雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Keiji Ueda[1,] *, Hiroko Omori	Successful Generation of Hepatitis B virus (HBV) Pseudotype Particle; a versatile tool for Identification of the HBV Receptor and Investigation of HBV infectivity	J. Liver	3 (5)	1000169	2014
Tanaka T, Kasai H, Yamashita A, Okuyama-Dobashi K, Yasumoto J, Maekawa S, Enomoto N, Okamoto T, Matsuura Y, Morimatsu M, Manabe N, Ochiai K, Yamashita K, Moriishi K	Hallmarks of hepatitis C virus in equine hepacivirus.	J. Virol.	88	13352-13366	2014
Salam KA, Furuta A, Noda N, Tsuneda S, Sekiguchi Y, Yamashita A, Moriishi K, Nakakoshi M, Tani H, Roy SR, Tanaka J, Tsubuki M, Akimitsu N	PBDE: Structure- Activity Studies for the Inhibition of Hepatitis C Virus NS3 Helicase	Molecules,	19	4006-4020	2014
Matsuzawa T, Kawamura T, Ogawa Y, Maeda K, Nakata H, Moriishi K, Koyanagi Y, Gatanaga H, Shimada S, Mitsuya H	EFdA, a Reverse Transcriptase Inhibitor, Potently Blocks HIV-1 Ex Vivo Infection of Langerhans Cells within Epithelium	J. Invest. Dermatol	134	1158-1161	2014
Furuta A, Salam KA, Hermawan I, Akimitsu N, Tanaka J, Tani H, Yamashita A, Moriishi K, Nakakoshi M, Tsubuki M, Peng PW, Suzuki Y, Yamamoto N, Sekiguchi Y, Tsuneda S, Noda N	Identification and biochemical characterization of halisulfate 3 and suvanine as novel inhibitors of hepatitis C virus NS3 helicase from a marine sponge.	Mar. Drugs	12	462-476	2014
Allen SJ, Mott KR, Matsuura Y, Moriishi K, Kousoulas KG, Ghiasi H	Binding of HSV-1 Glycoprotein K (gK) to Signal Peptide Peptidase (SPP) Is Required for Virus Infectivity	PLOS one	9	e85360,	2014

Yoshimoto N, Tatematsu K, Iijima M, Niimi T, Maturana AD, Fujii I, Kondo A, Tanizawa K, and Kuroda S.	High-throughput de novo screening of receptor agonists with an automated single-cell analysis and isolation system	Scientific Reports	4	4242 (E-pub 9 pages)	2014
Ohno M, Otsuka M, Kishikawa T, Shibata C, Yoshikawa T, Takata A, Muroyama R, Kowatari N, Sato M, Kato N, Kuroda S, and Koike K.	Specific delivery of microRNA93 into HBV-replicating hepatocytes downregulates protein expression of liver cancer susceptible gene MICA	Oncotarget	5	5581-5590	2014
Yoshimoto N., and Kuroda, S.	Single-cell-based breeding: Rational strategy for the establishment of cell lines from a single cell with the most favorable properties.	J. Biosci. Bioeng.	117	394-400	2014
Fukuhara T, Wada M, Nakamura S, Ono C, Shiokawa M, Yamamoto S, Motomura T, Okamoto T, Okuzaki D, Yamamoto M, Saito I, Wakita T, Koike K, and Matsuura Y.	Amphipathic α-Helices in apolipoproteins are crucial to the formation of infectious hepatitis C virus particles.	PLoS Pathog		doi: 10.1371/journal.ppat.1004534	2014
Shiokawa M, Fukuhara T, Ono C, Yamamoto S, Okamoto T, Watanabe N, Wakita T, and Matsuura Y.	Novel permissive cell lines for a complete propagation of hepatitis C virus.	J Virol	88	5578-5594	2014
鎌田　佳宏 三善　英知 竹原　徹郎	NAFLDの診断と検査法のupdate	日本消化器病学会雑誌	111(1)	25-34	2014
鎌田　佳宏 三善　英知 吉田　雄一 竹原　徹郎	サイトカインとNASH	最新醫學	69(9)	35-41	2014
Azuma K Shinzaki S Asazawa H Kuroki E Kawamoto S Kamada Y Hayakawa K and Miyoshi E	Twin studies on the influence of genetic factors on serum agalactosyl immunoglobulin G level.	Biomedical Reports	2	213-216	2014

