CORRESPONDENCE

Association of the Cannabinoid Receptor 2 (CB2) Gln63Arg Polymorphism with Indices of Liver Damage in Obese Children: An Alternative Way to Highlight the CB2 Hepatoprotective Properties

To the Editor:

We read with great interest the article by Teixera-Clerc et al.,[1] regarding the hepatoprotective properties displayed by cannabinoid receptor 2 (CB2) agonists in a mouse model of carbon tetrachloride (CCl_4)-induced liver injury. Acute hepatitis induced by CCl_4 was accelerated in CB2 knockout mice, resulting in increased serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels compared to wild-type (WT) animals. Conversely, the ALT and AST levels were reduced in CCl_4-treated WT mice following the treatment with the CB2 agonist JWH-133.

In apparent disagreement with these results, the same group previously demonstrated that, in the liver of obese mice which were fed a high-fat diet, CB2 stimulation potentiated insulin resistance and nonalcoholic fatty liver disease (NAFLD), whereas CB2 inactivation reduced liver inflammation.[2] In obese children, NAFLD ranges from simple liver steatosis to steatosis associated with inflammation and increased liver enzymes, which may eventually progress to liver cirrhosis.[3]

To highlight the role of CB2 in NAFLD progression, we took advantage of a functionally relevant CB2 polymorphism. We studied the rs35761398 variant of the *CNR2* (cannabinoid receptor 2; GeneID 1269) gene, encoding for the CB2 receptor, which causes the substitution of a glutamine with a positively charged arginine at codon 63, in the first intracellular signaling loop. Receptor carrying arginine shows reduced function when activated by an endogenous cannabinoid.[4]

The variant was screened by direct sequencing in 438 Italian obese children with liver steatosis at ultrasound imaging in order to evaluate the association of the CB2 receptor Gln63Arg (Q63R) functional variant with serum AST and ALT levels. Results from this analysis (Table 1) demonstrate that the less functional R63 variant is statistically associated with higher ALT and AST levels independent of sex, age and pubertal stage. The variant is not associated with Body Mass Index, insulin resistance (assessed by Homeostasis Model Assessment of Insulin Resistance score) and size of abdominal fat (assessed by waist-to-hip ratio).

To be homozygous for the R63 allele results in a significant risk factor for liver damage, with an odds ratio for ALT >40 of 2.7 (95% confidence interval = 1.65-3.92, $P = 0.001$).

Table 1. Clinical and Laboratory Characteristics of 438 Obese Children with Liver Steatosis Stratified by Cannabinoid Receptor 2 Q63R Genotype

Characteristic	QQ	QR	RR	*P* Values
N (%)	27 (7.5)	222 (50.7)	189 (41.8)	
Females (%)	14 (53)	115 (52)	102 (54)	
Prepubertal (%)	13 (48.1)	111 (50)	93 (49.2)	
Age (years)	10.7 ± 1.9	11.3 ± 2.6	11.4 ± 3.2	0.29
BMI SDS	2.9 ± 0.4	3 ± 0.6	3.1 ± 0.7	0.043
W/Hr	0.65 ± 0.03	0.64 ± 0.05	0.65 ± 0.07	0.16
HOMA-IR	5.7 ± 3.4	6.5 ± 4.8	6.6 ± 4.3	0.78
ALT (U/L)	24.4 ± 8.6	32.3 ± 16.4	37.2 ± 21.4	0.0001
AST (U/L)	22.5 ± 6.5	24.5 ± 7.0	27 ± 13.2	0.002

Values are expressed as means ± standard deviations. General linear model (GLM) analysis, including sex, pubertal stage, and age as covariates, has been used to compare continuous variables. Abbreviations: BMI-SDS, body mass index standard deviation scores; W/Hr, waist circumference to height ratio; HOMA-IR, homeostatic model of assessment of insulin resistance index.

These data, obtained using an approach (i.e., association study in humans) completely different from that of previously published studies (i.e., analysis in murine models), showing that the partially inactive R63 CB2 variant is associated with liver damage progression in obese children with liver steatosis, corroborate the idea of a hepatoprotective role for CB2 receptors.

Francesca Rossi, M.D., Ph.D.[1*]
Giulia Bellini, Ph.D.[2*]
Bruno Nobili, M.D.[1]
Sabatino Maione, M.D.[2]
Laura Perrone, M.D.[1]
Emanuele Miraglia del Giudice, M.D.[1]
[1]*Department of Pediatrics*
[2]*Department of Experimental Medicine*
Second University of Naples
Naples, Italy
**These authors contributed equally to this work.*

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Reply:

We thank Rossi and colleagues for their interest in our experimental findings delineating the impact of CB2 receptors in experimental models of liver injury. We reported recently that the hepatic cannabinoid receptor 2 (CB2) displays beneficial hepatoprotective and antifibrogenic properties.[1,2] However, as stressed by Rossi and colleagues, we also demonstrated that treatment of obese mice with the CB2-selective agonist JWH-133 enhances insulin resistance and steatosis.[3] Deleterious effects of CB2 agonism in obese mice were attributed to a proinflammatory effect of adipose tissue CB2 receptors.[3] However, our recent findings in a model of alcoholic liver injury indicate that stimulation of hepatic CB2 receptors reduces the development of fatty liver.[4] Altogether, our experimental data demonstrate the protective effects of hepatic CB2 receptors against liver injury, steatosis, and fibrosis.

In their cohort study of over 400 obese children, Rossi and colleagues show a positive correlation between the presence of a CB2 variant with reduced activity, as assessed in culture studies, and the degree of liver injury estimated by serum aminotransferase levels. These interesting findings constitute the first evidence supporting the clinical relevance of hepatoprotective effects of CB2 receptors. Further studies should be conducted to examine the impact of CB2 polymorphism on the severity of liver lesions (steatosis, necroinflammation, and fibrosis) in patients with nonalcoholic steatohepatitis.

