are currently approved for the treatment of MAFLD, this is a hot area for clinical trials. Both systemic "metabolic" drugs and specific liver targeted agents are in various phases of clinical development, with the former being the most promising. It is likely that in the next 2-3 years, some of these agents will be available for clinical use, particularly for patients with the most aggressive forms of the disease. However, for the vast majority of patients with advanced disease in most regions of the world, these therapies will remain beyond the reach of health systems. The therapy for ALD remains an area of unmet need and requires judicious use of psychosocial and pharmacological approaches.

Dr. Atsushi Miyajima
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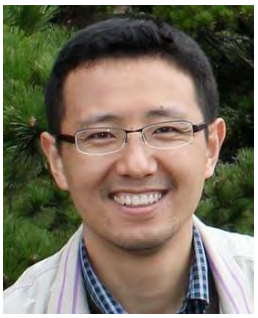
University of Tokyo, Japan

Generation of Quiescent Hepatic Stellate Cells from Human iPSCs and Development of Anti-fibrotic Drugs

Hepatic stellate cells (HSCs) play a central role for the progression of liver fibrosis by producing extracellular matrices. Although anti-fibrotic drugs are urgently needed, the development of drugs to suppress liver fibrosis has been hampered by the lack of an appropriate in vitro model that faithfully recapitulates the HSC activation. Because freshly isolated HSCs are easily activated in culture, we have developed a culture system to generate quiescent HSCs (qHSCs) from human iPSCs. Those qHSCs can be converted into activated HSCs (aHSCs) in culture. Furthermore, to monitor the activation process of HSC, we have developed a reporter iPS cell line by inserting the fluorescent protein (RFP) gene to the downstream of an activation marker gene, ACTA2. The qHSCs derived from the reporter hiPS cells expressed RFP along with the activation of HSC in culture. Using this reporter system, we screened a repurposing chemical library and identified therapeutic candidates that suppressed liver fibrosis in a mouse model of liver fibrosis. By modifying one of the lead compounds, we have developed novel anti-fibrotic drugs.

Session 6: Use of Pluripotent Stem Cells and Reprogrammed Cells for Therapy



Dr. Lijian Hui
State Key Laboratory of Cell Biology, Shanghai Institute of Biochemistry and Cell Biology, Center for Excellence in Molecular Cell Science, Chinese Academy of Sciences, Shanghai, China

Cell Identity Conversion and Liver Regeneration

To understand the mechanistic regulation underlying tumorigenesis of normal cells is a long-term interest of our lab. Recently, taken hepatocytes as the experimental system, our lab has initiated studies on cell lineage conversion for regeneration, namely transdifferentiation and dedifferentiation in vitro and in vivo. Striving to understand these two seemingly different phenomena, we find ourselves in querying the essential scientific question: How is cell identity maintained through preventing the conversion of terminally differentiated cells to other cell types, including cell lineage conversion and transformation to tumor cells; or in a reversed term, how is the cell plasticity regulated? In this talk, I will present our latest findings to demonstrate a role of hepatocyte reprogramming in liver regeneration and tumorigenesis.

Dr. Hideki Taniguchi
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Graduate School of Medicine, Yokohama City University,
Department of Regenerative Medicine, Japan

Generation of Human Liver Using iPS Cells for Regenerative Therapies

A critical shortage of donor liver for treating end-stage liver failure highlights the urgent need for generating liver from human induced pluripotent stem cells (iPSCs). Despite many reports describing functional cell differentiation, no studies have succeeded in generating a three-dimensional vascularized organ such as liver. Here we show the generation of vascularized and functional human liver from human iPSCs by transplantation of liver buds created in vitro (iPSC-LBs). Specified hepatic cells (immature endodermal cells destined to track the hepatic cell fate) self-organized into three-dimensional iPSC-LBs by recapitulating organogenetic interactions between endothelial and mesenchymal cells. Immunostaining and gene-expression analyses revealed a resemblance between in vitro grown iPSC-LBs and in vivo liver buds. Human vasculatures in iPSC-LB transplants became functional by connecting to the host vessels within 48 hours. The formation of functional vasculatures stimulated the maturation of iPSC-LBs into tissue resembling the adult liver. Highly metabolic iPSC-derived tissue performed liver-specific functions such as protein production and human-specific drug metabolism without recipient liver replacement. Furthermore, mesenteric transplantation of iPSC-LBs rescued the drug-induced lethal liver failure model. To our knowledge, this is the first report demonstrating the generation of a functional human liver from pluripotent stem cells. Although efforts must ensue to translate these techniques to treatments for patients, this proof-of-concept demonstration of organ-bud transplantation provides a promising new approach to study regenerative medicine.

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IL-17 Signaling in Steatotic Hepatocytes Promotes Alcoholic Liver Disease-induced Hepatocellular Carcinoma

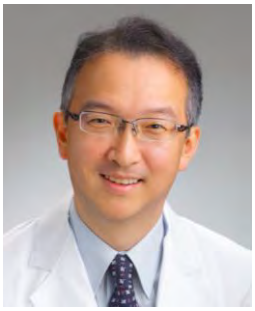
Chronic alcohol (EtOH) consumption is a leading risk factor for development of hepatocellular carcinoma (HCC), which is associated with marked increase of hepatic expression of pro-inflammatory IL-17A and its receptor IL-17RA. We and the others have shown that IL-17 signaling regulates TGF-β1 production by Kupffer cells/macrophages and directly stimulates activation and collagen production in HSCs. Here we have studied the role of IL-17 signaling in metabolically injured hepatocytes and demonstrated that IL-17 drives progression of alcohol-induced steatohepatitis to fibrosis and hepatocellular carcinoma (HCC). IL-17 signaling regulates de novo lipogenesis in steatotic hepatocytes via regulation of TNF-Caspase2-SREBP1/2-DHCR7 pathways, which activate chemokine secretion and cholesterol production in steatotic hepatocytes. Steatotic IL-17RA-deficient hepatocytes downregulated expression of Cxcl1 and other chemokines, exhibited a striking defect in TNF-TNFR1-dependent Caspase-2-SREBP-1/2-DHCR7-mediated cholesterol synthesis, and upregulated production of anti-oxidant Vitamin D_3. Pharmacological blocking of IL-17A/Th-17 cells using anti-IL-12/IL-23 Ab suppressed progression of HCC (by 70%) in alcohol-fed mice, indicating that targeting IL-17 signaling might provide novel strategies for treatment of alcohol-induced HCC.

Session 7: Gut-Liver-Brain Axis in Hepatic Pathophysiology and Clinical Medicine

Dr. Hiroshi Ohno
Laboratory for Intestinal Ecosystem,
RIKEN Center for Integrative Medical Sciences, Japan

Gut Microbiota and Autoimmune Diseases – Type 1 Diabetes and Multiple Sclerosis –

Resides in our gut is a numerous commensal bacteria, gut microbiota, exceeding the number of human somatic cells making our bodies. Accumulating evidence in the past decade has suggested that gut microbiota deeply impacts the pathogenesis of various diseases including autoimmune diseases; nevertheless, the underlying mechanisms are still largely unclear. Regarding type 1 diabetes mellitus (T1D), by employing the streptozotocin-induced T1D mouse model and human patients, we have found that Ruminococcus species in the gut could increase systemic Foxp3+CD8+ Tregs to ameliorate the disease, both in mice and humans. We have also studied multiple sclerosis (MS), another autoimmune inflammatory disorder influencing the brain and spinal cord. By using experimental autoimmune encephalomyelitis (EAE), a mouse model of MS, we have found that two small intestinal commensal bacteria, having distinct immunomodulatory ability, act together to exacerbate the pathogenesis of EAE. I will also discuss the impact of oral bacteria on the gut microbiota and liver functions.



Dr. Takanori Kanai
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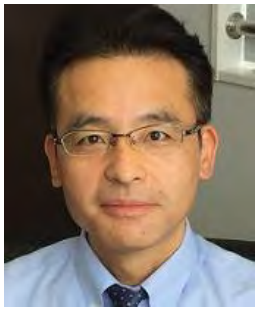
The Liver-brain-gut Neural Reflex Maintains the Niche of Gut Regulatory T cells

Peripheral Foxp3+ regulatory T cells (pTregs) are most abundant in the mucosal tissues, especially the colon lamina propria, and maintain immune homeostasis in the gut. The generation of pTreg is promoted by combinations of local factors, such as cytokines, microbial and dietary signals. The gut-brain axis, a reciprocal interaction between central nervous system (CNS) and peripheral intestinal functions, is conceptually feasible from recent clinical and experimental evidence that has shown mutual interactions between CNS and gut microbiota closely associated with the bidirectional effects of IBDs and CNS disorders. Despite the recent advances in understanding neuro-immune interactions, it remains unknown how the gut and brain communicate to control the induction and maintenance of pTregs. Here, we found a novel liver-brain-gut neural circuit ensures proper differentiation and maintenance of gut pTregs. The hepatic vagal afferent nerve is responsible for indirectly sensing the gut microenvironment and relays the sense inputs to the nucleus tractus solitarius of the brainstem, and ultimately to the efferent vagal nerves and enteric neurons. Surgical and chemical perturbation of cholinergic signals at the hepatic afferent level significantly impaired colonic pTreg, which is attributed to impairment of aldehyde dehydrogenase (ALDH) expression and retinoic acid synthesis from intestinal antigen-presenting cells (APCs). Muscarinic Ach receptor (mAChR) activation directly induced ALDH gene expression both in human and mouse colonic APCs, whereas genetic ablation of mAChRs or chemical perturbation of hepatic vagal afferent nerves abolished the excitement of APCs *in vitro*. Using colitis models, disruption of vagus afferents from the liver to the brainstem diminished colonic pTreg pool, resulting in increased susceptibility to colitis. These results demonstrate that the novel liver-brain-gut reflex arc regulates the number of pTreg and maintains the gut homeostasis. Our work highlights the essential roles of liver-brain-gut neural arc that specificates the immunoregulatory niche and fine-tunes immune responses in the intestine. Intervening of liver-brain-gut neural arc could provide broad applications to promote the treatment of IBD, infectious diseases, and cancer in the gut.

Session 7: Gut-Liver-Brain Axis in Hepatic Pathophysiology and Clinical Medicine



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High-throughput Screening of New Compounds that Remove Senescent Cell toward Prevention of HCC Development

Although cellular senescence has a well-established role in tumor suppression, emerging evidence is revealing that the accumulation of senescent cells *in vivo* exerts deleterious side effects due to inflammatory and tumor-promoting factor secretion. The clearance of senescent cells *in vivo* has alleviated such unwanted side effects, in various aging and/or disease mouse models. Thus, the development of new drugs that specifically eliminate senescent cells, termed “senolytic drug”, is anticipated. Here, by combining an unbiased high-throughput screening of chemical compound libraries of 47,000 small molecules and bio-functional analysis, we have identified a chemical compound as a promising new class of senolytic drug. This compound robustly provokes autophagy-associated apoptosis in senescent cells through two independent but integrated pathways: (1) exacerbation of DNA double-strand breaks (DSBs) by blocking non-homologous end joining (NHEJ) repair, and (2) up-regulation of autophagic gene expression. Notably, the treatment with this compound prevented the accumulation of senescent hepatic stellate cells in obese mouse livers, accompanied by the attenuation of obesity-associated hepatocellular carcinoma (HCC) development. Furthermore, the elimination of chemotherapy-induced senescent cells by the treatment with this compound substantially increased the efficacy of chemotherapy against xenograft tumor growth in immunocompromised mice. These results reveal cellular vulnerability of senescent cells, and provide valuable novel insights into the resistance of senescent cells to death and open up new possibilities for its control.

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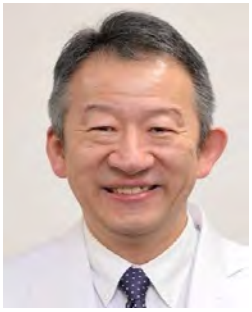
Whole Genomics and Precision Oncology for Hepato-Biliary Cancer

Cancer is essentially a "disease of the genome" which develops and evolves with the accumulation of a variety of mutations, based on the background of its genomic instability and germline variants, and some driver mutations were successfully targeted for treatment. Cancer also has been proved to have a feature of "immune reaction" and have been affected by immune editing in carcinogenic steps. To explore comprehensive genomic features of cancer, we have been addressing cancer whole genome sequencing (WGS) analysis for liver cancers (ICGC, Nat Gent 2016) and biliary tract cancer (BTC) including ICC and cHCC/CC.

Several mutated driver genes and pathways in HCC were identified, including *TERT*, *TP53*, Wnt/*CTNNB1* pathway, and chromatin regulators. WGS analysis detected virus integrations of HBV and AAV, which preferentially occurred in *TERT* promoter and open chromatin regions, leading to aberrant gene expression and genomic instability. Their whole genome mutation patterns estimated cell-of-origin of liver cancers, indicating some ICCs can be originated from hepatocyte (J Hepatol 2018). *TP53* mutations were associated with cellular plasticity of cHCC/CC (Cancer Cell 2019). RNA analysis characterized their immunological features, and classified liver cancers into 4 sub-immune groups. Immune suppressive signatures within liver cancer tissues were significantly associated with mutations of *CTNNB1* and *ARID2* (EBioMed 2020).

For BTC, we analyzed genome and RNA expression of 200~ BTCs and, by using Asian knowledgebase, annotated several actionable mutations such as *FGFR2* or immune signature related with ICB sensitivity. Totally, 50% of BTC cases were evaluated to have any actionability of molecular therapy or ICB. Furthermore, we searched for germline variants associated with hereditary cancer in 1300 Japanese BTC cases and *BRCA1/2* pathogenic variants were significantly associated with BTC development. WGS analysis also detected HRD (homologous recombination deficiency) signatures in somatic genome of BTCs, indicating cancer hereditability and actionability of PARP inhibitor for BTC.

These genomic approaches combined with immunology and knowledgebase can clarify the underlying carcinogenesis and achieve molecular sub-classification of cancer, which facilitates discovery of genomic biomarkers and personalized treatment for hepatobiliary cancer.



Dr. Naoya Kato
Department of Gastroenterology,
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Biomarkers in the Treatment of Liver Cancer

In the treatment of advanced hepatocellular carcinoma (HCC), combined immunotherapy with atezolizumab plus bevacizumab recently became the first-line treatment option. Four tyrosine kinase inhibitors (TKI), sorafenib, lenvatinib, regorafenib, and cabozantinib, and a VEGFR2 antibody, ramucirumab, can be used for second and/or later lines of treatment. However, all the evidence of second-line treatment to date has been established with sorafenib as the first-line treatment, and thus, the evidence for second-line treatment has been reset after the approval of atezolizumab plus bevacizumab as the first-line treatment. Under such circumstances, how to use these six drugs will greatly affect the prognosis of patients with advanced HCC. It is not realistic to conduct prospective randomized trials for all drugs, and real-world data with a high level of evidence requires a large number of cases, which is also not easy. Therefore, rationale is important for treatment selection, and biomarkers are necessary for treatment selection based on rationale. To date, candidate biomarkers for treatment selection include liver function, etiology, AFP, VEGF signaling activation, FGF signaling activation, and WNT/β-catenin pathway activation.

FGFR2 fusion or other rearrangement is shown to be a driver in 5-10% of intrahepatic cholangiocarcinoma (ICC). Pemigatinib, a small molecule inhibitor of FGFR1/2/3, was recently approved for the treatment of adults with previously treated, unresectable, locally advanced or metastatic cholangiocarcinoma with FGFR2 fusion or other rearrangement.

In this presentation, I would like to review biomarkers that are useful in selecting the treatment for liver cancer.

