United States — Japan Cooperative Medical Sciences Program

2024 International Conference on Emerging Infectious Diseases (EID) in the Pacific Rim

And meetings of the Acute Respiratory Panel, Cancer Panel, AIDS Panel, Hepatitis Panel, Viral Diseases Panel, Immunology Board, and the 2nd International Symposium for Infectious Diseases Research Institutes Cooperation (IDRIC)

March 5-8, 2024
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Japan Agency for Medical Research and Development

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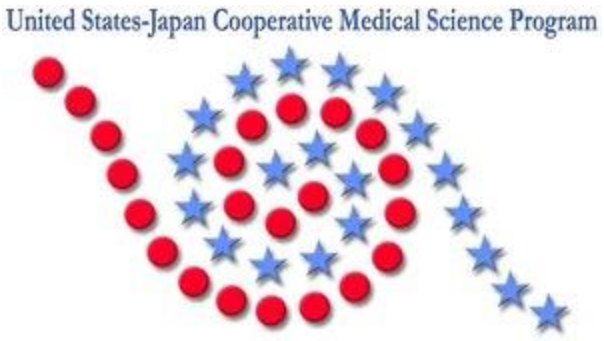

The Joint 24th International Conference on Emerging Infectious Diseases in the Pacific Rim of the U.S.-Japan Cooperative Medical Sciences Program (USJCMSP)

ABSTRACT E-BOOK

March 7 and 8: Hepatitis Panel

[in order of presentation]

TOPIC ONE: Progress in Development of New Therapies for Chronic HBV Infection and HBV+HDV Co-infection

Koichi Watashi – Japan

Structure of HBV preS1/NTCP complex and its implication to HBV entry

Hepatitis B virus (HBV) infection to host hepatocytes is initiated by binding of its preS1 domain in the viral surface antigen to sodium taurocholate cotransporting polypeptide (NTCP/SLC10A1), a hepatic bile acid transporter. We and other groups recently solved the structure of apo-state of NTCP, which was revealed to be a nine transmembrane protein (Park et al. Nature 2022). Here we solved the cryo-electron microscopy structure of preS1/NTCP complex (Asami et al. Nat Struct Mol Biol 2024). The overall structure of NTCP in complex with preS1 was similar to that of the outward-facing form of apo-NTCP, but preS1 enlarged the width of bile acid tunnel of NTCP by insertion. PreS1 formed a lasso-like structure in its N-terminal 30 amino acid region that embeds deeply into the bile acid tunnel of NTCP, followed by an extension with more C-terminal region that additionally contacts on the extracellular surface of NTCP. Based on the solved structure, HBV infection assays confirmed the importance of transmembrane 1 and 8b as well as 5 on the bile acid tunnel and a part of the extracellular face of NTCP for preS1 binding. These areas were overlapped but wider than the functional region for bile acid transport, suggesting that the viral receptor function involved larger face of NTCP than that for bile acid transporter function. Thus, the intrinsically disordered preS1 domain adopts to form a structure that produces large area of contact sites to NTCP. These results reveal a mechanism for establishing high-affinity virus-host attachment and provide information for rational design of anti-HBV entry inhibitors.

Kyun-Hwan Kim – Korea

Kazuaki Chayama, Japan

Biological Characterization of Various Hepatitis B Virus Strains Using Human Hepatocyte Chimeric Mice

The Hepatitis B virus (HBV) is commonly perceived as non-cytopathic, as healthy carriers exhibit no liver cell damage despite high viral loads. However, mutations within the precore (PC) and basal core promoter (BCP) regions of the HBV genome are linked to severe hepatitis and HBV reactivation. Yet, the direct impact of these mutations on liver damage remains poorly understood. To address this, we explored the mechanisms of direct cytopathic effects caused by PC/BCP mutants in vitro and in vivo, without interference from the immune response. Utilizing a hepatocyte humanized mouse model and human hepatocytes derived from these mice, we infected subjects with wild-type or mutant-type PC/BCP HBV, assessing HBV replication and hepatocyte damage. We observed drustic HBV proliferation in PC/BCP-mutant-infected mice, accompanied by significant human hepatocyte loss and slight elevation of human alanine transaminase (ALT) levels specifically in mutant-infected mice. Furthermore, in PC/BCP mutant infection, the accumulation of HBsAg in humanized livers coincided with endoplasmic reticulum (ER) localization, triggering apoptosis via the unfolded protein response in HBV-infected hepatocytes. RNA-sequencing unveiled the molecular underpinnings of the PC/BCP mutant infection phenotype in the humanized mouse model. The observed relatively lower ALT elevation and higher HBV DNA levels align with HBV reactivation traits, suggesting that the hepatocyte damage in this model mirrors HBV reactivation under immunosuppressive conditions. Our findings indicate that PC and BCP mutations enhance viral replication and induce cell death through ER stress in HBV infection models. This underscores the need for novel strategies to suppress viral replication and reduce HBsAg levels to improve patient survival in cases of fulminant hepatitis or HBV reactivation.

SF Akbar – Japan

Sustained Anti-viral and Anti-fibrotic Effect of HBV-specific Immune Modulator for Treatment of CHB Patients: Stoppage of NUCs in Sight

In treatment-naive CHB patients (HBV DNA in sera and sustained elevated ALT), a therapeutic vaccine containing HBsAg and HBcAg (NASVAC) has exhibited an excellent safety profile and profound antiviral and liver-protective capacities at the end of therapy (EOT), 2, 3, 5, and 10 years after EOT. In head-to-head comparison, patients receiving NASVAC did not progress to advanced fibrosis as compared to patients receiving pegylated interferon. In nucleoside-experienced CHB patients with elevated ALT or normal ALT, NASVAC reduced serum HBsAg levels. Recently, a group of patients who had been taking nucleoside analogs (NUCs) for years discontinued NUCs, and the patients were provided with NASVAC. There was no hepatitis flare when NASVAC replaced NUC, and they maintained low HBV DNA and ALT in the sera for 72 weeks. NASVAC seems to be a novel drug for treating different types of CHB, and the usage of NASVAC may be pertinent to achieving the goal of “Elimination of Hepatitis" by 2030, as NASVAC can be taken by nasal route and NASVAC is patients-friendly for developing countries.