Sophie Lotersztajn, Ph.D.[1,2]
Fatima Teixeira-Clerc, Ph.D.[1,2]
Vanessa Deveaux, Ph.D.[1,2]
Sylvie Manin[1,2]
Ariane Mallat, M.D., Ph.D.[1,2]
[1]*INSERM, U955, Créteil France*
[2]*Université Paris Est, Créteil France*

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Stem Cells or Macrophages: Which Contribute to Bone Marrow Cell Therapy for Liver Cirrhosis?

To the Editor:

The experimental studies and clinical trials of bone marrow (BM) cell therapy for severe liver diseases have been studied extensively, although the benefit is controversial, and the underlying mechanisms remain undefined.[1] With great interest, I read the article by Thomas et al.,[2] who demonstrated that BM-derived differentiated macrophage cell therapy improves structural and functional parameters of experimental chronic liver injury. Their results have identified defined single cell types from BM that have beneficial effects on liver fibrosis. Their study can be hailed as an original contribution in terms of understanding the mechanisms of BM cell therapy for liver fibrosis.

However, other previous studies on BM cell therapy for liver cirrhosis have focused on stem cells that may work by hepatic differentiation or paracrine effects.[3] Which kind of cells in the BM may contribute to cellular therapy for liver cirrhosis: stem cells, macrophages, or both? Here, we would like to offer our concerns regarding this proposed therapy. First, in the report by Thomas et al., the stem cells therapy group was not set to be compared with groups treated with differentiated macrophages. Thus, the different effects of these two kinds of cells on liver injury cannot be known under the same conditions. Furthermore, animal experimental studies have indicated that BM cell-derived stem cell therapy for liver fibrosis is under debate. Nevertheless, some optimistic results can be found in recent clinical trials reports,[4] although safety and efficacy should be further demonstrated. Because there are many differences between mice and humans,[5] more rational and legalized clinical trials based on BM-derived stem cells or macrophages should be encouraged. In addition, BM is composed of a variety of cell types such as hematopoietic stem cells, mesenchymal stem cells, and macrophages. BM cell therapy function may depend on synergistic effects of several kinds of cells, rather than defined single cell types. Thus, experimental studies based on single and combinations of cell types also need to be performed. Moreover, both posttransplant BM-derived differentiated macrophages and stem cells are in transient cell states that may only give short-term relief for liver fibrosis. Thus, repeated cell therapy may be required to acquire definitive therapeutic effects.

Tao Liu, M.D.[1,2]
Yingjie Wang, M.D.[1]
Chenting Wen, M.D.[3]
Shichang Zhang, M.D.[4]
Chunmei Zhang, M.D.[2]
[1]*Department of Infectious Diseases, Southwest Hospital Third Military Medical University, Chongqing, China*
[2]*Department of Internal Medicine 3 The Northern Region of No. 401 Hospital Qingdao, Shandong, China*
[3]*Dongfeng General Hospital, Hubei University of Medicine Shiyan, Hubei, China*
[4]*State Key Laboratory of Trauma Burns and Combined Injury, Department 4 Institute of Surgery Research, Daping Hospital Third Military Medical University, Chongqing, China*

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Reply:

We thank Liu and colleagues for their comments; however, it is important to clarify a number of points.

This was not a comparative study of stem cells versus differentiated cells.[1] Bone marrow (BM) contains a wide range of cell types with potentially diverse actions when delivered during liver injury. Positive effects have been detected following the application of stem/progenitor cell containing BM fractions of varying purity and potency.[2,3] In our experiments, unfractionated BM delivery caused a net increase in murine liver fibrosis.[1] This phenotype is likely to be the result of several (interacting and opposing) BM cell populations. BM stem cells, including hematopoietic and mesenchymal stem cells, constitute a numerically small minority of BM cells. Importantly, injected mesenchymal stem cells can give rise to profibrogenic liver cell populations,[4,5] highlighting the potential risks of uncharacterized cell populations.

The advantages of a single differentiated cell type in terms of predictability and mechanistic clarity do not preclude the potential therapeutic benefits of mixed cell fractions. However, for the field to progress, we need to understand the precise activities of individual donor cell lineages before we can make sense of the potentially diverse effects of mixed BM fractions.

BM stem cells or their derivatives may have benefit in chronic liver disease. However, to date, clinical studies have been small or uncontrolled, hence larger, randomized controlled trials are now required to determine whether there are positive or negative clinical effects. Indeed, adverse events have already been detected following autologous BM cell infusion.[6] To this effect, we are part of a multicenter randomized trial testing CD133+ hematopoietic stem cells in cirrhosis that has already opened in the United Kingdom (ISRCTN: 91288089).

Regarding the possibility of BM stem cells transdifferentiating *in vivo* into hepatocytes, this has repeatedly been shown not to occur to any meaningful degree, if at all. Therefore, paracrine mechanisms of action are more plausible.[7,8]

The chronology of human cirrhosis is measured in years, so clinically useful disease-modifying treatments may require repeat administration. Furthermore, it is possible that cell-based treatments will ultimately be tailored to parameters such as disease etiology and stage of disease, necessitating a broad and well-understood therapeutic arsenal.

James A. Thomas, B.M., B.Sc., M.R.C.P.
John P. Iredale, B.M.(Hons), D.M., F.R.C.P., F.Med.Sci.
Stuart J. Forbes, M.B.Ch.B., Ph.D., F.R.C.P.(Edin)
MRC/University of Edinburgh
Center for Inflammation Research
The Queen's Medical Research Institute
Edinburgh, United Kingdom

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Virological Breakthrough and Resistance in Patients with Chronic Hepatitis B Receiving Nucleos(t)ide Analogues in Clinical Practice

To the Editor:

We read with interest the article by Hongthanakorn et al. published in a recent issue of Hepatology.[1] The authors reported a very high incidence of virological breakthrough (VBT) in patients receiving five different nucleos(t)ide analogues (NUCs) in clinical practice: 26% (39 patients). They reported that 7% of NUC-naïve patients receiving entecavir (ETV) experienced VBT, and that the cumulative probability of experiencing VBT at 3 years was 13%.