Dr. Masatoshi Kudo
Professor and Chairman,
Department of Gastroenterology and Hepatology,
Kindai University Faculty of Medicine, Japan

Nobel Treatment Strategy for Intermediate-stage Hepatocellular Carcinoma

Transarterial chemoembolization (TACE) is the only guideline-recommended global standard of care for intermediate-stage hepatocellular carcinoma (HCC). However, TACE is not beneficial for three subgroups of patients with the following characteristics: (1) conditions that easily become refractory to TACE, (2) conditions in which TACE causes deterioration of hepatic functional reserve to Child-Pugh class B, and (3) conditions that are unlikely to benefit from TACE. In the first subgroup, which includes HCCs beyond the up-to-seven criteria, the response to TACE is typically poor, and hepatic functional reserve is further impaired. This may hinder the switch to molecular targeted therapy, resulting in a worse prognosis and shorter overall survival. Recent evidence indicates that lenvatinib-TACE (LEN-TACE) sequential therapy markedly improves overall survival in this TACE-unsuitable subpopulation. Furthermore, atezolizumab + bevacizumab combination therapy followed by curative therapy such as resection, ablation and TACE provide cancer free with drug free even in poor prognostic tumor type of HCC. Thus, LEN-TACE sequential therapy and AMC conversion therapy will become the standard of care for patients who do not benefit from TACE.

Dr. Massimo Colombo

Liver center IRCCS San Raffaele Hospital Milan,

Italy

HCC Surveillance in Patients with Pharmacologically Treated Viral Hepatitis

The excess risk of hepatocellular carcinoma (HCC) that remains in both HBV and HCV treated patients, makes biannual surveillance with abdominal ultrasound (US) recommended by all societies in order to improve early detection of HCC and reduce cancer related mortality in this setting. Recommendations show nuances in add on serum alfa-foetoprotein, second level imaging with abbreviated MRI, and screening beyond the classical target of cirrhosis. In HBV suppressed patients, the annual HCC risk is 0.5%-1.4% during chronic hepatitis and up to 5.4% in cirrhosis, both meeting the cut off for cost-effective screening, ie 0.2% in chronic hepatitis and 1.5% in cirrhosis.(Sarrazin 1996)Risk stratification scores help identify a 3 to 5 yr window of no HCC risk in Caucasians treated for at least 5 yrs [SAGE-B (age, gender + liver stiffness) score =/< 5 points, Papatheodoridis 2020) and in similar Asians with =/< 8 points of modified PAGE-B(age, gender, platelets, albumin, Yip 2020), to prioritize screening, for instance during the Covid crisis. However, the six monthly interval of screening should not be relaxed in patients at intermediate risk due to the faster doubling volume time of HBV HCC compared to other etiologies(Nathani 2020)whereas in high risk patients intensified screening is challenged by the low PPV of the scores.(Voulgaris 2020).Poorly measured is confounding by metabolic and life style factors. In HCV patients, SVR reduces HCC risk by 80% (Beng 2017), the annual risk spanning from 0.24 in non cirrhotics to 1.22 in cirrhotics classified by non invasive tests(NIT). (Ioannou 2019)Onset of de-novo HCC is predicted by pre-treatment unmeasured non malignant nodules at CT or MR(Sangiovanni 2020),male sex, diabetes and disease severity.(Degasperi 2020; Auderau 2020)Recurrence and mortality after SVR are better than in viremic patients. (Toyoda 2018 and 2021) Risk of recurrence after curative therapy is a matter of controversy: it was low in a meta-analysis in the US(Singal 2019) but high in a single patient data analysis in EU and Asia(Sabena 2021), possibly in consequence of substantial differences in demography and study design. By modelling, surveillance is cost effective in patients with cirrhosis, bridging fibrosis (MetavirF3) or FIB-4 >3.24 (Zangneh 2019) as it is recommended by EASL.AASLD recommends screening in cirrhosis (histology or NIT) only, whereas APASL recommends surveillance in older patients, those with cirrhosis (even if regressed) or with diabetes.

APASL Single Topic Conference 2021 Osaka

"Molecular and Cell Biology of the Liver: Recent Evolution to Clinical Application"

Abstracts

Sponsored Seminars

Luncheon Seminar 1 by Eisai Co. Ltd. / MSD K.K.

Dr. Sadahisa Ogasawara

Department of Gastroenterology, Graduate School of Medicine,

Chiba University, Japan

Consideration of the Decision Process of Treatment in Patients with Hepatocellular Carcinoma

Hepatocellular carcinoma (HCC) is a malignant tumor that develops in the liver with decreased liver function and high potential of carcinogenesis. These two features are not found in other malignancies and are the most significant factors that complicate the development of treatment strategies for HCC. As with other cancers, complete removal of tumor from the liver can significantly improve patient prognosis in HCC. However, we must pay close attention to the liver function that we sacrifice in curative treatment for the liver. Additionally, liver function at the time of recurrence should be taken into account in treatment selections, as HCC shows high recurrence rate due to synergy between the occurrence of new lesions associated with carcinogenesis with the background of liver and metastatic recurrence from a primary lesion. Recent advances in systemic therapies with high potential for tumor shrinkage will increase the chance of complete removal (so called “cancer free”) of tumor in patients with HCC. On the other hand, if complete removal of tumor is not expected, the primary goal of treatment in HCC is to control tumor and prevent progression. In this phase, maintaining liver function increases the possibility of treatment sequencing and contribute significantly to prolong survival outcome in patients with HCC. Lately, we demonstrated that the emergence of multiple molecular target agents evolved the treatment by sequencing agents from one to the next and prolonged the prognosis of advanced HCC patients significantly. In considering treatment strategies for HCC, it is essential to determine whether the patient in the phase of aiming for complete removal (“cancer free”) at the expense of liver function, or the phase of controlling tumor while maintaining liver function. As these two phases are often interchanged during the clinical course of recurrence and progression, the specialist of HCC must be able to recognize which phase the patient belongs to. The treatment strategy for HCC is changing drastically as systemic therapies make significant progress. The positioning of conventional treatment, such as hepatic resection, local ablation, and transcatheter arterial chemo embolization (TACE) will also shift accordingly.

Luncheon Seminar 1 by Eisai Co. Ltd. / MSD K.K.

Dr. Kaoru Tsuchiya
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Musashino Red Cross Hospital, Japan

Clinical Outcome of Lenvatinib Therapy in Japanese Patients with Unresectable HCC

Lenvatinib was approved in Mar 2018 and has been widely used in Japanese patients with unresectable HCC. We performed a retrospective nationwide multicenter study about the clinical outcome of lenvatinib in real-world practice and reported the results in Cancers, 2021. A total of 343 patients were enrolled in this study, and the median observation period was10.5 months. Among the patients, 246 patients (72%) received lenvatinib as a 1st-line molecular targeted therapy (MTT). Median overall survival (OS) and progression-free survival (PFS) in Child-Pugh A (n=276) were 21.0 and 8.8 months. The median OS and PFS in MTT naïve patients with BCLC stage B and modified ALBI 1 or 2a (n=68) were 25.3 and 12.3 months. The median OS and PFS in MTT naïve patients with BCLC stage C and modified ALBI 1 or 2a (n=64) were 20.9 and 12.7 months. The objective response rate (ORR) and disease control rate (DCR) according to modified RECIST criteria were 42.1% and 82.1% in 280 patients. The significant factors associated with OS in all patients were AFP ≥ 400ng/mL, modified ALBI grade 2b or 3, major vascular invasion, PS >0, and MTT experience. The median OS and PFS in the patients (n=174) who completely met the inclusion criteria of the REFLECT study were 25.3 and 10.0 months, while the median OS and PFS in the patients (n=71) who met the inclusion criteria except for the experience of previous systemic therapy for HCC were 15.2 and 7.1 months. Major adverse events during lenvatinib therapy were hypertension, decreased appetite, and fatigue. TACE during lenvatinib therapy was performed in 27 patients to achieve complete response (CR) or maintain DCR. Among them, median OS and PFS were 25.4 and 15.0 months. Lenvatinib therapy for Japanese patients with unresectable HCC in real-world practice showed similar clinical outcomes compared to the REFLECT trial. The HCC patients with BCLC stage B revealed the potential to achieve long survival by lenvatinib-TACE sequential therapy.

Evening Seminar 1 by Chugai Pharmaceutical Co., Ltd.

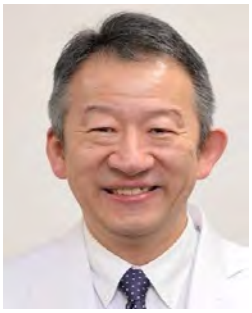
Dr. Masatoshi Kudo
Professor and Chairman,
Department of Gastroenterology and Hepatology,
Kindai University Faculty of Medicine, Japan

A New Era of Cancer Immunotherapy in Hepatocellular Carcinoma

The systemic therapy for hepatocellular carcinoma (HCC) has changed since sorafenib, first molecular-targeted drug, showed to prolong the survival of patients with HCC in the SHARP and Asia-Pacific trials in 2007. Since then, several drugs were investigated during the ten years from 2007 to 2016; all of them failed to show their survival benefit. In 2017 and 2018, four drugs, regorafenib, lenvatinib, cabozantinib, and ramucirumab showed efficacy in the clinical trials and are now available in the clinical practice. Atezolizumab + bevacizumab (Atezo+Bev) combination therapy for unresectable or metastatic hepatocellular carcinoma became available in Japan in September 2020. The IMbrave150 study, a global phase III trial, compared the Atezo+Bev combination therapy with sorafenib; Atezo+Bev combination therapy was approved in Japan based on the results of this trial. The primary efficacy endpoints were overall survival (OS) and progression-free survival (PFS). Atezo+Bev combination therapy was superior to sorafenib in both OS [hazard ratio (95% confidence interval): 0.58 (0.42-0.79), p=0.0006, (stratified log-rank test)] and PFS as assessed by the Independent Review Facility based on RECIST v1.1 [hazard ratio (95% confidence interval): 0.59 (0.47-0.76), p<0.0001, (stratified log-rank test)]. Regarding safety, 329 patients received at least one dose of Atezo+Bev combination therapy and were included in safety analyses. Treatment-related adverse events were reported by 276 patients (83.9%) who received Atezo+Bev combination therapy. Grade 5 events occurred in 6 patients (1.8%) and serious adverse events occurred in 56 patients (17.0%). The common adverse reactions reported in the Atezo+Bev group were hypertension, occurred in 78 patients (23.7%), proteinuria, occurred in 62 patients (18.8%), and fatigue, occurred in 50 patients (15.2%). The percentage of patients who discontinued any treatment component because of adverse events was 42 patients (12.8%) in the Atezo+Bev group.

Overall, the advances in immunotherapy for HCC have brought change to clinical practice. In addition to the various trial results, including those of GO30140 and IMbrave150 studies, a number of prescribing experiences in the clinical practice have been reported. In this seminar, I am going to talk about the results of clinical trials and clinical experience of Atezo+Bev combination therapy, as well as the latest information on cancer immunotherapy in HCC.

Morning Seminar by Otsuka Pharmaceutical Co., Ltd.



Dr. Naoya Kato
Department of Gastroenterology,
Graduate School of Medicine,
Chiba University, Japan

Management of Liver Cirrhosis: Summary of JSGE & JSH Evidence-based Clinical Practice Guidelines for Liver Cirrhosis 2020

HCV is still the leading cause of cirrhosis in Japan. DAA treatment is shown to be effective in decompensated cirrhosis. SVR improves not only liver damage but also restores liver function even in decompensated cirrhosis. Nevertheless, the prognosis of HCV- HCC has improved significantly in the era of DAA treatment. In fact, SVR is now achieved in about 80% of patients with HCV-HCC, and the 5-year survival rate for this population has reached 84% in our hospital.

The prognosis of patients with cirrhosis depends on the liver function. Nutritional therapy for cirrhosis not only improves serum albumin level, ascites, encephalopathy, and sarcopenia, but also improves overall survival.

1) For the treatment of ascites, it is recommended to start vasopressin V2 receptor antagonist (tolvaptan) after poor control with anti-aldosterone and loop diuretics in order to maintain renal function.
2) For the treatment of hepatic encephalopathy, BCAA, carnitine, synthetic disaccharides (lactulose and lactitol) and nonabsorbable antibiotics (rifaximin) are used. Minimal/covert hepatic encephalopathy is a growing problem, and early therapeutic intervention is recommended.
3) Sarcopenia affects the condition and prognosis of patients with cirrhosis and thus should be evaluated and appropriate therapeutic interventions is recommended. BCAA and carnitine are shown to be useful.
4) In patients with cirrhosis, muscle clamp interferes with their QOL and cause sleep disturbance. Shakuyakukanzoto, carnitine, BCAA, and zinc preparations (zinc acetate dihydrate) are useful.

A high rate of carcinogenesis is observed in cirrhosis. Recent advances in molecular targeted therapy are improving the prognosis of patients with advanced HCC. Unlike other cancers, background liver function is significantly related to the prognosis of HCC patients. To maximize prognosis of these patients, hepatologist need to consider not only treating HCC but also maintaining background liver function.

Luncheon Seminar 2 by AbbVie GK

Dr. Toshifumi Tada
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Clinical Outcome of Antiviral Therapy in Patients with Hepatitis C Virus Infection

(1): present the changes in liver stiffness and steatosis in patients with HCV who received direct-acting antiviral (DAA) therapy and achieved sustained virological response (SVR). A total of 198 patients infected with HCV who achieved SVR after DAA therapy were analyzed. The median (interquartile range) liver stiffness values at baseline and SVR24 were 3.10 (2.70–4.18) kPa and 2.80 (2.40–3.77) kPa, respectively ($p<0.001$). The magnetic resonance imaging–determined proton density fat fraction (PDFF) values at baseline and SVR 24 were 2.4 (1.7–3.4) % and 1.9 (1.3–2.8) %, respectively ($p<0.001$). In addition, approximately 70% (19/28) of patients with fatty liver at baseline (PDFF ≥ 5.2%; n=28) no longer had fatty liver (PDFF < 5.2%) at SVR24. Viral eradication reduces both liver stiffness and steatosis in patients with chronic HCV who received DAA therapy. (2): present the long-term prognosis of liver disease in patients with HCV eradication after antiviral therapy versus those with persistent HCV infection. Four hundred and eighty patients who received interferon-based therapy and achieved SVR and 848 patients with persistent HCV infection were included. In the analysis of 1-year liver disease state transition probability matrices using Markov chain models, progression to cirrhosis from the chronic hepatitis state was observed (0.00%–0.63%) in patients with HCV eradication. Among patients with chronic hepatitis or cirrhosis and HCV eradication, hepatocellular carcinoma (HCC) development was observed in males aged ≥50 years (0.97%–1.96%) and females aged ≥60 years (0.26%–5.00%). Conversely, in patients with chronic hepatitis and persistent HCV infection, progression to cirrhosis was observed in males aged ≥30 years and female aged ≥40 years (0.44%–1.99%). HCV eradication can reduce the risk of developing cirrhosis or HCC in patients with chronic HCV infection. (3) present the impact of HCV eradication on all-cause mortality, especially non–liver-related mortality in patients with HCV. We enrolled 309 patients with HCV who achieved SVR by IFN-based therapy (IFN-SVR patients) or who did not receive IFN-based therapy (non-IFN patients) using propensity score matching. The eradication of HCV (IFN-SVR) increased survival from all-cause mortality (hazard ratio (HR), 0.265; 95% confidence interval (CI), 0.058–0.380) and reduced liver-related mortality (HR, 0.149; 95% CI, 0.058–0.380), non–liver-related mortality (HR, 0.439; 95% CI, 0.231–0.834), and the incidence of HCC (HR, 0.275; 95% CI, 0.156–0.448). Eradication of HCV reduced not only liver-related mortality but also non–liver-related mortality in patients with chronic HCV.