Hiroshi Yotsuyanagi – Japan

HBV epidemiology after nationwide vaccination in Japan

In 1986, Japan initiated a project to prevent mother-to-child transmission of HBIG and HB vaccine to infants born to HBeAg-positive pregnant women; in 1995, this project was expanded to include all infants born to HBsAg-positive pregnant women. Epidemiological data in young adults revealed the need to prevent horizontal transmission, so nationwide HB vaccination was initiated in 2016.

Pediatric HBV infections have decreased dramatically since the start of nationwide immunization, and as of 2020, the anti-HBc positive rate among children (1~3 years old) is 0.45%. However, the anti-HBs positive rate has dropped to 61% at age 3 years. Therefore, additional vaccination may be necessary for high-risk children.

Georg Lauer – United States

Immune responses mediating HBV functional cure

Functional cure, i.e. long-term stable control of HBV replication and antigenemia in the absence of therapy, should be a feasible goal of advanced HBV therapies, given that it is the outcome of almost all adult infections. More importantly it can still be observed even after decades of chronic infection, in patients on treatment or without. However, no current treatment protocol achieves substantial rates of functional cure. Despite wide agreement that induction of functional cure will require a combination of antiviral and immunomodulatory drugs with or without the addition of therapeutic vaccines. there is significant uncertainty about the best therapeutic combinations that should go forward into future trials. This is a result of insufficient investments into HBV research after the availability of prophylactic vaccines, leaving us with limited data on the immune correlates of functional cure but also on key immune deficiencies preventing functional cure in the different stages of chronic HBV infection. As a consequence, it is not exactly clear which arms of the immune system require modification and in what CHB populations immune-based therapeutic intervention might be most easily achieved. In addition, a lack of detailed molecular immune analyses in the context of clinical trials with novel HBV agents means that their impact on immune responses also remains unclear.

In this presentation I will discuss the significant challenges posed by HBV as the most complicated of all chronic infections in humans, our current knowledge base of the immune correlates of HBV functional cure, and what I see as the most pressing immunological questions in both HBV natural history and HBV treatment that should be answered to inform novel treatment approaches and the combination of different treatment modalities.

Takaji Wakita – Japan

Viral genome analysis and in vitro propagation of Genotype1b HCV

Since the discovery of the hepatitis C virus (HCV) in 1989, it has been recognized as a serious medical and public health problem worldwide. Research progress of HCV replicon and virus culture systems enabled various achievement in the HCV research field. We have developed virus cell culture system with different genotypes although genotype1b HCV propagation has been difficult. In addition, HCV genome accumulates nucleotide substitutions during its persistent infection due to its low viral RNA polymerase fidelity and high viral replication ratio. This intra-host variability, i.e. “quasispecies”, has been considered to contribute for viral escape from the selective pressure exerted by the host immune system or anti-viral drug exposure. Here, we analyzed the full-length viral genome RNA species from chronic hepatitis C patient (genotype1b) serum combined NGS and capillary sequencing. Using this method, we determined three independent dominant species co-existed in one patient serum. The full-length sequences were quite different depend on the core polymorphisms. Using identified 3 full-length genotype 1b sequences, we synthesized each HCV cDNA clone and cloned into the plasmid. Using these 3 HCV clones, we transfected synthesized viral RNAs into cultured cells and recovered infectious virus. This system will be available for further characterization of genotype 1b HCV and analysis of the mechanism of co-existence of multiple HCV species.

Naranbaatar Dashdorji – Mongolia

Junko Tanaka – Japan

Epidemiological study towards elimination of HBV and HCV

Hepatitis C virus (HCV) and hepatitis B virus (HBV) are transmitted via blood contact, and if they progress to chronic infection, they can cause liver cirrhosis and liver cancer. In Japan, approximately 3.0-3.7 million (HBV 1.3-1.5 million / HCV 1.7-2.2 million) individuals were estimated to be chronically infected with HBV or HCV in 2000. However, this number reduced to approximately 2.0-2.5 million (1.1-1.2 million / 0.9-1.3 million) in 2015 and further to 1.4-2.0 million (1.0 million / 0.4-1.0 million) in 2020 by our predictions.

Japan is one of the most advanced countries globally in implementing measures against viral hepatitis. A national project utilizing HBIG and vaccines to prevent HBV transmission from mother to child has been in place since 1986, along with the implementation of the HCV screening system among blood donors since 1990. Additionally, free screening among residents over 40 years old was introduced in 2002, followed by the implementation of a subsidy system in 2008, and the establishment of the Basic Act on Hepatitis Measures in 2009.

This presentation will showcase some epidemiological research on HBV and HCV, as well as the current progress and challenges for HBV and HCV elimination in Japan.

Chunkyu Ko – Korea

Targeting capsid forming ability of hepatitis B virus core protein with small-molecule inhibitors

Chronic infection with the hepatitis B virus (HBV) affects approximately 296 million individuals globally, resulting in approximately 800,000 deaths annually. Individuals living with these viruses face a high risk of developing liver cirrhosis and liver cancer. While current nucleos(t)ide analogue therapy effectively controls HBV replication, it cannot eradicate the virus. Therefore, a comprehensive understanding of the life cycles of HBV is imperative to identify new antiviral targets and develop innovative therapeutics, ultimately aiming for the eradication of HBV infections. Capsid assembly modulators (CAMs) are small molecules that bind to HBV core proteins and modulate capsid assembly process, resulting in aberrant or HBV genome-free capsids. In this talk, antiviral potency and detailed mode-of-action of some new CAMs will be introduced.

Masaya Sugiyama – Japan

Identification of novel cell populations associated with liver fibrosis in hepatitis B.

Aim: Liver fibrosis is an unmet medical need with no effective treatment. We have previously used hepatitis B virus-infected human hepatocyte-replacing chimeric mice (chimeric mice) as a model of liver fibrosis. In this study, single cell analysis using the same mouse model and human samples was used to identify novel cell populations associated with liver fibrosis and to explore novel therapeutic targets for liver fibrosis.

Method: Single-cell RNA-seq analysis was performed on liver tissue from HBV-infected chimeric mice and hepatitis B patients. From these data, we extracted cell populations associated with fibrosis development and searched for characteristically expressed genes. To confirm the presence of these cell populations, tissue staining and FACS analyses were performed; anti-GPNMB antibodies were administered to HBV-infected chimeric mice and liver fibrosis levels were assessed three months later.