The VBT rates reported by Hongthanakorn et al. are higher than described previously. In our population of 69 NUC-naïve chronic HBV patients treated in routine clinical practice with ETV, we found that 100% achieved undetectable HBV DNA after 96 weeks of treatment.[2] We did not perform tests to evaluate genetic resistance, but we found no evidence of clinical resistance to treatment or VBT. Other studies in clinical practice have shown high efficacy of treatment with low rates of VBT, around 1%.[3-5] In phase 3 randomized clinical trials, VBT rates with ETV treatment were 1.6%.[6,7]

Hongthanakorn et al. argue that "medication adherence is likely to be lower in clinical practice than in phase 3 clinical trials, where highly motivated patients are recruited and closely monitored" and that "patients with CHB receiving NUC therapy who experienced VBT should be counseled on medication adherence."[1] We agree that adherence is crucial in the efficacy of treatment, but we have demonstrated that a high adherence rate and treatment efficacy can be obtained in clinical practice.

Ezequiel Ridruejo, M.D.[1,3]
Raúl Adrover, M.D.[2]
Marcelo O. Silva, M.D.[3]
[1]*Hepatology Section, Department of Medicine, Centro de Educación Médica e Investigaciones Clínicas Norberto Quirno "CEMIC", Ciudad Autónoma de Buenos Aires, Argentina*
[2]*Centro de Hepatología La Plata, Prov. de Buenos Aires, Argentina*
[3]*Hepatology and Liver Transplant Unit Hospital Universitario Austral Pilar Prov. de Buenos Aires, Argentina*

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Potential conflict of interest: Nothing to report.

Reply:

We appreciate Dr. Ridruejo and colleagues' interest in our article. We would like to clarify that the high rate of virological breakthrough we observed in our practice does not mean that nucleos(t)ide analog treatment is ineffective in suppressing hepatitis B viral (HBV) replication. Table 2 showed that 71% of patients (53% HBeAg-positive) had undetectable HBV DNA at 1 year, similar to what had been reported in clinical trials. The point that we wish to make is that during long-term treatment, transient virological breakthroughs are common and some of these episodes of breakthrough are related to medication nonadherence rather than antiviral drug resistance, particularly when antiviral drugs with high genetic barrier to resistance are used. Confirmation of virological breakthrough and/or testing for drug resistance mutation would avoid unnecessary changes to the treatment regimen. Because of the transient nature of some of these breakthroughs, the frequency with which they are observed depends on the frequency of HBV DNA monitoring. In our practice, HBV DNA is monitored every 3 months throughout the course of treatment. The rates of breakthrough would likely be lower if our patients were monitored every 6 months or less often.

Anna S. Lok, M.D.
University of Michigan Health System Ann Arbor, MI

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Clinical Correlation of miR-375 and Alpha-Fetoprotein in Hepatocellular Carcinoma: Comparison in Mice and Humans

To the Editor:

We read with great interest the article by Kowalik et al.,[1] recently published in Hepatology. The authors found that hepatocellular carcinomas (HCCs) developed in mice that were administered diethylnitrosamine and then repeatedly treated with the mitogen 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene. These mice showed increased expression levels of the transcriptional coactivator Yes-associated protein (YAP), and these increased expression levels were associated with the down-regulation of miR-375 expression, which is known to control YAP expression,[2] and with enhanced levels of alpha-fetoprotein (AFP), which is encoded by the target gene of YAP. However, the inverse association between miR-375 and AFP expression was not dose-dependent. We describe our single-center experience with 157 HCC patients who underwent primary resection. The patients were divided into two groups on the basis of the mean levels of miR-375 expression in all tumor tissues, which were determined using an miRNA array and validated by real-time polymerase chain reaction (PCR) analysis. Ten samples from living donor livers (mean [SD] miRNA expression, 13.85 [0.57]) were used as controls. miR-375 expression was down-regulated in HCCs. The clinicopathologic characteristics of the patients are summarized in Table 1. We found that preoperative serum AFP levels in the group showing less reduction in miR-375 expression were significantly higher than those in the group showing higher levels of reduction in miR-375 expression. The findings for the other factors were comparable in both groups. In summary, our results suggest that AFP expression in HCCs was not solely regulated by the axis of miR-375-YAP-AFP.

Cheng-Maw Ho, M.D.
Po-Huang Lee, M.D., Ph.D.
Rey-Heng Hu, M.D., Ph.D.
Department of Surgery National Taiwan University Hospital Taipei, Taiwan

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Table 1. Clinicopathologic Characteristics of HCC Patients

Parameter	High Reduction in miR-375 Expression n = 71	Low Reduction in miR-375 Expression n = 86	*P* Value
miR-375 expression, mean ± SD	10.52 ± 1.02	13.19 ± 0.74	0.003
Mean age ± SD (years)	59.4 ± 12.6	59.0 ± 12.3	1.00
Sex (male)	55/71	67/86	1.00
Tumor size ± SD (cm)	4.7 ± 3.0	4.2 ± 2.9	0.63
Cirrhosis	30/71	42/86	0.41
HBV	49/71	63/86	0.6
HCV	18/71	24/86	0.86
Histology grade			0.65
I	7	12	
II	59	68	
III	4	6	
IV	0	0	
TNM staging			0.55
I	43	50	
II	8	15	
III	20	20	
IV	0	1	
Portal vein thrombosis	20/70	22/86	0.72
Preoperative AFP expression, mean ± SD (ng/mL)	2089.3 ± 11347.4	4748.2 ± 16552.6	0.047

Abbreviations: HCC, hepatocellular carcinoma; SD, standard deviation; HBV, hepatitis B virus; HCV, hepatitis C virus; AFP, α-fetoprotein.

enlargement and hepatocellular carcinoma development in mice. HEPATOLOGY 2011;53:2086-2096.
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Reply:

We read with great interest the letter by Ho et al., commenting on our study about the increased expression levels of Yes-associated protein (YAP) in chemically induced mouse hepatocellular carcinomas (HCCs).[1] These increased expression levels were associated with down-regulation of microRNA-375 (miR-375) expression, which is known to control YAP expression,[2] and with enhanced levels of alpha-fetoprotein (AFP), which, in two independent studies, was found to be a target gene of YAP.[3,4] Ho et al. in their letter show that in 157 HCC, divided into two groups on the basis of the mean levels of miR-375 expression, serum AFP levels were significantly higher in the group showing less reduction in miR-375 than in the group showing stronger reduction of miR-375. Based on these findings, the authors suggest that AFP expression in HCCs is not solely regulated by the axis of miR-375-YAP-AFP. We totally agree with this conclusion. Indeed, as shown in our article, while an anti-correlation exists between miR-375 and YAP expression, such an inverse relation could not be observed between miR and AFP, and nowhere in the text did we imply that a YAP increase could be the main mechanism responsible for AFP regulation. AFP is a well-known marker of HCC and its increase is considered the result of a loss of differentiation of cancer cells. Thus, it seems clear that the increased levels of AFP cannot be due simply to up-regulation of YAP, but rather to a general change in the several factors regulating this protein. Interestingly, the article that originally described AFP as a target gene of YAP was based on a transgenic mouse model where YAP overexpression was induced in newborn (3-week-old) animals.[3] Thus, it is possible that the increased AFP expression observed in YAP-overexpressing mice could also be due to YAP-dependent alteration of hepatocyte differentiation.

In conclusion, we agree with Ho et al. that AFP expression in HCCs is not solely regulated by the axis of miR-375-YAP-AFP. As with most genes, many pathways and many mechanisms (both at the transcriptional, posttranscriptional, and posttranslational levels) can regulate the expression of a particular protein and this is surely true also for AFP. What we said in our article is that YAP is among the many factors that can contribute to this regulation.

MARTA A. KOWALIK, PH.D.[1]
GIOVANNA M. LEDDA-COLUMBANO, PH.D.[1]
SILVIA GIORDANO, M.D., PH.D.[2]
AMEDEO COLUMBANO, PH.D.[1]
[1]*Department of Toxicology*
University of Cagliari, Italy
[2]*Institute for Cancer Research and Treatment*
University of Torino, Italy

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Human Hepatic Stellate Cells Are Liver-Resident Antigen-Presenting Cells

To the Editor:

We read with great interest the article published in this journal by Schildberg et al.[1] In this study, the investigators demonstrated that hepatic stellate cells (HSCs) veto naïve cluster of differentiation (CD)8$^+$ T-cell priming through a cell-contact–dependent mechanism, and that this novel HSC function is dependent on their activation state. High expression levels of CD54 on HSCs correlated with impaired expression of interleukin (IL)-2 receptor and IL-2 production in T cells. The take-home message of this study is the identification of a direct role of HSCs in controlling non-major histocompatibility complex (MHC)-restricted dependent immune tolerance.

Although this study reveals a novel, interesting immunological function of HSCs, here, we want to call attention also to their potential role as antigen-presenting cells (APCs). Winau et al.[2] demonstrated that HSCs are intrahepatic APCs activating T cells and eliciting a multiplicity of T-cell responses. More recently, Bomble et al.[3] demonstrated that, even though the function of HSCs as APCs might be secondary in healthy liver and reduced during epithelial-mesenchymal transition, *in vitro* stimulation with interferon gamma (IFN-γ) induces the expression of MHC class II and restores their antigen-presenting functionality.

Here, we reinforce the data on the potential role of HSCs as APCs by demonstrating that these cells express high levels of two components of the antigen processing machinery: the endoplasmic reticulum (ER) aminopeptidases, ERAP1 and ERAP2, which have been shown to optimize precursor peptides for binding to MHC class I molecules.[4] As expected, the human HSC line, LX-2, in basal conditions expresses high levels of surface MHC class I and low levels of surface MHC class II molecules (Fig. 1A). IFN-γ stimulation resulted in the surface enhancement of both MHC class I and MHC class II molecules. Interestingly, MHC class I overexpression, also confirmed by immunoblotting, was associated with the up-regulation of both ERAP1 and ERAP2 (Fig. 1B).

Altogether, these results, in agreement with previous studies,[2,3] demonstrate that HSCs are liver-resident APCs with the capability to induce hepatic T-cell immunity by MHC-restricted interaction.

Finally, these findings, as well as those presented by Schildberg et al., suggest that therapeutic strategies aiming to recover or increase MHC-restricted and/or non-MHC-restricted antigen-presenting functionality of HSCs may provide opportunities to devise immunotherapy strategies toward enhancing the ability of T lymphocytes to recognize and kill tumor cells.

Anna Alisi, Ph.D.[1]
Paolo Romania, Ph.D.[2]
Valerio Nobili, M.D.[1]
Franco Locatelli, M.D.[2,3]
Doriana Fruci, Ph.D.[2]
[1]*Liver Unit and* [2]*Oncohaematology Department Bambino Gesù Children's Hospital and Research Institute, Rome, Italy*
[3]*University of Pavia, Pavia, Italy*

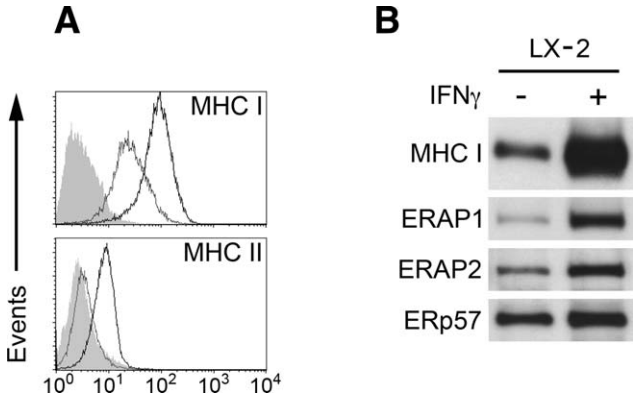

Fig. 1. IFN-γ enhances antigen-processing/presenting functionality of LX-2 cells. (A) Flow cytometric analysis of MHC class I and MHC class II molecules in LX-2 cells grown in serum-free medium in the absence (gray lines) and presence (black lines) of 500 U/mL of IFN-γ for 6 days. Shaded histograms, isotype-matched negative controls. (B) MHC class I, ERAP1, and ERAP2 immunoblotting in LX-2 cells treated as in (A) ERp57 was used for normalization.

