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利用 B 型肝炎-昆蟲桿狀重組病毒以偵測急性感染時肝細胞的基因表現

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The Gene Expression Profiles of Hepatocytes when Hepatitis B Virus Genes are Carried in by Baculovirus-A Possible Acute Infection Model

Yen-Hsuan Ni, Chun-Hsien Yu, Hong-Yuan Hsu, Huey-Ling Chen, Mei-Hwei Chang

Department of Pediatrics, College of Medicine and Hospital, National Taiwan University, Taipei, Taiwan

ABSTRACT

Background/Aim: The hepatocyte gene expression profile at the time they respond to hepatitis B virus (HBV) acute infection was rarely studied. We aimed to describe this profile, which may help to delineate signal transduction pathway when virus enters inside the hepatocytes. Methods: We adapted baculo-HBV recombinant virus system to transfect HBV into non-primate, mammalian hepatocytes. This system was used to infect rat hepatocytes with HBV through portal vein injection of baculo-HBV recombinant virus. The control was the rat undergoing the same procedure but with the baculovirus that did not contain HBV. The hepatocyte RNAs of both rats were extracted for microarray and made for comparison. An in vitro study was done simultaneously. We also adapted the baculo-HBV recombinant virus and the baculovirus without HBV insert as the control to infected HepG2 cell cultures. The hepatoma cell RNAs of both cultures were also extracted for microarray and compared. Results: There were three differently expressed genes commonly found in both *in vivo* and *in vitro* studies: Stat1, Zinc finger protein 83 (ZNF83), and RNA binding motif protein 3 (RBM3). Conclusion: In the future, these genes of interest will become our foci to study their roles in the acute phase of HBV-hepatocyte interaction. Obviously, they are key cellular factors to allow the replication of HBV

inside the hepatocytes. Based on these findings, we may develop antiviral therapies by targeting these factors.

Key words: hepatitis B virus, baculo-HBV recombinant virus, microarray, Stat1, Zinc finger protein 83, RNA binding motif protein 3

INTRODUCTION

Hepatitis B virus (HBV) is a hepatotropic DNA virus that infects humans and certain nonhuman primates. The major obstacles in the study of HBV have been the inability of the virus to infect cells *in vitro*, and the lack of good animal model systems due to a strict virus-host range. The chimpanzee system is very expensive and difficult to handle, and is only available in few centers in the world. The duck system and the woodchuck system are valuable models for studying HBV lifecycle; however, many significant differences still existed between animal hepadanviruses and human HBV.

To overcome the above problems, an HBV recombinant baculovirus system was established (1). Baculovirus, *Autographa californica* multiple nuclear polyhedrosis virus (AcMNPV),

is an insect cell virus and had been used widely for large-scale recombinant protein production. It is unable to replicate in mammalian cells. However, some previous reports showed this virus can express foreign genes under appropriate promoter control in mammalian cells, especially interesting in its hepatotropic property (2,3).

The previous study showed that by way of its endogenous promoter, a 1.3x genome of HBV construct inside a baculovirus could be successfully generated and transfected HepG2 cells. This HBV recombinant baculovirus had advantages over

the previous cell lines: (i) High-level of HBV expression. (ii) HBV replication level can be regulated over a wide range simply by changing the baculovirus multiplicity of infection (MOI). (iii) HBV replication in this system is detectable one day after infection and persisted for at least 11 days. (iv) The superinfection with this baculovirus can enhance or extend the infection period of the cell line. For these advantages, we decided to set up this novel system.

We have now established the HBV recombinant baculovirus system. A 1.5X HBV genome was inserted into a baculovirus to construct this recombinant baculovirus. This system was well demonstrated to be successful in terms of the production of HBsAg, the dose-dependent manner of the HBsAg production, and the sustained production of HBsAg by a superinfection of the recombinant virus. We also know the temporal profile of HBsAg production in this system. In the future, this system can be applied to: (i) The model for acute response to HBV infection in hepatocytes; we may check the molecular profiles of hepatocytes before and after infected by HBV recombinant baculovirus. (ii) The evaluation of efficacy of antiviral therapies. (iii) The model of co-infection of HBV plus other viruses or superinfection of other viruses upon an HBV-infected hepatocyte cell lines. In the current study, we will focus on the first goal.