Results: Single-cell RNA-seq analysis of liver tissue from HBV-infected chimeric mice identified a population of macrophages characteristic of sites of liver fibrosis. A similar macrophage population was also identified in liver resection fragments from hepatitis B patients. This cell population consisted of C1Q-positive/GPNMB-positive/CD68-positive macrophages. The proportion of this cell population correlated positively with the degree of liver fibrosis; this correlation was not observed in liver tissue from hepatitis C. Inhibition of function using anti-GPNMB antibodies was tested in HBV-infected chimeric mice. Evaluation of liver tissue at 3 months showed that the degree of fibrosis and the expression of fibrosis-related genes were suppressed ($p < 0.05$).

Conclusions: Functional inhibition of GPNMB-positive macrophages by antibody administration showed inhibition of liver fibrosis. The cell population is expected to be useful as a therapeutic target, leading to the development of novel therapeutic agents, including antibody therapies.

Mehrangiz Dezhbord – Korea

The interferon gamma (IFN-γ) induced MHC class II transactivator as a novel and potent anti-HBV factor

MHC class II transactivator (CIITA) is a transcription factor induced by IFN-γ that promotes the expression of MHC class II in antigen presenting cells. Here, we report a non-canonical function of CIITA as a novel antiviral factor that can inhibit the transcription of HBV. Initially, RNA sequencing analysis revealed that CIITA level was significantly increased by TNFα and IFNγ treatment in PHH isolated from two HBV infected and two Not infected donors. Accordingly, the antiviral activity of CIITA against HBV were assessed. Ectopic expression of CIITA effectively reduced HBV DNA and RNA, as well as HBeAg and HBsAg levels in vitro. In addition, the antiviral effect of cytokines was attenuated by silencing endogenous CIITA. Notably, in vivo experiments demonstrated that HBV DNA and secreted antigen levels significantly decreased in mice injected with the CIITA construct. Mechanistically, CIITA down regulated HNF1α and HNF4α expression through activation of the ERK1/2 pathway. Therefore, the transcriptional activity of HBV enhancers was significantly suppressed. Interestingly, HBx (amino acid sequences 51 to 154) binds to CIITA and diminished its antiviral activity. In summary, we revealed a new host factor that can regulate HBV transcription and we presented evidence of a new HBx mediated immune-evasion strategy for HBV.

TOPIC TWO: Protective Immune Responses to HCV in Aid of Vaccine Development - Joint with Immunology Board

Andrea Cox – USA

Lessons learned from natural immunity and a hepatitis C virus vaccine efficacy trial.

Risk factors for hepatitis C virus (HCV) infection vary globally, but there are an estimated 1.75 million new cases worldwide annually. A safe and effective vaccine to prevent chronic hepatitis C virus (HCV) infection is essential to reduce transmission. Although highly effective vaccines could prevent infection altogether, immune responses that increase the rate of HCV clearance and prevent chronic infection may be sufficient to reduce disease burden. Strategies to generate an effective HCV vaccine are diverse, but only a small subset, including adjuvant envelope or core protein and virus-vectored non-structural antigen vaccines, has advanced to testing in healthy volunteers who are not at risk for HCV infection. Despite development challenges, a prophylactic vaccine is necessary for global control of HCV. Potential strategies for advancing HCV vaccine design will be discussed.

Masanori Isogawa – Japan

Interleukin-2 Produced by Type 2 Peripheral T Helper Cells Contribute to the Durability of Recall Antibody Responses

Serum anti-SARS-CoV-2 neutralizing antibody titers represent one of the most reliable and convenient correlates of protection against COVID-19 onset. However, the longevity of neutralizing antibody responses varies significantly among vaccinees. Conducting a comprehensive spike-specific immune profiling after homologous BNT162b booster vaccinations, we here demonstrate that the pre-booster capacity of spike-specific CD4+ T cells to produce interleukin (IL)-2 and Th2 cytokines closely correlates the longevity of antibody responses. A newly developed in vitro analysis underscores the essential role of IL-2 produced by CD4+ T cells in supporting the antigen-specific production of antibodies and Th2 cytokines. High dimensional cytometric and transcriptomic analyses revealed that IL-2-producing CD4+ T cells lack expression of prototypical molecules associated with circulating T follicular helper cells (CXCR5 and BCL6) or peripheral helper T cells (CXCL13 and Blimp). Nonetheless, IL-2-producing T cells highly express helper molecules, including CD40LG, ICOS, and IL-21. Furthermore, they express the transcription factor BACH2, which is shown to prevent T cell senescence. Thus, the current study introduces a novel helper T cell subset, herein termed “type 2 peripheral T helper” (Tph2) cells and identify IL-2 produced by Tph2 cells as a key determinant of antibody responses.

Bali Pulendran – USA

Hideki Ueno – Japan

CD4+ T cell Responses Against Human Coronaviruses

The primary goal of the COVID-19 vaccination is to prevent the infection by SARS-CoV-2 by inducing their neutralizing antibodies. However, the continuous emergence of escape mutants and relatively short-lived antibody responses has rendered the current COVID-19 vaccine strategy insufficient in preventing breakthrough infections. Nonetheless, COVID-19 vaccination can reduce the disease severity during the acute phase of SARS-CoV-2 infection. Accumulating evidence shows T cells play the central roles, however, our understanding of the mechanism of how T cells contribute to the reduction of disease severity remains incomplete. Notably, CD4+ T cell responses exhibit considerable diversity. What type of CD4+ T cell responses are induced by SARS-CoV-2 infection and COVID-19 vaccinations? How do the T cell responses differ among different ages and sex? Which CD4+ T cell subsets contribute to the reduction of disease severity? How do they contribute to the induction of durable immune memory? What is the contribution of cross-reactive T cells with human common-coronaviruses (H-CoVs)? To address these questions, we have been analyzing SARS-CoV-2 and H-CoV-specific T cells induced by SARS-CoV-2 infection and vaccinations by ultra-multicolor flow cytometry, scRNAseq, and scTCRseq. In my talk, I will delve into the intricate heterogeneity of CD4+ T cell responses across subjects, their impact on disease severity, and their role in fostering durable immune memory.

Yoshimasa Takahashi – Japan

Vaccine-elicited memory B cell responses for tackling virus variants

Enormous efforts have been made to understand vaccine-induced immunity against SARS-CoV-2 virus and their potential relevance to the vaccine effectiveness. Vaccination using mRNA technology elicits memory B and T cells which play important roles on the durable and broad protection against SARS-CoV-2 variants. Memory B cells persist for a long period with the increased breadth and potency over time. Moreover, memory B-cell subset composition undergoes significant changes, concordant with the increased antibody recall upon boosters after extended time intervals. Repeated vaccination further enhances the breadth, potency, and durability of recalled antibodies, resulting in the elicitation of Omicron-neutralizing antibodies following the additional dose of Wuhan-based vaccines.