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Prominent Regulatory But Weak Antigen-Presenting Cell Function of Hepatic Stellate Cells

To the Editor:

In their commentary, Alisi et al. emphasize the function of hepatic stellate cells (HSCs) as antigen-presenting cells (APCs) besides their regulatory function.[1] As indicated by Alisi et al., HSCs can, in principle, act as APCs for cluster of differentiation (CD)4, CD8, and natural killer T cells.[2] It remained unclear how efficiently HSCs function as APCs relative to other hepatic cells, in particular being located in the Dissé space next to liver sinusoidal endothelial cells (LSECs), a well-documented liver-resident APC.[3]

During conditions of direct competition *in vivo*, HSCs were less efficient than LSECs in the uptake of circulating antigen from the blood (Fig. 1A). Only dendritic cells (DCs) bear the capacity to function as APC after the uptake of small amounts of antigen. They employ antigen targeting through receptor-mediated endocytosis into intracellular compartments dedicated to cross-presentation in combination with antigen-persistence within these compartments for efficient, prolonged antigen presentation.[4,5] Other cells, such as macrophages, or LSECs need more antigen uptake to cross-present antigen in a similar fashion,[6,7] thus indicating that antigen processing is less efficient, compared to DCs, but compensated by superior antigen uptake. Thus, we next investigated the relative efficiency of HSCs to cross-present antigen to CD8 T cells. Clearly, HSCs were again inferior to LSECs in cross-presentation of circulating antigen ingested *in vivo* (Fig. 1B). These results demonstrate that HSCs do not possess antigen-processing capability similar to DCs, suggesting that if already antigen uptake is inefficient, downstream mechanisms, such as peptide trimming in the endoplasmic reticulum (ER), are unlikely to improve APC function. A recent report also showed that primary HSCs have little, if any, APC function for CD4 T cells, even after stimulation with exogenous interferon gamma.[8] Taken together, these results demonstrate a hierarchy in the performance of APC function for DCs being most efficient in antigen processing, macrophages and LSECs compensating for inefficient antigen processing by high antigen uptake, and HSCs showing low antigen uptake and inefficient antigen processing. These data call into question a prominent role for HSCs as liver-resident APCs.

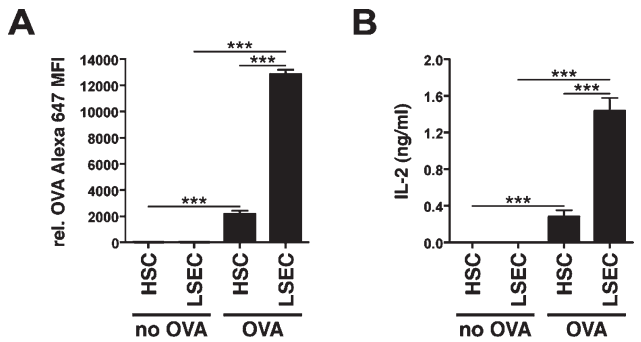

Fig. 1. (A) Antigen-uptake by LSECs or HSCs isolated 1 hour after challenge with fluorochrome-labeled ovalbumin (OVA; 4 μg). (B) IL2 release from B3Z-cells recognizing OVA-derived SIINFEKL on H-2K^b after 18 hours coculture with LSECs or HSCs, isolated from mice 1 hour after injection of 1 mg of OVA.

FRANK A. SCHILDBERG
CHRISTIAN KURTS, M.D.
PERCY A. KNOLLE, M.D.
Institutes of Molecular Medicine and Experimental Immunology
Friedrich-Wilhelms-Universität Bonn, Bonn, Germany

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Outcome of Patients Suffering from a Spontaneous Reactivation of Hepatitis B Presenting as Acute-On-Chronic Liver Failure and Treated with Antivirals

To the Editor:

We read with great interest Garg et al.'s article[1] published in HEPATOLOGY. The authors conducted a randomized study to compare the efficacy of tenofovir disoproxil fumarate (TDF) therapy and placebo therapy in patients suffering from a severe spontaneous reactivation of chronic hepatitis B (CHB) presenting as acute-on-chronic liver failure. They reported a high overall mortality rate of 63.0% (17/27), with rates of 43% and 85% in the TDF and placebo arms,

respectively. TDF significantly reduced the mortality rate and hepatitis B virus DNA levels and improved the Child-Turcotte-Pugh and Model for End-Stage Liver Disease (MELD) scores in these patients at 3 months.

It is noteworthy that some patients with cirrhosis were enrolled. First, we consider 3 months of observation to be too short for determining the survival of these patients. We reexamined 96 patients with liver decompensation treated with lamivudine in our previous study in Taiwan.[2] Eight patients (8.3%), two patients (2.1%), and three patients (3.1%) died within 1, 1 to 3, and 3 to 6 months of lamivudine treatment, respectively. In other words, 23.1% of the patients (3/13) who did not survive for 6 months died after 3 months of antiviral treatment. Villeneuve et al.[3] reported that 25% of their patients (1/4) without hepatocellular carcinoma died from hepatic failure within 3 to 6 months of the initiation of lamivudine treatment. Fontana et al.[4] reported that patients with decompensated cirrhosis were still dying even after the first 6 months. Hence, the mortality rate is possibly underestimated in Garg et al.'s study.[1] The mean baseline MELD scores (27 and 25 in the TDF and placebo arms, respectively) reflect the fact that the patients enrolled in their study had more severe liver disease. In HEPATOLOGY, Liaw et al.[5] reported lower mortality rates for patients with CHB and decompensated liver disease who were treated with TDF (4.4% or 2/45) or entecavir (9.1% or 2/22) by 48 weeks. The lower mortality rate is likely related to the milder severity of the patients' liver disease (the median baseline MELD scores were 11 and 10.5 for the TDF and entecavir arms, respectively). Hence, the earlier initiation of nucleot(s)ide analogue therapy seems critical for these patients.