Onuki K Sugiyama H Ishige K Kawamoto T Ota T Ariizumi S Yamato M Kadota S Takeuchi K Ishikawa A Onodera M Onizawa K Yamamoto M Miyoshi E Shoda J	Expression of N-acetylglucosaminyltransferase V in the subserosal layer correlates with postsurgical survival of pathological tumor stage 2 carcinoma of the gallbladder.	J Gastroenterology	49(4)	702-714	2014
Sato M Kamada Y Takeda Y Kida S Ohara Y Fujii H Akita M Mizutani K Yoshida Y Yamada M Hogaku K Takehara T Miyoshi E	Fetuin-A negatively correlates with liver and vascular fibrosis in nonalcoholic fatty liver disease subjects.	Liver International			in press
Tomita Y Azuma K Nonaka Y Kamada Y Tomoeda M Kishida M Tanemura M Miyoshi E	Pancreatic fatty degeneration and fibrosis as predisposing factors for development of pancreatic ductal adenocarcinoma.	Pancreas			in press
Fujita K Shimomura M Umemura M Nakata W Sato M Nagahara A Nakai Y Takamatsu S Miyoshi E Nonomura N	Serum fucosylated haptoglobin as a novel prognostic biomarker predicting a high Gleason score of prostate cancer	The Prostate	74(10)	1052-1058	2014

Furukawa K Kawamoto K Eguchi H Tanemura M Tomimaru Y Akita H Hama N Wada H Kobayashi S Nonaka Y Takamatsu S Shinzaki S Kumada T Satomura S Ito T Serada S Naka T Mori M Doki Y Miyoshi E Nagano N	Clinico-pathological significance of leucine-rich alpha-2-glycoprotein-1 in sera of patients with pancreatic cancer.	Pancreas	44(1)	93-98		2015
Asazawa H Kamada Y Takeda Y Takamatsu S Shinzaki S Kim Y Nezu R Kuzushita N Mita E Kato M Miyoshi E	Serum fucosylated haptoglobin in chronic liver disease as a potential biomarker of hepatocellular carcinoma development.	Clin Chem Lab Med.	53(1)	95-102		2014
Kamada Y Sato M Kida S Akita M Mizutani K Fujii H Sobajima T Yoshida Y Shinzaki S Takamatsu S Takehara T Miyoshi E	N-acetylglucosaminyltransferase V exacerbates concanavalin A-induced mouse hepatitis.	Molecular Medicine Reports				in press
Azuma K Serada S Takamatsu S Terao N Takeishi S Kamada Y Naka T Miyoshi E	Identification of sialylated glycoproteins in doxorubicin-treated hepatoma cells with glycoproteomic analyses.	J Proteome Research	13(11)	4869-4877		2014

Ohkura T Fujioka Y Nakanishi R Shiochi H Sumi K Yamamoto N Matsuzawa K Izawa S Ohkura H Ueta E Kato M Miyoshi E Taniguchi S Yamamoto K	Low level galectin-3 concentrations are associated with insulin resistance in patients with type 2 diabetes mellitus.	Diabetology & Metabolic Syndrome			in press
Tanida T Tanemura M Miyoshi E Nagano H Furukawa K Nonaka Y Akita H Hama N Wada H Kawamoto K Kobayashi S Eguchi H Mori M Doki Y	Pancreatic cancer immunotherapy using a tumor lysate vaccine, engineered to express α-gal epitopes, targets pancreatic cancer stem cells.	Int J Oncology			in press
Honma R Kinoshita I Miyoshi E Tomaru U Matsuno Y Shimizu Y Takeuchi S Kobayashi Y Kaga K Taniguchi N Akita DH	Expression of α1,6-fucosyltransferase is associated with prognosis and histology in non-small cell lung cancers.	Oncology			in press
Sobajima T Yoshimura S Iwano T Kunii M Watanabe M Atik N Mushiake S Morii E Koyama Y Miyoshi E Harada A	Rab11a is required for apical protein localization in the intestine.	Biol Open	4(1)	86-94	2014
Wang Y Fukuda T Isaji T Lu J Gu W Lee HH Ohkubo Y Kamada Y Taniguchi N Miyoshi E Gu J	Loss of α1,6-fucosyltransferase suppressed liver regeneration: implication of core fucose in the regulation of growth factor receptor-mediated cellular signaling.	Scientific Reports	5	8264	2015