The improved recall responses after repeated vaccination and extended time intervals has played a crucial role in countering emerging SARS-CoV-2 variants through Wuhan-based vaccines. However, it may also impede the induction of de novo memory B cells specific to the latest circulating variants after Omicron-adapted vaccines, a phenomenon known as immunological imprinting. Therefore, unveiling the vaccine-elicited memory B cell responses is of paramount importance for future vaccine strategy and development for tackling emerging variants. I will present recent updates of humoral immune memory in humans revealed by COVID-19 vaccination.

Bette Korber – USA (virtual)

Epigraph vaccines for highly variable pathogens.

We developed the concept of using a bioinformatic approach we call “Epigraphs” to optimize the design of vaccine antigens to create a small set of vaccine antigens that “look and feel” like natural proteins, but are artificial designs that combination provide optimized coverage of linear epitopes. I will review the approach and some promising studies in animal models for both a pan-filovirus vaccine and influenza virus vaccines. Then I will briefly describe how we went about designing a T-cell epigraph vaccine for HCV, and considerations for a possible epigraph-based HCV antibody vaccine design.

Eui-Cheol Shin – Korea

Liver sinusoidal T cells in healthy and pathologic livers

The liver provides a unique niche of lymphocytes enriched with CD8+ T and NK cells. To examine characteristic features of liver sinusoidal CD8+ T cells, we obtained liver sinusoidal mononuclear cells from the liver perfusate of healthy donors and recipients during liver transplantation. First, we examined liver-enriched CD69+CD103-CD8+ T cells. Liver sinusoidal CD69+CD103-CD8+ T cells typically exhibited HIF-2a upregulation with a phenotype of tissue residency and terminal differentiation. CD69+CD103-CD8+ T cells comprised non-hepatotropic virus-specific T cells as well as hepatotropic virus-specific T cells, whereas CD69+CD103+CD8+ T cells exhibited only hepatotropic virus specificity. An HIF-2a inhibitor suppressed the effector functions and survival of CD69+CD103-CD8+ T cells. In addition, these T cells were activated and expressed higher levels of HIF-2a in liver pathologies.

Using liver sinusoidal mononuclear cells, we also identified a distinct CD56hiCD161-CD8+ T cell population that is characterized by upregulation of various NK receptors. CD56hiCD161-CD8+ T cells highly respond to innate cytokines, such as IL-12/18 and IL-15, in the absence of TCR stimulation. In summary, we discovered unique subpopulations among liver sinusoidal CD8+ T cells in healthy and pathologic livers.

Tetsuro Suzuki – Japan

Involvement of phospholipase A2 group 4C induced by hepatitis C virus infection in hepatic lipid accumulation

The aim of this study was to determine how the phospholipid pathway is involved in the pathogenesis of hepatic steatosis induced by HCV infection. Cellular lipid droplets (LDs) have a core of neutral lipids protected from the aqueous environment by protein-containing phospholipid monolayers. Accelerated LD formation in hepatocytes is a common feature of liver pathology in chronic HCV infection. Based on the experimental results obtained, we propose the following model as a mechanism by which HCV infection causes an increase in LD size in infected cells.

HCV infection induces transcription of the phospholipase A2 group 4C (PLA2G4C) gene by activating NF-kB and c-Myc, which are known to be activated via oxidative stress. Increased expression of PLA2G4C increases PLA2 activity in HCV-infected cells, leading to degradation of PC species into lysophosphatidylcholines (LPCs); PCs are the most abundant phospholipids in LD lipid monolayers. Since PLA2G4C is localized to cytoplasmic membranes, including LD membranes, its increased expression may decrease the amount of membrane PC and alter the dynamic phospholipid-protein interactions on LD membranes. Changes in the PC/LPC ratio are presumed to lead to changes in the hydrophobicity of the LD surface. As a result, membrane associations of triglyceride (TG) degradation-related factors that localize at LD membranes, such as ATGL, PLIN1, and ABHD5, possibly decrease, reducing the level of LD membrane localization of these factors. This leads to decreased TG degradation in the LDs and to stabilization and enlargement of LDs in the viral infected cells.

Oxidative stress, the main factor of NF-kB and c-Myc activation in liver diseases, is known to be induced not only by HCV infection but also by lifestyle-related hepatic steatosis, such as NASH. Analyses of whether PLA2 activity is increased in alcoholic or nonalcoholic fatty liver disease as well as viral hepatitis, whether the phospholipid composition of LD membranes is altered, and whether the LD localization of ATGL and its cofactors is decreased in the process to fatty liver development should be important in the future to elucidate the pathogenesis of liver diseases that involve changes in LD formation.

Ken J. Ishii – Japan

Science and design for nucleic-acid-based vaccine and adjuvant

Vaccine science was a field that had received little attention prior to the COVID-19, but this changed with the pandemic, and the ripple effect has been far beyond expectations, spreading widely from molecules (academia) to ethics (social contact). The practical application of RNA vaccines is expanding the possibilities of nucleic acid medicine design and science. The practical application of RNA vaccines is expanding the possibilities for the design and science of nucleic acid medicine for the sake of their efficacy as well as safety. It is common knowledge that nucleic acids are genetic information inside the cell, but they are also released outside the cell, where they exhibit specific activities that differ from those inside the cell. In other words, nucleic acids act on immune cells and various other cells as extracellular microparticles, which not only act as adjuvants but also have a wide range of effects on biological phenomena in vivo such as inflammation, cancer, allergy, neurodegeneration, aging, and fibrosis. In this presentation, we will focus on extracellular nano- to micro-particles containing nucleic acids or groups of microparticles that induce the release of nucleic acids to explore the mechanisms of biological responses and their physiological significance and will present the results of our research and development of techniques for measuring and controlling extracellular nucleic acids.