Another issue is the design of this study of patients with cirrhosis and CHB-related acute-on-chronic liver failure: a placebo arm was included. With a lack of facilities for liver transplantation, Garg et al.[1] observed a high mortality rate, and most deaths (82%) occurred because of progressive liver failure that led to the development of multiorgan failure.[1] With a mortality rate of 4% to 30% 6 to 12 months after lamivudine, telbivudine, and entecavir therapy[3-8] and with significant improvements in the long-term survival of patients with hepatic failure,[4] it does not seem appropriate to include a placebo arm in studies enrolling patients with cirrhosis and critical liver failure. In the era of nucleot(s)ide analogue therapy with more potent anti–hepatitis B virus effects, we look forward to the results of more large-scale studies seeking to clarify whether the efficacy can be improved, particularly in patients with cirrhosis, CHB, and acute exacerbation, who have a poorer prognosis.

CHIA-YEN DAI, M.D., PH.D.[1–3]
MING-LUN YEH, M.D.[1]
CHUNG-FENG HUANG, M.D., M.S.[1,2,4]
JEE-FU HUANG, M.D.[1,3,5]
MING-LUNG YU, M.D., PH.D.[1,3,4]
WAN-LONG CHUANG, M.D., PH.D.[1,3]
Departments of [1]Internal Medicine (Hepatobiliary Division) and [2]Occupational Medicine
Kaohsiung Medical University Hospital, Kaohsiung, Taiwan
[3]Faculty of Internal Medicine, College of Medicine
Kaohsiung Medical University, Kaohsiung, Taiwan
[4]Department of Internal Medicine, Kaohsiung Municipal Ta-Tung Hospital, Kaohsiung, Taiwan
[5]Department of Internal Medicine, Kaohsiung Municipal Hsiao-Kang Hospital, Kaohsiung, Taiwan

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Lack of an Association Between an Apolipoprotein C3 Genetic Variant and the Liver Fat Content in Patients with Type 2 Diabetes

To the Editor:

We read with great interest the article by Kozlitina et al.,[1] who found no causal relationship between apolipoprotein C3 (APOC3) variants and hepatic triglyceride contents in middle-aged men and women. These results are not in accordance with a recent publication by Petersen et al.,[2] who demonstrated that C-482T and T-455C polymorphisms in APOC3 are associated with nonalcoholic fatty liver disease (NAFLD) and insulin resistance. Even though NAFLD is well known to be associated with insulin resistance and diabetes mellitus, the link between certain genetic polymorphisms, NAFLD, and insulin resistance is quite complex. Indeed, the patatin-like phospholipase domain containing 3 (PNPLA3) polymorphism is strongly associated with NAFLD but not with obesity or insulin resistance.[3,4] In contrast, Petersen et al. found that genetic variants in APOC3 are associated with the liver fat content and insulin resistance; their results, however, have not been confirmed by Kozlitina et al. We recently published a

Table 1. Clinical and Metabolic Characteristics of Patients With Type 2 Diabetes According to the APOC3 rs2854117 Polymorphism

Characteristic	Wild-Type Homozygote (n = 124)	Variant-Allele Carriers (n = 129)	*P* Value
Age (years)	62.1 ± 10.7	61.6 ± 9.5	0.79
Body mass index (kg/m^2)	34.5 ± 6.5	33.9 ± 6.9	0.90
Aspartate aminotransferase (IU/L)	24.1 ± 18.1	22.9 ± 11.4	0.89
Alanine aminotransferase (IU/L)	37.1 ± 21.6	40.1 ± 32.6	0.44
Plasma triglyceride level (mmol/L)	2.25 ± 1.57	2.13 ± 1.53	0.24
Adiponectin plasma level (μg/mL)	6.2 ± 4.7	5.9 ± 5.0	0.70
Visceral fat area (cm^2)	267 ± 109	273 ± 89	0.42
Liver fat content (%)	10.4 ± 8.5	10.7 ± 8.9	0.84
Steatosis: liver fat content > 5.5% [n (%)]	76 (61.2)	82 (63.5)	0.79

study confirming that in people with type 2 diabetes, the liver fat content was related to the rs738409 PNPLA3 polymorphism.[5] In this discordant context, we set out to determine whether the liver fat content, evaluated with proton magnetic resonance spectroscopy, was associated with the rs2854117 APOC3 polymorphism in this population. The study involved 253 patients with type 2 diabetes. One hundred fifty-eight patients (62.4%) had steatosis (hepatic triglyceride content >5.5%). In comparison with patients without steatosis, patients with steatosis had a higher body mass index, a higher visceral fat area, higher plasma alanine aminotransferase levels, higher plasma triglyceride levels, and lower plasma adiponectin levels.

The APOC3 rs2854117 variant was not associated with the liver fat content in our population (Table 1). No associations were found between this APOC3 variant and either the plasma triglyceride levels or the visceral fat area. In accordance with the study reported by Kozlitina et al.,[1] our data for a specific population of patients with type 2 diabetes and a high prevalence of NAFLD suggest that the rs2854117 APOC3 genetic variant has little or no impact on the liver fat content.

Jean Michel Petit, M.D.
Boris Guiu, M.D.
David Masson, Ph.D.
Jean-Pierre Cercueil, M.D.
Patrick Hillon, M.D.
Bruno Verges, M.D.
Institut National de la Santé et de la Recherche Médicale Unité 866 (Centre de Recherche), Centre Hospitalier Universitaire du Bocage Université de Bourgogne, Dijon, France

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Carnitine Palmitoyltransferase as a Potential Target for Treating Diabetes: Is Inhibition or Activation Preferable?