Yamada R, Hiramatsu N, Oze T, Morishita N, Harada N, Yakushijin T, Iio S, Doi Y, Yamada A, Kaneko A, Hagiwara H, Mita E, Oshita M, Itoh T, Fukui H, Hijioka T, Katayama K, Tamura S, Yoshihara H, Imai Y, Kato M, Miyagi T, Yoshida Y, Tatsumi T, Kasahara A, Hamasaki T, Hayashi N, Takehara T; the Osaka Liver	Impact of alpha-fetoprotein on hepatocellular carcinoma development during entecavir treatment for chronic hepatitis B virus infection.	J Gastroenterol		In press	2014
Aoki, Y., Sugiyama, M., Murata, K., Yoshio, S., Kurosaki, M., Hashimoto, S., Yatsuhashi, H., Nomura, H., Jong-Hon, K., Takeda, T., Naito, S., Kimura, T., Yamagiwa, Y., Korenaga, M., Imamura, M., Masaki, N., Izumi, N., Kage, M., Mizokami, M., and Kanto, T.	Association of serum IFN-□3 with inflammatory and fibrosis markers in patients with chronic hepatitis C virus infection.	J Gastroenterol			2014 (in press)
Nishikawa J, Yoshiyama H, Iizasa H, Kanehiro Y, Nakamura M, Nishimura J, Saito M, Okamoto T, Sakai K, Suehiro Y, Yamasaki T, Oga A, Yanai H, Sakaida I.	Epstein-Barr virus in gastric carcinoma.	Cancers	6	2259-2274	2014

Okamoto T, Hayashi Y, Mizuno H, Yanai H, Nishikawa J, Nakazawa T, Iizasa H, Jinushi M, Sakaida I, Yoshiyama H.	Colonization of an acid resistant Kingella denitrificans in the stomach may contribute to gastric dysbiosis by Helicobacter pylori.	J Infect Chemother	20	169-174	2014
Sai Y, Nishimura T, Muta M, Iizasa H, Miyata T, Nakashima E.	Basic fibroblast growth factor is essential to maintain endothelial progenitor cell phenotype in TR-BME2 cells.	Biol Pharm Bull	37	688-693	2014
Kanda T, Miyata M, Kano M, Kondo S, Yoshizaki T, Iizasa H.	Clustered microRNAs of the Epstein-Barr Virus cooperatively downregulate an epithelial cell-specific metastasis suppressor.	J Virol	89	2684-2697	2015

IV. 研究成果の刊行物・別刷

Change in Cellular Gene Expression by hepatitis B virus (HBV)

Keiji Ueda

Division of Virology, Department of Microbiology and Immunology, Osaka University Graduate School of

Medicine,

2-2 Yamada-oka, Suita, Osaka 565-0871, Japan

Tel; +81-6-6789-3783, Fax; +81-6-6879-3789, Email; kueda@virus.med.osaka-u.ac.jp

ABSTRACT

Gene expression profiles of hepatitis B virus (HBV) producing and non-producing cells were investigated to know how HBV affects cellular gene expression profiles. In this report, we tested the gene expression profile of an HBV producing cell line called HB611 cells and its parental counterpart Huh6 cells. The HB611 cells have been confirmed to express all HBV-related gene products except X gene and produce HBV-like particles that supposed to be infectious into the medium. Gene expression profile analyses were performed using a DNA microarray and it was found that not a few genes was changed for their expression in the presence of HBV related products. Among them, 2,063 genes out of 51,000 were expressed in HB611 cells eight times or more than in Huh6 cells and 1014 genes were expressed in Huh6 cells eight times or more than in HB611 cells. Interestingly, alpha-fetoprotein (*AFP*), which is an immature hepatocyte factor, and *CD24* (a small cell lung carcinoma cluster 4 antigen) were extremely highly expressed in HB611 cells. Some cell cycle genes such as cyclin E (*CYCE*) and cdk2 (*CDK2*) were expressed higher in HB611 cells than in Huh6 cells. This analysis was performed with the cells expressing HBV related genes and producing HBV artificially in the hepatocellular originated cell line. Therefore, the gene expression profile may be somewhat different from that of natural HBV infection to normal hepatocytes. Nonetheless, it was suggested that HBV infection and its gene expression should made the cellular gene expression change and some of them should be relevant to blastic change of differentiated hepatocytes.