Justin Bailey – USA

Neutralizing antibodies exploit vulnerable sites in HCV E2 and mediate spontaneous clearance of infection

Individuals who clear primary hepatitis C virus (HCV) infections clear subsequent reinfections more than 80% of the time, but the mechanisms are poorly defined. Here, we used HCV variants and plasma from individuals with repeated clearance to characterize longitudinal changes in envelope glycoprotein E2 sequences, function, and neutralizing antibody (NAb) resistance. Clearance of infection was associated with early selection of viruses with NAb resistance substitutions that also reduced E2 binding to CD81, the primary HCV receptor. Later, peri-clearance plasma samples regained neutralizing capacity against these variants. We identified a subset of broadly-neutralizing antibodies (bNAbs) for which these loss-of-fitness substitutions conferred resistance to unmutated bNAb ancestors but increased sensitivity to mature bNAbs. These data demonstrate a mechanism by which neutralizing antibodies contribute to repeated immune-mediated HCV clearance, identifying specific bNAbs that exploit fundamental vulnerabilities in E2. The induction of bNAbs with these specificities should be a goal of HCV vaccine development.

Aska Tobuse – Japan

Sachiyo Yoshio – Japan

Comprehensive analysis of immune pathogenesis of acute hepatitis A with or without HIV co-infection

Hepatitis A (HAV) usually recovers within one to two months, but in HIV-1-infected patients, it is reported to be prolonged. This study aimed to identify immune factors involved in HAV persistence in HAV/HIV-1 co-infected patients. Seventy cytokines and chemokines were measured in 49 HAV/HIV-1 co-infected subjects, 26 HAV mono-infected subjects, and 8 healthy adults. No differences were observed in peak ALT, total bilirubin, and PT levels in the HAV co-infected subjects compared with those infected with HAV alone. On the other hand, the duration before ALT normalization was longer (98.7 vs. 31 days) in HAV/HIV-1 co-infected patients. CXCL9, CXCL10, CXCL11, CXCL13, and IL-18 were elevated at the ALT peak and decreased with recovery. On the contrary, CCL22 and CCL17 were decreased at the ALT peak both in HAV mono-infected and HAV/HIV-1 co-infected patients. ALT levels and the number of activated CD8+ and CD4+ T cells were positively correlated with CXCL9, CXCL10, CXCL11, and CXCL13, and negatively correlated with CCL22. Multivariate analysis showed that high CXCL13 and low CCL22 were independent contributors to hepatitis A persistence in HIV-infected patients. Activation of T cells, reflected by high CXCL13 and low CCL22 might impede the normalization of ALT at acute HAV infection with HIV-1 positive patients.

So-Young Kim – Korea

TOPIC 3: Hepatitis Virus-related and Non-viral Liver Cancer - Joint with Immunology Board and Cancer Panel

Severin Gudima – USA

Sera of individuals chronically infected with hepatitis B virus (HBV) contain diverse RNA types produced by HBV replication and derived from integrated HBV DNA

This study aimed to better characterize the repertoire of serum hepatitis B virus (HBV) RNAs during chronic HBV infection in humans, which remains understudied. Using reverse transcription-PCR (RT-PCR), real-time quantitative PCR (RTqPCR), RNA-sequencing, and immunoprecipitation, we found that (i) >50% of serum samples contained different amounts of HBV replication-derived RNAs (rd-RNAs); (ii) a few samples contained RNAs transcribed from integrated HBV DNA, including 5'-HBV-human-3' RNAs (integrant-derived RNAs or id-RNAs) and 5'-human-HBV-3' transcripts, as a minority of serum HBV RNAs; (iii) spliced HBV RNAs were abundant in <50% of analyzed samples; (iv) most serum rd-RNAs were polyadenylated via conventional HBV polyadenylation signal; (v) pre-genomic RNA (pgRNA) was the major component of the pool of serum RNAs; (vi) the area of HBV positions 1531 to 1739 had very high RNA read coverage and thus should be used as a target for detecting serum HBV RNAs; (vii) the vast majority of rd-RNAs and pgRNA were associated with HBV virions but not with unenveloped capsids, exosomes, classic microvesicles, or apoptotic vesicles and bodies; (viii) considerable rd-RNAs presence in the circulating immune complexes was found in a few samples; and (ix) serum relaxed circular DNA (rcDNA) and rd-RNAs should be quantified simultaneously to evaluate HBV replication status and efficacy of anti-HBV therapy with nucleos(t)ide analogs. In summary, sera contain various HBV RNA types of different origin, which are likely secreted via different mechanisms. In addition, since we previously showed that id-RNAs were abundant or predominant HBV RNAs in many of liver and hepatocellular carcinoma tissues as compared to rd-RNAs, there is likely a mechanism favoring the egress of replication-derived RNAs

Tatsuhiro Shibata – Japan

Genetic and epigenetic analysis of HBV genome integration in HCC

Hepatocellular carcinoma (HCC) is the sixth most common cancer and the third most common cause of cancer-related death4. Approximately 300,000 new cases each year are associated with hepatitis B or C virus (HBV and HCV) infection. HBV, a DNA virus that integrates its genome into the genome of infected human hepatocytes, is the most common and highest risk factor for HCC. Virus genome insertions and stepwise somatic accumulation of genetic and epigenetic alterations during clonal evolution promote hepatocarcinogenesis.

We have reported the correlation between the occurrence of epigenetic features and genetic aberrations by whole-genome bisulfite, whole-genome shotgun, long-read and virus capture sequencing of 373 liver cancers. HBV genome integration sites are frequently detected within inactive chromatin regions in cancer cells, as a consequence of negative selection for integrations in active chromatin regions. Long and short read sequencing identified ultra-high structural instability and preserved unmethylation of integrated HBV genomes.

Kohji Moriishi – Japan

Regulatory mechanism of polycomb repressive complex1 by HCV infection

Hepatitis C virus (HCV) infection causes liver pathologies, including hepatocellular carcinoma (HCC). Homeobox (HOX) gene products regulate embryonic development and are associated with tumorigenesis, although the regulation of HOX genes by HCV infection has not been clarified in detail. Polycomb repressive complex (PRC1) catalyzes monoubiquitination of histone H2A K119 to silence several developmental genes including HOX genes and plays roles in cell lineage commitment, stem cell identity, tumorigenesis and so on. In this study, we examined the effect of HCV infection on PRC1-dependent HOX gene expression. When Huh7 cells or PHHs were infected with HCV, HCV infection induced about 70% of the HOX genes and reduced the level of histone H2A monoubiquitination on lysine (K) 119 (H2Aub), which represses HOX gene promoter activity. HCV infection also promoted the proteasome-dependent degradation of RNF2, which is an E3-ubiquitin ligase mediating H2A monoubiquitination as a main component of PRC1. Expression of the core protein also reduced the amounts of RNF2 and H2Aub and induced HOX genes in a smilar way to the infeciton. The chromatin immunoprecipitation assay revealed HCV infection-dependent reductions in H2Aub located in HOX gene promoters. These results suggest that HCV infection or the core protein induces HOX genes by impairing histone H2A monoubiquitination via a reduction in the RNF2 level. We next investigated the mechanisms by which the core protein activates proteasome activity for RNF2 degradation. The RNF2-interacting host protein was identified by yeast two hybrid system. HCV infection or the core protein expression potentiated acetylation of the RNF2-interacting protein and then induced multimer formation of the host protein to upregulate 20S proteasome activity for RNF2 degradation. These findings reveal a novel mechanism of HCV-related histone modification and may provide information about new targets for diagnosis and prevention of HCC occurrence.

