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The Intracellular Changes and the Localization of Core particles by Hepatitis B Virus P Protein-Deficient Mutant

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The Intracellular Changes and the Localization of Core Particles by Hepatitis B Virus P Protein-Deficient Mutant

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- ABSTRACTS-

The Intracellular Changes and the Localization of Core Particles by Hepatitis B Virus P Protein-Deficient Mutant

Purpose: Hepatitis B virus (HBV) core particles move to ER or recycle back to the nucleus. This moving mechanism is thought to be important in HBV replication and HBV life cycles. P protein-deficient mutant can form core particles in the absence of P protein and pregenomic RNA (pgRNA). In this study, the intracellular changes of P protein-deficient mutant expressing stable cells and the localization of P protein-deficient mutant core particles were observed.

Materials & Methods: HBV replicating and non-replicating stable cells had been established in HuH7 hepatoma cell lines. Using Northern blot and Southern blot analyses, HBV transcription and replication were examined in HBV replicating and non-replicating stable cells. Distribution of intracellular organelles was examined by immunofluorescene assay. Mitochondria were stained using Mito-Tracker. Core particles were visualized using anti-HBc antibody. Microtubules, vimentin, nuclear pore complex (NPC) were visualized using mouse anti-tubulin, mouse anti-vimentin, and mouse anti-NPC antibody, respectively.

Results: P-deficient mutant core particles co-localized with microtubule, NPC, and vimentin, like HBV wild type (wt) core particles. Core particles of priming- and reverse transcriptase-deficient mutants that only consist of immature core particles, were not co-localized with microtubule, NPC, and vimentin. The mitochondria of P-deficient mutant expressing stable cells aggregated into peri-nuclear region like those of HBV wt expressing stable cells.

Conclusion: These results indicated that P protein-deficient mutant core particles act like HBV wt core particles, suggesting that P protein-deficient mutant and mature core particles might share similar properties.

Key Words: Hepatitis B virus, P-protein deficient mutant, immature core particle, mature core particle.

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I. INTRODUCTION

A. HBV replication

Hepadnaviruses are small, enveloped DNA viruses that replicate preferentially in liver cells and are involved in hepatocellular carcinoma. Hepatitis B virus (HBV), a prototype of hepadnavirus, has a partially duplex relaxed circular DNA genome. After entry into hepatocytes and transportation of the virus into the nucleus, viral DNA is converted to covalently closed circular (ccc) DNA, which serves as the template for viral messenger RNA (mRNA) transcription. Pregenomic RNA (pgRNA) serves as mRNA for capsid (C) and polymerase (P) proteins and as a template for reverse transcription. The subgenomic 2.4kb RNAs serve as mRNAs for the expression of the large HBsAg, the 2.1kb mRNA for the middle and small HBsAg and the 0.7kb mRNA for the HBx protein. The pgRNA and reverse transcriptase are packaged into viral core particles in cytoplasm to form immature core particles. Encapsidated pgRNAs are reverse transcribed to form partially duplex relaxed circular DNA by reverse transcriptase. Mature core particle containing the partially duplex relaxed circular DNA is either undergoes further processing in the endoplasmic reticulum (ER) and Golgi for virion assembly, or recycle back to the nucleus. This recycling mechanism is thought to be important in maintaining cccDNA pool in the nucleus. These fates of HBV core particles in host cytoplasm are important for HBV life cycle but the mechanism is not elucidated yet (Blum et al., 1989; Seeger and Mason).

B. HBV C protein and core particle

The icosahedral HBV core particle is formed by multiple copies of C protein consisting of 183 or 185 amino acid residues (aa) depending on the genotype. The C protein can be efficiently expressed in different cell types like. bacteria, yeast, frog oocytes, or mammalian cells and self-assemble in into core particles in the absence of other HBV components in these cells Assembly starts with the formation of homodimers (Zhou and Standring, 1992) by a disulfide bridge between cystein 61. Higher intermediate C protein complexes containing chaperons have been found but have not been defined in any detail. The core particle is held together by rather weak interdimer interactions (Ceres and Zlotnick, 2002). The particles appear as two types (Crowther et al., 1994): one type has a diameter of 30 nm and consists of 90 dimers arranged according to an icosahedral $T = 3$ symmetry, the other type is slightly larger (diameter 34 nm) and contains 120 dimers organized with a $T = 4$ symmetry. Both particle species can also be found in infected human liver (Kenney et al., 1995). C-terminal region of C protein contained rich-arginine residues. This domain is similar to protamine and involved in packaging of the pregenome reverse transcriptase complex. Upon heterologous expression of the C protein in the absence of P protein and pregenome, unspecific host mRNA is packaged into core particles. Deletion of the arginine-rich domain abolishes nucleic acid packaging but still allows efficient core particle assembly (Gallina et al., 1989). In *E. coli*, the C-terminally truncated variant

generated predominantly the larger particle form and can be produced in high quantity. This material was suitable to define the folding of the C protein by cryo-electron microscopy at lower resolution and finally at a resolution of 3.3Å after crystallization (Wynne et al., 1999).

C. Movement of core particles

For DHBV model, it is suggested that nuclear targeting of core particle influenced by the envelop protein (L-protein) and nuclear localization (NLS) signal on the core particle surface (Summer et al, 1990). Various expreriments suggest that phosphorylation may paly a major role in regulating signal-mediated nucleocytoplasmic transport of the hepadnaviral core protein (Liao, W., and J.H. Ou. 1995). After assembly, HBV core particles either go to the endoplasmic reticulum (ER) or Golgi to release virion, or recycle back to nucleus. Two fates of HBV core particles are important for HBV life cycle. Directly after the HBV core particles has formed, it contains an pgRNA, whereas secreted virions contain circular, partially double-stranded DNA synthesized by reverse transcription in the lumen of the core particles. In fact, core particles showing early stages of the viral DNA synthesis could be found in infected cells but not in virions (Mason et al., 1982; Weiser et al., 1983). Therefore, it was proposed that the immature RNA-containing core particles is excluded from the releasing pathway and that the viral DNA synthesis is associated with a structural change in the core particle allowing only mature core particle to be released (Summers and Mason, 1982). This

model gained support from genetic experiments. Several C-terminal truncations of the duck HBV (DHBV) core protein blocking viral DNA synthesis at a step prior to the formation of circular partially double-stranded DNA also inhibited core particle envelopment (Yu and Summers, 1991). Point mutations in the active centre of the HBV and DHBV reverse transcriptase destroying the enzymatic activity yielded core particle "frozen" in an immature state. In accordance with the model, the maturation signal of these core particles was responsible for core particle localization (Gerelsaikhan et al., 1996; Wei et al., 1996).

