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Subcellular localisation of internally deleted HBV core proteins

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Abstract

Hepatitis B virus (HBV) variants with core internal deletions were isolated from renal graft recipients in whom an association with end stage liver disease was observed. The aim of this work was evaluation of intracellular localisation of these HBV core variants with internal deletions. Infection of baby hamster kidney cell culture BHC-21 C13 with recombinant Semliki Forest viruses encoding the deleted variant and undeleted wild type HBV core (HBc) genes led to the detection of HBc protein within the nucleus only in the case of deletion variants. Deleted HBc protein in all probability may be partially unfolded, hence unstable. As a consequence, deleted HBc proteins are not part of HBV nucleocapsids, but are left unassembled and are able to cross the nuclear membrane. We suggest that the presence of deleted HBc proteins within the cell nucleus may be of pathogenic importance.

Key words: deletion mutants, hepatitis B virus, HBV core protein, Semliki Forest virus, subcellular localisation.

Introduction

Hepatitis B virus (HBV) causes an inflammatory liver infection whose clinical course ranges from acute to chronic forms, which may lead to cirrhosis and hepatocellular carcinoma.

HBV has a small 3.2 kb DNA genome of four partially overlapping open reading frames. The 21 kDa HBV core (HBc) protein, encoded by the HBV C gene (precore-core), is the structural material for viral nucleoprotein capsids, which are composed of 180 or 240 HBc subunits and organised in T = 3 or T = 4 icosahedrons, respectively. The HBc protein consists of an amino-terminal self-assembly domain (amino acid residues 1 to 150) and a carboxy-terminal protamine-like arginine-rich domain. The latter contains a nuclear localisation signal (Eckhardt et al. 1991).

The HBc protein has been detected in chronic HBV carriers in both nucleus and cytoplasm (Mondelli et al. 1986). A nuclear distribution pattern was found to correlate

with mild hepatitis, whereas cytoplasmic localisation was found to be associated with chronic active hepatitis (Chu, Liaw 1987; Hsu et al. 1987). A positive correlation between the level of HBV DNA in serum and the degree of expression of HBc protein in the nucleus has been observed (Chu et al. 1997). It is supposed that the shift of intracellular HBc protein from the nucleus to the cytoplasm is caused by the regeneration of hepatocytes (Chu et al. 1995). Furthermore, an association of basal core promoter (BCP) mutations with cytoplasmic localisation of the HBc protein was demonstrated by Kawai et al. (2003). The precore sequence is considered to be essential for nuclear localisation of HBc protein (Aiba et al. 1997).

Subcellular localisation of the HBc protein is regulated by phosphorylation of serine residues within the nuclear localisation signal (Liao, Ou 1995) and is dependent on the cell cycle, with its nuclear localisation increased during the G1 phase (Yeh et al. 1993). Cytoplasmic HBc protein is phosphorylated, whereas nuclear HBc protein is unphosphorylated. Phosphorylated HBc protein binds to the nuclear pore complex (Kann, Gerlich 1994; Kann et al. 1999) to release the genome at the nuclear membrane; its uptake is mediated by viral polymerase, but is independent of HBc protein (Kann et al. 1997).

With a particle size of 30 or 34 nm for the T = 3 or T = 4 particles, respectively, HBc capsids exceed the functional diameter of the nuclear pore complex (Dworetzky, Feldherr 1988). Therefore, HBc capsids have to be dissociated for import of HBc protein into the nucleus. It has been shown in the transgenic mouse model that HBc capsids do not cross the nuclear membrane and that they are formed *de novo* within the nucleus (Guidotti et al. 1994).

Variants of the HBV gene C with internal deletions (CID) are frequently present in chronic hepatitis B carriers (Marinos et al. 1996). The internal deletion variants of the HBc gene were also isolated from renal graft recipients in whom an association with a severe course of the illness leading to end stage liver disease (ESLD) was observed (Günther et al. 1996). They are mostly in frame, located in the central part of the HBc protein and incompetent for autonomous replication (Günther et. al. 2000).

We cloned a set of HBV C gene variants with internal deletions into a Semliki Forest virus (SFV)-derived vector pSFV1 (Liljeström, Garoff 1991). The variants were derived from chronic carriers, mostly from patients with liver cirrhosis that led to ESLD, apart from one patient with mild hepatitis. The intracellular distribution of wild type HBc and CID variants was investigated by immonocytochemical methods in infected BHK and Huh7 cells. HBc protein was found in the nucleus of infected cells only in the case of HBc deletion variants and not in the case of wild type HBc.