Luncheon Seminar 2 by AbbVie GK

Dr. Hidenori Toyoda
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Hepatocellular Carcinoma Developing after the Eradication of Hepatitis C Virus: Surveillance, Characteristics, and Prognosis

With the use of direct-acting antiviral agents for chronic hepatitis C virus (HCV) infection, most patients with HCV can achieve sustained virologic response (SVR): the eradication of HCV. This dramatic increase in patients with SVR, the number of patients who developed hepatocellular carcinoma (HCC) after SVR is increasing rapidly. Here, I report the incidence, characteristics, and prognosis of HCC detected after SVR.

Most post-SVR HCC emerged as a hypervascularization of a non-hypervascular hypointense nodule (NHHN) depicted in hepatobiliary phase of gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid–enhanced magnetic resonance imaging, and patients in whom NHHN was present before SVR had 10-fold higher risk of developing post-SVR HCC when patients had no history of HCC before SVR. In contrast, HCC could often develop after SVR in the absence of NHHN in patients with a history of HCC before SVR.

Most post-SVR HCC were detected and diagnosed in early stage under surveillance. When compared with HCC that developed in patients with persistent HCV infection, patients with post-SVR HCC had less deteriorated liver function. Survival rate after HCC diagnosis was significantly higher in HCC patients with SVR than those with persistent HCV. In contrast, recurrence rates after curative treatment were identical between SVR patients and patients with persistent HCV. Whereas liver function improved during the interval between initial HCC diagnosis and the first recurrence in SVR patients, it was impaired during the same period in patients with persistent HCV infection. As a result, more than 80% of patients with SVR underwent curative treatment for recurrence, only 50% of patients with persistent HCV could undergo curative treatment for recurrence. Therefore, survival rate after the first recurrence was significantly higher in SVR patients, which contributed to the higher overall survival in this patient population.

Evening Seminar 2 by Gilead Sciences K.K.

Dr. Man-Fung Yuen
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The University of Hong Kong,
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Novel Agents for HBV Therapy

To date, long-term use of nucleos(t)ide analogs (NA) is the mainstay of treatment of chronic hepatitis B (CHB) infection. They lead to profound HBV DNA suppression leading to normalization of liver biochemistry, regression of liver fibrosis/ cirrhosis and reduction of liver-related complications and mortality. However, residual risk of development of long-term complications still persist. To achieve better disease outcome, functional cure of the disease defined by HBsAg seroclearance is currently proposed to be the next treatment goal for CHB after long decades of using NA. Although functional cure can be achieved spontaneously or by NA or pegylated interferon, it occurs at a very low rate. It is estimated to be <1% annually for spontaneous functional cure and <10% over 5-10 years for NA-induced functional cure.

Because of this drawback, many novel therapeutic agents have been investigated with initial promising results. They either act directly or indirectly to restore the immune dysfunction induced by CHB and hence results in better host control on the virus. The former group is mainly the immunotherapeutics which include toll like receptor agonists, therapeutic vaccines and immune checkpoint inhibitors. The latter group include different kinds of direct antiviral agents. For instance, agents which compete with HBV for binding with hepatocyte receptor (sodium taurocholate cotransporting polypeptide) can result in inhibiting the viral entry into the hepatocytes. Short interfering RNAs or anti-sense oligonucleotides through RNA inhibition would silence viral mRNA transcriptions and hence removing the immune repressive effect exerted by high load of viral antigens, in particular the HBsAg. There are also other groups of agents explored in different studies, e.g. core protein assembly modulators (CpAM), which primarily inhibit viral capsid encapsidation and secondarily reduce cccDNA formation; HBsAg transporting inhibitor including nucleic acid polymers (NAP) and S-antigen Transport-inhibiting oligonucleotide polymers (STOPs) which block HBsAg release from infected hepatocytes; and neutralizing antibodies to HBsAg. With all these novel treatments, achieving a higher rate of functional cure of CHB is eagerly awaited.

APASL Single Topic Conference 2021 Osaka

"Molecular and Cell Biology of the Liver: Recent Evolution to Clinical Application"

Abstracts

Oral Free Papers

S1-4
#10061

Toll-like Receptor (TLR)-3 and TLR-9 Genes Polymorphism with Hepatitis C Virus Specific Cell Immunity Outcomes in Egyptian Health-care Workers

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Variations in the immune response could explain resistance to hepatitis C virus (HCV) infection. Toll like receptor gene (TLR) 3 is an innate detector of dsRNA viruses, and the TLR-9 gene recognizes bacterial and viral unmethylated cytosine phosphate guanosine motifs. We previously reported that the TLR3.rs3775290 CC genotype was associated with HCV chronicity and that the TLR9 gene played no major role in this infection. We enrolled 265 HCWs in this study and divided them into four groups. Group 1: 140 seronegative aviraemic HCWs; group 2: 20 seronegative viraemic HCWs; group 3: 35 subjects with spontaneously resolved HCV infection; and group 4: 70 chronic HCV HCWs (patients). All subjects were genotyped by polymerase chain reaction restriction fragment length polymorphism analysis for the TLR3.rs3775290, TLR9.rs5743836 and TLR9.rs352140 single nucleotide polymorphisms (SNPs). We also quantified HCV specific CMI in the four groups using an interferon gamma enzyme linked immunospot assay in response to nine HCV genotype 4a, overlapping 15mer peptide pools covering the whole viral genome. No statistically significant difference was found between CMI responding subjects with different HCV states and TLR3.rs3775290 or TLR9.rs352140 genotypes. However, there was a significant relationship between the outcome of the HCV-specific CMI and the TLR9.rs5743836 genotype among the responding subjects and the chronic HCV patients. In conclusion, TLR9.rs5743836 SNP, but not TLR3. rs3775290 or TLR9.rs352140 genotypes, could predict the outcome of HCV specific CMI responses among Egyptians infected with genotype 4

S1-5
#10060

The New Immunological Strategy of HCC Prevention to Suppress MICA Shedding

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Jun Arai[1], Kaku Goto[2], Yumi Otoyama[1], Ikuya Sugiura[1], Yoko Nakajima[1], Atsushi Kajiwara[1], Yuki Ichikawa[1], Shojiro Uozumi[1], Yuu Shimozuma[1], Manabu Uchikoshi[1], Masashi Sakaki[1], Ryo Nakagawa[3], Ryosuke Muroyama[3], Naoya Kato[3], Hitoshi Yoshida[1]

Background: In our previous genome-wide association study, we demonstrated the association between MHC class I-related chain A (MICA) and hepatocellular carcinoma (HCC) development in patients with chronic hepatitis C. Increasing membrane-bound MICA (mMICA) in cancer cells by reducing MICA sheddases facilitates natural killer (NK) cell-mediated cytotoxicity. Our recent study clarified that A disintegrin and metalloproteases 9 (ADAM9) is MICA sheddase in HCC, and that the suppression of ADAM9 increases mMICA, demonstrating the rationality of mMICA-NK targeted therapy. Furthermore, we showed that regorafenib suppresses ADAM9 transcriptionally and translationally. In this study, we aimed to identify new inhibitors of ADAM9 from a library of FDA-approved drugs in vitro.
Methods: A library of FDA-approved drugs (636 species) was screened for efficient inhibitors of ADAM9. Human HCC cell line PLC/PRF/5 and HepG2 cells were used to measure sMICA and mMICA levels were measured by ELISA and FACS, respectively.
Results: The in vitro assays confirmed that two leukotriene receptor antagonists (LTRAs) inhibited ADAM9 in a dose-dependent manner. The expression of mMICA after treatment with various candidate drugs identified LTRAs as potential ADAM9 inhibitors. Furthermore, LTRAs alone or in combination with regorafenib upregulated mMICA, which was in turn downregulated by leukotriene C4 and D4 via ADAM9 function.
Conclusions: Our study demonstrates that LTRAs could be developed as novel drugs for immunological control and suppression of ADAM9 in HCC. Further, LTRAs should be explored as combination therapy partners with conventional multi-kinase inhibitors for developing therapeutic strategies with enhanced efficacies for HCC management and treatment.

S2-4
#10022

A robust Cell Culture System for Anti-hepatitis B Virus Drug Study via Epigenetic Reprogramming

Liver Cancer Prevention Research Unit, RIKEN Cluster for Pioneering Research, Wako, Japan
Luc Gailhouste, Yutaka Furutani, Soichi Kojima

Chronic hepatitis B (HBV) infections remain a health burden affecting approximately 250 million people worldwide. Because available interferon-alpha (IFN&alpha)-based therapies show unsatisfactory cure rates, alternative therapeutic molecules are required. However, their development has been hampered because accessible cell models supporting relevant HBV replication and appropriate antiviral activity are lacking. We previously reported that hepatic cell lines treated with low concentrations of the DNA-demethylating reagent 5-azacytidine (5-AZA) recovered gene expression profiles and functional features similar to hepatocytes. We hypothesized that this procedure, named epigenetic reprogramming, could restore a consistent antiviral response in HepG2-NTCP cells and could provide a cell culture system suitable for antiviral drug evaluation. First, our results showed a significant inhibition in HBV DNA levels in epigenetically reprogrammed HepG2-NTCP-C4 (REP-HepG2-NTCP) cells after IFN&alpha treatment, while IFN&alpha had no effect in non-reprogrammed HepG2-NTCP cells. This inhibitory effect was associated with an augmentation of major interferon-stimulated genes (ISGs), known to be associated with an IFN&alpha antiviral response in patients. A methylation analysis revealed the epigenetic unmasking of critical ISGs in REP-HepG2-NTCP cells, which were silenced by hypermethylation before reprogramming. Next, we demonstrated the utility of REP-HepG2-NTCP cells by assessing the therapeutic action of the IFN-like compound CDM-3008. In summary, HepG2-NTCP cell epigenetic reprogramming could represent a valuable and accessible tool to generate robust cell culture experimental systems for anti-HBV drug studies.

S2-5
#10064

Zinc Chloride Enhances dsRNA-induced Beta-interferon Promoter Activity through the Inhibition of Mitogen-Activated Protein Kinase Kinase 3 Expression

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Tatsuo Kanda[1], Reina S. Tanaka[1], Ryota Masuzaki[1], Naoki Matsumoto[1], Hiroaki Okamoto[2], Mitsuhiko Moriyama[1]

Background and Aim: Interferon are involved in collagen gene expression and suppresses experimental hepatic fibrosis in vivo and in vitro. Mitogen-activated protein kinase (MAPK) signaling are also involved in the activation of interferon activation in hepatocytes. Zinc compounds have been shown to enhance the action of interferon. In the present study, we examine the effects zinc chloride on the dsRNA-induced beta-interferon promoter activity.
Methods: (1) The effects of zinc chloride on TLR signaling pathways in human hepatoma cell lines were examined by a human TLR signaling target RT-PCR array. (2) Expression of mitogen-activated protein kinase kinase 3 (MAP2K3) was also examined by Western blotting. (3) The activation of beta-interferon promoter were examined by reporter assay in the presence or absence of polyinosinic-polycytidylic acid [poly(I:C)]. (4) We examined the effects of zinc chloride on HAV subgenomic replicon and HAV HA11-1299 replication.
Results: (1) Zinc chloride inhibits MAP2K3 expression. (2) Silencing of MAP2K3 or inhibitor of MAP2K3-p38 MAPK signal transduction pathways could negatively regulate HAV replication in human hepatocytes. (3) Silencing of MAP2K3 or inhibitor of MAP2K3-p38 MAPK signal transduction pathways enhances beta-interferon promoter activities stimulated by poly(I:C).
Conclusion: MAP2K3 could be one of the modulation factors for dsRNA PAMP triggers that produce beta-interferon. MAP2K3 may be an promising target in the regulation of viral replication and the progression of hepatic fibrosis.

S3-4
#10002

COLCA1 and *COLCA2*, the Effector Genes Driven by rs1944919 in Primary Biliary Cholangitis (PBC) Susceptibility Locus Chromosome 11q23.1 in the Japanese Population

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Primary biliary cholangitis (PBC) is a chronic and cholestatic autoimmune liver disease caused by the destruction of intrahepatic small bile ducts. Our previous genome-wide association study (GWAS) identified chromosome 11q23.1 as a susceptibility gene locus for PBC in the Japanese population. Although candidate genes with well-known functions that are located in disease susceptibility loci have frequently been reported as disease susceptibility genes in GWASs, the identification of effector genes regulated by primary functional variants is necessary to understand the contribution of susceptibility loci to pathogenesis.
Here, in order to analyze the disease susceptibility of all genetic variations in this locus, we carried out high-density association mapping of chromosome 11q23.1 based on single nucleotide polymorphisms (SNPs) imputation using data from a whole-genome sequence reference panel of 1,070 Japanese individuals and our previous GWAS. Subsequent *in silico* and *in vitro* functional analyses identified rs1944919 as the primary functional variant. Expression-quantitative trait loci (e-QTL) analyses showed that rs1944919 was significantly associated with expression levels of *COLCA1* and *COLCA2* ($P=3.8X10^{-29}$ and $P=6.5X10^{-32}$, respectively) whose function have not been fully elucidated. Additionally, the effects of rs1944919 on *COLCA1/COLCA2* expression levels were confirmed using genotype knock-in versions of cell lines constructed using the CRISPR/Cas9 system and differed between rs1944919-G/G clones and -T/T clones ($P<0.01$).
This is the first study to demonstrate that *COLCA1* and *COLCA2* are the effector genes regulated by the primary functional variant rs1944919, and that increased expressions of *COLCA1/COLCA2* might be involved in the pathogenesis of PBC.

S3-5
#10062

Palmitate Disrupts the Circadian Rhythm of Mitochondrial Sirtuins in Non-neoplastic Hepatocytes (PH5CH8), While it Restores the Rhythm in Hepatocellular Carcinoma Cell Line (HepG2)

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Background and Aims: The Sirtuins (SIRT1-SIRT7) belong to class of NAD+-dependent deacetylases and regulate fatty acid metabolism. As dysregulated circadian rhythm is implicated in pathogenesis of fatty liver, we evaluated the effects of palmitate, on rhythmic oscillations of Sirtuins and the Clock genes in hepatocytes in an in vitro culture system.
Method: Hepatocyte cell lines PH5CH8, HepG2 were cultured in presence or absence of palmitate. Lipid uptake was quantified by oil red staining. Growth kinetics was monitored and quantitative PCR was done for gene expression. For evaluating circadian rhythm, cells were subjected to synchronization by an initial serum shock followed by release in fresh media with or without palmitate and RNA was extracted from cells at regular intervals upto 48hrs.
Results: Palmitate induced microsteatosis and growth inhibition at 400 µ dose in both the cell lines. However, a three-fold decline in mitochondrial Sirtuins (SIRT4 and SIRT5) was seen in PH5CH8 and HepG2 cells. Following serum synchronization PH5CH8 cells showed a rhythmic pattern of gene expression for SIRT4, SIRT5 and CLOCK1. However, this rhythmic oscillation of gene expression was abrogated in presence of palmitate. On the other hand, HepG2 showed either minimal or no oscillatory patterns for Sirtuin isoforms and Clock genes following serum shock. Further, in HepG2 cells both the Clock genes regained their rhythmic expression following palmitate treatment.
Conclusion: Palmitate abrogates the circadian rhythm of gene expression of mitochondrial Sirtuins and Clock gene in non-malignant hepatocytes, while it restores the lost circadian expression of mitochondrial Sirtuins in cancerous cells.