D. Viral infection and change of intracellular motility

During viral life cycles, virus modifies host system in various ways. Virus induces the cytoskeletal reorganization and organelle distribution in the host cell during their life cycles in order to facilitate their spread during entry and exit from the host (Cudmore et al., 1997; Dramsi and Cossart, 1998; Ploudibou et al., 2000). For example, adenovirus infection activated protein kinase A and p38/MAPK pathway and increased viral nuclear targeting (Ploudidou and Way, 2001). Virus increased infection efficiency through reorganization of cytoskeleton, regulation of motor activity and regulation of function of centrosome (Ploubidou et al., 2000).

E. Function of cytoskeleton

Distribution of and intracellular organelles, including mitochondria, are under the strong influence of the cytoskeleton (Summerhayes et al., 1983). The cytoskeleton is a dynamic network of intracellular proteinaceous structural elements responsible for cell shape, motility, migration, polarity, and maintenance of intercellular contacts involved in tissue architecture. Cytoskeletons are composed of three cytosolic filaments: microtubules, intermediated filaments, and microfilaments. Distribution of intracellular organelle has determined by interaction of three cytoskeletons (Pluobidou and Way, 2001). Among others, microtubule play an important role in maintaining cellular morphology, mitotic processing and intracellular trafficking. Also, microtubules serve as tracks for the movement of mitochondria, endoplasmic reticulum and various other organelles such as endocytotic or exocytotic vesicles (Sodeik, 2000).

Hepatitis B virus (HBV) core particles move to ER or recycle back to the nucleus. This moving mechanism is thought to be important in HBV replication and HBV life cycles. P protein-deficient mutant can form core particles in the absence of P protein and pregenomic RNA (pgRNA). In this study, the intracellular changes of P protein-deficient mutant expressing stable cells and the localization of P protein-deficient mutant core particles were observed.

II. MATERIALS AND METHODS

A. Use the DNA constructs and stable cell lines

The partially redundant HBV wild type (wt) construct designated pPB from the 1.3 length of the HBV subtype adw R9 (Blum et al., 1991)had been subcloned into pcDNA3 (Invitrogen^{tm}) to express HBV under the cytomegalovirus immediate early (CMV IE) promoter (Kim et al, 2004). Priming deficient TP Tyr65 Phe mutant involved the alteration of Tyr65 to Phe in the TP domain of P protein (Kim et al, 2004). P protein-deficient mutant was prepared, in which the AUG start codon was changed and frame-shifted. RT reaction-deficient RT YMHA mutant was constructed, in which the conserved YMDD reverse transcriptase motif was modified to YMHA (Kim et al, 2004). C protein-deficient mutant of HBV had been generated by introducing stop codon (TAA) at the 8th amino acid (Glu, GAA) of the C protein. RNase H reaction-deficient RNaseH mutant have been constructed by alterating Glu750 to Val in the RH domain of P protein. HBV replicating stable cell line PUB9 had been established by the trasfection of pPB. HBV non-replicating stable cell lines, PUF12, YMHA46, RH27, and P-def30 have been established by the transfection of TP Tyr65 Phe, RT YMHA, RNase H, and P-deficient mutants, respectively. Stable cell lines were selected with 1mg/ml of G418 sulfate (geneticin®) and maintained with 200ug/ml of G418 sulfate. As positive control, PEB8 was prepared by the transfection of pPB into HepG2 cell line and maintained with 200ug/ml of G418 sulfate.

B. Cell culture and transfection

HuH7 hepatoma cell lines were used for the transfection of HBV wt and mutant clones. HuH7 cells were maintained in Dulbecco's modified eagle's medium supplemented with penicillin and streptomycin, and 10% fetal bovine serum. HBV wt and mutant plasmids (8ug) were transfected into HuH7 cells on a 10-cm plate using polyethyleneimine (1ug/ul). HBV replicating stable cell line PUB9 had been established by the trasfection of pPB. HBV non-replicating stable cell lines have been established by the transfection of each mutants. Stable cell lines were maintained in Dulbecco's modified eagle's medium supplemented with 100ug/ml of penicillin and streptomycin, 200ug/ml of G418 sulfate, and 10% fetal bovine serum.

C. RNA isolation

Total RNA was extracted using RNA-STAT-60 (TEL-TEST INC, Friendswood, Texas, U.S.A). Cells were lysed by the addition of 1ml of RNA-STAT-60 per 5×10^6 cells and 200ul chloroform was added. After vigorous shaking, the cell lysate was incubated at room temperature for 3min and centrifuged at 12,000 ×g for 15min at 4℃. The aqueous phase was transferred to the fresh ependorf tube, and the RNA was precipitated by adding the equal volume of isopropanol. After centrifugation, RNA was dried and then dissolved in RNase-free distilled water.

D. Northern blot analysis

To analyze HBV mRNAs, total RNA was fractionated on 1% agarose gel containing formaldehyde and transferred onto a nylon membrane. RNA on the membrane was hybridized with random-primed ^{32}P-labeled probe specific for the HBV sequence at 68℃ with overnight incubation. The hybridized RNA were visualized by autoradiography.

E. Isolation of core particles

Three days after transfection, HuH7 cells were lysed in 1ml TNE (10mM Tris-HCl [pH8.0], 50mM NaCl, 1mM EDTA)- 1% Nonidet P-40. The clarified lysate was adjusted with 10mM $MgCl_2$ and 8mM $CaCl_2$, and incubated 1 hr at 37 ℃ with 20 U DNase I (Sigma) and 20ug of RNase A. Cytoplasmic core particles were precipitated with 6.5% polyethylene glycol.