To the Editor:

We have read with interest the recent article by Orellana-Gavaldà et al.,[1] who report that a long-term increase in hepatic fatty acid oxidation (FAO) leads to a beneficial effect in a mouse model of obesity and diabetes. This effect of increased FAO is induced by the overexpression of carnitine palmitoyltransferase 1A (CPT1A) or its malonyl–coenzyme A insensitive mutant isoform (CPT1AM). With the reduction in hepatic steatosis, there should be an accompanying increase in insulin sensitivity. CPT1AM overexpression is the more effective treatment in this obese/diabetes phenotype animal model. An important finding is that an increase in FAO during the postprandial phase contributes to the overall effect; during this time, endogenous CPT1A activity is reduced by a concomitant increase of malonyl–coenzyme A, its physiological inhibitor. Greater FAO activity during the phase in which the liver is primarily engaged in the synthesis of fatty acids and triglycerides (TAGs) may lead to important long-term changes in metabolic intermediates (i.e., acyl coenzyme A). These intermediates may mediate the observed improvements in glucose and lipid metabolism by affecting the complex network

of transcriptional factors (i.e., peroxisome proliferator-activated receptors, hepatocyte nuclear factor, and sirtuins).[2] Furthermore, the lowering of liver TAG levels may be associated with a reduction of TAG metabolic intermediates, which are known to counteract insulin signaling.[3] This article describes a remarkable decrease in plasma glucose levels in CPT1AM$^{+/+}$ db/db mice (genetically obese and diabetic mice). This counterintuitive effect occurs in a murine model in which the severe hyperglycemic condition is primarily dictated by an increased rate of gluconeogenesis (GNG). Indeed, this metabolic pathway is strongly dependent on increased FAO activity according to the following observations: it provides adenosine triphosphate and reducing equivalents, and it increases the intramitochondrial levels of acetyl coenzyme A, which is an obligate allosteric activator of the key enzyme pyruvate carboxylase in the GNG pathway.[4]

Metformin, a first-line therapy for type 2 diabetes, depresses GNG via the reduction of intracellular adenosine triphosphate contents.[5] Also, an efficient way of reducing hepatic GNG is the inhibition of CPT1A. We have recently shown that selectively inhibiting CPT1A depresses GNG both *in vitro* and *in vivo* and results in improvement in the diabetic phenotype for db/db mice; this is also accompanied by improved insulin sensitivity in diet-induced obese mice.[6] These effects have been observed despite concomitant increases in liver TAG levels. According to these metabolic considerations, promoting FAO seems to conflict with the antidiabetic outcomes reported in db/db mice expressing CPT1AM in the liver. An analysis of the molecular mechanism or mechanisms underlying the antidiabetic effects resulting from the greater activity of liver FAO perhaps may open new approaches to modulating GNG in type 2 diabetes. One scientific inaccuracy in this work is the erroneous report by the authors that β-hydroxybutyryl coenzyme A in the liver is an intermediate of ketone body metabolism (see Table 1 and p 825 of their article). D-β-Hydroxybutyrate is a ketone body enantiomer oxidized through the formation of acetoacetate and not through its esterification to coenzyme A, whereas the enantiomer L-β-hydroxybutyl coenzyme A is an intermediate of FAO.[7,8]

Arduino Arduini, M.D.[1]
Mario Bonomini, M.D.[2]
[1]*R&D Department*
CoreQuest Sagl
Bioggio, Switzerland
[2]*Institute of Nephrology*
Department of Medicine
G. d'Annunzio University
Chieti, Italy

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Vitamin D Deficiency May Link Diabetes and Liver Neoplasms

To the Editor:

I read with great interest the article by Chen et al.,[1] who confirmed the association of diabetes with liver neoplasms and found that the incidence of primary malignant neoplasms of the liver was significantly higher in patients with diabetes versus control subjects. Even though multiple factors should be responsible for the association between diabetes and an increased risk of malignant neoplasms of the liver, I propose that vitamin D deficiency potentially links the two disorders for the following reasons.

First, vitamin D levels have been found to be significantly lower in diabetic populations versus subjects without diabetes.[2,3] It has been reported that vitamin D deficiency predisposes individuals to type 1 diabetes and type 2 diabetes and may be involved in the pathogenesis of both forms of diabetes.[3,4] Second, vitamin D deficiency has been proposed to contribute to high risks for various types of cancers. An increasing number of epidemiological studies support the hypothesis that vitamin D plays a role in cancer prevention.[5,6] Therefore, it seems rational to infer that vitamin D deficiency may account in part for the experimental finding of a higher incidence of malignant neoplasms of the liver in patients with diabetes versus age- and sex-matched control subjects. Moreover, if this hypothesis is verified in future studies, vitamin D status optimization in patients with diabetes may represent a potential strategy not only for improving the condition of patients with diabetes but also for lowering the associated risk of malignant neoplasms of the liver.

Hong-Fang Ji, Ph.D.
Shandong Provincial Research Center for Bioinformatic Engineering and Technique, Shandong University of Technology, Zibo
People's Republic of China

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Profound Differences of MicroRNA Expression Patterns in Hepatocytes and Hepatoma Cell Lines Commonly Used in Hepatitis C Virus Studies

To the Editor:

In their recent letter to the editor, Bensadoun et al. describe a divergent *IL-28B* (interleukin-28B) genotype of hepatoma cell lines including Huh7 and its derivatives.[1] Because these cell lines are commonly used in cell culture studies of hepatitis C virus (HCV) and the *IL-28B* gene polymorphism is a predictive marker for the outcome of HCV infection, their observations emphasize the importance of cautiously interpreting infection studies performed *in vitro*. Here, we extend their conclusions and report profound differences in the microRNA (miRNA) expression between primary human hepatocytes (PHHs) and Huh7 cell lines.