S4-4
#10029

Behavior of Reactive Cholangiocytes in the Tissue Repair Stage from Chronic Liver Injury

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Background: Chronic liver injury causes the proliferation and expansion of atypical cholangiocytes, known as the ductular reaction, and liver fibrosis. The ductular reaction contributes to liver regeneration in part through reconstructing complementary bile-excreting conduit systems for the lost bile canalicular networks. However, it is unclear how the reactive cholangiocytes behave in the tissue repair from chronic liver injury. In the present study, we examined the fate of reactive cholangiocytes during the recovery phase.
Methods: Chronic liver injury was induced in wild type and $CK19^{CreERT/+}Rosa26^{LSL\text{-}tdTomato/+}$ mice by drinking thioacetamide (TAA)-containing water for 8 weeks. After injury, these animals were administered with normal water (recovery phase) for up to 24 weeks. Excised liver specimens were subjected to 3-dimentional immunofluorescent staining. Inhibition of focal adhesion kinase (FAK) or Notch signaling was induced by repeated intraperitoneal injections of PF-573228 or gamma secretase inhibitor IX, respectively, for 1 week starting from the 3rd week of the recovery phase.
Results: The expanded ductular network gradually diminished with the bile canalicular network reconstitution during 24 weeks of recovery from TAA-induced liver injury. The reactive cholangiocytes rarely trans-differentiated into hepatocytes and gradually disappeared by cell apoptosis. The collagen fiber was accumulated adjacent to the reactive cholangiocytes, and fibrosis also gradually decreased during the recovery phase. Inhibition of FAK or Notch signaling promoted elimination of the reactive cholangiocytes concomitantly with accelerated regression of fibrosis.
Conclusion: These results suggest that the elimination of reactive cholangiocytes may contribute to rapid tissue repair in recovery stage from chronic liver injury.

S4-5
#10037

Capacity of Extracellular Globins in Suppression of Collagen Production from Activated Hepatic Stellate Cells via Scavenging ROS and Promoting MMP-1 Secretion

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Background: Anti-fibrotic therapy remains an unmet medical need in human chronic liver diseases. We aim to study anti-fibrotic property of 4 human “Globins”: tetramer Hemoglobin (Hb), monomer-Myoglobin (Mb), -Cytoglobin (Cygb), and -Neuroglobin (Ngb).
Methods & Results: We produced recombinant human 6-His-tagged Cygb and Ngb and obtained commercial Mb and Hb. We traced their biodistribution and assessed their biological activities by administrating these globins into Human hepatic stellate cells (HHSteCs) under spontaneous, ROS, or TGFβ1-induced activation. In a cell-free system, all globins demonstrated antioxidant capacity (Hb equals 4.5, Ngb 3.4, Cygb 2, Mb 0.7 mM of Trolox) and superoxide-scavenging activity (IC50 of 0.43, 25.2, 2.4, 0.38 μM, respectively). Extracellularly added Mb, Ngb, and Cygb, but not Hb, penetrated the cells, clearly scavenged intracellular ROS induction, and dose-dependently suppressed type I Collagen production in spontaneous or TGFβ1-stimulated HHSteCs, in which Ngb showed lowest IC50 (0.6 μM), compared to Mb (IC50 of 1.27 μM) and Cygb (IC50 of 2.4 μM). Mutations of a disulfide bond between Cys-46 and Cys-55 in Ngb decreased heme activity and superoxide-scavenging activity, accompanied by the attenuation in their capacity on inhibiting collagen production. Besides, upregulation of matrix metalloproteinase (MMP)-1 at both mRNA and protein levels was found in response to all globin treatment. MMP-1 knockdown in HHSteCs reversed partially the globin effect in decreasing collagen secretion into medium.
Conclusions: These findings revealed a profound role for Mb, Ngb, and Cygb in maintaining HSCs in deactivated status. Our study proposed "globin therapy" to combat fibrotic liver disease.

S5-5
#10014

Possible Repurposing of the Analgesic Neurotropin for NAFLD/NASH Treatment

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Background: The prevalence of non-alcoholic fatty liver disease (NAFLD) has been increasing over the last decade, with 20 to 25% developing to NASH. Despite many clinical trials, no effective drugs have been approved by the Food and Drug Administration. Hence, a drug for NAFLD/NASH is highly sought-after. Existing drugs, with safety data available, can be repurposed for new indications, thereby saving time and cost for development. We investigated if the drug Neurotropin (NTP) can be used for NAFLD treatment through an animal model.
Methods: We divided C57BL/6 WT mice into two groups: (1) normal chow diet (control); (2) high-fat diet plus high fructose/glucose drinking water, and three treatment groups: (1) vehicle control; (2) Obeticholic acid (OCA); (3) NTP. The mice were fed for total 12 weeks. The drugs were administered daily by oral gavage for 6 weeks after 6 weeks of diet feeding.
Results: NTP treatment decreased serum ALT and AST levels, showing the protection against the diet-induced hepatocellular damage. NTP also inhibited liver steatosis as assessed by serum lipids, Oil red O, and H&E staining. Finally, reticulin staining revealed that NTP suppressed fibrosis. Consistent with this observation, mRNA levels of CXCL1, Timp1, collagen1a1, collagen4a1, Dgat-1 and 2 were suppressed by NTP treatment.
Conclusions: Our data demonstrated that NTP can be a useful intervention for treating NAFLD/NASH. Because NTP has been widely used for over 50 years in Japan and China without significant side-effect reported, NTP can be an effective and safe drug for NAFLD/NASH management.

S5-6
#10042

Gut-liver Axis-mediated Mechanism of NASH-associated Hepatocellular Carcinoma Progression

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Recently, nonalcoholic steatohepatitis (NASH) is recognized as a major cause of hepatocellular carcinoma (HCC). We previously identified that the hepatic stellate cells (HSCs) exposed to deoxycholic acid (DCA), a gut microbial metabolite, undergo senescence-associated secretory phenotype (SASP), a phenotype that senescent cells secrete a variety of inflammatory cytokines, chemokines and proteases. We found that the obesity-associated hepatic translocation of lipoteichoic acid (LTA), a gram positive gut microbial component, enhanced SASP phenotype in hepatic stellate cells through Toll like receptor 2. We previously showed that IL-1β, a SASP factor, secreted from senescent HSCs, played a crucial role in HCC development. In this study, we found that IL-33 was highly induced in the liver tumor areas particularly in the senescent HSCs in an IL-1β-dependent manner in high fat diet-induced NASH model in vivo. Interestingly, IL-33-null mice developed significantly less tumors compared with those in wild-type mice, indicating that IL-33 plays an important role in HCC development. Moreover, a short and active form of IL-33, which was cleaved by elastase family called CELA1, was detected in the HSCs in the senescent HSCs in the liver tumor areas. The released active form of IL-33 suppressed the antitumor immunity, thereby contributing to the progression of the NASH-associated HCC. Interestingly, IL-33 overexpression was detected in HSCs in human NASH-associated HCC tumor areas, implying that similar mechanism could be involved in human liver cancer progression. We suggest here a novel tumor-promoting axis by multiple SASP factors (IL-1β, IL-33 and CELA1) secreted from senescent HSCs.

S6-4
#10063

The Therapeutic Effect of Human Placenta Mesenchymal Stem Cell-Derived Exosomes on Primary Sclerosing Cholangitis

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Background: Primary sclerosing cholangitis (PSC) is a chronic autoimmune liver disease, biliary fibro-inflammation and cholangiocyte senescence are its partial characteristics. Human placenta mesenchymal stem cell (hPMSC)-derived exosomes proven anti-aging effects on senescent-related diseases. We intend to apply PSC mouse model to investigate the protective effects of hPMSC-derived exosomes, and use senescent cholangiocyte organoids (cho-orgs) as an in vitro model of PSC to verify the anti-aging effects.
Methods: The exosomes were gained by ultra-centrifuge process, which were characterized and injected into MDR2-/- mouse through tail vein for four weeks. One week after final injection, the mice were killed and serum ALT, AST levels were examined. Senescent mouse cho-orgs were induced by oxidative stress, which were co-cultured with 0.1 μg/ml exosomes for 120 h, then the expression of the senescence markers and the mRNA and protein expression of chemokines and senescence-associated secretory phenotype (SASP) components of organoids were identified.
Results: hPMSC-derived exosomes were well distributed, expressing CD9 and CD81. The liver/weight ratio of MDR2-/- mouse injected with exosomes were decreased, as well as the serum ALT, AST levels. The liver fibrosis and bile duct reaction were also improved by exosome. Besides, exosomes reduced the senescent markers of cho-orgs and the levels of SASP components.
Conclusions: hPMSC-derived exosomes have protective effects to PSC mouse model and senescent cholangioids, which might have therapeutic potential for PSC.
Key words: Exosomes, Primary sclerosing cholangitis, Organoids, MSC

S6-5
#10044

Kruppel-like Factor 15 Induces the Development of Mature-type Hepatocytes from Progenitor Cells

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Background: Hepatocytes acquire various metabolic functions by the maturation step during the fetal period; however, the molecular mechanism inducing maturation of hepatic progenitor cells is still unclear. Kruppel-like factor 15 (KLF15) was one of transcriptional regulators whose expression level was different between mouse fetal hepatic progenitor cells and adult hepatocytes. We revealed KLF15 as a novel factor involved in the regulation of hepatic progenitor cells.
Methods: We performed overexpression of KLF15 using a retroviral vector and knockdown by siRNA in mouse hepatic progenitor cells, and analyzed the expression of liver functional genes. In addition, we performed overexpression of KLF15 in hepatic progenitor cells differentiated from human induced pluripotent stem cells (hiPSCs) and measured the expression of functional genes and the changes in the expression of Cdk inhibitors.
Results: Overexpression of KLF15 in mouse hepatic progenitor cell culture increased the expression of mature hepatocyte markers, while the suppression of KLF15 expression by shRNA downregulated hepatic maturation. Similar induction of maturation was observed in hiPSCs-derived hepatic progenitor cell culture overexpressing KLF15. In addition, KLF15 suppressed the proliferation of human iPSC-derived hepatoblasts and expression of p57cdkn1c was increased in the KLF15-overexpressing cells.
Conclusions: KLF15 induced hepatic maturation in both mouse and human hepatic progenitor cells, and it is useful for the generation of functional human hepatocytes *in vitro* for regenerative medicine and drug discovery.

S7-5
#10050

Nimbolide Restores Gut Dysbiosis and Prevents Bacterial Translocation by Improving Tight Junction Proteins Expression in an Experimental Hepatocarcinogenesis

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Background and Aim: Gut dysbiosis plays a key pathological role in hepatocellular carcinoma (HCC) progression; however, the mechanism is poorly understood. Here, we aimed to investigate the effect of Nimbolide on regulating gut dysbiosis and bacterial translocation in an experimental hepatocarcinogenesis.
Methods: Male CD-1 mice were induced HCC by a single intraperitoneal injection of diethylnitrosamine (DEN) followed by N-nitrosomorpholine (NMOR) in drinking water for 28 weeks. Nimbolide (6mg/kg) was administered orally for four consecutive weeks to HCC mice from week 28. All the mice were sacrificed at week 32, blood, intestinal and hepatic tissues were collected for molecular analysis.
Result: Nimbolide treatment to HCC mice significantly reduces tumor volume and tumor size by 51.06%. Nimbolide treatment also lowered plasma level of bacterial translocation marker LBP, sCD14 and procalcitonin in HCC mice. Furthermore, significantly increased Escherichia coli, Enterococcus spp., Bacteroides spp., reduced Bifidobacterium spp., and Lactobacillus spp. 16s rRNA levels were observed in the small intestine and hepatic tissue of HCC mice compared to naive mice. Treatment with Nimbolide to HCC mice significantly inhibited small intestinal bacterial overgrowth and prevented Escherichia coli, Enterococcus spp. and Bacteroides spp. translocation to the liver.
Conclusion: Our study showed for the first time that Nimbolide treatment to HCC mice restored gut dysbiosis and prevented gut bacterial translocation by improving intestinal TJ protein expression and suppressing tumor growth. Consequently, Nimbolide showed a promising natural therapeutic agent for HCC treatment; however, further clinical studies are warranted.

S7-6
#10054

Dysbiosis of the Gut Microbiota Associated with Plasma Levels of IFN-&gamma and Viral Load in Patients with Acute Hepatitis E Infection

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In order to explore the relationship between gut microbiota and the occurrence of acute hepatitis E (AHE), we conducted a comparative study from 58 enrolled patients including 33 AHE patients and 25 healthy controls (HC) using high-throughput 16S rRNA gene sequencing. Shannon and Simpson indices showed bacterial diversity had no significant differences between the AHE and HC groups. Proteobacteria, Gammaproteobacteria and Enterobacteriaceae were most abundant in the AHE group, which contributed to the difference between the gut microbiota of AHE and HC groups, and also the same conclusion of HEV RNA positive group and HEV RNA negative group. Functional prediction analysis showed that the top 3 metabolic pathways were Ribosome, Purine metabolism, Two-component system are the top three metabolic pathways. Compared with IFN-&gamma normal AHE group, Proteobacteria, Gammaproteobacteria, Xanthomonadaceae and Enterobacteriaceae were most abundant in the IFN-&gamma high group. The abundance of Gammaproteobacteria genus was positively correlated with the level of serum ALT and TBIL, respectively. Gammaproteobacteria genus could discriminate AHE from HC, and also could be better predicted severity of AHE patients. We believe that our findings will contribute to propose a novel treatment strategy for AHE.

S8-5
#10008

Analysis of Tumor Microenvironment in Patients with Advanced Hepatocellular Carcinoma Eligible for Systemic Therapy

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Background and Aims: Nowadays, immune checkpoint inhibitors and combination for those and other agents have been available in advanced hepatocellular carcinoma (HCC). Although exploring tumor microenvironment has essential clinical implications, tumor microenvironment in advanced HCC is unclear. The present study was aimed to analyze the tumor microenvironment by using needle biopsy samples obtained prior to initiation of systemic therapy in patients with advanced HCC. Methods: Tumor microenvironment was evaluated by PD-L1, CD8, VEGF, and HLA-class1 immunohistochemical analyses.
Results: 1) PD-L1-positive tumor was not dominant (28.6%). Positive PD-L1 expression correlated with high infiltration of CD8-positive lymphocytes. As the expression of VEGF upregulated, levels of infiltrating CD8-positive lymphocytes showed a tendency to decrease. 2) Positive PD-L1 expression was associated with high AFP levels in advanced HCC. Advanced HCC patients with macro vascular invasion indicated high levels of infiltrating CD8-positive lymphocytes. 3) Of the 70 patients, 20 patients were able to analyze samples obtained immediately before systemic therapy as well as those obtained before. 40.0% had no change in PD-L1 expression or infiltration of CD8-positive lymphocytes at two different time points, 20.0% had a change in PD-L1 expression to positive, and 35.0% had a change in infiltration of CD8-positive lymphocytes to highly infiltrated.
Conclusions: Since almost half of HCC patients changed tumor microenvironment status during clinical courses, assessments of tumor microenvironment should be conducted before starting systemic therapy including immunotherapy.

S8-6
#10027

Soluble PD-1 as a Potential Biomarker for Predicting Response to the Immune Checkpoint Inhibitor in HCC Patients

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Background and Aims: As multiple systemic agents, including immune checkpoint inhibitor (ICI), have been introduced to treat hepatocellular carcinoma (HCC), there is a growing demand for biomarkers that can predict a favorable response to treatment. In other types of cancers, immune checkpoint molecules in peripheral blood are reported to predict clinical outcome of ICI. This study aimed to reveal the utility of soluble immune checkpoint molecules for predicting the outcome of HCC patients who were treated with ICI.
Method: Between October 2020 and March 2021, 33 unresectable HCC patients treated with ICI were enrolled in this study. All patients underwent at least two cycles of atezolizumab and bevacizumab combination therapy, and we analyzed blood samples taken at pre-treatment and 6±2 weeks after the initiation of treatment. Serum sPD-1, sPD-L1, and sCTLA-4 were measured using chemiluminescence enzyme immunoassay (CLEIA).
Results: Among 33 patients, 21 patients achieved either partial response or stable disease (disease control; DC) evaluated by RECIST version 1.1. Baseline sPD-1 was significantly higher in DC patients than non-DC patients (277 vs. 192 pg/mL, $p=0.04$). Whereas, both sPD-L1 and sCTLA-4 did not correlate to DC. Regarding dynamics, sPD-1 (237 vs. 352pg/mL, $p<0.001$) and sCTLA-4 (1.95 vs. 3.2 pg/mL, $p<0.001$) increased after 2 cycles of the treatment, and sPD-L1 decreased significantly (339.5 vs. 59 pg/mL, $p<0.001$). In DC patients, ratio of post-treatment to pre-treatment sPD-1 was significantly lower than non-DC patients (1.24 vs. 1.56, $p=0.01$).
Conclusions: Soluble PD-1 can be a biomarker to predict response to ICI treatment in HCC patients.