The other important tool in our proposal is the implantation of a catheter in the

portal vascular system for infusion. By this method, we may easily infect the hepatocytes by injecting the recombinant virus into the portal system. We can enhance the virus dosage by repeating the injection. This technique was well established in large animal models such as baboons, dogs, pigs, and rabbits (4-7). However, the technique for catheterization of the portal vein in large animals requires surgical expertise and a whole set of surgical equipment. Therefore, in order to perform more extensive experiments, a simplified portal cannulation technique needs to be developed for small-sized animals. Actually, we now have already set up a rat model for the development of long-term cannulation in the main portal vein (detail in the Method section). The portal cannula remained secure without perturbing portal circulation and allowed for both noninvasive infusions of baculovirus.

Although the host range of HBV is thought to be controlled at the stage of viral entry, the hepatic tropism of HBV is also due to tissue-specific viral gene expression (8). In the past, the studies focusing on the hepatocyte gene expression during acute phase were rarely seen. A woodchuck model showed the recovery from acute hepatitis was preceded by a significantly greater hepatic expression of interferon-γand CD3 (9). These cytokines can help the host to overcome HBV infection. In a mouse model, mouse hepatitis virus-induced hepatitis could be protected by a liver-specific α2-interferon (10). A recent report also demonstrated an increased

concentration of HNF-1αis coincident with decreased HBV transcription in an HBV transgenic mice (11,12). All the above results may help to elucidate the pathways that regulate the viral life cycle and suggested additional approaches for the treatment of chronic HBV infection.

In this study, we plan to use the HBV-recombinant baculovirus to infect human hepatocytes *in vitro* and rat hepatocytes *in vivo*. The RNA will be extracted from the human and rat hepatocytes after the HBV-recombinant baculovirus infection. The RNA will then be subjected to microarray study (the strategy is listed below). Based upon the microarray results, we are likely to find out some possible candidate genes that may regulate the antiviral responses. It is also likely for us to find out possible viral receptor(s) in the mammalian hepatocytes. As the duck model system, it was shown the overexpression of gp180 (receptor) could enhance the duck hepatitis B virus entry into the hepatocytes (13). The microarray possibly will find out overexpression of candidate receptor(s) genes when the recombinant HBV-baculovirus enters the hepatocytes, either *in vitro* or *in vivo*. By the bioinformatics analysis, we might zero in on several candidates and do further research on these ones.

MATERIALS AND METHODS

Cell culture. Sf21 insect cells (kindly provided by Prof. Bor-Leung Chiang) were maintained in complete TNM-FH medium (Grace's insect medium supplemented with 10% fetal bovine serum and 1% PS) in non-humidified incubator at 27℃ without CO_2. Huh7 cells were maintained in Dulbecco's modified essential medium supplemented with 10% fetal bovine serum and 1% PS and were grown in humidified incubator at 37℃ and 5% CO_2. (All medium, serum, and antibiotic are from Gibco, Life Technology).

Construction of Baculovirus Transfer Vector. A recombinant transfer vector was constructed by excising a PvuII/PvuII fragment containing 1.5×HBV DNA (~5 kb) from pGEM3Z4.8 (kindly provided by Dr. Hui-Ling Wu) and cloned into the SmaI site of the multiple cloning region of pBlueBac4.5 (Invitrogen, Chatsworth, CA, USA). The insertion and orientation of the recombinant transfer vector were then confirmed by HindIII/KpnI enzyme digestion and DNA sequencing (Fig. 1).

Generation of Recombinant Baculovirus Containing 1.5× HBV DNA. Seed 2×10^6 Sf21 cells in a 60 mm dish, and gently wash twice with Grace's insect medium without FBS to remove the serum. Six μg of the purified recombinant transfer vector, 0.5μg of the Bac-N-Blue™ DNA (linear *AcMNPV* baculovirus DNA), and 20μl of Cellfectin® reagent were then co-transfected into the Sf21 cells according to the manufacturer's instructions (BAC-N-BLUE transfection kit, Invitrogen). After

4-hour of incubation at room temperature, 1 ml of complete TNM-FH medium was added. The dish was then sealed with paraffin and incubated at 27℃ for 72 hours before harvesting the recombinant baculovirus.

Harvest the Recombinant Baculovirus by Plaque assay. Seed 5×10^6 Sf21 cells to 100 mm plate and infect the cells with 10^2, 10^3, and 10^4 dilutions of the transfection viral stock in TNM-FH medium at room temperature for one hour. The medium was then aspirated and an agarose/medium/X-gal mixture, consisting of 2.5 ml of 2.5% agarose solution (47℃), 2.5 ml of complete TNM-FH medium (47℃), and 5 ml of complete TNM-FH medium with X-gal (concentration: 150μg/ml, room temperature) was gently poured into the plate to overlay the virus-infected Sf21 cultured cells. The plates were sealed and incubated at 27℃ for 5 days until the distinguishable blue plaques are formed.