INTRODUCTION

Hepatitis B virus (HBV) is a causative agent for acute, chronic hepatitis leading to liver cirrhosis and finally to hepatocellular carcinoma, which are serious problems where HBV is prevalent such as east and south-east Asia, middle east countries, Africa, the equator of south America, Alaska, the coast area of Greenland [17].

HBV was identified as an Australian antigen firstly by Blumberg about a half century ago[1]. It is obvious that HBV infection is involved in the several diseases mentioned above [10]. The pathophysiology, however, that HBV causes, and the viral life cycle itself, have not been yet to be clarified, largely due to limited propagation system of this virus [20]. Even though there are several animal models similar to HBV such as avihepadnaviruses (duck hepatitis B virus [DHBV] and heron hepatitis B virus [HHBV] etc.,) and the other or-thohepadnaviruses like grand squirrel hepatitis virus (GSHV) and woodchuck hepatitis virus (WHV), they are still inconvenient for daily research activity [17]. Of course, orthohepadnaviruses have been identified in pri-mates; wooly monkey hepatitis B virus (WMHBV), gibbon ape hepatitis B virus (GAHBV), orangutan hepatitis B virus (OHBV) and chimpanzee hepatitis B virus (CHBV) [17], but their usage in the species are beset with ethical issues .

Molecular studies of HBV clarified many functions of HBV related gene products. Such data, however, should be verified in the natural life cycle *in vitro* and/or *in vivo* and further more, there are a lot of mysteries about HBV including the covalently closed circular DNA (cccDNA) formation and its regulation, the entry and the egress [4]. HBV infects efficiently primary human hepatocytes (PHH) in vitro [5, 16] and tupaia primary human hepatocytes [23]. PHH is now commercially available from human liver-uPA-SCID mouse [13], but too expensive for ordinary research. A hepatocellular carcinoma-originated cell line called HepaRG was reported to become permissive for HBV several weeks after differentiation induction with 2% dimethyl sulfoxide (DMSO) [6]. Nonetheless, its infectivity is limited as much as twenty to thirty percent and the Hep-aRG is also commercially sold and restricted for its use and propagation. Therefore, more useful and conven-ient HBV infection system must be established.

Thus, it is very difficult to understand accurate HBV life cycle and we only test HBV amplification mechanism by transfecting cloned HBV genome into hepatocellular-originated cell lines such as HepG2, HuH6, HuH7 and so on. The limited transfection efficiency to these cell lines seems not to be appropriate how HBV affects the cellular gene expression profile. And there have been only two reports about cellular gene expression profiles under HBV producing and infecting models [2, 7].

In this report, we analyzed cellular gene expression profiles comparing HBV producing cells to its parental HBV non-producing cells and recognized that considerable numbers of gene expression level was altered by HBV amplification. We also picked up several characteristic gene expression level involved in cell cycle and interferon system.

MATERIALS AND METHODS

CELL. HuH6 and HB611 cells were grown in DMEM (low glucose; 1.0g/L) (Nakalai tesque®) supplemented with 10% fetal bovine serum, 10IU/ml penicillin G and 10μg/ml streptomycin (Nakalai tesque®). In case of HB611 culture, G418 (Nakalai tesque®) was added in the media at 0.5mg/ml.

RNA PURIFICATION. HuH6 and HB611 cells were grown on the 10 cm cell culture dish (Iwaki®) at almost confluent condition whose cell number was about 2~3 x 10⁶/dish. The cells were washed with phosphate buffered saline (PBS) twice and then lysed directly in 3ml Trizol® (Invtrogen-Life Technologies). Each lysed solution was transferred into a 15ml centrifugation tube (BD™), respectively and total RNA was purified according to the manufacturer's protocol. The RNA was finally solved in TE solution (10mM Tris-HCl pH7.6, 1mM EDTA) and the concentration was measured with a UV Spectrophotometer (DU800™, Beckman)