Materials and methods

Cell cultures

BHK-21 C13 (Baby hamster kidney, Syrian or golden hamster, *Mesocricetus auratus*, ATCC CCL-10) were grown in BHK–21 medium (GIBCO-BRL) containing 5 % fetal calf serum, 10 % tryptose phosphate broth, 20 mM HEPES, and 2 mM glutamine, 100 U ml^{-1} of penicillin/streptomycin (final concentration). The human hepatoma cell line Huh7 (our laboratory stock) was grown in RPMI medium (GIBCO-BRL) supplemented with 2 mM L-glutamine, 2 % sodium selenite (GIBCO-BRL), 100 U ml^{-1} of penicillin/ streptomycin (final concentration) and 10 % foetal bovine serum. Cells were incubated in

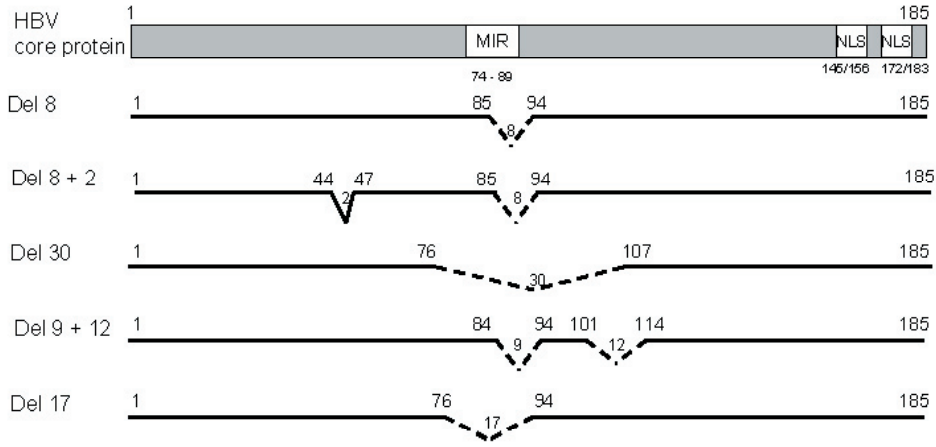

Fig. 1. Internally deleted variants of HBV core protein: the positions of the deletions are indicated by broken lines and the amino-acid positions are given. The numbers within the broken lines indicate the respective length of the deletions in amino acids (MIR, major immunodominant region; NLS, nuclear localisation signal).

a 5 % CO_2 atmosphere at 37 °C. For morphological evaluation and immunocytochemistry, cells were grown on sterile tissue culture chamber slides (Nagle Nunc International), infected with recombinant SFV and incubated at 37 °C (5 % CO_2). Cells were harvested at 4, 12, 24 and 36 h of growth or after infection for morphological evaluation and at 4 and 24 h for immunocytochemistry.

Plasmid construction

HBV core deletion variants were originally derived from serum of immunosuppressed renal transplant recipients (Günther et al. 1996). The HBc variants used in this work are presented in Fig. 1. All deletion variants were amplified by PCR using the following primers: 5'-GCGGATCCATGGACATTGACCCTTATA-3' to introduce a restriction site for BamHI and 5'-CGCCCGGGTAAAGTTTCCCACCTTATG-3' to introduce a restriction site for SmaI. The PCR fragments were cut out with SmaI and BamHI and ligated into the pSFV1 vector (Liljestrom, Garoff 1991) restricted with the same enzymes. Additionally a HBV pregenome (gene bank accession number: NCBI: 329640) of genotype D was cloned into the SmaI site of pSFVdelStNruI (gift of H. Garoff) after PCR amplification with the primers 3'-CCGGAAAGCTTGAGCTCTTCTTTTTCACCTCTGCCTAATCA-5', and 5'-CCGGAAAGCTTGAGCTCTTCAAAAAGTTGCATGGTGCTGG-3', restriction with Hind III and reconstruction of the blunt ends by T4 polymerase.

The pSFV helper 1 plasmid used for recombinant virus stocks preparation was a gift from H. Garoff.

Core genes

The following core genes were cloned into pSFV-1: (i) an 8 amino acid deletion, deleted from amino acid 86 to 93 (del 8); (ii) a variant comprising two deletions of eight and two amino acids in length from amino acid 45 to 46 and 86 to 93 (del 8+2); (iii) a 30 amino acid deletion, deleted from amino acid 77 to 106 (del 30); (iv) a variant comprising two

deletions of nine and twelve amino acids in length from amino acid 85 to 93 and 102 to 113 (del 9+12); (v) a 17 amino acid deletion, deleted from amino acid 77 to 93 (del 17) and (vi) an undeleted wild type core gene of genotype A (corewt). With the exception of variant del 9+12, which was derived from the serum of a patient with mild hepatitis, all variants were derived from the serum of patients suffering from liver cirrhosis that led to end-stage liver disease.