Tatsuya Kanto – Japan

Immunoglobulin-like transcript 2 (ILT2) as an exhaustion marker of NK cells in patients with hepatocellular carcinoma

Natural killer (NK) cells play a pivotal role in immune surveillance in the liver. We aimed to identify potential targets for NK cell-mediated immune intervention by revealing the functional molecules on NK cells in HCC patients. To evaluate the impact of aging on NK cell phenotypes, we examined NK cells from healthy volunteers (HVs) of various ages. Because ILT2 expression on CD56dim NK cells increased with increasing age, we enrolled age-matched HCC patients and HVs. We determined the NK cell phenotypes in blood mononuclear cells (PBMCs) and intrahepatic lymphocytes (IHLs) from cancerous and non-cancerous tissues. We evaluated cytotoxicity and antibody-dependent cellular cytotoxicity (ADCC) of NK cells in vitro. ILT2-positive CD56dim NK cells in PBMCs were increased in HCC patients compared with HVs. In HCC patients, ILT2-positive CD56dim NK cells were increased in cancerous IHLs compared with non-cancerous IHLs and PBMCs. We examined the impact of macrophage migration inhibitory factor (MIF) on ILT2 expression in co-cultures of HCC cells and NK cells. The enhanced expression of ILT2 on CD56dim NK cells from HCC patients was inhibited by masking antibodies against MIF and CXCR4. ILT2-positive CD56dim NK cells exhibited lower capacities for cytotoxicity and ADCC than ILT2-negative cells, which were partially restored by ILT2 blockade.

In conclusion, ILT2 is a signature molecule for cancerous CD56dim NK cells with impaired cytolytic capacity in HCC patients. The MIF-CXCR4 interaction is associated with ILT2 induction on CD56dim NK cells and ILT2 serves as a target for functional NK cell restoration.

Xin Wei Wang – USA

Molecular landscape of liver cancer and its clinical implications

Liver cancer is among the top five deadliest cancers in the world, especially in Southeast Asia and Sub-Saharan Africa, and its incidence rates continue to increase in recent decades. Liver cancer mainly consists of two clinical types, i.e., hepatocellular carcinoma (HCC) and cholangiocarcinoma (CCA), each of which could be further divided into several clinical and molecular subtypes. Chronic liver diseases due to viral hepatitis, alcohol consumption, chemical carcinogens, or nonalcoholic fatty liver disease/nonalcoholic steatohepatitis, are major global health burdens that increase the risk of HCC while parasitic infections may be linked to CCA. It is unclear how complex etiological factors determine tumor subtypes and whether common features may be shared among HCC and CCA. Given the presence of so many clinical and molecular variables, it is imperative to develop well-defined cohorts that include diverse etiologies, race/ethnicities, sex and age, which provides an unbiased platform by minimizing confounding factors to study liver cancer types. Accordingly, we have established several national and international collaborative projects, including the NCI-CLARITY study, the NCI-UMD cohort study, the NCI-Mongolian cohort study, and the TIGER-LC (Thailand) consortium. We have applied molecular-based technologies such as genomics, transcriptomics, metabolomics and microbiomics, including single-cell omics to comprehensively analyze biopsies from diverse populations, in order to better characterize heterogeneity among and within patients, to further define tumor molecular subtypes with unique tumor biology and to understand tumor evolution in response to treatment. Recently, we have developed a paradigm-shift approach by determining individuals' virome as an early onset of HCC to improve risk prediction and early diagnosis of liver cancer. Our past and future ability to translate our research findings towards patient management through the identification of molecular-based knowledge for understanding of liver cancer pathophysiology with the application of early detection and treatment of liver cancer may have a considerable impact on clinical practice and public health.

Su-Hyung Park – Korea

4-1BB Co-Stimulation, a Promising Strategy for Treating HCC

4-1BB expression on CD8+ TILs represents a distinct activation state among highly exhausted CD8+ T cells in HCC. 4-1BB co-stimulation with agonistic antibodies may be a promising strategy for treating HCCs exhibiting prominent T-cell activation. However, dose-dependent hepatotoxicity was observed in clinical trials with monoclonal anti-4-1BB agonistic antibodies due to the activation of 4-1BB signaling in liver resident Kupffer cells. To avoid this on-target liver toxicity, we developed a novel bispecific antibody (4-1BB×PD-L1 bispecific antibody, termed 'ABL503') uniquely designed to activate 4-1BB signaling only in the context of PD-L1, while also blocking PD-1/PD-L1 signaling. The anti-4-1BB×PD-L1 bispecific antibody augmented T-cell activation in in vitro assays, and further enhanced the anti-PD-L1-mediated reinvigoration of tumor-infiltrating CD8+ T cells. Furthermore, in humanized PD-L1/4-1BB transgenic mice challenged with huPD-L1-expressing tumor cells, the bispecific antibody induced superior anti-tumor activity and maintained an anti-tumor response against tumor rechallenge. In conclusion, the novel anti-4-1BB×PD-L1 bispecific antibody may exert a strong anti-tumor therapeutic efficacy with a low risk of liver toxicity through the restriction of 4-1BB stimulation in tumors.

Antonio Bertoletti – Singapore

mRNA-based T cell engineering for treatment of virus-related cancer and virus infection.