F. Western blot analysis

Isolated core particles were electrophoresed on a 1% native agarose gel. Core particles were transferred to polyvinylidene fluoride (PVDF) membranes as described for Northern blot analysis. Immunoblotting was performed using an anti-HBc antibody

(DAKO). Horseradish peroxidase-conjugated anti-rabbit secondary antibody and enhanced chemical luminescence (ECL) were employed to visualize HBV core particles.

G. Southern blot analysis

To identify HBV replication in transfected cells, HBV DNA isolated from the cytoplasmic core particles was separated on a 1% agarose gel and transferred to nylon membrane. The HBV DNA on membrane was hybridized with random-primed ^{32}P-labeled probe specific for the HBV sequence at 68℃ with overnight incubation. The hybridized DNA were visualized by autography.

H. Immnofluorscence assay

Stable and transiently trasfected cells were incubated for 24-48hrs, washed with PBS, fixed in 4% formaldehyde for 7min, and then permeabilized in 0.15% Triton X-100 in PBS for 5min. Samples were incubated with 1% bovine serum albumin (BSA) for 1hr and then with the first antibody. Anti-HBc antibody (DAKO) was used to detect core particles. To detect microtubule, vimentin, and nuclear pore complex (NPC), mouse- anti α-tubulin, mouse anti vimentin, and mouse anti- NPC antibody were used, respectively. The first antibodies in the 1% bovine serum albumin were incubated at room temperature for 1hr. cells were incubated with FITC conjugated anti-mouse antibody or rhodamin conjugated anti-mouse antibody for 1hr. Mito-tracker were used

to detect mitochondria. Cover slips were applied to the slides in mounting solution and the cells were examined with a confocal laser scanning microscope (LSM510, Zeiss).

III. RESULTS

A. HBV transcription of HBV wt or mutants expressing cells.

HBV wild-type (wt) subtype adw R9 (pPB) and mutant constructs were prepared, from which the transcription of pgRNA is controlled by a cytomegalovirus immediate early (CMV) promoter (Fig. 1) (Kim et al, 2004).

The establishment of HBV expressing stable cells was prepared by the transfection of wt or mutant constructs into HuH7 cells and the G418-selection. Priming deficient TP Tyr65 Phe mutant involved the alteration of Tyr65 to Phe in the TP domain of P protein. In P protein-deficient mutant, the AUG start codon was changed and frame-shifted. P protein-deficient mutant except P protein were express all viral proteins. In RT reaction-deficient RT YMHA mutants, the conserved YMDD reverse transcriptase motif was modified to YMHA (Kim et al, 2004). C protein-deficient mutant of HBV had been generated by introducing stop codon (TAA) at the 8th amino acid (Glu, GAA) of the C protein. RNase H reaction-deficient RNaseH mutant have been constructed by alterating Glu750 to Val in the RH domain of P protein. Each mutant transfected cells were selected by antibiotic selections with 1mg/ml and then maintained with 200ug/ml of G418 sulfate. HBV replicating and non-replicating stable cells in HuH7 human hepatoma cell lines were named as PUB9 and RH27, PUF12, YMHA46, P-def30, respectively. The production of pgRNA and surface mRNAs from HBV wt and mutant constructs were analyzed by Northern blot analysis from transiently transfected cells

and stably expressed cells (Fig. 2). Upon transfection, pgRNA was expressed by the action of the CMV promoter. Subgenomic RNA were synthesized under the control of their authentic promoters. The 3.5kb pgRNA were detected from wt or mutant transiently and stably expressed cells. But Subgenomic RNA were detected weak signal. There were no significant differences in RNA expression levels between wt and mutants when transfected transiently. But there are differences in RNA expression levels between wt and mutants expressed stably. In addition, RNA from PEB8 cells, the HBV replicating stable cell in HepG2 cell, was included as positive control, since HBV replication signal is more intense in PEB8 cells than PUB9 cells.