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Abstracts

Poster Free Papers

P-01 #10028

Combination Therapy of Juzentaihoto and Mesenchymal Stem Cells Attenuates Liver Damage and Regresses Fibrosis in Mice

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Introduction: Liver cirrhosis is an end-stage multiple liver disease. Mesenchymal stem cells (MSCs) are an attractive cell source for reducing liver damage and inhibiting inflammation and hepatic fibrosis.; additional therapies accompanying MSCs can potentially enhance their therapeutic effects. Kampo medicines exhibit anti-inflammatory and anti-oxidative effects. Herein, we investigate the therapeutic effect of MSCs combined with the Kampo medicine Juzentaihoto (JTT) as a combination therapy in a carbon tetrachloride (CCl4)-induced cirrhosis mouse model.
Method: C57BL/6 Mice were administered JTT (orally) and/or MSC (one time, intravenously). Concentrations of liver proteins were measured in sera. Sirius Red staining and hydroxyproline quantitation were conducted on hepatic tissues and immune cells and their associated properties were also evaluated. Liver metabolomics was performed on liver tissues.
Result: JTT monotherapy attenuated liver damage and increased serum albumin levels, but did not effectively induce fibrolysis. JTT rapidly reduced liver damage, in a dose-dependent, after a single dose CCl4 administration. Further, JTT-MSC combination therapy synergistically attenuated liver damage, improved liver function, and regressed liver fibrosis. While both the MSC and JTT groups had significantly decreased serum AST and ALT levels relative to the Ctrl group, the JTT-MSC group exhibited an additional decrease in these levels. While the effects against immune cells in the liver by JTT and MSC were different, JTT-MSC combination therapy synergistically affected the immune response, inducing the production of anti-inflammatory macrophages.
Conclusion: The addition of JTT enhanced the therapeutic effects of MSCs; this combination could be a potential treatment strategy against cirrhosis therapy.

P-02 #10057

The Anti-fibrotic Effect of *ex vivo*-expanded CD34$^+$ Cell Transplantation in a Mouse Model of NASH

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Ex vivo expanded autologous cell transplantation is a promising treatment for chronic disease including liver cirrhosis. We investigated the anti-fibrotic effect of cell transplantation with *ex vivo*-expanded mouse CD34$^+$ cells on a diet-induced NASH model. C57BL/6J mice were fed a control or a choline-deficient amino acid-defined, high-fat diet (CDAHFD) for up to 40 weeks. The 12-week feeding group and the 20-week feeding group was used as a mild fibrosis and a severe fibrosis model, respectively. Each model was transplanted only once (CD34s) or twice (CD34d) with expanded CD34$^+$ cells, and was infused saline as a control. After 7-days culture, mouse CD34$^+$ cells were effectively expanded in a serum-free culture medium. CDAHFD feeding increased the percentage of Sirius-red positive area in the hepatic lobule up to 20 weeks, however no significant difference was observed between 20 and 40 weeks. In both fibrosis models, the progression of liver fibrosis was significantly inhibited in a dose-dependent manner in expanded CD34$^+$ cell-transplanted livers compared to saline-treated livers. Gene ontology analysis using RNA-seq data showed that “defense response to virus” was the most altered category, among which *Stat1/Cxcl10* axis was suppressed in CD34d livers.
Conclusion: We demonstrated that transplantation of *ex vivo*-expanded CD34$^+$ cells inhibited liver fibrogenesis in NASH model mice regardless of the severity of liver fibrosis. Furthermore, RNA-seq analysis suggested that the mechanism is inhibition of migration of T lymphocytes and macrophages to the liver by suppressing *Stat1/Cxcl10* axis.

P-03 #10056

Cell Membrane-mediated Direct Crosstalks between Hepatocytes and HSCs

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Compared to other organs, extracellular cellular matrices are scarce in the liver, its occupancy being ~0.6% of the total liver mass. Histologically, a single quiescent hepatic stellate cell (HSC) entangle 30-40 hepatocytes (heps) with their protrusions called spikes. We recently found that disruption of cell-cell interactions between heps and HSCs can be the main trigger for a series of pathological reactions. In this study, we investigated the presence of cell membrane-mediated intercellular crosstalks on the functional stability of heps and HSCs. We first observed that primary HSCs co-cultured with heps were rich in protrusions compared to those cultured alone. Next, oil red O staining showed that the co-culturing synergistically promoted the LD formation in HSCs compared to single cultures. 3H-retinol uptake in vitro experiments showed that HSCs and heps in coculture incorporated 3H-retinol at ~1.8-fold and 7.5-fold higher rates than those in corresponding single cultures, respectively. This stimulation of uptakes in heps seems to be specifically induced by HSCs, as no such stimulus occurred when heps were co-cultured with dermal fibroblast. HSCs cultured with digoxin-treated, but intact cell membrane-possessing heps increased their protrusions compared to those in single cultures. Furthermore, we found that HSCs cultured on isolated heps-membranes did not undergo spontaneous activation. Based on these results we conclude that cell membrane-mediated direct crosstalks between heps and HSCs play crucial roles in their physiology and pathology. Our study provides unprecedented insight into mechanism of the interactions of heps and HSCs and might lead to new therapeutic strategies for liver diseases.

P-04 #10009

Bioinformatics Study of Genes E1 and E2 from Hepatitis C Virus (HCV) with Genotypes 1, 2, 3 and 6 as Vaccine Candidates for Virus-like particles (VLPs)

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Hepatitis C virus (HCV) is the cause of hepatitis. This disease is dangerous because of the large number of sufferers of chronic hepatitis infection. Chronic hepatitis infection can progress to cirrhosis and liver cancer and even death. HCV vaccine is very necessary to prevent transmission of infection. The difficulty faced is the number of HCV genotypes in circulation. In Indonesia, there are genotypes 1, 2, 3, and 6. The HCV vaccine must be able to provide protection against infection with several genotypes. Therefore, the development of the HCV vaccine takes a long time. One vaccine approach is the Viral-like Particles (VLPs) vaccine. The E1E2 protein found on the surface of the virus is a very good candidate to be used as a VLPs vaccine material because it has high immunogenicity. In this study, bioinformatics analysis was carried out on genes E1 and E2 from genotypes 1, 2, 3, and 6. The sequences of these genes were obtained through the GenBank and further analyzed using some software such as Bio Edit Sequence Alignment Editor, BLAST and BLAST Primer. The results of this study were successful in obtaining consensus sequences of E1-E2 genes derived from genotypes 1, 2, 3, and 6 which have a length of 1672 bp. This sequence forms the basis for the primary pair design and the primers are obtained. Primary X sequence has been confirmed using BLAST and X primary sequence has a high homology rate (> 90%).

P-05 #10052

Improvement of Liver Fibrosis in Patients Achieving a Sustained Virological Response to DAA Treatment for Hepatitis C

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Background: Direct-acting antiviral (DAA) treatment achieves a sustained virological response (SVR) in almost all patients with hepatitis C virus (HCV) infection. This study compared the degree of liver fibrosis improvement after treatment in patients with and without persistently normal alanine transaminase (PNALT).
Methods: We enrolled 108 HCV patients who achieved an SVR using DAAs and were followed for 1 year (1yr) after 24 weeks of SVR (SVR24). Changes in laboratory values at the start of DAAs (Pre), at SVR24, and at 1yr were measured in relation to Mac2-binding protein glycan isomer (M2BPGi) in PNALT (ALT<30 U/L) and non-PNALT (ALT>30 U/L) patients.
Results: Pre M2BPGi was significantly lower in the PNALT group (n=43) than in the non-PNALT group (n=65) (1.15 vs. 2.29 COI, P<0.01). In PNALT patients, M2BPGi decreased in a stepwise manner (Pre, SVR24, and 1yr: 1.15, 0.86, and 0.83 COI, respectively; Pre vs. SVR24: P<0.01 and SVR24 vs. 1yr: P=0.04). Similar results were observed in the non-PNALT group (2.29, 1.18, and 0.99 COI, respectively; P<0.01 and P<0.01, respectively). The increase rate of M2BPGi from SVR24 to 1yr was -3.7% (range: -5.3-4.6%) in the PNALT group and -17.4% (range: -54.9-7.4%) in the non-PNALT group, which was a significant difference (P=0.01), although M2BPGi at 1yr was comparable between the groups (0.83 vs. 0.99 COI, P=0.25).
Conclusions: Achieving a SVR with DAAs regardless of PNALT status appears desirable since fibrosis improvements may be enhanced for patients in the chronic hepatitis stage during the year following SVR24.

P-07 #10048

microRNA-6126 Reduces the Stability of NTCP Messenger RNA and Suppresses its Expression in Hepatocytes

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Background: Hepatitis B virus (HBV) infection is one of the most serious health problems worldwide. In our study, we found that an increase in serum miR-6126 levels during 1 year of peginterferon treatment predicted a decrease in HBs antigen. The aim of this study was to demonstrate that miR-6126 decreases the expression level of sodium taurocholate cotransporting polypeptide (NTCP), a host cell receptor required for hepatitis B virus (HBV) entry.
Methods: HepG2-NTCP and PXB cells were used to evaluate the expression level of NTCP. mRNA expression level of NTCP was evaluated by real-time qPCR. protein expression level of NTCP was evaluated by Western Blot analysis, Immunostaining, PreS1- TAMRA attachment assay.
Results: Transfection with miR-6126 decreased NTCP by Western Blot analysis and immunostaining. In addition, miR-6126 promoted the phosphorylation of mTOR and Erk.
Conclusion: miR-6126 may inhibit the infection of HBV activity into hepatocytes.

P-08 #10017

Molecular Characterization of Hepatitis B Virus in Young People of Vietnam

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The present study aims to molecularly characterize HBV isolates from young residents of districts Thai Nguyen and Da Nang, Vietnam.
Material and Methods: A cross-sectional survey was carried out on young people aged 19-22 years from two districts that are different in socio-economic development and climatic conditions. HBV DNA positive samples (n=21) were subjected to whole-genome sequencing. HBV genotype and subgenotype, recombinants, were determined.
Results: Of 21 sequenced isolates, the predominant genotype was subgenotype B4 (81%,17/21), followed by subgenotype C1 (19%,4/21). Phylogenetic analysis was shown high heterogeneity of Vietnamese HBV isolates of subgenotype B4. The genetic diversity of the HBV genome is not only related to resistance to antiviral therapies and associated with the clinical characteristics of HBV infection. It is known that HBV genotype C is more virulent and has a high risk of developing HCC. It was shown that 16 out of 17 HBV isolates identified as subgenotype B4 were recombinant variants in the preC/C region of the genome, formed by widely circulating variants of genotypes B and C.
Conclusion: Our findings demonstrate HBV subgenotypes B4 and C1 are prevalent among young people in northern and central Vietnam. Understanding the viral variations and mutations is important in diagnostic, prevention, and therapy development. It is necessary to improve the control measures against viral hepatitis to protect the young population.

P-09 #10043

Emergence of Novel Resistance-associated Substitutions against Pibrentasvir in Genotype 1b HCV-Infected Mice

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Background: We evaluated the efficacy of glecaprevir plus pibrentasvir therapy (GLE/PIB) for patients with a history of direct acting antivirals (DAAs) therapies and emergence of NS5A resistance-associated substitutions (RASs) against PIB using hepatitis C virus (HCV)-infected mice.
Methods: Fifty-six patients who have previously failed to respond to DAA therapies (Genotype 1/2: 39/17 patients) were retreated with GLE/PIB for 12 weeks. Human hepatocyte transplanted mice were infected with genotype 1b HCV, and then orally administered either 12.5 or 25 mg/kg/day of PIB. The nucleotide and amino acid sequences of the NS5A region were determined by sanger sequence analysis. Antiviral activity of PIB was analyzed using HCV subgenomic replicon cells.
Results: Although 53 patients (95%) achieved SVR, three genotype 1b HCV-infected patients with NS5A-L31I/Y93H, L31F/P32del and L31V/32del, respectively failed to achieve SVR by GLE/PIB retreatment. Mice serum HCV RNA levels transiently decreased but followed by relapse during PIB treatment, and direct sequence analysis showed emergences of various mutations in the NS5A region, including L31V/P32del, L31F/P32del/Y93H, NS5A-P29del/Y85C, and NS5A-F37Y at HCV relapse. PIB was less effective in mice with NS5A-F37Y mutations compared to mice with wild-type HCV. NS5A-F37Y showed 5.4-fold resistance to PIB relative to wild-type based on analysis using HCV subgenomic replicon systems.
Conclusion: The present study identified NS5A-F37Y as a novel RAS against PIB and showed the possibility of emergence of various NS5A RASs including P29del, P32del and F37Y following PIB treatment. These mutations might emerge and lead to failure to respond to DAA therapies including PIB-based regimens in HCV-infected patients.

P-10 #10023

B-cell Activating Factor Promotes Lipid Synthesis in Murine Hepatocytes

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Background: Previously, we have reported that the B-cell activating factor (BAFF) was preferentially expressed in visceral adipose tissue, and the serum concentration of BAFF was associated with the severity of nonalcoholic fatty liver disease (NAFLD). In this study, we aimed to identify the effect of BAFF on hepatocyte lipid synthesis.
Methods: Primary cultured hepatocytes were isolated from wild-type (WT) C57BL/6 mice or BAFF mice fed a high-fat diet (HFD) for 24 weeks. The expression of lipid synthesis-related genes was analyzed by real-time RT-PCR. Additionally, primary hepatocytes from WT mice or BAFF mice were cultured with 0.3 mM palmitate for 18 hours in vitro, and triglyceride content in hepatocytes was measured.
Results: The expression of genes related to lipid synthesis, such as SREBP-1, ACC, FAS, and SCD1 was significantly downregulated in hepatocytes from HFD-fed BAFF mice compared to those from HFD-fed WT mice ($p<0.01$). When cultured with palmitate, triglyceride accumulation in hepatocytes from BAFF mice was lower compared to WT mice ($p<0.01$).
Conclusion: BAFF induces hepatic steatosis via the promotion of de novo lipogenesis. These results indicate that BAFF may serve as a therapeutic target for NAFLD treatment.