DNA MICROARRAY ANALYSES. Each total RNA (250ng) was labeled with GeneChip® 3′IVT Express Kit aRNA amplification prodedure according to the manufacturer's protocol (GeneChip® 3′IVT Kit User Manual, P/N 702646 Rev.1; Chapter 2 aRNA Amplification Protocol and Chapter 3 Evaluation and Fragmentation of aRNA). The probe was hybridized on a DNA microarray, Human Genome U133 Plus2.0® (Affymetrix™)

with a hybridization oven (Hybridization Oven 640 110V®; Affymetrix 800138) and washed with Fluidics Station 450® (Affymetrix 00-0079).   The microarray was scanned with GeneChip Scanner 3000® (Affymetrix 00-0074) and analyzed with GeneChip Operating Software ver1.4® (Affymetrix 690036).   The analysis protocol was GeneChip Expression Analysis Data Fundamentals® (Chapter 4 First-Order Data Analysis and Data Quality Assesment and Chapter 5 Statistical Alogorithms Reference) with GeneChip Operating Software ver1.4®.

RESULTS

HB611.   This cell was established by transfecting three tandemly arranged HBV genomes (adr4) in a plasmid containing the G418 resistance gene into HuH6 cells (Fig. 1) [19, 21].   HB611 cells maintains one copy of three tandemly arranged HBV genomes and is supposed to reflect all pathways of the HBV life cycle except the attachment and the entry process and the covalently closed circular DNA (cccDNA) formation (Fig.1. and Fig.2).   The integrated HBV genome functions as cccDNA and produces HBV-related transcripts.   The difference between HB611 and HuH6 cells is that the HBV genome is integrated into a host chromosome with transcription competence and there is no other system affecting cell physiology such as an immune system and therefore it could be possible to compare gene expression profiles purely by HBV production.

Global differences of gene expression profiles between HB611 and HuH6.   This time, we utilized the Affymetrix Human Genome U133 Plus2.0™.   This microarray loaded more than 50,000 probes.   The scatter plot shows that 95% genes are expressed at the same level within eight folds difference (Fig. 3A, and 3B).   Ten to twenty genes are drastically changed for their expression (arrows in the Fig. 3, Table 1, Table 2).   Typical ones were picked up and drawn on the graph.   Interestingly, some genes such as steroid sulfatace (microsomal) isozyme S, microsomal triglyceride transfere protein and fatty acid binding protein involved in fatty acids metabolism were highly expressed in HB611.   And cell-growth related genes such as *CD24* (gi; 180167), platelet-derived growth factor receptor α (*PDGFRα*) and *myc* (*N-myc* related) were also highly expressed in HB611 (Table 2., Fig. 7).

Not a few genes seem to be changed by HBV production for their expression.   1014 genes (~2.0%)

were highly expressed in the HB611 cells more than eight times and 2063 genes (~4%) were highly expressed in the HuH6 cells more than eight times. These data suggest that HBV production should affect cellular gene expression program.

Cell cycle control genes. It is interesting how HBV production affects cell cycle since HBV is a major cause of liver cancer in the world. We mined the data related to cell cycle control; cyclins, cyclin dependent kinases (*CDKs*) and cyclin dependent kinase inhibitors (*CKIs*). In these cells, cyclin D1(*CYCD1*, gi; 12652656), cyclin A2 (*CYCA2*. gi; 4502612), cyclin B1 (*CYCB1*, gi; 1443518) and cyclin B2 (*CYCB2*, gi; 1093801) were modestly expressed, but the expression level did not show much difference (Fig.4). Among *CDKs*, these cells expressed *CDK4* that functions in G0-G1 phase. *CDK1*, *CDK2* and anther G0-G1 cyclin, *CDK6* were modestly expressed and their expression level was not different between HB611 cells and HuH6 cells (Fig. 5). As for *CKIs*, *p21^{Cip1}* and *p27^{Kip1}* were mainly expressed in these cells and suggest that they should probably work as major cell cycle controllers (Fig. 5). We have to take into consideration that 80 ~90 % cells were in the G1 in the ordinary culture condition. In this term, it is interesting that S phase and M phase cyclins were relatively highly expressed and *CDK4* was fairly highly expressed in these cells. This might represent that these cells were transformed cell lines.