Adoptive therapy with T cells engineered with a Chimeric Antigen Receptor (CAR) or a classical T Cell Receptor (TCR) -indicated as CAR/TCR-T cells- transitioned from an experimental approach to a main strategy of care in cancer treatment. However, while clinical success has been remarkable in some hematological cancers (i.e., B cell lymphoma), efficacy has been limited in solid tumors, and the production cost can limit its usage in clinical practice. We have already utilized the flexibility of mRNA electroporation technology in engineering mRNA TCR-T cells for the production of HBV- and SARS-CoV-2-specific T cells. mRNA-HBV-specific TCR-T cells have also been used in clinical trials for the treatment of primary and secondary HBV-related hepatocellular carcinoma (HCC). I will discuss the advantages (no genetic modifications, escalating doses to limit side effects, cost) and limitations (transient functionality, multiple infusions) of this approach. I will also present novel data that suggest that adoptive transfer of mRNA HBV-TCR-T cells in patients with HBV-related HCC can trigger the induction of novel T cell specificities with ability to target the tumor and/or HBV.

Ikuo Shoji – Japan

Oxidative stress sensor Keap1 recognizes HBx protein to activate the Nrf2/ARE signaling pathway, thereby inhibiting hepatitis B virus replication

Hepatitis B virus (HBV) infection promotes reactive oxygen species production while paradoxically inducing the expression of antioxidant enzymes. HBV-induced disorder of redox homeostasis is closely associated with development of hepatic diseases. However, the molecular mechanisms underlying HBV-induced antioxidant response are poorly understood. The NF-E2-related factor 2 (Nrf2)/antioxidant response element (ARE) signaling pathway is an intrinsic defense mechanism against oxidative stress. We here aimed to elucidate the role of the Nrf2/ARE signaling pathway in the HBV life cycle. ARE-driven reporter assays revealed that expression of HBx, but not HBc, large HBs, or HBV polymerase, strongly enhanced ARE-luciferase activity, suggesting that HBx plays an important role in the HBV-induced antioxidant response. Knockdown of Nrf2 resulted in a marked decrease in HBx-induced ARE-luciferase activity. Immunoblot analysis and immunofluorescence staining suggested that HBx activates Nrf2 by increasing Nrf2 protein levels and enhancing Nrf2 nuclear localization. The oxidative stress sensor Keap1 is required for ubiquitin-dependent degradation of Nrf2. Coimmunoprecipitation analysis revealed that HBx interacted with Keap1, suggesting that HBx competes with Nrf2 for interaction with Keap1. A cell-based ubiquitylation assay showed that HBx promoted polyubiquitylation of Nrf2 via K6-linked polyubiquitin chains, and that this action may be associated with Nrf2 stabilization. A chromatin immunoprecipitation assay suggested that Nrf2 interacts with the HBV core promoter. Overexpression of Nrf2 strongly suppressed the HBV core promoter activity, resulting in a marked reduction in viral replication. Based on the above, we propose that Keap1 recognizes HBx to activate the Nrf2/ARE signaling pathway upon HBV infection, thereby inhibiting HBV replication.

Anuradha Budhu – USA

Epidemiology and genomic applications detect risk factors and discern treatment response in liver cancer.

Primary liver cancers (PLC), encompassing hepatocellular carcinoma (HCC), and biliary tract cancers (BTC), including intra- and extra-hepatic cholangiocarcinoma (iCCA/eCCA), are leading causes of cancer mortality worldwide. Thailand is among countries with the highest global incidence of PLC and a rising incidence has been observed in the U.S. In national and international cohorts, we have utilized molecular epidemiology and functional genomics to aid in the discovery of preventative, diagnostic, prognostic and predictive PLC biomarkers. In a Thailand-based consortium study (TIGER-LC), we have identified multi-omic molecular subgroups as well as risk factors of PLC and explored their association with biological pathways and patient survival. In a U.S.-based multisite clinical study (NCI-CLARITY) with retrospective and prospective arms, we have profiled the transcriptome and genomic alterations among PLC patients, prior to and following immune checkpoint inhibitor treatment, and shown that patients with heterogenous liver cancer may be stratified by molecular status related to aggressive tumor biology and microenvironmental features. These molecular indicators of biological and outcome patterns suggest approaches to stratify patients to reduce exposures and/or improve anticipated survivorship and treatment response.

Burcu Temizoz

Immune resilience to cancer by repurposing Mycobacterium tuberculosis-specific CD4 memory Th1 cells

Mycobacterium tuberculosis (Mtb) hot water extract (MX) holds anti-tumor and anti-microbial potentials mediated via yet to be elucidated mechanisms. Here, we revealed that MX exerted a strong anti-tumor effect only when mice were pre-immunized with BCG, or MX plus Th1 adjuvant. Particularly, Mtb-derived lipoprotein antigen and Mtb-derived STING agonist c-di-AMP found in MX robustly elicited Mtb-specific CD4 T cell-derived IFN-γ that acted directly on tumor cells to halt tumor growth. Yet, administration of this lipoprotein antigen to BCG-immunized mice, or tumor-unrelated antigen administration to the mice with Th1 memory response to that tumor-unrelated antigen, such as OVA, also inhibited tumor growth. Moreover, Mtb antigen-reactive Th1 cells in human PBMCs were also reactive to MX, indicating that these cells could be responsible for the anti-tumor effect of MX in humans. Thus, MX might demonstrate therapeutic potential for cancer via IFN-ɣ provided by MX-boosted Mtb-specific CD4 T cells in individuals with BCG immunization or tuberculosis history.

Shuntaro Shimizu

Takahiro Kodama – Japan

A novel mechanism driving intrahepatic cholangiocarcinoma

The tumor immune microenvironment (TIME) has received great attention as a therapeutic target for primary liver cancers. The latest large-scale single-cell transcriptome-based TIME classification identified a myeloid cell-enriched immunosuppressive subtype in intrahepatic cholangiocarcinoma (ICC), showing poor prognosis. However, it is unclear how such TIME subtype is formed and drives ICC development. We showed herein the novel link between an endoribonuclease REGNASE-1 (REG1) and oncogenic myeloid cells, which promoted ICC carcinogenesis. Phenotypic analysis of REG1 KO mice with scRNA-seq and lineage tracing revealed that REG1 deletion in hepatocytes recruited unique CXCR2+CD11b+ myeloid cells in the liver, which in turn induced hepatocyte transdifferentiation into proliferative cholangiocytes via CSF1 and TNF signaling, leading to ICC development. Mechanistically, REG1 in hepatocytes directly and negatively regulated CXC chemokines and CSF3, furnishing oncogenic myeloid cells in the liver. Clinically, intratumor Reg1 expression was negatively correlated with the number of infiltrated CD11b+ myeloid cells and poor prognosis in ICC patients. CSF1+TNF+ CXCR2+CD11b+ cells were specifically present surrounding the tumor cells in patients with low REG1 ICC. In conclusion, REGNASE-1 is a novel ICC tumor suppressor and its down-regulation drives ICC development via creating TIME enriched in oncogenic myeloid cells