P-11 #10026

Development of Human Hepatic Stellate Cell Activation/Deactivation Culture Condition Aiming the Liver Fibrosis Valuation

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Background: Nonalcoholic steatohepatitis (NASH) is associated with the accumulation of extracellular matrix (ECM) proteins produced by activated hepatic stellate cells. We have already reported that culturing hepatic stellate cells in spheroids led to their deactivation. In this study, we investigated the degree of activation of hepatic stellate cells in order to construct an evaluation system for their activation/deactivation based on change of cell morphology.
Methods: Human hepatic stellate cell line LI90 cultured on a spheroid-forming culture plate EZSPHERE (AGC) to form spheroids. These spheroids were cultured in normal culture plates as adherent culture or in low attachment culture plates as non-adherent culture. Activation was assessed by morphology and filamentous actin (F-actin) formation. Gene expressions of activation markers were measured by quantitative PCR. In addition, activation of spheroids by TGF-β stimulation were evaluated in each culture condition.
Results: Spheroid-form LI90 cells showed reduced gene expressions of activation markers. When spheroids were cultured in adherent culture, elongated cells and F-actin were observed, and the gene expressions of activation markers increased. On the other hand, in non-adherent culture, the spheroid morphology was maintained and the gene expressions of activation markers remained low. These results indicated change of LI90 cells from deactivated phenotype to activated one. Moreover, stimulation of these cells by TGF-β induced proliferation and increased the gene expressions of activation markers.
Conclusion: The results indicated that spheroid culture of stellate cells could be useful for the evaluation system of their activation/deactivation and liver fibrosis.

P-12 #10025

Ezetimibe Suppresses the Progression of NASH and NASH-associated HCC

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Background: Hypercholesterolemia is a feature of patients with nonalcoholic steatohepatitis (NASH). Recent reports have demonstrated that statins could prevent the progression of NASH and HCC in humans. However, we have little information whether ezetimibe prevents the progression of NASH and NASH-associated HCC.
Methods: We investigated effects of ezetimibe in a mouse NASH model. Hepatocyte-specific phosphatase and tensin homolog-deficient (Pten KO) mice were fed standard diet or high fat (HF) diet for 40 weeks.
Results: In the standard diet group, Pten KO mice at 48 weeks of age showed steatohepatitis as well as multiple tumors in the liver. A HF diet feeding promoted the severity of steatohepatitis and liver tumors. Serum cholesterol levels showed a mild elevation in standard diet group and marked elevation in the HF diet group. Ezetimibe reduced serum cholesterol levels and the development of liver tumors in HF diet group but not standard diet group, in which serum cholesterol levels were not decreased. The HF diet feeding increased serum levels of VEGF, a crucial component of angiogenesis as well as liver fibrosis. The number of VEGF-positive cells and vascular endothelial cells increased in the liver of Pten KO mice fed HF diet. Ezetimibe decreased serum VEGF levels and proliferation of tumors cells. In the liver, Kupffer cells increased expression of VEGF in response to fat overloading both in vivo and in vitro experiments.
Conclusion: Ezetimibe prevents the progression of NASH and NASH-associated HCC s in Pten KO mice fed a HF diet.

P-13 #10038

Analysis of Liver Fibrosis Progression, Lipid Profile, and Atherosclerosis in NAFLD

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Background: High-density lipoprotein (HDL)-C is reportedly decreased in non-alcoholic fatty liver disease (NAFLD), although its function and clinical implications are unknown. This study analyzed the correlations among fibrosis progression, HDL function, and atherosclerosis in NAFLD using the novel HDL-cholesterol-uptake-capacity (HDL-CUC) method.
Methods: Among 215 NAFLD patients who visited our hospital between 2015 and 2019, 141 (median age: 56 years; 59 male) were retrospectively enrolled after excluding patients with lipid-lowering therapy or concomitant hepatocellular cancer or liver failure. HDL function was calculated as HDL-CUC/HDL-C, and aortic calcification (AC) was judged as calcification in the aorta on ultrasonography or CT. Statistical comparisons were made among clinical parameters and AC.
Results: HDL function correlated positively with M2BPGi ($r=0.42$, $P<0.001$) and FIB-4 Index ($r=0.22$, $P=0.009$). Next, advanced and mild fibrosis groups were established based on FIB-4 Index scores. LDL-C was significantly lower (109 vs. 126 mg/dL, $P<0.001$), HDL function was significantly higher (1.74 vs. 1.45, $P=0.012$), and AC frequency tended to be higher in the advanced fibrosis group. Patients with AC (n=58) were significantly older than those without AC (n=83) (66 vs. 48 years, $P<0.001$) and exhibited significantly higher M2BPGi (1.11 vs. 0.85 COI, $P=0.005$) and FIB-4 Index (2.0 vs. 1.0, $P<0.001$). LDL-C was significantly lower in the AC group (116 vs. 124 mg/dL, $P=0.004$), whereas HDL function was comparable (1.56 vs. 1.51, $P=0.99$).
Conclusion: In NAFLD, aging and liver fibrosis progression appears to be complicated by AC, which may be accompanied by the compensatory lipid profile changes of lower LDL-C and higher HDL-CUC.

P-14 #10030

Glycine Ameliorates Steatohepatitis via Reduction of Oxidative Stress in Hepatocyte-specific PTEN-Deficient Mice

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Background: We investigated the effect of amino acid glycine on hepatocyte-specific phosphatase and tensin homolog deleted on chromosome 10 (PTEN) deficient mouse, which exhibits pathological findings of NASH.
Methods: TG (AlbCre) $Pten^{flox/flox}$ male aged 11 to 15 weeks were fed a normal diet (KO group) or a 5% glycine-containing diet (KO+Gly group) for 2 weeks. TG (-) $Pten^{flox/+}$ mice fed normal chow were used as control. Oxidative stress was evaluated by immunohistochemical staining for 4-hydroxynonenal (4HNE), and mRNA was quantified by RT-PCR.
Results: The serum concentration of glycine was not different between control and KO, and increased more than 5 times in KO+Gly. There was no difference in body weight among groups. KO showed severe steatosis with ballooning hepatocytes and inflammatory cell infiltration, and serum ALT level of KO significantly increased to 163±30 IU/L compared to 41±4 IU/L in control, whereas in KO+Gly, lipid droplet decreased and serum ALT levels decreased to 23±10 IU/L ($p < 0.05$). In liver tissue of KO, the expression of 4HNE was enhanced predominantly in Zone 3, and suppressed in KO+Gly. The expression of mRNA for tumor necrosis factor α in liver was increased more than 2-fold in KO, and decreased to the same level as control in KO+Gly. Expression of catalase and superoxide dismutase 1 mRNA was significantly reduced in KO and increased in KO+Gly to the same level as in control ($p < 0.05$).
Conclusion: Our findings indicated that glycine enhanced the expression of antioxidant enzymes decreased by PTEN deficiency, inhibited oxidative stress and ameliorated steatohepatitis.

P-15 #10015

Synergistic Regulation of Hepatic Fsp27b Expression by HNF4α and CREBH

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The CIDE (cell death-inducing DFF45-like effector) family composed of CIDEA, CIDEB, CIDEC/FSP27 (fat-specific protein 27), has a critical role in growth of lipid droplets. Of these, CIDEB and CIDEC2/FSP27B are abundant in the liver, and the steatotic livers, respectively. Hepatocyte nuclear factor 4α (HNF4α) has an important role in lipid homeostasis because liver-specific HNF4α-null mice (*Hnf4a*$^{\delta Hep}$ mice) exhibit hepatosteatosis. We investigated whether HNF4α directly regulates expression of CIDE family genes. Expression of Cideb and Fsp27b was largely decreased in *Hnf4a*$^{\delta Hep}$ mice, while expression of Cidea was increased. Similar results were observed only in CIDEC2, the human orthologue of the *Fsp27b*, in human hepatoma cell lines in which HNF4α expression was knocked down. Conversely, overexpression of HNF4α strongly induced CIDEC2 expression in hepatoma cell lines. Furthermore, HNF4α transactivated *Fsp27b* by direct binding to an HNF4α response element in the *Fsp27b* promoter. In addition, *Fsp27b* is known to be transactivated by CREBH that is regulated by HNF4α, and expression of CREBH was induced by HNF4α in human hepatoma cells. Co-transfection of HNF4α and CREBH resulted in synergistic transactivation and induction of *Fsp27b* compared to that of HNF4α or CREBH alone. These results suggest that HNF4α, in conjunction with CREBH, plays an important role in regulation of *Fsp27b* expression.

P-16 #10040

A Novel Isolation Method of Hepatic Stellate Cells from Tumor Tissue of Obesity-associated Hepatocellular Carcinoma

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Background: Hepatic stellate cells (HSCs) have been identified as a prominent hub for inter-cellular and inter-molecular crosstalk in liver cirrhosis and NASH (non-alcoholic steatohepatitis) and also play a role as CAFs (cancer-associated fibroblast) in hepatocellular carcinoma (HCC) tissues. However, the efficient isolation methods of HSCs from tumor tissues in less heat-stressed conditions that minimize heat-associated artifacts have not been established yet.
Methods: An isolation method for HSCs that can preserve the cell status in obesity-associated HCC tumors in mice is developed by Pronase E-Collagenase perfusion at 6 degrees Celsius, followed by Nycodenz-based density gradient centrifugation to exclude the contamination of other types of cells.
Results: Primary HSCs isolated by this cold enzymatic method were analyzed by flow cytometry and single-cell RNA sequencing analysis, which represented the HSC features in the obesity-associated HCC microenvironment. Besides, our analysis indicated CD49a as an excellent marker of HSCs in both normal and HCC-bearing mouse livers which allowed efficient purification by CD49a-based cell sorting of HSCs. Moreover, we confirmed that this method was also extended to other obese mouse models and human liver tumor tissues with minor modifications.
Conclusions: We developed a novel cold isolation method showing the CD49a-high HSCs with extraordinarily high purity. This method enables us to isolate HSCs from both healthy and HCC livers, which provides the foundation for investigating the role of HSCs in the obesity-associated HCC microenvironment.

P-17 #10036

Type IV Collagen 7S is the Most Accurate Test for Identifying Advanced Fibrosis in Non-alcoholic Fatty Liver Disease with Type 2 Diabetes

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This study aimed to examine whether the diagnostic accuracy of four noninvasive tests (NITs) for detecting advanced fibrosis in non-alcoholic fatty liver disease (NAFLD) is maintained or is inferior to with or without the presence of type 2 diabetes. Overall, 874 patients with biopsy-proven NAFLD were enrolled. After propensity score matching by age, sex, and the prevalence of dyslipidemia, 311 patients were enrolled in each group of with or without diabetes. To evaluate the effect of diabetes, we compared the diagnostic accuracy of the fibrosis-4 (FIB-4) index, the NAFLD fibrosis score (NFS), the AST to platelet ratio index (APRI), and type IV collagen 7S (COL4-7S) in patients with NAFLD with and without diabetes. The AUROCs for identifying advanced fibrosis in patients without diabetes were 0.879 for the FIB-4 index, 0.851 for the NFS, 0.862 for the APRI, and 0.883 for COL4-7S. The AUROCs in patients with diabetes were 0.790 for the FIB-4 index, 0.784 for the NFS, 0.771 for the APRI, and 0.872 for COL4-7S. The AUROC of COL4-7S was significantly larger than that of the other NITs in patients with NAFLD with diabetes than in those without diabetes. The optimal high and low cutoff points of COL4-7S were 5.9 and 4.8 ng/mL, respectively. At the low cutoff point, the accuracy of COL4-7S was better than that of the other NITs, especially in patients with diabetes.
Conclusion: COL4-7S measurement might be the best NIT for identifying advanced fibrosis in NAFLD, especially in NAFLD with diabetes.

P-18 #10055

Systemic Inflammation Predict 30-day Bacterial Infection Post-liver Transplantation

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Objective: The relationship between aseptic systemic inflammation and postoperative bacterial infection is unclear. We investigated the correlation of systemic inflammation biomarkers with 30-day clinically significant bacterial infections (CSI) after LT.
Methods: This retrospective study enrolled 940 patients who received LT and followed for 30 days. The primary end point was 30-day CSI events. The cohort was divided into exploratory (n=508) and validation sets (n=432) sets according to different centers. Area under the receiver operated characteristic (AUROC) and Cox regression models were fitted to study the association between baseline systemic inflammation levels and CSI after LT.
Results: A total of 255 bacterial infectious events in 209 recipients occurred. Among systemic inflammation parameters, baseline C-reactive protein (CRP) was the best predictor of 30-day CSI (AUROC, 0.712). The predictive ability of CRP was comparable with MELD score, and was independently associated with 30-day CSI in multivariable analysis. These results were confirmed in a validation cohort. Additional, CRP levels were correlated with bacterial product lipopolysaccharide.
Conclusions: Our study suggests that pre-transplantation CRP is independent of other prognostic factors for 30-day CSI post-LT, and can be integrated into tools for assessing the risk of bacterial infection post-LT or as a component of prognostic models.

P-19 #10021

Elevated Alpha Fetoprotein in the Absence of Hepatic Malignancy in a Patient with Acute Hepatitis

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Objective: To describe a case of elevated AFP in a case of acute hepatitis and its pathophysiology
Materials and Methods: Case report
Results: The patient is a 26-year-old Asian male presenting with jaundice. He had no personal or family history of liver disease, occasional alcohol use, and denies drug or supplement use. Laboratory workup revealed hepatocellular type of liver injury (total bilirubin of 478 umol/L, direct bilirubin 401 umol/L, indirect bilirubin 77 umol/L, AST 1135 U/L, ALT 1592 U/L, LDH 53 U/L8, ALP 139 u/L), elevated AFP (3337 IU/ml), elevated INR, positive autoimmune panel (ANA, Anti-Sm, Anti-TPO, Anti-TG). Serological examinations revealed past infection with EBV and CMV. Imaging tests did not show ductal obstruction or the presence of mass lesions. Subsequent liver biopsy demonstrated interface hepatitis. The patient was treated as a case of autoimmune hepatitis and was started on glucocorticoids with clinical and biochemical improvement including normalization of AFP levels.
Conclusion: This study demonstrated the low specificity of AFP as it relates to these unusually elevated levels even as compared to other studies on non-malignant cases of liver disease. In this patient with acute hepatitis, AFP elevation may represent hepatic regeneration.

P-20 #10005

Nanoparticle-mediated Delivery of 2-deoxy-D-glucose Induces Antitumor Immunity and Cytotoxicity in Liver Tumors in Mice

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Background: Immune checkpoint inhibitors have shed light on the importance of antitumor immunity as a therapeutic strategy for hepatocellular carcinoma (HCC). The altered glucose metabolism known as the Warburg effect is recently gathering attention as a cancer immune resistance mechanism. Considering glycolysis inhibitors as therapeutic agents, their specific delivery to cancer cells is critical not to induce adverse effects. Thus, we investigated antitumor effects of a glycolysis inhibitor, consisting of 2-deoxy-D-glucose (2DG)-encapsulated poly (lactic-co-glycolic acid) (PLGA) nanoparticles (2DG-PLGA-NPs), against HCC in mice.
Methods: The antitumor effects of 2DG-PLGA-NPs were examined using hepatoma cell lines, xenograft tumors, and hepatocarcinogenic and syngeneic mouse models.
Results: The 2DG-PLGA-NPs induced cytotoxic effects and antitumor immunity through enhanced T cell trafficking. Additionally, 2DG-PLGA-NPs induced decreased lactate production and increased IFN-gamma-positive T cells in liver tumors. Human CD8+ T cells cocultured with 2DG-PLGA-NP-treated Huh7 cells revealed their increased IFN-gamma production and glucose uptake compared to the CD8+ T cells cocultured with PLGA-NP-treated Huh7 cells. Chemotaxis of CD8+ T cells was suppressed by lactate and enhanced by glucose. IFN-gamma enhanced CD8+ T cell chemotaxis in both an autocrine and paracrine manner. Notably, the 2DG-PLGA-NPs augmented chemokine (CXCL9/10) production in liver tumors via IFN-gamma-Jak-STAT1 pathway and AMPK-mediated suppression of histone H3 lysine 27 trimethylation. These 2DG-PLGA-NPs not only amplified antitumor effects induced by sorafenib or an anti-programmed death-1 (anti-PD1) antibody but also suppressed anti-PD1-resistant tumors.
Conclusion: The newly developed 2DG-PLGA-NPs demonstrated antitumor immunity and cytotoxicity in liver tumors in mice, suggesting the potential of 2DG-PLGA-NPs for future.