Select the Recombinant Baculovirus by PCR. The putative recombinant viruses were amplified from blue plaques. 72 hrs after infection, 0.75 ml of the medium was mixed with the same volume of 20%polyethylene/1M NaCl to precipitate the released virions from the blue plaques. The medium were then collected and labelled as P-1 stocks and store at 4℃. Viral DNA was purified by the standard Proteinase K digestion, phenol/chloroform extraction, and isopropanol precipitation. The DNA was used as templates for polymerase chain reaction (PCR) (Primers P1: 5'-TCA

CCA TAT TCT TGG GAA CAA GA-3' and P8: 5'-TTA GGG TTT AAA TGT ATG CCC-3') to detect if the virus isolates contain the 1.5× HBV DNA.

Preparation of High-Titer Viral Stocks. Seed 25 cm^2 flask with 2×10^6 log-phase Sf21 cells and add 20 μl of the P-1 viral stock in 5 ml complete TNM-FH medium. Incubate at 27℃ for 7 days until all the cells were lysed. Centrifuge at 1000g for 20 minutes to remove the cell debris and store at 4℃ as P-2 stock. Virus titres were determined by the plaque assay using 10^6, 10^7, and 10^8 dilutions of P-2 stock.

Analysis of Secreted Hepatitis B Surface Antigen (HBsAg). Detection of HBsAg was performed by enzyme linked immunoassay kit (EIA, Abbott). The media from Huh7 cells were collected at several time points after infection and stored at -80℃ until analysis.

Hepatocyte harvest from human liver fragment. A surgical specimen of liver (10 g) was put in 50ml test tube containing 15 ml icy cold Ringer, and minimized the ischemic time as short as possible. Then, the specimen will be transferred to a 6 cm petri dish, hold the specimen with gauze, and find a best vessel to cannulate, and perfusion at least 10 min with EGTA and collagenase. The perfusion will be continued till the liver is pale yellow. After the perfusion， the liver specimens is incubated at 37℃ for 10 minutes. The hepatocytes are then filtered through 100-um nylon mesh into DMEM, a low speed centrifuge will be repeated three times (50g, 4

C, 3 min) to isolate the single hepatocytes. The viability of the isolated hepatocytes will be counted by trypan blue dye exclusion. At least 80% viability should be achieved and available to go further study.

Infection of HepG2 cell cultures with Recombinant Baculovirus. Seed 6-well culture plates with 10^5 HepG2 cells per well and the cells were grown for 16 to 24 hours before infection. On the day of infection, P-2 virus stock was diluted with DMEM/FBS/PS according to their titers to achieve the desired multiplicity of infection (moi) and adjust the final infection volume to1 ml. After 1 hour of infection at 37℃, the inoculum was aspirated and the cells were gently washed with HBSS (Gibco, Life Technology) for 3 times to remove all the virus-containing supernatant. 2.5 ml of DMEM/FBS/PS was re-fed and maintained at 37℃ incubator.

Animals and cannula insertion into portal vein. F-344 rats (160-200 gm) were fed a laboratory diet with water and food *ad libitum* until use and were kept under constant environmental conditions with a 12-hour light-dark cycle. The animals received humane care in compliance with the guidelines of the National Science Council (NSC 1997). Animals were maintained in separate cages. The cannula was an 18 cm length of polyethylene tubing (PE-50, ID 0.58 mm, OD 0.96 mm; Becton Dickinson, Sparks, MD). One end was sheathed with a 2.5 cm length of PE-160 tubing (ID 1.14 mm, OD 1.59 mm; Becton Dickinson, Sparks, MD). The two tubings

were fused at one end with heat. The other end of the PE-50 cannula was beveled. A 3 mm section of a 20G A BD Vialon catheter (ID 1.1 mm; Becton Dickinson, Sandy, Utah) was placed 7 mm from the tip of the bevel as a collar. The finished cannula was sterilized with ethylene oxide. A novel spring-guide-wire introducer needle was created by the modification of the spring-wire guide of the pediatric central venous catheterization set (0.46 mm x 45 cm, Arrow International, Inc). The 25 GA introducer needle was cut 6 cm from its point, and the cut edge was smoothed. This needle was adapted to the straight-tip end of the spring-wire guide.