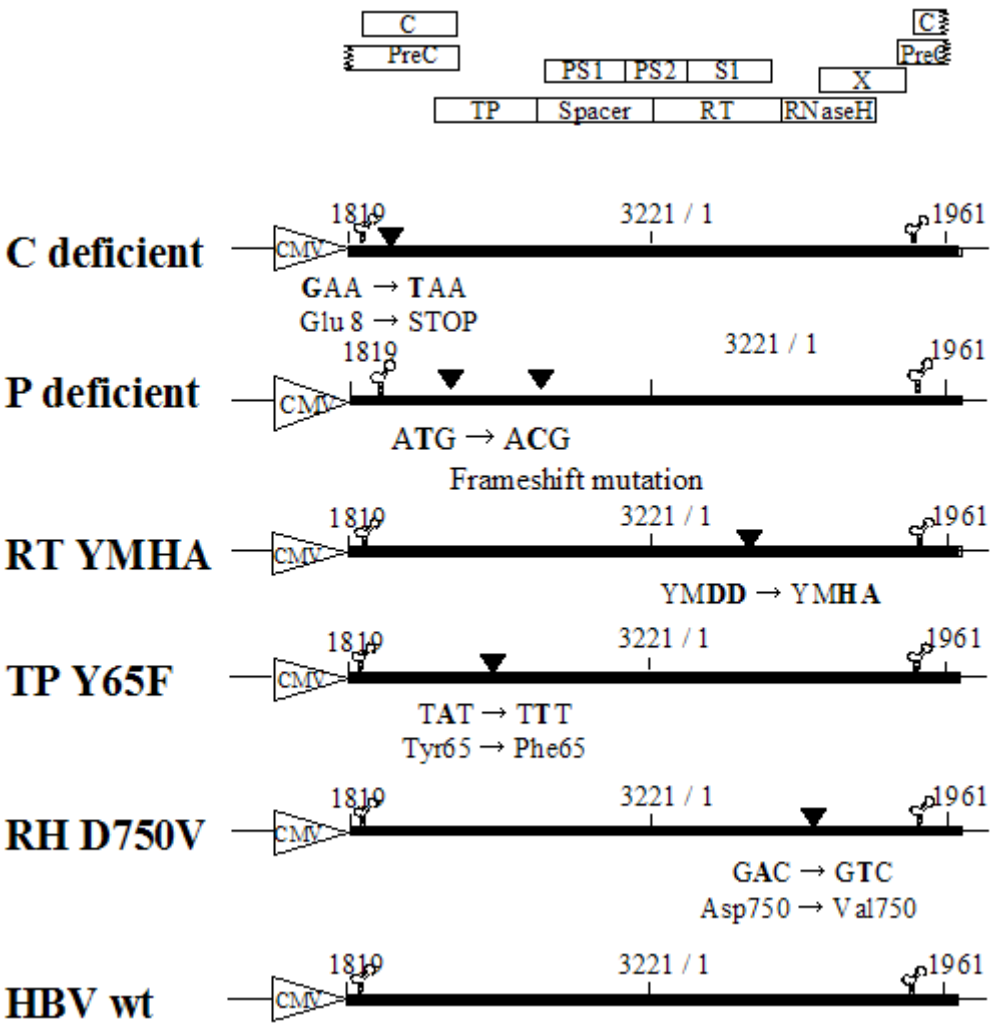

Fig. 1. Schematic diagram of HBV wild type and mutant constructs. HBV sequences are depicted as thick lines and the ε sequences at 5` and 3`ends are marked. The ORFs of C, P, S, and X genes are presented as open boxes. Each domain (TP, spacer, RT, and RNase H) of the P protein is indicated. The cytomegalovirus immediate early (CMV) promoter is represented by an open arrow. Mutant constructs, C protein-deficient, P protein-deficient, RT YMDD to YMHA, TP Tyr65 Phe mutant, and RNaseH Glu750 Val in a wt HBV adw R9 background are designated C-deficient, P-deficient, RT YMHA, TP Y65F, and RH D750V, respectively. The position of point mutations are indicated as closed arrowheads.

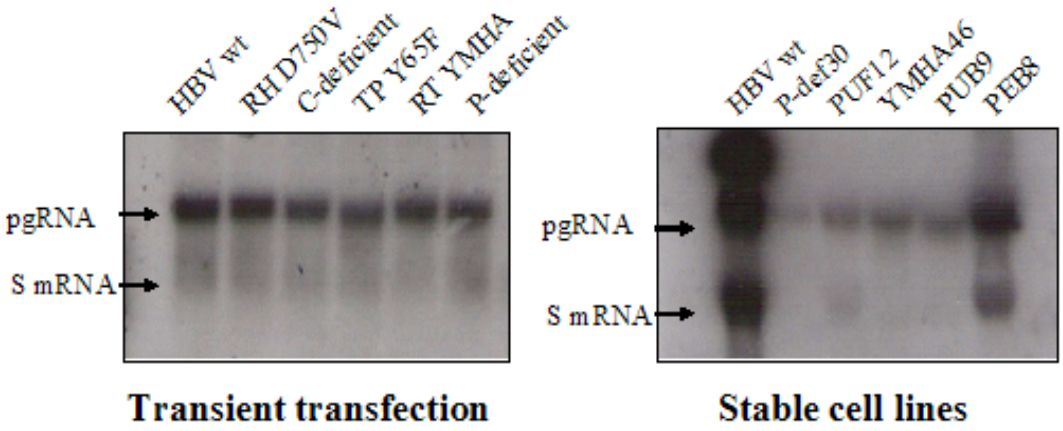

Fig. 2. Northern blot analyses to detect HBV mRNA expression. 3 days after transfection, total RNA was extracted by RNA STAT60. 10ug of total RNA was resolved in 1% formaldehyde gel electrophoresis and transferred to nylon membrane. The HBV mRNA on the membrane was hybridized with random-primed 32P-labeled HBV specific probes. The 3.5kb of pgRNA or 2.1/2.4kb of surface mRNAs were indicated. PUB9 and PEB8 are HBV wt replicating cell lines. PUF12, YMHA46, P-def30 are HBV non-replicating cell lines that are stably expressed by the transfections of TP Y65F, RT YMHA, and P-deficient mutants, respectively.

B. HBV DNA synthesis of HBV wt and mutants expressing cells

To investigate HBV DNA replication, cytoplasmic core particles were isolated from HuH7 cells transfected with HBV wt or mutants. Following extraction of HBV DNA from isolated core particles, Southern blot analysis was performed to detect replicating HBV DNA in HBV wt or mutants transfected cells and in stably expressed cells. In the left panel of Fig. 2, when cells were transfected transiently, replicating HBV DNA as single stranded, double-stranded, and partially double-stranded relaxed circular HBV DNA was observed in wt transfected cells (Fig. 3), but not in TP Y65F, RT YMHA, P-deficient, and C-deficient mutant transfected cells. Due to the loss of RNaseH activity in RNaseH mutant, the plus-strand DNA synthesis was blocked in RNaseH mutant transfected cells and consequently DNA-RNA hybrid was formed. In right panel of Fig. 3, we analyzed HBV DNA from stably expressed cells and transfected cells. In first lane, transfected HBV wt was used as a positive control. HBV DNA synthesis was only observed HBV DNA in PUB9 cells that have integrated HBV wt in the genome of HuH7 cell as weak signals, but not detects in PUF12, YMHA46, P-def30 cells.