Cellular miRNAs are key regulators in posttranscriptional regulation of gene expression. They can also directly participate in virus replication or act indirectly by determining the expression level of replication cofactors. To analyze whether the commonly used Huh7 cell lines differ in their miRNA expression patterns, we screened 883 human miRNAs using the Geniom Biochip miRNA *Homo sapiens* (febit holding gmbh, Heidelberg, Germany). PHHs isolated from human liver resections of two different patients were used as reference. Figure 1 and Supporting Table 1 show the profound differences not only between PHHs and hepatoma cell lines but also between the cell lines. Remarkably, the liver-specific miRNA122 that directly influences HCV replication both in cell culture and in infected chimpanzees[2-4] was one of the most differentially expressed miRNAs. Furthermore, predictions of the gene targets of the miRNAs from Fig. 1A by the Genetrail program (freely accessible at http://genetrail.bioinf.uni-sb.de) identified a number of host proteins whose differential expression is known or expected to influence HCV replication (see Supporting Table 2 for the complete target list). Because studies on host factor requirements for virus replication nowadays involve small interfering RNA–mediated down-regulation of candidate proteins, and the level of knockdown is influenced by protein abundance and

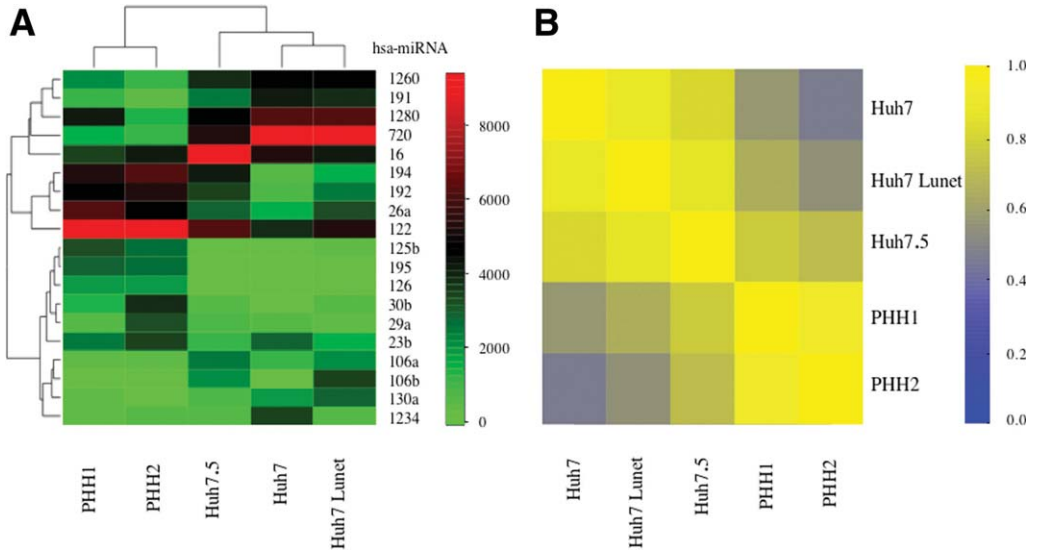

Fig. 1. MicroRNA patterns of primary human hepatocytes and HCV-permissive hepatoma cell lines. (A) Heat map of a hierarchical clustering of miRNAs and samples. The analyzed cell type is indicated on the *x*-axis and the 19 most different human miRNAs are indicated on the *y*-axis. Red color represents a high expression whereas green corresponds to a low expression of miRNAs. Relative expression levels are given in a relative scale from 0 to 10,000. Samples can mainly be separated into two clusters. The cluster on the left includes PHHs from two different donors without liver disease (PHH1 and PHH2), and the cluster on the right includes the three analyzed hepatoma cell lines Huh7, Huh7.5, and Huh7 Lunet. (B) Color-coded correlation of the overall miRNA expression pattern of PHHs and three hepatoma cell lines. Strong resemblance is indicated in yellow (1.0 equals 100%); no resemblance is given in dark blue.

turnover and thus miRNA composition, one would expect that the respective experimental results would be influenced by the host cells used. The recent observations of non-overlapping screening results for HCV host factors[5] may well be related to corresponding miRNA differences in the host cells.

Michael Ehrhardt[1]
Petra Leidinger, Ph.D.[2]
Andreas Keller, Ph.D.[2]
Thomas Baumert, M.D.[3,4]
Juana Díez, Ph.D.[5]
Eckart Meese, Ph.D.[2]
Andreas Meyerhans, Ph.D.[1,6]
Departments of [1]Virology and [2]Human Genetics, Saarland University, Homburg, Germany
[3]Institut National de la Santé et de la Recherche Médicale, Unité 748 and [4]Université de Strasbourg, Strasbourg, France
[5]Molecular Virology Group and [6]ICREA Infection Biology Group, Department of Experimental and Health Sciences, Universitat Pompeu Fabra, Barcelona, Spain

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Updated Management of Hepatocellular Carcinoma

To the Editor:

I read with interest the updated practice guidelines for the management of hepatocellular carcinoma (HCC) by the American Association for the Study of Liver Diseases.[1] It is undeniable that the Barcelona Clinic Liver Cancer staging system has become widely accepted in clinical practice. However, the management options for each stage appear to be too rigidly restricted. For example, percutaneous ethanol injection (PEI) can be considered for patients with liver nodules that are inaccessible to radiofrequency ablation, but in comparison with either treatment as monotherapy, the combination of radiofrequency ablation and PEI has been shown to be more effective for long-term survival.[2] For intermediate-stage patients, transarterial chemoembolization (TACE) seems to be the treatment of choice. However, in comparison with TACE alone, the combination of TACE and PEI has been demonstrated to prolong survival in patients with small lesions and in patients with advanced lesions.[3,4] On the other hand, although the use of sorafenib in patients with advanced HCC has been proven to prolong survival, the reported partial response rate is only 2%, and no patients have achieved a complete response.[5] Our group has demonstrated that an intra-arterial infusion of chemotherapy can lead to a complete response in 9.4% of patients with advanced HCC and to a partial response in 18.9%.[6] Thus, for patients with advanced HCC, an intra-arterial infusion of chemotherapy is a viable alternative to sorafenib.

Gin-Ho Lo, M.D.
Digestive Center, Department of Medical Education
E-Da Hospital, I-Shou University, Kaohsiung, Taiwan

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