Liver specific genes. The analysis was based on the hepatocellular carcinoma (HCC) originated cells with or without HBV production and therefore liver specific gene expression could be maintained modestly but fairly reduced. As expected, some liver specific transcription factors such as hepatocyte nuclear factor 1A (*HNF1A*, gi; 184264), 1B (*HNF1B*, gi; 4507396), 4A (*HNF4A*, gi; 3250320) and 4G (*HNF4G*, gi; 5636455) were at low expression (Fig. 6). Albumin (*ALB*, gi; 7959790) is one of the typical liver specific genes [9]and its expression was a kind of repressed in these cell lines, which suggests that these HCC originated cell lines or otherwise, HCC itself were dedifferentiated in the course of transformation (Fig. 6). Interestingly, alphafetoprotein (*AFP*, gi; 4501988) was extremely highly expressed in HB611 cells. This might suggest that HBV production should drastically changed the expression program of this gene. As shown in Table 2, a couple of genes were much highly expressed in the HB611 cells. It seems to be interesting how the gene expression was controlled, in a

similar way or in a different way?

Other interesting genes.   While we were mining data, we found interesting expression profiles of some genes. Sodium taurocholate co-transporting polypeptide (*NTCP* or *NTCP1*, gi; 4506970) was reported to be an HBV receptor last year [25].   The report pointed out that HCC originated cultured cell line such as HepG2 and HuH7 did not express this gene and correction of this gene expression by transfection endowed these cell lines with HBV infectivity and viral amplification.   We checked the gene expression in this study.   Beyond my expectations, HB611 cells expressed moderately NTCP (Fig. 7), which suggests that HBV produced from the HB611 cells could reinfect the cells.   But so far, cccDNA was not observed in the HB611 [19, 21], which suggests that there was some obstruction to HBV infection in this system.   HBV membrane protein, large S (LS), middle S (MS) and small S (SS or simply HBs) produced from the cells might interfere the HBV reinfection. On the other hand, another similar gene, *NTCP2* (gi; 456972) was at no expression level.

We checked about hundreds interferon related genes including INFs, IFN receptors and IFN regulatory factors (IRFs) but there was almost no difference between HB611 cells and HuH6 cell, expressed or not expressed (data not shown).   Likewise, Janus kinase (JAK)/ signal transduction and transcription factors) STATs related genes were not changed (data not shown), although it was reported that HBV polymerase (HBVpol) inhibited the activities of the STAT proteins [24].

DISCUSSION

In this report, we analyzed how HBV production affected cellular gene expression profiles using an artificially HBV producing system based on an HCC-originated cultured cell line.   HBV never infects cultured cell lines and experimental animals.   Needless to say, this kind of analysis should be done in natural infection system. HBV, however, has strict species specificity and there is no very convenient and useful infection system for HBV.   HepaRG, a HCC derived cell line, and PHH separated from SCID-hu hepatocyte are commercially available.   Even though PHH shows high competency for HBV infection, both cell lines are very expensive and inconvenient for daily HBV study.   Therefore, more convenient and useful systems for HBV infection

must be explored and currently we can utilize an HBV production system designed artificially in HCC derived cultured cell lines [18, 19]. Nevertheless, it was clear that such analysis was very informative and HBV production affected remarkably the cellular gene expression profiles and expression level of some genes appeared to be drastically changed by HBV production.

Considerable numbers of gene seemed to be affected for their gene expression. Since it was reported that hepatitis C virus infection affected lipid metabolism [15], it is very interesting that the expression level of some genes such as fatty acid binding protein (gi; 4557584) involved in lipid metabolism was remarkably changed in the presence of HBV related products in the cells. Change in lipid metabolism might lead to cellular phenotypic change.

Some genes related to cell growth such as PDGFR α and myc (N-myc related in this case, gi; 33877057) were extremely overexpressed in the HB611 cells. N-myc overexpression was observed in woodchuck hepatitis virus (WHV) involved hepatocarcinogenesis [8, 22]. WHV genome was found near or in the N-myc locus, which resulted in overexpression of N-myc gene [22]. In our case or human HCC, there has been no evidence that the HBV genome integrated in or near the N-myc locus. Therefore, N-myc overexpression in our system is not the case and maybe there could be different mechanism. HBV infection might increase the gene expression and facilitate hepatocyte growth.

Cell cycle engine is accelerated by cyclins (CYCs) and cyclin dependent kinase (CDKs) which are checked and controlled by CKI. Although CYCs and CDKs should be activated in immortalized and transformed cells, CYCD1 (gi; 12652656) as a G1 phase cyclin, CYCA2 (gi; 4502612) as a S phase cyclin and CYCB2 (gi; 1093801) and less CYCB1 (gi; 1443518) as an M phase cyclin were relatively activated in both HB611 and HuH6 cells. CDK4 rather than CDK6 was favored in these cell lines. p57^{Kip2} (gi; 854949) is thought to be a CKI unique for stem cells and p21^{Cip1} (gi; 11386202) and p27^{Kip1} (gi; 12805034) were main CKIs in this case as expected.