Rats were anesthetized intramuscularly with 75 mg/kg ketamine hydrochloride and 5 mg/kg xylazine. A midline laparotomy was performed, and the portal vein was exposed (14). One stitch was made through the superficial part of the main portal venous wall with a 7-0 silk suture armed with a BV-1 needle. The spring-guide-wire introducer needle was introduced through the cannula. Using this introducer needle, we punctured the portal vein about 1 mm down-stream from the stitch, advanced the needle 5 mm into the vein, and slid the cannula through the guide-wire into the portal vein up to the Vialon collar. The cannula was secured with a triple knot over the Violon collar with another triple knot just behind the collar. The guide-wire introducer needle was then withdrawn. After free blood returned into the cannula, the cannula was cleared with 0.1 ml heparinized saline (50 U/ml) and stopped with a blunt-ended

23 GA needle. Ampicillin (40 mg) and gentamicin (4 mg) were administered intraperitoneally. The abdominal wall incision was closed with 4-0 chromic surgical guts. The cannula was led through the subcutaneous tunnel to the interscapular incision (Fig. 2). The distal end of the cannula was anchored to the interscapula by suturing the cannula around the PE-160 tubing sheath to the skin. The patency of the cannula was reconfirmed and checked twice a week. The rats were allowed to recover from the surgery, and each was maintained in a separate cage for at least one week before use in the experiments.

RNA extraction from the cultured hepatocytes and rat liver. From cell culture: The tissue cultured cells lysate will be collected 48-72 hours after recombinant virus infection. Use a cell scraper or trypsin to free cells from flask and transfer the cell suspension to a centrifuge tube. Spin the cells for 10 minuets at 1000rpm at 10°C. Discard as much supernatant as possible. Store immediately on ice. Resuspend cells in equal volume of PBS and spin again at the same speed and temperature as above. Again, discard as much supernatant as possible. Return sample to ice.

From tissues: The rats will be infused the recombinant virus through the portal cannula. In the meantime, the other rats will be infused with the wild type baculovirus to serve as the control group. The rats, both the experimental group and the control group will be sacrificed 72 hours after the infusion of virus. When sacrificed, blood sample will be

collected through superior vena cava and sent for HBsAg assay (see section below) and a piece of liver tissue will be sent for histologic examination; the specimens were serially sectioned in 10-µm slices and stained with H&E to define the analyzed regions. Then, we will use a mini plastic tissue homogenizer to homogenize the tissues. Ensure that no large pieces are visible. Pipetting up and down to disrupt any clumps and incubate at room temp for 2-3 min. It is best that the samples are kept on ice as much as possible while working with Trizol (Qiagen, Chatsworth, CA, USA). Add 20% volume of chloroform. A milk-like liquid should be seen. Spin in a microcentrifuge at 4°C, max speed, for 15 min to get several layers. While storing samples on ice, immediately remove the top supernatant ensuring that the pipet does not touch the lower layers. Transfer small amounts at a time to a fresh 1.5ml tube. Add equal volume of room temperature 70% ethanol. Mix and transfer immediately to the mini spin column. Spin at 10,000rpm for 1 min. If any liquid left on the top of the filter, spin again. Discard flow-through and save the collection tube for the next step. Pipet 700µl Buffers three times and then pour onto the RNeasy column. At 20-25˚C, centrifuge for 2 min at max speed to dry the RNeasy membrane. Transfer the column to a 1.5 ml tube and add 30 µl of DEPC water to elute RNA with 1 min spin at 10,000rpm. Repeat two more times to make 90µl of collection for each mini column. Discard the filter.

Microarray to identify genes responsible for acute phase viral interaction. The overall study protocol is listed in Figure 2. The preparation of probes, hybridization, and scanning was performed as described previously (15). The fluorescence intensities of Cy5 (nontumor) and Cy3 (tumor) for each target spot were adjusted so that the mean Cy5 and Cy3 intensities of 52 housekeeping genes for each slide were equal. The relative expression of each gene (Cy3:Cy5 intensity ratio) into one of four categories: up-regulated (ratio, >2.0), down-regulated (ratio, <0.5), unchanged (ratio, between 0.5 and 2.0), and not expressed (or slight expression but under the cutoff level for detection). To detect differentially expressed genes, we recorded the number of samples in each category within each subgroup, for each gene. Then we calculated the *U* values of Mann-Whitney tests, which measured how the sample distributions between subgroups overlap. The number of samples within each group is counted and, according to the order of the category, the number of overlapped samples is incorporated into the *U* value. A small *U* shows that the sample distribution of the two groups is clearly separated, *e.g.*, commonly up-regulated in the recombinant group or down-regulated in the control group.

RESULTS

Construction, Generation, and Purification of Recombinant Baculovirus

Containing 1.5× HBV DNA. The baculo-HBV recombinant virus was constructed and harvested (Fig 3) as described in the methods and the viral titer was determined after we proved that it could produce HBsAg (Fig 4).