Hyung-Don Kum – Korea

Differential T cell and monocyte responses in hepatocellular carcinoma treated with regorafenib plus nivolumab

In the phase 2 REBNOBATE trial, we evaluated the efficacy and safety of regorafenib-nivolumab as a front-line treatment in patients with unresectable hepatocellular carcinoma (uHCC). From the subjects of the RENOBATE trial, single-cell RNA sequencing was performed using peripheral blood mononuclear cells collected at baseline and early on-treatment from patients showing progressive increase in tumor burden (early progressors) and response or stable disease for at least 10 months (long-term responders). Upon regorafenib-nivolumab, diversification of T-cell receptor repertoire and enrichment of genes representing immunotherapy-responsiveness and cytotoxicity in MKI67+ proliferating CD8+ T cells were noted in long-term responders. Relative abundance and prominent transcriptomic changes of classical monocytes were observed in long-term responders. Monocytic populations from long-term responders had a preferential M1-directed polarization as well as regorafenib-induced transcriptomic reprogramming. In contrast, those from early progressors were featured by M2-directed transcriptomic changes and insufficient up-regulation of inflammasome-related genes. Interaction through IFN-γ pathways between proliferating CD8+ T cells and classical monocytes was exclusively observed in long-term responders. In conclusion, differential T cell and monocytes responses were associated with distinct clinical outcomes of HCC patients treated with regorafenib plus nivolumab, suggesting a potential to develop biomarkers associated with these cells or novel immunotherapies to overcome resistance in uHCC patients.

Kouki Nio – Japan

Laminin y2 Monomer, a Novel Biomarker for Hepatocellular Carcinoma in Patients with Chronic Hepatitis Virus Infection

Chronic liver diseases associated with hepatitis B virus (HBV) and hepatitis C virus (HCV) infections are major causes of hepatocellular carcinoma (HCC), the most prevalent primary liver malignancy and the third leading cause of cancer-related death worldwide. Therefore, regular surveillance for HCC is recommended in patients with chronic liver disease associated with HBV and HCV infections, especially those with cirrhosis, to detect the tumors at an early stage when curative treatments are still possible.

In addition to imaging studies, the use of biomarkers is also recommended for HCC surveillance in those patients. Several serum biomarkers, including alpha-fetoprotein (AFP), AFP-L3, and des-gamma-carboxy prothrombin (DCP), have been proposed as potential tools for the early detection of HCC. However, the sensitivity and specificity of these biomarkers remain suboptimal, and their clinical utility in HCC surveillance is still being evaluated. Thus, the development of useful biomarkers for HCC surveillance in chronically hepatitis virus infected patients would be meaningful for the early detection of HCC and improved patient outcomes.

T he laminin γ2 monomer (LG2m) protein has been identified as a potential biomarker for HCC surveillance due to its upregulation in HCC tissues. In our previous multicenter prospective cohort study, we demonstrated the utility of serum LG2m measurement for predicting HCC in chronic hepatitis C patients who achieved sustained virological responses after treatment with direct-acting antivirals. Recently, we also observed the clinical utility of LG2m as a biomarker for HCC surveillance in patients with chronic HBV infection in a retrospective study. Our findings provide insights into the potential clinical application of LG2m as a predictive biomarker for HCC in patients with chronic liver diseases associated with HBV and HCV infections.

Kazuyoshi Ohta – Japan

Role of hepcidin upregulation and proteolytic cleavage of ferroportin 1 in hepatitis C virus-induced iron accumulation

Background: Although iron deposition has been found in hepatocytes in the liver of chronic hepatitis C patients, the molecular mechanism underlined Is not fully clarified. The iron-transporting membrane protein ferroportin 1 (FPN1), the only known cellular exporter of iron, and hepcidin, a 25-amino acid peptide hormone (hepcidin-25) produced mainly in the liver, play major roles in maintaining iron homeostasis. In this study, we focused on investigating the regulation of gene expression of hepcidin and proteolytic cleavage of FPN1 caused by HCV infection.

Methods: Intracellular iron content was measured using the metal assay-Ferrozine method. Transcriptional regulation was analyzed by the luciferase reporter assay, chromatin-IP and RT-qPCR. Proteolytic processing of FPN1 was assessed by Western blotting.

Results: In Huh7 cell-based HCV infection, hepcidin mRNA expression was increased from 2 up to 21 days post-infection and higher iron levels in cells was maintained accordingly. Regarding the transcriptional regulation of hepcidin expression in response to HCV infection, we demonstrated that activation of cAMP responsive element binding protein hepatocyte specific (CREBH) triggered by endoplasmic reticulum stress with HCV Core-NS2 protein has a key stimulatory role in hepcidin expression via not only its recruiting to hepcidin promoter but up-regulation of the BMP/SMAD pathway.

A cleaved form of FPN1 was detected in HCV-infected cells and was stabilized by addition of proteasomal and lysosomal inhibitors. The consensus sequence for NS3-4A-mediated proteolytic cleavage was found to be present in the central part of FPN1 and the N-terminal sequence analysis revealed that the cleavage site by NS3-4A is between Cys (aa284) and Ala (aa285) matched consensus sequence. Intracellular iron content was higher in cells expressing NS3-4A compared to cells without its expression, suggesting that FPN1 cleavage by NS3-4A leads to inactivation of FPN1 function. Further, mouse FPN1, which is highly homologous to human FPN1, was expectedly processed in the liver tissues of mice when a recombinant adenovirus carrying HCV NS3-4A gene was injected.

Iron deposition in the liver of HCV protein-expressing mice was significantly higher in HCV Core-NS2 and NS3-4A co-expressing mice than in HCV Core-NS2 expressing mice.

Conclusion: The hepcidin-FPN1 system plays a central role in iron regulation and in the pathogenesis of common iron disorders. Our study demonstrated that not only transcriptional upregulation of hepcidin but proteolytic cleavage of FPN1 induced by HCV

infection potentially contribute to decreased level of FPN1, leading to iron accumulation in the infected cells.