RNA transcription, generation of recombinant virus and cell infection

RNA transcripts were produced in vitro from 3 μg of SpeI-linearised pSFV1/del 8, pSFV1/del 8+2, pSFV1/del 30, pSFV1/del 9+12, pSFV1/del 17, pSFV1/HBVcorewt and NruI-linearised pSFV1delStNruI/HBVpgRNA, using SP6 RNA polymerase (Fermentas). For *in vivo* packaging of recombinant RNA into SFV particles, 5 - 10 μg *in vitro*-transcribed RNA was electroporated into BHK cells together with SFV helper 1 RNA at 850 V, 25 μF, pulsed twice (Bio-Rad Gene Pulser). Electroporated cells were diluted into 15 ml complete BHK medium, transferred into tissue culture flasks and incubated at 37 °C (5 % CO_2). After 20 h, SFV particles in the culture medium were collected and frozen rapidly to be stored as a virus stock. The infection of BHK cells was carried out in serum-free medium with an appropriate dilution of virus stock, which ensured that 100 % of the cells was infected. One hour after infection, BHK-21 medium containing 1 % fetal calf serum and 2 mM L-glutamine were added and incubation was performed overnight at 37 °C in an atmosphere of 5 % CO_2.

Cell lysis and ELISA (Enzyme-Linked Immunosorbent Assay)

At 16 to 20 h after infection, cell monolayers (on 3 cm ø plates) were washed with PBS, overlaid with 300 μl of lysis buffer (1 % Nonidet P-40, 50 mM Tris-HCl pH 7.6, 150 mM NaCl, 2 mM EDTA, 1 μg ml^{-1} PMSF) and let stand on ice for 10 min. The lysates were transferred to microcentrifuge tubes and centrifuged (3000 × g for 5 min) to remove the cell nuclei. The undiluted supernatants were used for specific HBcAg immunodetection by a ELISA kit for HBeAg (Diasorin), as described by the manufacturer.

Panoptic staining (for morphological estimation of the cells)

The cells grown on chamber-slides, dried at room temperature were fixed in 0.1 % Leischman's solution in 96 % methanol for 3 min, rinsed and stained with freshly prepared solution of 0.1% azure II / 0.1% eosin K (10:30 v/v) for 15 - 20 min. The cell morphology was evaluated and cell number counted and calculated per 100 μm^2.

Immunocytochemical detection of intracellular HBc variants by an indirect method using monoclonal antibodies (Mabs)

BHK cells that were grown on sterile tissue culture chamber slides and infected with recombinant SFV after drying at room temperature, fixed with ethanol/acetic acid (3:1) for 20 min and rinsed thoroughly (3 ×) in distilled water. Then the slides were immersed in PBS for 10 min., rinsed with PBS supplied with 0.25 % Triton X-100 and incubated for 24 h in a humidity chamber at 4 °C with anti-HBc Mab at a dilution of 1:200 (in PBS + 0.25 % Triton X-100 + 0.25 % BSA), and after that rinsed in PBS + 0.25 % Triton X-100. Two types of Mab C1-5 (HBcAg epitope aa 78-82) and 10C-6 (aa 134-140) (Bichko et al. 1993) were used. The site of the antigen-antibody reaction was revealed by

application of alkaline phosphatase conjugated anti-mouse IgG (whole molecule, Sigma) at dilution 1:200 (room temperature for 1 h, in the dark). After rinsing with PBS, the alkaline phosphatase activity was developed for 30 min at room temperature by Sigma FAST where Fast Red TR/Naphtol AS-MX is the immunohistology substrate of choice for alkaline phosphatase conjugated antibodies as it produces an intense red stain.

The slides were mounted in glycerol gelatin (Sigma). The evaluation was done by light microscopy.

In the fluorescent variant the site of the antigen-antibody reaction was revealed by FITC-conjugated anti-mouse IgG (Fab specific, Sigma) and evaluated under a fluorescent microscope Leitz MPV 3 using the appropriate filters.

Results

Expression of HBc deletion variants

The analysed HBc deletion variants were originally derived from sera of immunosuppressed renal transplant recipients (Günther et al. 1996). HBc variants, which have been analysed earlier for their stability and self-assembly competence in *E. coli* (Preikschat et al. 1999), were cloned into the mammalian expression vector pSFV-1 (Liljeström, Garoff 1991). Five HBc variants (Fig. 1) and an undeleted wild type (wt) HBc gene of genotype A were expressed in BHK cells after infection with recombinant SFV viruses. Additionally, a whole HBV pregenome of genotype D (HBVpg) was cloned into vector pSFV1delStNruI.