Microarray by RNA extraction from the cultured hepatocytes and rat liver. The cellular RNAs extracted from *in vitro* cell cultures of either baculo-HBV recombinant virus infection or the control baculovirus infection were extracted to process for microarray. Totally we found 55 differentially expressed genes between the control and the experimental settings (Table 1). As for the *in vivo* study, we extracted the RNA from the rat liver which was infected the baculo-HBV recombinant virus through the portal vein and the RNA from the control rat liver which was infected with the baculovirus, also through the portal vein injection. We also performed the microarray study to compare the differentially expressed RNAs and found 1823 genes with either 2 folds up-regulated (n=758) or two-folds down regulated (n=1065) (Fig. 5). We then compared the 55 and 1823 genes and found only three genes were overlapped in both settings. These three genes are: Signal transducer and activator of transcription 1 (Stat1), Zinc finger protein 83 (ZNF83), and RNA binding motif protein 3 (RBM3).

DISCUSSION

This study utilized a baculo-HBV recombinant virus system to deliver the HBV DNA into the hepatocytes. By way of comparing with the control baculovirus, we performed the microarray to detect the differentially expressed genes. The transfection routes were through the portal vein in rats and directly infected the cultured HepG2 cells *in vitro*.

This study design indeed contains several default drawbacks: (1) this is not a natural route of infection, either *in vivo* or *in vitro*. Thus, it hardly simulated the real condition in acute HBV infection. (2) The microarray gene chip used here is actually a mouse liver library, and very likely is different from the human and rat ones. However, because HBV has very limited natural hosts and is hard to replicate in vitro, this model is an option for the study of hepatocyte gene expression profile.

We summarized the results and picked up the three genes that were differentially expressed in both *in vitro* and *in vivo* experiments. These three genes: Stat1, ZNF83, and RBM3, are actually important molecules in the signal transduction pathway. They may be not the specific viral receptor or the very first several ligands for viral entry, but they may play some roles in the successive steps. Initially, we aimed to find out any viral receptors or acute phase reatants of HBV infection by this study. All these three molecules may not be the very first receptor(s) existing in the cell surface, but there are some possibilities that they are the secondary or tertiary

messengers.

When cytokines and growth factors combining with their respective receptors in cell surface and transmit the stimulation across the cell membrane, Stat1 can be activated (16). We believed that STAT1 was also playing the same role when hepatocytes were infected with the virus, even though this virus may not be transfected through the natural receptor. After the transfection, HBV likely induces some cytokine reaction intracellularly and triggers the activation of STAT1. STAT1 protein was shown to be essential for cell growth suppression in response to interferon-γ (IFN-γ). The molecular mechanism mediating this growth inhibition involves the regulation of the gene encoding the cyclin-dependent kinase inhibitor p21 WAF1/CIP1 by STAT1 (17). In summary, STAT1 is important in the signal pathway inside hepatocytes after HBV enters into the cells, but it seems not a receptor for virus.

Our laboratory had already demonstrated that RBMY gene, a RNA binding motif gene on Y chromosome had an oncogenic implication and related to HBV-associated hepatocellular carcinoma (18). This study also showed the RBM3, a family of RNA binding motif gene, had a differential expression between the baculo-HBV recombinant virus infected hepatocytes and the control group *in vivo* and *in vitro*. However, the significance of the RBM3 should lay more on the proliferative cellular

reaction rather than the receptors themselves.

A previous report revealed that most of the liver genes up-regulated by HBxAg expression can be clustered into three major groups, including genes encoding ribosomal proteins, transcription factors with zinc-finger motifs, and proteins associated with protein degradation pathways (19). These results led to the hypothesis that HBxAg may function as a major regulator in a common cellular pathway that, in turn, regulates protein synthesis, gene transcription, and protein degradation. In this study, we did not specify the role of HBxAg, instead, we cloned the whole HBV genome and transfected to the hepatocytes. We may divided the four open reading frames of HBV genes and check their respective differential expressions by this model.

In summary, HBV and hepatocytes may interact with each other through several pathways (Fig . Our study demonstrated three of them. These three may be the most pivotal factors in the later development of chronic infection events, like the fibrogenesis and cancer development. We have to dissect the genes into detail to further elucidate the mechanisms.