Liver specific gene expression could be important to show how much such cell lines maintained well-differentiated hepatocyte characters. Although normal liver gene expression profiles were not investi-

gated, liver specific transcription factors such as *HNF1A, 1B, 4A* and *4G* were expressed at low level but not absent. *ALB* (gi; 7959790) is one of the characteristic liver specific genes and was at low expression level. In contrast, *AFP* (gi; 4501988) was upregulated in HB611 cells, the mechanism of which could not be assessed in this report but HBV production might cause blastic change in hepatic cells. *NTCP* (gi; 4506970) is a notable factor as an HBV receptor [25]. Considering that cultured HCC derived cell lines never permit HBV infection, NTCP expression had not been expected. HB611 cells, however, showed moderate expression of *NTCP*. This fact let us expect that HB611 cells should competent for HBV infection and reinfection circuit could go around. So far, we have not observed cccDNA formation in the HB611 cells and this is not the case perhaps because S related membrane proteins secreted from HB611 cells should block the receptor and the ligand interaction.

We found a big difference of three genes influencing cell growth. EGF as well as hepatocyte growth factor (HGF) is a hepatocyte growth factor [12, 14] and therefore its receptor, *EGFR* (gi; 6228471) expression should be important for tumor growth dependent on EGF. HBV production, however, could change drastically growth factor usage to platelet derived growth factor (PDGF) by increasing PDGFR α (gi; 5453869).

It is fascinating how gene expression profiles are altered by HBV production. Core protein, polymerase and X protein could be good candidates that change the gene expression and these phenomenon have to be assessed in a natural HBV infection system using PHH. Furthermore, we should pay attention to non-coding short RNA (ncsRNA) expression status in presence or absence of HBV. ncsRNA including miRNA basically have a negative effect on the target gene [3]. Such RNA molecules should have remarkable effects on cellular and viral gene expression program and might be expressed from HBV genome as well as host genome (Fig. 8), though ten or more miRNAs have been reported to regulate HBV gene expression [11, 26].

REFERENCES

1.   Blumberg, B. S., Alter, H. J., and Visnich, S. (1965) A "New" Antigen in Leukemia Sera, *JAMA 191*, 541-546.

2.   Fletcher, S. P., Chin, D. J., Ji, Y., Iniguez, A. L., Taillon, B., Swinney, D. C., Ravindran, P., Cheng, D. T., Bitter, H., Lopatin, U., Ma, H., Klumpp, K., and Menne, S. (2012) Transcriptomic analysis of the woodchuck model of chronic hepatitis B, *Hepatology*.

3.   Gebauer, F., and Hentze, M. W. (2004) Molecular mechanisms of translational control, *Nature reviews. Molecular cell biology 5*, 827-835.

4.   Glebe, D., and Urban, S. (2007) Viral and cellular determinants involved in hepadnaviral entry, *World J Gastroenterol 13*, 22-38.

5.   Gripon, P., Diot, C., Theze, N., Fourel, I., Loreal, O., Brechot, C., and Guguen-Guillouzo, C. (1988) Hepatitis B virus infection of adult human hepatocytes cultured in the presence of dimethyl sulfoxide, *J Virol 62*, 4136-4143.

6.   Gripon, P., Rumin, S., Urban, S., Le Seyec, J., Glaise, D., Cannie, I., Guyomard, C., Lucas, J., Trepo, C., and Guguen-Guillouzo, C. (2002) Infection of a human hepatoma cell line by hepatitis B virus, *Proc Natl Acad Sci U S A 99*, 15655-15660.

7.   Hajjou, M., Norel, R., Carver, R., Marion, P., Cullen, J., Rogler, L. E., and Rogler, C. E. (2005) cDNA microarray analysis of HBV transgenic mouse liver identifies genes in lipid biosynthetic and growth control pathways affected by HBV, *J Med Virol 77*, 57-65.