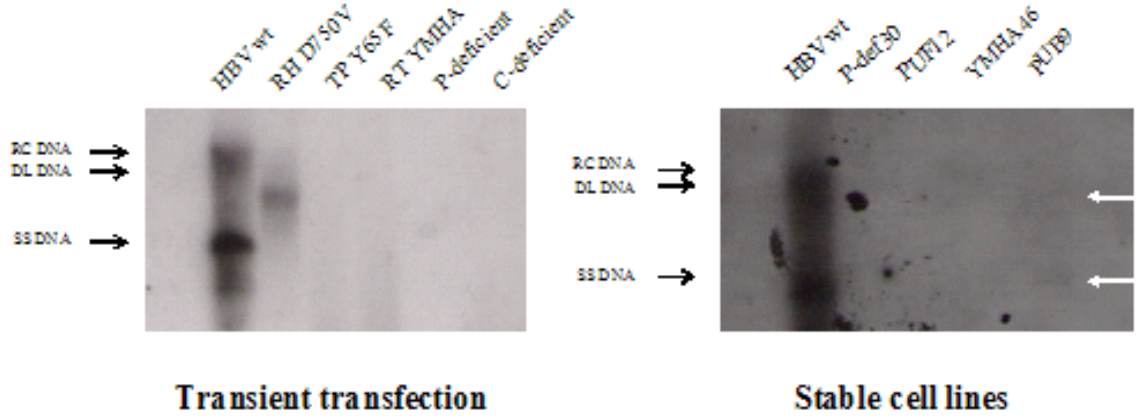

Fig. 3. Southern blot analysis to detect HBV DNA synthesis. HBV DNA from isolated core particle was resolve in agarose gel and transfered to nylon membrane and HBV DNA on the membrane was hybridized with random-primed 32P-labeled HBV specific probes. Relaxed circular (RC), double-stranded linear (DL), and single stranded (SS) forms of HBV DNA are indicated.

C. Detection of HBV core particle in transiently transfected cells.

To detect core particles in transiently trasfected cells, after isolation of core particles were isolated and Western blot analysis was performed using HBc-antibody. Core particles were detected from wt or mutant transfected cells that displayed similar mobility on a native agarose gel (Fig.4). Since C-deficient mutant can not express C protein, C-deficient transfected cells can not formed core particle. P-deficient mutant can form core particles in the absence of P protein and pgRNA, previously described. Core particles from P-deficient mutant transfected cells show stronger signal than HBV wt or other HBV mutant transfected cells.

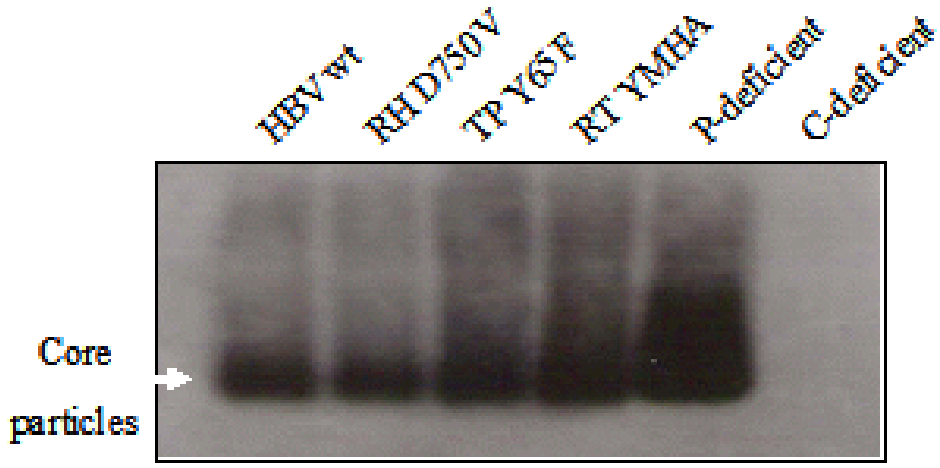

Fig. 4. Western blot analysis to detect core particles from a native agarose gel. 3 days after transfection, isolated core particles were transferred to PVDF membranes and incubated with HBc antibody. HRP-conjugated secondary antibody and ECL were used to visualize proteins. Core particles were detected from HBV wt and RH D750V, TP Y65F, RT YMHA, P-deficient mutants-transfected HuH7 cells, but not in C-deficient mutant-transfected cells.

D. Intracellular localization of HBV core particles in transiently transfected cells

To detect intracellular localization of HBV core particle, IFA was performed 2 days after transfection. Anti-HBc antibody (DAKO) was used to detect core particles. To detect microtubule, vimentin, and nuclear pore complex (NPC), Mouse- anti α-tubulin, mouse anti vimentin, and mouse anti- NPC antibody were used, respectively. In HBV wt or mutants transfected cells, the mature core particles from HBV wt tend to be clustered at peri-nuclear region but not the immature core particles from HBV RNaseH G750V, TP Y65F, RT YMHA. The mature core particles from HBV wt partially co-localized with microtubule, nuclear pore complex, and vimentin but not immature core particles from replication incompetent HBV mutants. In P-deficient mutant transiently transfected cells, core particles tend to co-localize with microtubule, nuclear pore complex, and vimentin like wt HBV core particles. From this result, we suggested replication competent HBV core particles interact with host cytoskeleton while moving inside of cell to target nucleus and then nuclear pore complex (NPC). And P-deficient mutant core particles might act like mature HBV core particles.

A.