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Table 1. The 55 differentially expressed genes in the HepG2 cells detected by microarray

Gene	Description
PIK3R1	Phosphoinositide-3-kinase, regulatory subunit 1 (p85 alpha)
FH	Fumarate hydratase
UPP1	Uridine phosphorylase 1
LIPE	Lipase, hormone-sensitive
SCO1	SCO cytochrome oxidase deficient homolog 1 (yeast)
	Homo sapiens gastric-associated differentially-expressed protein YA61P (YA61) mRNA, com
GART	Phosphoribosylglycinamide formyltransferase, phosphoribosylglycinamide synthetase, phosph synthetase
DCK	Deoxycytidine kinase
TOP2A	Topoisomerase (DNA) II alpha 170kDa
TOMM70A	Translocase of outer mitochondrial membrane 70 homolog A (yeast)
ZNF83	Zinc finger protein 83 (HPF1)
NT5M	5',3'-nucleotidase, mitochondrial
	Homo sapiens mRNA; cDNA DKFZp434I1820 (from clone DKFZp434I1820); partial cds.
RBM3	RNA binding motif (RNP1, RRM) protein 3
PPIG	Peptidyl-prolyl isomerase G (cyclophilin G)
HSPD1	Heat shock 60kDa protein 1 (chaperonin)
ROCK1	Rho-associated, coiled-coil containing protein kinase 1
PRKDC	Protein kinase, DNA-activated, catalytic polypeptide
TTK	TTK protein kinase
TRIP12	Thyroid hormone receptor interactor 12
UBQLN2	Ubiquilin 2
C20orf6	Chromosome 20 open reading frame 6
LOC400966	RAN-binding protein 2-like 1 short isoform
PIK3C2A	Phosphoinositide-3-kinase, class 2, alpha polypeptide
ERBP	Estrogen receptor binding protein
FLJ39207	C219-reactive peptide
IL16	Interleukin 16 (lymphocyte chemoattractant factor)
HSPCA	Heat shock 90kDa protein 1, alpha
ch-TOG	KIAA0097 gene product
TIPARP	TCDD-inducible poly(ADP-ribose) polymerase
RBBP9	Retinoblastoma binding protein 9
MSH6	MutS homolog 6 (E. coli)

SMC2L1	SMC2 structural maintenance of chromosomes 2-like 1 (yeast)
SCC-112	SCC-112 protein
CUL4B	Cullin 4B
ANLN	Anillin, actin binding protein (scraps homolog, Drosophila)
ATF7IP	Activating transcription factor 7 interacting protein
ITGAV	Integrin, alpha V (vitronectin receptor, alpha polypeptide, antigen CD51)
APOB	Apolipoprotein B (including Ag(x) antigen)
APOB	Apolipoprotein B (including Ag(x) antigen)
CD2AP	CD2-associated protein
RGS13	Regulator of G-protein signalling 13
IPO7	Importin 7
STAT1	Signal transducer and activator of transcription 1, 91kDa
CLTC	Clathrin, heavy polypeptide (Hc)
MATR3	Matrin 3
FAM51A1	Family with sequence similarity 51, member A1
FLJ16517	FLJ16517 protein
BTBD9	BTB (POZ) domain containing 9
	CDNA FLJ10095 fis, clone HEMBA1002430
C14orf143	Chromosome 14 open reading frame 143
	NTera2D1 cell line mRNA containing L1 retroposon, clone P2
	Clone IMAGE:110218 mRNA sequence
	Hypothetical gene supported by BC040718
ABCC5	ATP-binding cassette, sub-family C (CFTR/MRP), member 5

FIGURE LEGENDS

Figure 1. The construction of baculo-HBV recombinant virus. The control virus contains the same backbone except the 1.5X HBV insert.

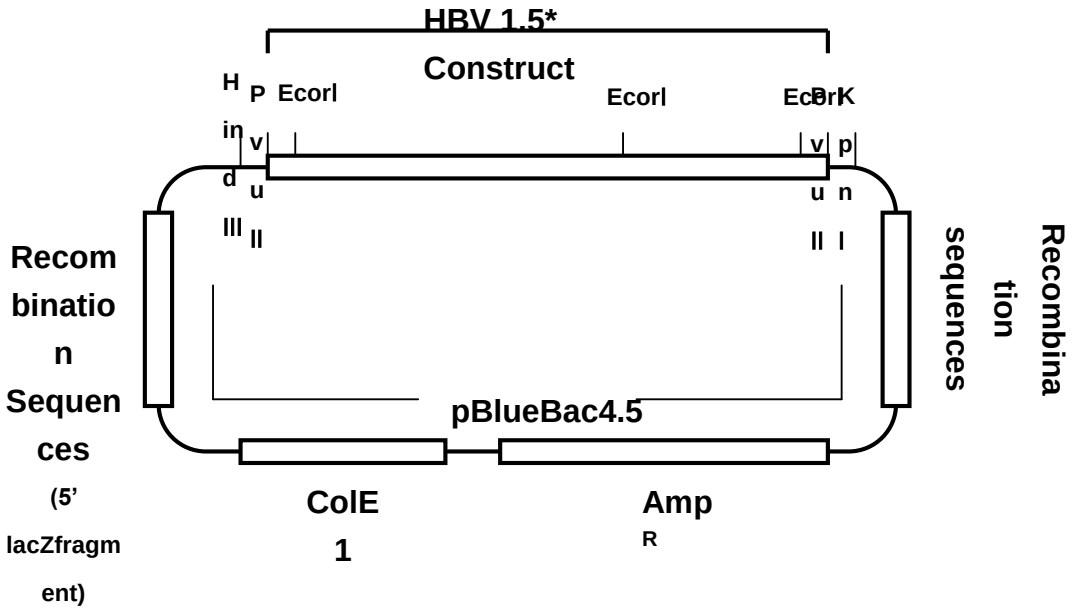
Figure 2. The overview of the study flow chart.