P-21 #10033

The Complication Rate and Clinical Significance of Extrahepatic Autoimmune Diseases (EHAIDs) in Primary Biliary Cholangitis (PBC)

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Background: Primary biliary cholangitis (PBC) was sometimes associated with extrahepatic autoimmune diseases (EHAIDs). The aim of this study is to clarify the prevalence and clinical significance of EHAIDs in PBC patients.
Methods: A total of 209 PBC patients who were measured all of the following marker (mitochondrial antibody, anti-nuclear antibody, anti-centromere antibody, anti-thyroid peroxidase antibody, anti-thyroglobulin antibody, anti-SSA antibody, anti-SSB antibody, RF, and anti-CCP antibody) were included. EHAID was defined as cases of systemic lupus erythematosus, systemic scleroderma, localized scleroderma, chronic thyroiditis, Sjogren's syndrome, and rheumatoid arthritis. The prevalence of EHAID and clinical markers at diagnosis were evaluated.
Results: Median age was 61 years old and 81% were female. EHAID were diagnosed in 54 (26%) patients (SLE: 1%, systemic scleroderma: 2%, localized scleroderma: 4%, chronic thyroiditis: 12%, Sjogren's syndrome: 6%, rheumatoid arthritis: 5%). There were more women in the EHAID (+) group than in the EHAID (-) group (96.3% vs 76.1%, p=0.001). EHAID (+) group have lower T-Bil (0.7 vs 0.8 mg/dL, p=0.004) and lower γGTP (103 vs 162 U/L, p=0.014). However, there was no difference in ALP (397 vs 464 U/L, p=0.112). The UDCA response score was higher in the EHAID (+) group (0.95 vs 0.94, p=0.045).
Conclusions: EHAID was associated with about one quarter of all PBC patients. Chronic thyroiditis was most common EHAIDs in our cohort. PBC patients with EHAIDs have a less aggressive clinical phenotype at presentation.

P-22 #10058

Role of CD40 in Hepatocellular Carcinoma

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Background: Hepatocellular carcinoma (HCC) is one of the most common primary liver malignancy and is a leading cause of cancer-related death worldwide. This study aimed to analyze the role of CD40, a member of the TNF-receptor superfamily, in tumor progression.
Methods: 168 HCV-infected patients including 47 HCC and 121 non-HCC were enrolled in this study. The level of soluble (s) and membrane bound (m) CD40 were detected in plasma and tissue samples, respectively. CD4+T cells were isolated from peripheral blood of healthy donor, activated, and co-cultured with HLF, a HCC cell line.
Results: The plasma level of sCD40 was increased significantly in HCC compared to non-HCC group. mCD40 was found highly expressed in B cells, macrophages, and cancer cells in tumor but not in non-tumor areas. In vitro, three HCC cell lines expressed CD40 including HLE, HLF, and SNU-387. After activation, CD4+T cells isolated from healthy donor expressed CD40 ligand. Co-cultured HLFs with activated CD4+T cells resulted in elevation of CD40 at both RNA and protein level compared to single-cultured HLFs in both ratio- and time-dependent manners, with the more number of CD4+T cells over cancer cells, the higher CD40 expression level. In addition, Cyclin D1 was also increased in co-cultured cancer cells after 1 hour onwards. Level of CD40 and CyclinD1 did not change when HLFs were co-cultured with inactivated CD4+T cells that lack CD40 ligand.
Conclusion: HCC patients have higher level of sCD40 compared to those without HCC. Interaction of CD4+T cells with HCC induced CD40 expression.

P-23 #10049

Changes of Soluble Immune Checkpoint Proteins following Antiviral Treatment in Chronic Hepatitis C Patients and the Roles of the CD27-CD70 Pathway in Hepatocellular Carcinoma Development

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Immune checkpoint proteins (ICPs) are being studied extensively in various cancers such as melanoma, non-small lung cancer, but not hepatocellular carcinoma (HCC). This study investigated the role of soluble ICPs. 168 chronic hepatitis C (HCV) patients have enrolled, who has developed HCC (n = 47) and not (n = 121). Plasma samples were collected at baseline, end of treatment, SVR, and endpoint (time of HCC occurrence) and measured levels of 16 ICPs. Interestingly, at baseline, HCC group showed higher levels of sCD27, sCD28, sTIM-3, sHVEM, sCD40, sTLR-2, sPD-1, sCD80, sCD86 compared with non-HCC group. During antiviral treatment, the differences of ICPs were depleted. However, at endpoint, levels of sCD27, sCD28, sCD40, sCD86 in HCC group returned higher than non-HCC group. By Kaplan-Meier analysis, patients with high baseline levels of sCD27 and sCD40 indicated greater HCC cumulative rate. Next, we observed that CD27 was located dominantly in T cells, partly in B cells and macrophages, whereas CD70 was strongly expressed in HCC tissues and all 6 HCC cell lines. In vitro, stimulation of CD27-CD70 axis in HepG2 cells revealed the cell proliferation escalated in time dependent manner. Moreover, we have found the up-regulated transcriptions of cell division stimulators including cyclin A2, B1, E2, CDK1/2, while p21, a cell cycle inhibitor, dropped. Anti-apoptosis genes, in contrast, clearly elevated including cIAP-1, -2. Our results suggested that baseline sCD27, sCD40 levels can be used as HCC prognostic factors in HCV SVR patients. sCD27 may promote cancer cell proliferation and protect them from apoptosis.

P-24 #10012

Case Report of Overlap Syndrome: Autoimmune Hepatitis and Primary Biliary Cholangitis, a Vietnamese Patient Diagnosing by Pathology

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In 1940, Waldenstrom reported the first clinical case of autoimmune hepatitis with the term chronic active hepatitis (CAH). In 1955, Joske discovered autoantibodies of lupus erythematosus in CAH patients. The term autoimmune hepatitis was used since 1965 and was officially published in 1993. Autoimmune hepatitis (AIH) is an autoimmune disease that occurs when the body's immune system fights against liver cells. Autoimmune hepatitis accounts for 11-23% of chronic hepatitis. The prevalence of autoimmune hepatitis in Asia - Pacific: Singapore is 4/100,000 people, in Brunei: 5.61/100,000 people, in Australia: 8.0/100,000 people. The coexistence of AIH and features of cholestatic liver diseases have been designated as either a cholestatic variant syndrome (CVS) or overlap syndrome (OS). The term OS implies the coexistence of AIH and either PBC or PSC. The term CVS indicates coexistence of AIH and cholestatic features resembling either PBC or PSC. The prevalence of PBC and AIH in one study ranged from 2.1% to 19% using the Paris criteria and IAIHG revised criteria. In patients with PBC who present with high transaminases and hypergammaglobulinemia, overlap syndrome should be suspected. As AIH-PBC overlap syndrome is a rare disease, timely and accurate diagnosis and early intervention may potentially improve long-term adverse outcomes in patients with overlap syndrome. On the other hand, over diagnoses can lead to unnecessary overtreatment and adverse medication effects. We report a clinical case, which was diagnosed PBC and AIH by liver biopsy.

P-25 #10051

Functional Analysis of Liver Cirrhosis-associated Gut Microbiota Uncovers a Unique Mechanism of Hyperammonemia in Hepatic Encephalopathy

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Hepatic encephalopathy (HE) is a serious condition of liver cirrhosis (LC) associated with neuropsychiatric impairment. Dysbiosis of the gut is associated with liver diseases such as LC and liver cancer as well as HE, because hyperammonemia could be caused by increase of ammonia producing gut microbiota. However, the key microbial species that could cause HE remain to be elucidated. We aimed to identify bacteria involved in the pathophysiology of HE, based on the difference of the gut microbial profiles between hyperammonemia patients including HE, and healthy controls (HC). A cohort study was conducted to collect the feces of HC and HE groups from April 2017 until March 2020, and the gut microbiota profile was compared using the data of 16S rRNA gene sequencing analysis and metagenomic analysis to identify causal bacterial species for HE. As a result, clear differences in the gut bacterial profiles were detected between HC and HE groups, identifying a strong candidate of bacterial species that was significantly abundant in the HE group. The oral administration of the identified bacteria in the CCl4-treated cirrhosis mouse model elevated the levels of blood NH3 and brain glutamine, showing that the candidate bacteria indeed induced HE phenotype. Moreover, Rifaximin was found to be effective for this HE-associated bacterial species. In this study, we identified a strong candidate of bacterial species involved in the pathophysiology of HE using patient samples, and confirmed the relationship between this bacterial species and hyperammonemia in mice.

P-26 #10035

Role of the FibroScan-aspartate Aminotransferase Score in Risk Stratification for a Japanese Cohort with Fatty Liver Diseases

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Aims: To prove that the FibroScan-aspartate aminotransferase (FAST)-score can be used to stratify disease severity in Japanese cohort with fatty liver diseases [metabolic dysfunction-associated fatty liver disease (MAFLD) and nonalcoholic fatty liver disease (NAFLD)].
Methods: All participants (n=2,254) underwent liver stiffness measurements and controlled attenuation parameter assessments. We compared the clinical characteristics of the patients with MAFLD and NAFLD using the FAST score. We also explored the independent determinants of FAST scores >0.35, which indicated possible progressive disease.
Results: MAFLD was diagnosed in 789 (35.0%), while NAFLD was diagnosed in 618 (27.4%) of the overall population. MAFLD had a higher proportion of patients with a condition that suspected progressive liver disease than NAFLD [68 (8.6%) vs 48 (7.7%)]. The area under the receiver-operating characteristic curve of the FAST score for diagnosing advanced fibrosis was 0.969 in MAFLD and 0.965 in NAFLD. Multivariate analyses determined that diabetes mellitus, alanine aminotransferase (ALT) level, fatty liver index (FLI), and Fibrosis-4 index (FIB-4) independently predict FAST scores >0.35 in patients with MAFLD. The ALT level had the highest correlation with the FAST score (ρ=0.7817).
Conclusion: The FAST score could stratify the disease severity in a Japanese cohort with both MAFLD and NAFLD.

P-27 #10039

Possible Involvement of ZNF641 with the Prognosis of Hepatocellular Carcinoma

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Background: We have identified hepatoma-derived growth factor (HDGF), which participates in the progression of hepatocellular carcinoma (HCC). Recently, we determined two microRNAs (miR-6072 and miR-3137) that were induced in response to the administration of HDGF and were related to the prognosis of HCC patients. We further searched for the target genes of these HDGF-related microRNAs.
Methods: Using the microarray method (Human Oligo chip 25k Ver. 2.10, Toray, Japan), we determined the mRNAs that increased (>1.5-fold) or decreased (<0.67-fold) after the administration of HDGF in two hepatoma-derived cell lines (HepG2 and SK-Hep1). Using an open-access databank, we determined the genes that were predicted to be the target genes of both miR-6072 and miR-3137. Finally, using the public cancer genomics database and the Kaplan-Meier method, we determined a gene that was associated with the prognosis of HCC patients.
Results: Among a total of 1132 genes which were found to be candidate target genes of the two HDGF-related microRNAs, we determined 7 mRNAs that were changed commonly after stimulation with HDGF in the 2 hepatoma-derived cell lines. Using the cancer genomics database, we evaluated 351 HCC patients and found that a high copy number of the ZNF641 (Zinc Finger Protein 641) gene was significantly related to an unfavorable prognosis with poorer survival (p=0.015, 0.007 and 0.028 for 1-, 3-, and 5-year survival, respectively).
Conclusion: Using a microarray method, ZNF641 was found to be a potential target gene of HDGF-related microRNAs, and it was also related to the prognosis of HCC.

P-28 #10004

The Long Non-coding RNA of RMRP is Repressed by PERK and Induces Apoptosis in Hepatocellular Carcinoma

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Backgrounds: Endoplasmic reticulum (ER) stress plays an important role in carcinogenesis and cancer progression. PKR like endoplasmic reticulum kinase (PERK) is a major ER stress molecule and is activated by ER stress. The aim of this study is to identify novel intercellular molecules that are affected by PERK and to investigate the role of these molecules in cancer progression.
Methods: We used siRNA to knockdown PERK in hepatocellular carcinoma cell lines and identified the molecule which increased expression by transcriptome analysis. We modulated PERK expression using a plasmid and tunicamycin against PERK, and then confirmed the target gene expression. We further analyzed the apoptotic function.
Results: Transcriptome analysis revealed that expression of the RNA component of mitochondrial RNA processing endoribonuclease (RMRP) was strongly correlated with PERK expression. RMRP was downregulated by PERK expression plasmid and endoplasmic reticulum stress. In hepatocellular carcinoma, endoplasmic reticulum stress suppressed RMRP through PERK. In addition, knockdown of RMRP induced apoptotic cell death. RMRP downregulation activates the endogenous apoptotic signaling pathway in hepatocellular carcinoma lines. The downregulation of RMRP by endoplasmic reticulum stress may activate the endogenous apoptotic signaling pathway and promote apoptosis induction.
Conclusion: The long noncoding RNA of RMRP was downregulated by PERK and induced apoptosis in hepatocellular carcinoma.

P-29 #10011

Identifying UGT1A1 Gene Mutations in Vietnamese Patients with Gilbert Syndrome

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Gilbert's syndrome (GS) is the most common inherited disorder of bilirubin metabolism, non-lethal, affecting 3-12% of the population. The genetic variants of the UDP- glucuronosyltransferase 1A1 (UGT1A1) gene might reduce the gene transcription activity and its enzyme expression, which affects the ability to conjugate glucuronidation in the liver. This study aimed to identify genetic variants of UGT1A1 in two Vietnamese sibling brothers with jaundice manifestations suspected GS. The peripheral blood samples of patients were used to extract genome DNA and sequence the enhancer, promoter, and coding all five exons of UGT1A1. Two pathogenic variants c.-3279T & gt G located in the phenobarbital responsive enhancer module (gtPBREM) and A(TA)7 TAA of the TATA box in the promoter region were identified. They are twice common pathogenic variants that were reported in almost hyperbilirubinemia individuals from different populations. The obtained results improved the accuracy of medical diagnosis and warned the patients to be cautious in case they have to use medical drugs in the future.

P-30 #10020

Validation of Platelets - Albumin - Bilirubin (PALBI) Score for Predicting Overall Survival of Hepatocellular Carcinoma at Hanoi Medical University Hospital, Vietnam

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Backgrounds: To validate PALBI score for predicting overall survival of hepatocellular carcinoma.
Methods: n a retrospective study from June 2017, 67 HCC patients at Hanoi Medical Hospital and Bach Mai Hospital were collected and followed up median overall survival until June 2021. The Platelets Albumin Bilirubin (PALBI) score was calculated based on three indexes: platelets, albumin, and total bilirubin as PALBI score= (2.02xLog10Bilirubin)+(0.37x(Log10Bilirubin)2+(0.04xAlbumin)+(3.48xLog10Platelets)+(1.01x(Log10Platelets)2.
PALBI score was classified into 3 groups: PALBI 1 <2.53; -2.53 <PALBI 2 <2.09 and PALBI 3 >-2.09. PALBI score, Child-Pugh classification, and BCLC classification were also calculated to compare the predictive value by the receiver operating curves (ROC).
Results: This study included 67 HCC patients in which the proportion of males is 92.5% The median age of the research group is 59 years. Hepatitis B accounts for 80.6% of HCC patients. The median overall survival of PALBI 1, PALBI 2, PALBI 3 is 35.14 months, 28.25 months, and 10.14 months, respectively. The difference in overall survival of these three groups was statistically significant with p = 0.000. Additionally, ROC analysis in the entire cohort revealed that the PALBI score had a better AUC than the Child Pugh score and BCLC classification. In HCC patients who have Child-Pugh A classification, the PALBI score divided into 3 groups which have statistically significant differences among median overall survival.
Conclusions: These results suggested that the PALBI score could be an alternative hepatic function score for stratifying HCC patients.