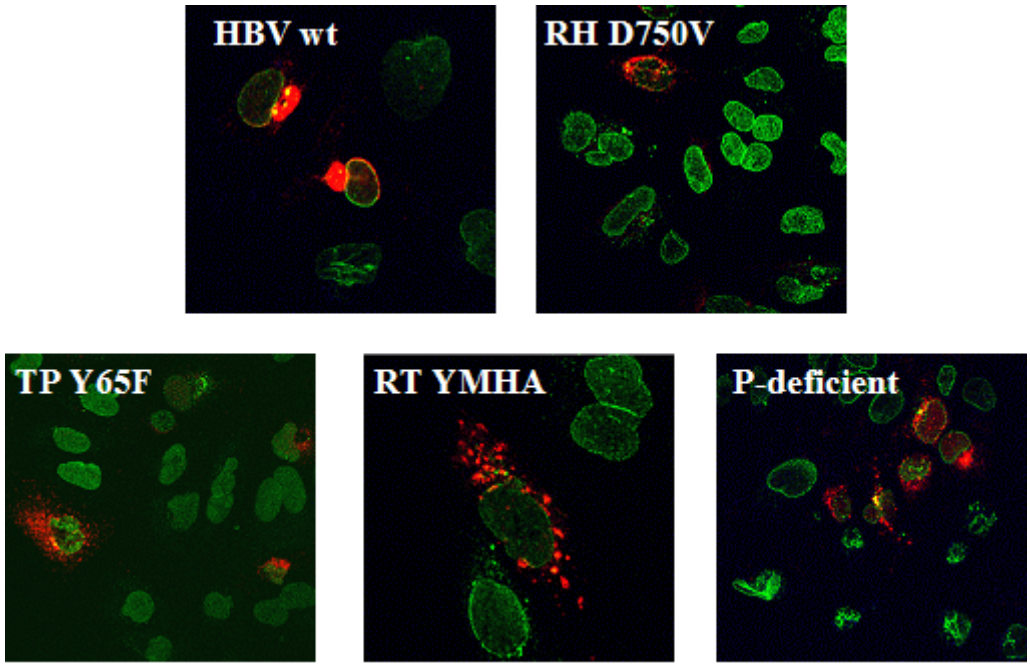

Fig. 5. Intracellular localizations of HBV core particles with nuclear pore complexes (A), microtubule (B), and (C) intermediate filament using confocal microscopy.

(A) The distribution of core particles of HBV wt or mutants-transiently transfected cells and nuclear pore complex (NPC). HBV wt and P-deficient mutant core particles (red) partially co-localized with NPC (green) shown as yellow by merging image, but not RH D750V, TP Y65F, and RT YMHA mutant core particles.

B.

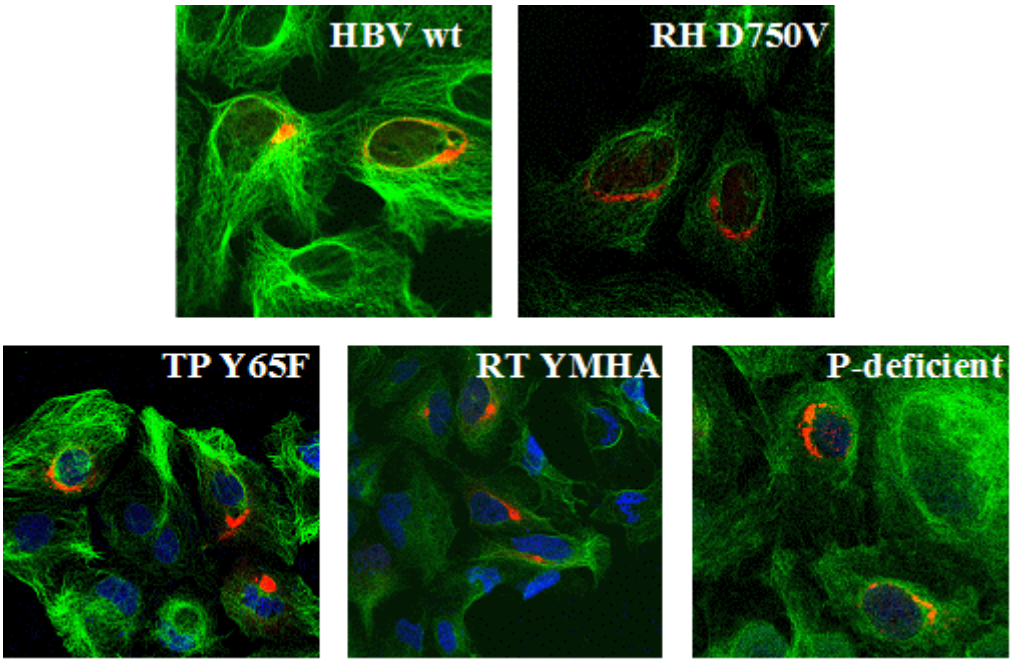

Fig. 6. Intracellular localizations of HBV core particles with nuclear pore complexes (A), microtubule (B), and (C) intermediate filament using confocal microscopy.

(B) Core particles (red) and microtubule (green) of HBV wt or mutants transiently transfected cell. HBV wt and P-deficient mutant core particles (red) partially co-localized with microtubule (green) shown as yellow by merging image, but not RH D750V, TP Y65F, and RT YMHA mutant core particles.

C.

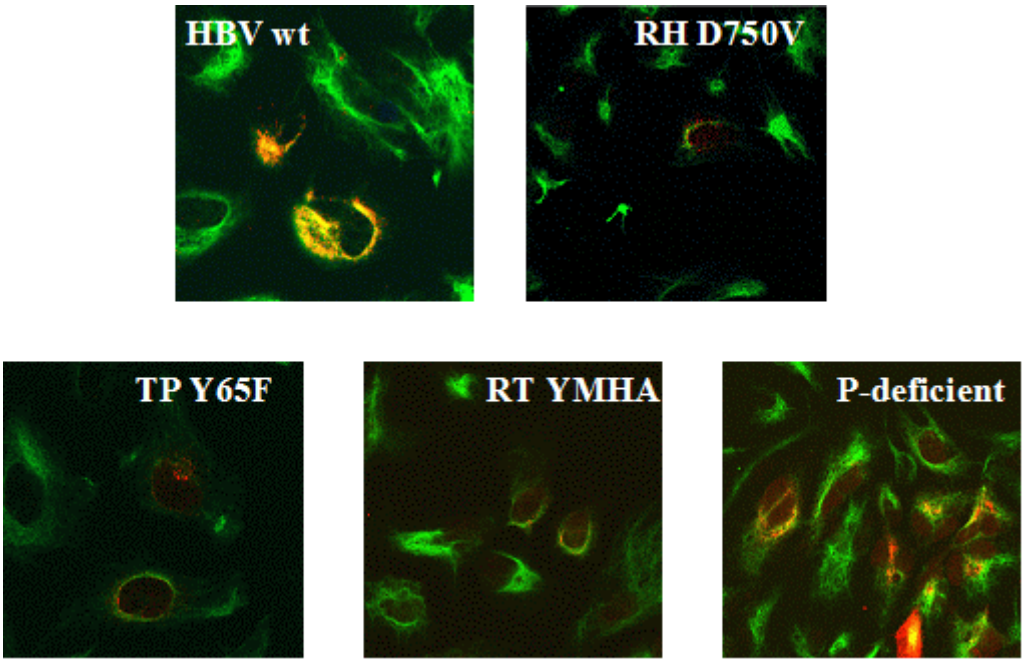

Fig. 6. Intracellular localizations of HBV core particles with nuclear pore complexes (A), microtubule (B), and (C) intermediate filament using confocal microscopy.