Figure 3. Successful recombinant baculovirus yield blue plaques.

Figure 4. Analysis of HBsAg in the medium of Huh7 cells infected with HBV baculovirus.

Figure 5. The illustration of microarray results in the *in vivo* experiment. We picked up total 1823 genes, 758 of which are 2 folds up-regulated while 1065 of them are 2 folds down-regulated.

Figure 6. The summary of a hypothetic HBV-hepatocyte interaction.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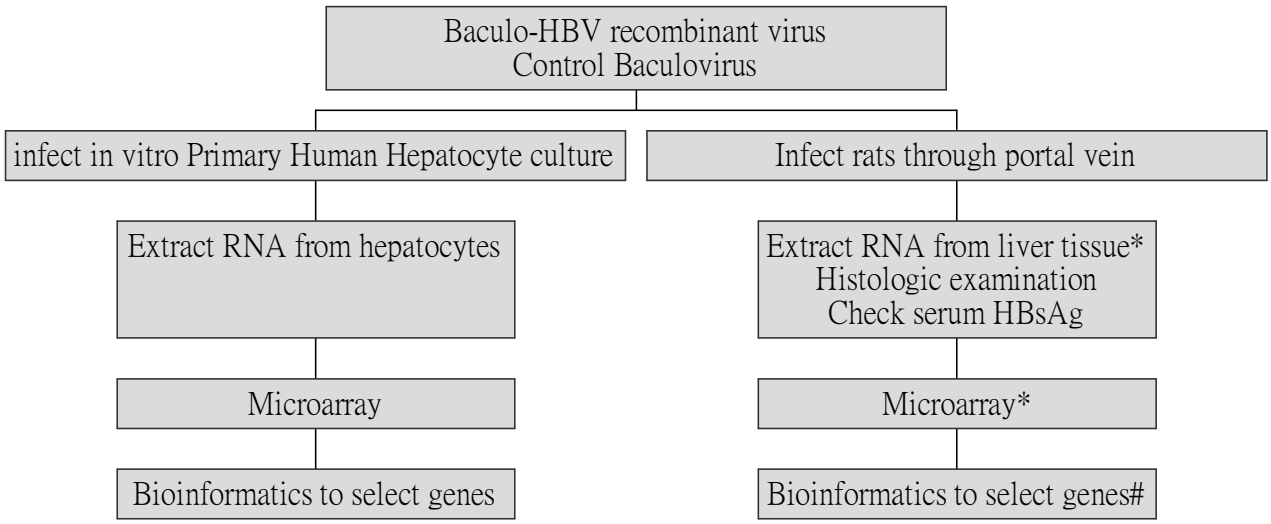
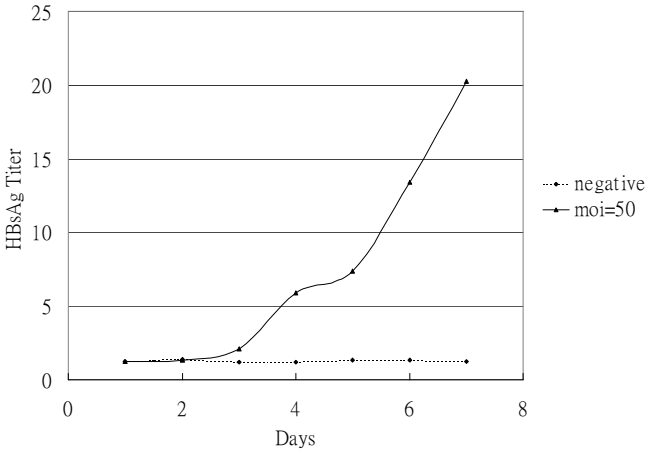
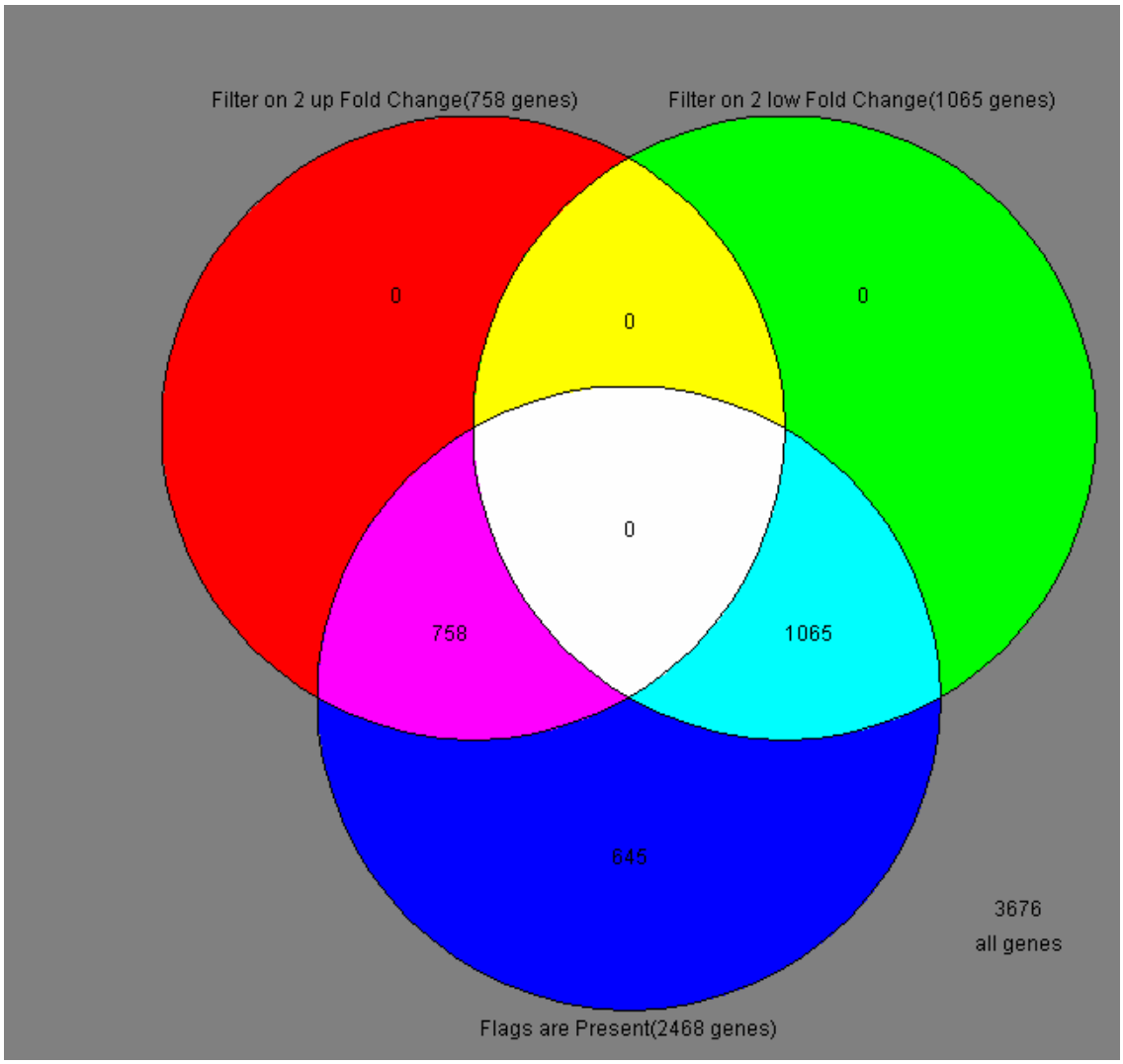
ColE1
Amp R


Baculo-HBV recombinant virus
Control Baculovirus
infect in vitro Primary Human Hepatocyte culture
Infect rats through portal vein
Extract RNA from hepatocytes
Extract RNA from liver tissue*
Histologic examination
Check serum HBsAg
Microarray
Microarray*
Bioinformatics to select genes
Bioinformatics to select genes#


25
20
15
10
5
0
HBsAg Titer
0
2
4
6
8
Days
negative
moi=50


Filter on 2 up Fold Change(758 genes)
Filter on 2 low Fold Change(1065 genes)
0
0
0
0
758
1065
645
3676
all genes
Flags are Present(2468 genes)

HBV and Hepatocytes