8.   Hansen, L. J., Tennant, B. C., Seeger, C., and Ganem, D. (1993) Differential activation of myc gene

family members in hepatic carcinogenesis by closely related hepatitis B viruses, *Mol Cell Biol 13, 659-667.*

9.   Knowles, B. B., Howe, C. C., and Aden, D. P. (1980) Human hepatocellular carcinoma cell lines secrete the major plasma proteins and hepatitis B surface antigen, *Science 209, 497-499.*

10.   Liang, T. J. (2009) Hepatitis B: the virus and disease, *Hepatology 49,* S13-21.

11.   Liu, W. H., Yeh, S. H., and Chen, P. J. (2011) Role of microRNAs in hepatitis B virus replication and pathogenesis, *Biochim Biophys Acta.*

12.   McGowan, J. A., Strain, A. J., and Bucher, N. L. (1981) DNA synthesis in primary cultures of adult rat hepatocytes in a defined medium: effects of epidermal growth factor, insulin, glucagon, and cyclic-AMP, *Journal of cellular physiology 108,* 353-363.

13.   Meuleman, P., and Leroux-Roels, G. (2008) The human liver-uPA-SCID mouse: a model for the evaluation of antiviral compounds against HBV and HCV, *Antiviral Res 80,* 231-238.

14.   Michalopoulos, G. K. (1990) Liver regeneration: molecular mechanisms of growth control, *FASEB journal : official publication of the Federation of American Societies for Experimental Biology 4,* 176-187.

15.   Moradpour, D., Englert, C., Wakita, T., and Wands, J. R. (1996) Characterization of cell lines allowing tightly regulated expression of hepatitis C virus core protein, *Virology 222,* 51-63.

16.   Ochiya, T., Tsurimoto, T., Ueda, K., Okubo, K., Shiozawa, M., and Matsubara, K. (1989) An in vitro system for infection with hepatitis B virus that uses primary human fetal hepatocytes, *Proc Natl Acad Sci U S A 86,* 1875-1879.

17.   Seeger, C., Zoulim, F., and MASON, W. S. (2007) *Hepadnaviruses,* Vol. II, 5 ed., Lippincott Williams and Wilkins, Philadelphia.

18.   Sells, M. A., Chen, M. L., and Acs, G. (1987) Production of hepatitis B virus particles in Hep G2 cells transfected with cloned hepatitis B virus DNA, *Proc Natl Acad Sci U S A 84*, 1005-1009.

19.   Tsurimoto, T., Fujiyama, A., and Matsubara, K. (1987) Stable expression and replication of hepatitis B virus genome in an integrated state in a human hepatoma cell line transfected with the cloned viral DNA, *Proc Natl Acad Sci U S A 84*, 444-448.

20.   Ueda, K. (2013) Start or End? One of the biggest Mysteries is Finally Solved, *J Infect Dis Ther 1*, 1.

21.   Ueda, K., Tsurimoto, T., Nagahata, T., Chisaka, O., and Matsubara, K. (1989) An in vitro system for screening anti-hepatitis B virus drugs, *Virology 169*, 213-216.

22.   Ueda, K., Wei, Y., and Ganem, D. (1996) Activation of N-myc2 gene expression by cis-acting elements of oncogenic hepadnaviral genomes: key role of enhancer II, *Virology 217*, 413-417.

23.   Walter, E., Keist, R., Niederost, B., Pult, I., and Blum, H. E. (1996) Hepatitis B virus infection of tupaia hepatocytes in vitro and in vivo, *Hepatology 24*, 1-5.

24.   Wu, M., Xu, Y., Lin, S., Zhang, X., Xiang, L., and Yuan, Z. (2007) Hepatitis B virus polymerase inhibits the interferon-inducible MyD88 promoter by blocking nuclear translocation of Stat1, *J Gen Virol 88*, 3260-3269.

25.   Yan, H., Zhong, G., Xu, G., He, W., Jing, Z., Gao, Z., Huang, Y., Qi, Y., Peng, B., Wang, H., Fu, L., Song, M., Chen, P., Gao, W., Ren, B., Sun, Y., Cai, T., Feng, X., Sui, J., and Li, W. (2012) Sodium taurocholate cotransporting polypeptide is a functional receptor for human hepatitis B and D virus, *eLife 1*, e00049.

26.   Zhang, Q., Pu, R., Du, Y., Han, Y., Su, T., Wang, H., and Cao, G. (2012) Non-coding RNAs in hepatitis B or C-associated hepatocellular carcinoma: potential diagnostic and prognostic markers and therapeutic targets, *Cancer letters 321*, 1-12.