(C). Core particles (red) and vimentin (green), the intermediate filament, of P-deficient mutant transiently transfected cell. HBV wt and P-deficient mutant core particles (red) co-localized partially with vimentin (green) show as yellow by merging image, but not RH D750V, TP Y65F, and RT YMHA mutant core particles.

D. Mitochondrial distribution in P-deficient mutant expressing cells.

To investigate the distribution of intracellular organelles in HBV wt and mutants expressing cells, mitochondrial localization were examined using mito-tracker and confocal microscopy. In HBV replicating cells, PUB9, there is a striking clustering of mitochondria near the nucleus. In contrast, mitochondria in non-replicating cells in which HBV mutants were expressed stably, were distributed throughout the cytoplasm. Surprisingly, mitochondria of P-deficient mutant expressing cells aggregated at peri-nuclear region like that of HBV replicating cells.

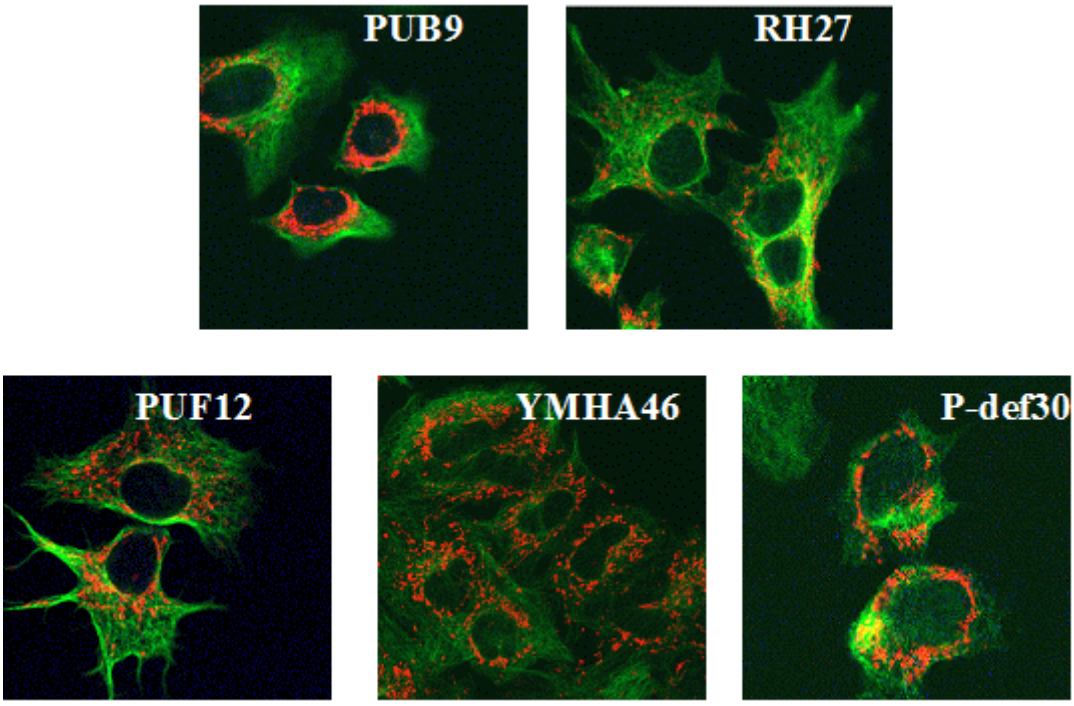

Fig. 5. Mitochondrial distribution of HBV wt and mutants-expressing stable cells. Mitochondria (red) and microtubule (green) were stained with Mito-Tracker and anti anti-α-tubulin antibody, respectively, and observed under confocal microscope. Mitochondria in PUB9 and P-def30 cells were gathered in the peri-nuclear region, but not in RH27, PUF12, YMHA46 cells.

IV. DISCUSSION

During HBV life cycles, virus modifies host system in order to facilitate their spread and replication. Up to date, various mechanisms underlying modification of host system by viruses were reported (Cudmore et al., 1997; Dramsi and Cossart, 1998; Ploubidou et al., 2000). Studies of hepatitis B virus were reported that it also modifies host system. However, knowledge about mature and immature core particle movement was lacking (Kann, 1999, Yoriko Lill, 2006). In this study, we researched for localization of mature and immature core particles for replication.

HBV core particles either go to the endoplasmic reticulum (ER) or Golgi to acquire viral envelop, or to nucleus to recycle back. These two fates of HBV core particles are important for HBV life cycle but the mechanism for movement of core particles is not clear yet. The mature core particles in HBV wt transfected cell can go to ER or Golgi, finally, change to virion form. But immature core particles from HBV RNaseH G750V, TP Y65F, and RT YMHA mutant transfected cells can not be virion. The nature of maturation signal of core particles is unknown. The phosphorylation state of the arginine rich-domain of the C protein might be part of the signal (Yu and Summers, 1994 a, b).

To elucidate how core particles of HBV wt and mutants are transported in cytoplasm or enter nucleus again, HBV replicating and non-replicating construct were prepared and HBV wt and mutants expressing stable cell lines were maintained.