P-31 #10016

Validation of ABCR and ART for Predicting Overall Survival in Patient with Hepato-cellular Carcinoma Treated with Transarterial Chemoembolism in Hanoi Medical University Hospital, Vietnam

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Objective: Validation of ABCR and ART score for predicting overall survival and re-treatment of hepatocellular carcinoma patient treated by conventional transarterial chemoembolization at Ha Noi Medical University Hospital.
Method: From January 2018 to December 2020, 30 consecutive HCC patients, mainly with the viral-induced disease, were treated with TACE. Using a regression model on the predictive variables of our population, we validated two scores designed to help for repeat TACE.
Result: In the multivariate analysis, three prognostic factors were associated with overall survival: BCLC and AFP (>200 ng/ml) at baseline and absence of radiological response. These factors were included in a score (ABCR, ranging from -3 to +6) which correlates with survival and identifies three groups. The ABCR score was validated and proved to perform better than the ART score in distinguishing between patients' prognoses.
Conclusion: The ABCR and ART score is a simple and clinically relevant index, summing several prognostic variables endorsed in HCC. An ABCR score of >3 and ART > of 1.5 before the second TACE identifies patients with dismal prognosis who may not benefit from further TACE.

P-32 #10024

A Novel Anticancer Therapy with Deferoxamine and Drugs Targeting Iron-chelation-modulated Metabolism

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Background: Iron is an essential trace element, and although iron chelation has garnered attention as a novel therapeutic strategy for cancer, there is a demand for a more efficient method of treatment is increasing. In the present study, we examined a combined anticancer therapy that targeted the metabolic changes forcibly induced upon iron chelation.
Methods: The deferoxiamine (DFO)-resistant cell lines were established by gradually increasing the DFO concentration. To delineate the mechanisms underlying resistance in a DFO-resistant strain, metabolomic analysis was performed. The synergistic effect of the drugs was examined by in vitro proliferation assay, and combination index was estimated with the CalcuSyn software program. A xenograft model in nude mice was used to evaluate the drug effects in vivo.
Results: Enhanced glycolysis, de novo nucleic acid synthesis, and reduced mitochondrial metabolism are found in DFO resistant HeLa cells. Synergistic effect of DFO and lactate excretion inhibitor (CHC) was observed in HeLa cells, but not in Huh7 cells. Metabolome analysis of the DFO-resistant Huh7 cells revealed enhanced glycolysis and salvage cycle, alters in glutamine metabolism, and accumulation of dipeptides. Huh7 cultured in the absence of glutamine showed enhanced sensitivity to DFO, and glutaminase inhibitor (CB839) showed a synergistic effect with DFO. Furthermore, the effect of DFO was enhanced by autophagy inhibitor (chloroquine) in both in vitro and in vivo xenograft model.
Conclusion: DFO-induced metabolic changes are specific targets for developing an efficient anticancer combinatorial therapy using DFO. These findings will be beneficial for the development of novel cancer chemotherapeutics.

P-33 #10059

Identification of Key Modules and Hub Genes Involved in Cholangiocarcinoma Progression and Prognosis

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The current diagnosis of Cholangiocarcinoma (CCA) by non-invasive methods has insufficient accuracy, and the key role of most tumorigenesis molecules in CCA remains unclear. Therefore, it is important to detect the tumorigenic mechanism and develop new prognostic biomarkers for clinical applications. The mRNA sequencing data and clinical information of patients with CCA in The Cancer Genome Atlas (TCGA) were analyzed by weighted gene co-expression network analysis (WGCNA). Using WGCNA, we identified 61 hub genes that regulate the progression and prognosis of CCA. Eight hub genes (VSNL1, TH, PCP4, IGDCC3, RAD51AP2, MUC2, BUB1, and BUB1B) were identified, which exhibited significant interactions with the tumorigenic mechanism and prognosis of CCA. In addition, GO and KEGG analysis revealed that blue and magenta modules were associated with chromosome segregation, mitotic and oocyte meiosis, cell cycle, and sister chromatid segregation. Four hub genes (VSNL1, PCP4, BUB1 and BUB1B) were also verified as biomarkers of progression and prognosis by GSE119336 cohort data and human samples. WGCNA enabled identification of two modules and eight hub genes, which were associated with CCA progression and prognosis. Four genes were verified experimentally. However, these results should be verified in laboratory experiments and large-scale cohort studies.

P-34 #10031

PD-L1 Promotes Cell Proliferation in Liver Cancer Cells

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Immune checkpoint inhibitor (ICI) is a powerful tool to treat advanced malignant tumors. However, it could induce so-termed hyperprogressive disease (HPD) in patients with such tumors due to unknown mechanism. In this study, we assessed possible direct action of ICIs on PD-L1-expressing liver cancer cells under immune cell-free condition. Among 11 liver cancer cell lines, the sarcomatous HAK-5 highly expressed PD-L1, while HepG2 did not. Consistently, sarcomatous hepatocellular carcinoma (HCC) tissues expressed profound PD-L1. When HAK-5 cells were treated with anti-PD-L1 antibodies (durvalumab (Dur) and atezolizumab (Ate)) for 48 h, about 20% increase in cell proliferation was found compared with those treated with IgG and pembrolizumab, an anti-PD-1 antibody. PD-L1-nonexpressing HepG2 cells did not respond to Dur or Ate. Gene silencing and overexpression of PD-L1 in HAK-5 and HepG2 cells led to significant decrease and increase in cell proliferation, respectively. Zenografted tumor model of NOD/SCID mice using PD-L1-overexpressing HepG2 cells demonstrated significantly highest increase in tumor volume in the Ate-treated mice than control groups. Akt and Erk signaling pathways were activated after the PD-L1 stimulation. It was suggested that anti-PD-L1 antibodies promoted cell proliferation via PD-L1 in specific types of cancer, including sarcomatous HCC.

P-35 #10003

Efficacy of Zinc Acetate in HCC Cell Lines via the Induction of Apoptosis

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Background: Several kinds of zinc compounds showed remarkable antitumor effect on various types of malignant diseases. However, no previous data were available on antitumor effect of zinc acetate. We investigated antitumor effects of zinc acetate on hepatocellular carcinoma (HCC) in vitro.
Methods: Five HCC cell lines were used to evaluate the antitumor effects of zinc acetate. Cell viability was determined by the Cell Counting Kit-8 (CCK-8) assay. The cell-cycle alteration was evaluated by a flow cytometric analysis and the detection of cell cycle-related proteins. Apoptosis was determined based on the caspase-cleaved cytokeratin 18 (CCK-18) levels. The microRNAs (miRNAs) related to an antitumor effect of zinc acetate were identified using microarrays.
Results: Zinc acetate suppressed the proliferation of HCC cells in a dose-dependent manner. The treatment with zinc acetate resulted in increased CCK-18 levels and enhanced the expression of heme oxygenase-1 (HO-1) in HCC cells. The flow cytometric analysis revealed an increase of HCC cells in the S and G2/M phases by the administration of zinc acetate, and the expressions of ccdk2 and cyclin E were increased. The miRNA expression profile of the HCC cells treated with zinc acetate was extremely different from that of the untreated HCC cells.
Conclusions: These results suggest that the zinc acetate supplementation causes the apoptosis of HCC cells, but does not affect the cell cycle progression. Upregulation of HO-1 and the alteration of miRNAs' profile may be associated with antitumor effects of zinc acetate in HCC cells.

P-36 #10007

Liver Biopsy Technique in the Era of Cancer Genomic Therapies: A Single Center Retrospective Analysis

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Background: With the evolution of personalized medicine in the field of oncology, including optimal treatment selection by next-generation sequencing-based companion diagnostics systems and tumor-agnostic treatments according to common biomarkers, establishment of a method of liver tumor biopsy that can obtain a sufficient amount of tumor specimen is strongly required. The aim of this study was to evaluate safety and availability of a liver tumor biopsy technique for multiple punctures coaxial using introducer needle and embolization by gelatin sponge particles.
Methods: Patients with primary or metastatic liver cancer who underwent liver tumor biopsies with puncture tract embolization using gelatin sponge were included in the study from October 2019 to September 2020. We estimated the complication and diagnostic rate, and evaluated whether amounts of samples were sufficient to perform FoundationOne CDx criteria.
Results: Total 96 patients were enrolled. Median total number of punctures per patients was 3 times (range: 1-8 times). The pathological diagnostic rate was 79.2%, and using the FoundationOne CDx criteria, 84.9% of cases had the potential of collection the amounts of samples needed for genomic medicine. The rate of bleeding complication was 4.1% (n = 4), and only 1.0% (n = 1) was major bleeding required transfusion.
Conclusion: The liver biopsy with puncture tract embolization by gelatin sponge may be a safe and useful method to collect sufficient volume of specimens required in modern cancer treatments.

P-37 #10041

Intracellular Gaps Formation of Liver Sinusoidal Endothelial Cells Facilitates Cancer Cell Engraftment in Liver

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Intracellular gap formation of LSECs results from the destruction of the fenestration under histopathological conditions, but their role in liver metastasis remains uncharacterized. Therefore, this study aims to clarify the molecular function of the gaps in liver metastasis. In electron microscopic observations, we found the intrasplenic injection of hepatoma cell line, Hepa1-6 increased the intracellular gaps in LSECs, and protrusion of Hepa1-6 extended toward the gaps. In thioacetamide-induced liver fibrosis model, intracellular gaps of LSECs appeared only in the non-fibrotic region (2.0±1.3, $p<0.001$) and not in the fibrotic region, and the injected Hepa 1-6 engrafted mainly in the non-fibrotic region. In vitro studies have found that mRNA level of cell-cell adhesion molecule-1 (1.5-fold) was increased in LSECs cocultured with Hepa 1-6 and were selectively expressed around the LSEC gap. In addition, MMP-9 mRNA levels were increased 30-fold and the number of gaps was enhanced ($p<0.05$) compared to single cultures. Interestingly, MMPs inhibitor, doxycycline-pretreatment significantly reduced number of gaps in the cocultured-LSECs ($p<0.05$). In vivo, monocrotaline- matrix metalloproteinases (MMP) 2, 9 inducer, stimulated the gap formation in LSECs along with an increasing number of hepatic metastases (52.8±15.3) compared to control (29.9±4.8) after 3 days of cancer cell injection, but this effect was attenuated when mice treated with doxycycline (28.8±10.3, $p<0.01$). Furthermore, three-dimensions tomographic reconstruction image showed that cancer cells broke through the LSEC wall. Our study demonstrated that the intracellular gaps in LSECs promote the cancer cells' metastatic ability, which may lead to novel strategies to prevent liver metastasis.

P-38 #10045

Role of SKI in the Suppression of Cholangiocarcinoma Cell Proliferation by Inducing G1-phase Arrest: Validation of Results from Clinical Specimens

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Background: The role of aberrantly expressed mRNA and its interaction with noncod-ing RNA in cholangiocarcinoma progression is unclear. We conducted a comprehensive search for microRNAs using resected cholangiocarcinoma specimens to discover new ther-apeutic targets (IRB-approved at our institution).
Methods: The subjects were tissue specimens from tumor and non-tumor areas of the intrahepatic cholangiocarcinoma from surgically resected 10 cases without HBV/HCV infection. We analyzed the relationship be-tween oncogenes and cell proliferation in experiments on human cholangiocarcinoma cell lines (OZ, KKU100) through microRNA microarrays, principal component analyses.
Results: We extracted the Sloan Kettering Institute (SKI) mRNA with a high target ef-fectiveness of microRNA-3648 by in silico screening. Immunostaining of human cholan-giocarcinoma tumor sites showed that endogenous SKI protein expression intensity was suppressed compared to non-tumor sites. Cell proliferation of both OZ and KKU100 cells was inhibited by SKI overexpression, and that of OZ cell was promoted by SKI knock-down. The expression level of mRNA/protein of cyclin-dependent kinase inhibitor p21waf/cip1 in OZ cell was increased by SKI overexpression and decreased by SKI knock-down. Cell cycle transition from G1 to S and nuclear translocation of the DNA replication factor CDT1 in KKU100 cell were inhibited by SKI overexpression and promoted by SKI knockdown. p21waf/cip1 promoter-luciferase activity was increased by SKI overexpression.
Conclusions: It is postulated that the SKI protein, a potential therapeutic target, is involved in the transcription of p21waf/cip1 and brakes G1 phase of cell cycle, resulting in the suppression of cholangiocarcinoma cell proliferation.

P-39 #10034

The Association between Plasma Free Amino Acids and Sarcopenia in the Course of Hepatocellular Carcinoma Recurrence

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Background: Sarcopenia impairs the prognosis of patients with hepatocellular carcinoma (HCC). The aim of this study is to elucidate the plasma free amino acids (PFAAs) associated with the sarcopenia in the course of HCC recurrence.
Methods: One hundred eighty-seven patients with HCC were enrolled retrospectively. The skeletal muscle index (SMI) was measured by CT scan image at the third lumbar vertebra (L3). The concentrations of 24 PFAAs of fasting patients were measured by HPLC. We defined the changes of each parameter in the same patient between recurrences as d-, and the associations between sarcopenia and PFAAs were evaluated by a logistic regression model. The d-SMI was compared between the subjects that received branched-chain amino acids (BCAAs) formulation and the subjects without the formulation.
Results: Sarcopenia and myosteatosis were observed in 276 (56%) subjects and 313 (63%) subjects, respectively. Sarcopenia significantly decreased the survival rate, the 1-, 3-, and 5-y survival rates were 85%, 42%, and 9%, respectively. Multivariate analysis revealed that, in 24 PFAAs, the level of total BCAAs was significantly associated with sarcopenia. Furthermore, d-BCAAs, especially d-Leucine, were significantly correlated with the d-SMI. In the Child-Pugh (CP) grade B or C, the decrease of SMI was significantly suppressed in the subjects with the BCAAs formulation than that without BCAAs formulation.
Conclusions: Among the PFAAs, the level of BCAAs, especially leucine, in peripheral blood was associated with sarcopenia in the course of HCC recurrence. The BCAAs formulation is useful for preventing sarcopenia of patients with HCC.

P-40 #10010

A Survey on Approaches towards Patients with Elevated Liver Enzymes before Surgery in Different Vietnamese Hospitals

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A questionnaire on Google form was designed to explore doctor's approaches towards patients with elevated liver enzymes before a selective surgery in Vietnamese hospitals. 114 doctors with the mean age of 32.8 ±6.9 years completed our survey. 42.1%, 22.8%, and 13.2% of respondents were anesthesiologists, internal gastroenterologists and hepatologists, and surgeons, respectively. The doctor's approaches were categorized into 3 main groups: assessing the severity and etiologies, using liver-enzymes-reducing medicines, and consulting with others. To assess the severity and etiologies, 71.9%, 70.2%, and 58.8% of respondents decided to do hepatitis virus tests, liver function tests, and imaging exploration, respectively. 63/114 respondents decided to indicate all three tests, this trend was significantly popular in doctors with working experience of more than five years. Doctors who were not hepatologists had a high prevalence of requiring consultancy response (85.2%). Most respondents believed that liver-enzymes-reducing drugs should be prescribed when the level of liver enzymes is above 2-5 times the upper limit of normal (54.4%). In terms of delaying surgery decision, most responses (56.1%) were chosen when liver enzymes level is above 5 times the upper limit of normal, while others (13.1%) would not delay if the liver functions remaining stable regardless of liver enzyme levels. There is a lack of conformity in Vietnamese doctors' approaches towards patients with elevated liver enzymes before elective surgery. More scientific evidence and guidelines are needed to support for doctors to have a better decision.

Keywords: liver enzymes, elevated liver enzymes, before surgery.

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