HBV transcription and HBV DNA replication rate in the HBV replicating or non-

replicating stable cells and the wt or mutant transfected cells were examined using Northern and Southern blot analyses (Fig. 2 and Fig.3). To identify HBV core particles in HBV wt or mutant transfected cells, Western blot analysis was performed using HBc antibody (Fig. 4). Surprisingly, core particles from P-deficient mutant transfected cells show stronger signal than that of other mutant transfected cells, such as TP Y65F, RT YMHA, since P protein or RNP complexes might downregulate core particle formation during pgRNA encapsidation (Kim et al, 2004).

Virus induces the changes in the cytoskeletal reorganization and the organelle distribution of the host cells during their life cycles in order to facilitate their spread during entry and exit from the host. To investigate the microtubule reorganization and mitochondria distribution in HBV replicating and non-replicating stable cells, microtubule and mitochondria was observed by immunofluorescence assay and confocal microscopy. Also, co-localization of cytoskeleton and NPC with core particles examined. In HBV replicating cells, PUB9, there is a striking clustering of mitochondria near the nucleus. In contrast, mitochondria in non-replicating cells in which HBV mutants were expressed stably, were distributed throughout the cytoplasm. Mature core particles from HBV wt transfected cell partially co-localized with microtubule, nuclear pore complex, and vimentin but not immature core particles from HBV mutant transfected cells. Surprisingly, mitochondria of P-deficient mutant expressing cells aggregated at peri-nuclear region like that of HBV replicating cells. And P-deficient core particles co-localized with microtubule, nuclear pore complex, and vimentin like core particle from HBV wt. From this result, it suggests that HBV induced the

cytoskeletal reorganization for the movement of mature core particles. And HBV core particle can enter into the nucleus throughout nuclear pore complex.

So far, core particle in wt and mutant transfected cells were reported. The immature, RNA or incomplete DNA-RNA hybrid form containing core particle is excluded from the secretory pathway and that the viral DNA synthesis is associated with a structural change (Gerelsaikhan et al., 1996;Wei et al., 1996). Point mutation in the active centre of the HBV reverse transcriptase destroys the enzymatic activity and priming deficient mutant yields immature core particles. Core particle of these mutants did not use microtubule, vimentin for intracellular movement. From our results, it suggests that the difference between immature and mature core particles might affect the localization of themselves.

It was speculated that P-deficient mutant core particles may not be localized with microtubule, nuclear pore complex, and vimentin like core particles from non-replicating construct. Even though P-deficient is non-replicating mutant, P-deficient mutant core particles mimicked mature core particles from HBV wt. And mitochondria of P-deficient expressing stable cells aggregated at peri-nuclear region liked that of wt expressing stable cells. These results suggest that P protein-deficient mutant and mature core particles might share similar properties. In HBV wt construct transfected cells, various stages of core particles exist which are immature and mature stages of core particles. So far, mature core particles only can not be examined in HBV wt transfected cells, since these cells contain various replication stages of core particles. With this P-deficient mutant, it might be possible to study the intracellular trafficking and changes

by mature HBV core particles.

V. CONCLUSION

P-deficient mutant core particles partially co-localized with microtubule, nuclear pore complex, and vimentin like HBV wt core particles. The mitochondria of P-deficient mutant expressing stable cells aggregated into peri-nuclear region like those of HBV wt expressing stable cells.

From this result, suggest that P-deficient mutant core particles act like mature HBV core particles.

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-국문요약-

DNA 중합효소가 없는 B 형 간염 바이러스 변이주를 발현하는 세포의 변화와 변이주 core 입자의 세포내 이동

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목적: B형 간염 바이러스의 core 입자는 소포체로 이동하거나 핵으로 다시 들어간다. 이러한 이동 메카니즘은 B형 간염 바이러스의 복제와 생활사에 중요하게 작용할 것이라고 생각된다. 복제가 불가능한 다양한 변이주 중 DNA 중합효소가 없는 B형 간염 바이러스 변이주는 DNA 중합효소와 pgRNA가 없는 core 입자를 형성한다. 이 연구에서는 DNA 중합효소가 없는 변이주를 발현하는 세포의 세포내 변화와 DNA중합효소가 없는 변이주 core 입자의 세포내 위치를 관찰하였다.

재료 및 방법: 간암세포에서 확립된 B형 간염 바이러스가 증식하는 세포와 증식하지 못하는 stable세포를 이용하였다. Northern blot 과 Southern blot 분석에 의해서 B형 간염 바이러스의 전사와 복제를 확인하였다. 면역형광염색법 및 confocal 현미경에 의해서 세포 내 소

기관의 분포를 관찰하였다. 미토콘드리아는 미토콘드리아에 특이적으로 염색되는 형광물질인 MitoTracker로 관찰하였다. 미세소관은 tubulin 항체, vimentin은 vimentin 항체, 핵공은 NPC 항체를 사용하여 관찰하였다. 또한 B형 간염 바이러스 core 입자는 core 항체를 이용하여서 관찰하였다.

결과: DNA 중합효소가 없는 B형 간염 바이러스 변이주가 발현하는 core 미세입자는 B형 간염 바이러스가 증식하는 세포의 core 입자와 마찬가지로 미세소관, 핵공, vimentin의 일부가 같이 위치하는 것을 관찰하였다. B형 간염 바이러스가 증식가능 하지 못하는 변이주가 만드는 미성숙한 core 입자는 미세소관, 핵공, vimentin과 세포내에서 같이 위치하지 않은 것으로 관찰되었다. 또한 DNA 중합효소가 없는 변이주를 발현하는 세포의 미토콘드리아 역시 B형 간염 바이러스가 증식하는 세포의 미토콘드리아처럼 핵 주변으로 응집되는 것을 알 수 있었다.

결론: 이상의 결과는 DNA 중합효소가 없는 변이주가 발현하는 core 입자는 B형 간염 바이러스가 발현하는 세포의 core 미세 입자와 비슷하게 행동하는 것으로 관찰되었다. 따라서, DNA 중합효소가 없는 변이주의 core 입자가 성숙한 core 입자와 비슷한 성질을 가질 것이라고 생각된다.

핵심 되는 말: B형 간염 바이러스, DNA 중합효소가 없는 변이주, 미성숙 core 미세 입자, 성숙 core 미세 입자