Synthesis of HBc deletion variants in cultivated cells

The potential of the deleted HBc genes to synthesise variant HBc proteins was monitored by commercial HBe ELISA (Fig. 2). Variants with small deletions (del 8, del 8+2, del 17)

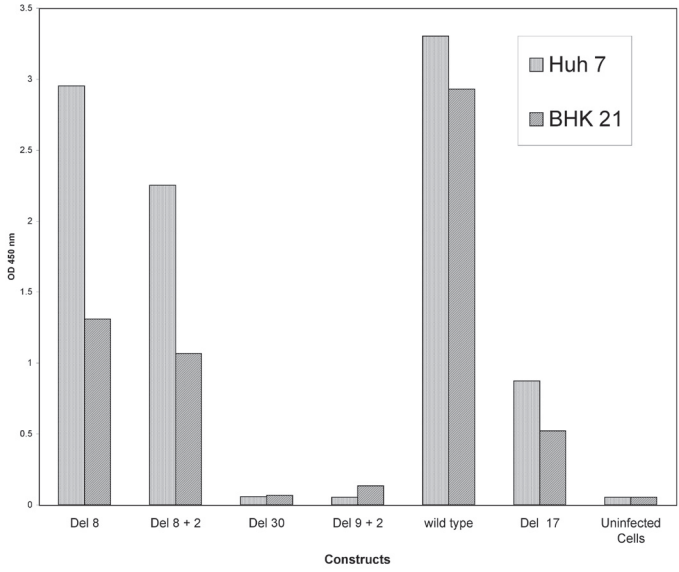

Fig. 2. Comparison of the expression level of different HBcAg variants. BHK-21 and Huh 7 cells were infected with recombinant SFV1/core viruses, and aliquots of cell lysates were analysed by ELISA (Diasorin) for HBeAg.

showed expression of variant HBc protein, albeit below the wt HBc level. Expression of HBc gene variants was not detected for mutants with larger deletions (del 9+12, del 30). Expression in cell lines BHK and Huh 7 did not reveal a difference in the relative expression level of the variants.

Intracellular localisation of HBc deletion variants

Attempts to detect the expressed proteins inside the BHK cells by indirect immunofluorescent assay with Mab 10C-6, whose epitope (aa 134-140) is preserved in all variants, led only to the detection of wild type HBc and of the highest expressed variant – HBc del 8.

Immunocytochemical analysis of HBc proteins with the same Mab by light microscopy, after reaction with anti-mouse IgG conjugated with alkaline phosphatase, showed the expression of all HBc deletion variants (Fig. 3). The expression of deletion variants del 8, del 8+2, del 9+12 and wild type was also confirmed by immunocytochemical staining with Mab C1-5. The epitope (aa 78 - 82) detected by Mab C1-5 was preserved only in these variants.

The numbers of stained cells and the intensity of staining varied greatly among HBc variants. The amount of stained cells was highest for the wt HBc construct, reaching values of 60 - 80 %, rather high (47 - 55 %) for del 8. The values for the other HBc deletion variants were lower. Only a few specifically stained cells were found in the case of the largest HBc deletion variant, del 30. Cytoplasmic staining was detected for all HBc deletion variants and wild type. Strong cytoplasmic staining was observed in the case of wt HBc, where HBc protein in form of densely packed granules accumulated mainly within the cell area around the nucleus or was dispersed throughout the cytoplasm. Cytoplasmic staining of the HBc deletion variants was much less intensive.

Further analysis of the intracellular distribution of the HBc variants revealed that a small amount of HBc protein was detectable in the nucleus of BHK cells only in the case of HBc deletion mutants (Fig. 3A). About 5 % of cells had HBc protein in the nucleus in the cases of the variants del 8 and del 8+2, less for variant del 30 and occasionally for variant del 9+12. The same expression pattern – cytoplasmic and nuclear staining – was observed for wt HBc protein expressed in the context of the HBV pregenome. Surprisingly, wt HBc protein expressed from SFV1/wt HBc showed strong cytoplasmic, but never nuclear staining.

Considering that the SFV expression system is cytopathic, a compromise has to be found between recombinant gene expression and cellular death. Therefore the synthesis of wt HBc and del 8 proteins was followed in a time course. The appearance of proteins

Table 1. Cell number per 100 μm^2 in 24 h BHK culture treated with HBc

Time of growth or after infection (h)	Control	SFV	wt HBc	Del8
4	170 ± 16	130 ± 13	130 ± 40	145 ± 13
12	358 ± 45	175 ± 28	155 ± 20	135 ± 50
24	425 ± 26	154 ± 27	96 ± 10	115 ± 38
36	549 ± 105	70 ± 20	92 ± 12	17 ± 14

could be noticed as early as 4 h after infection. Their level increased during the next 20 h. An insignificant decrease in the number of HBc-expressing cells 36 h after infection could either be connected with the injuring effect of SFV or with specific effects of the expressed HBc genes. To find out if any of the expressed HBc deletion variants exert an influence on cell growth potential or could provoke specific cytopathic effects on BHK cells, the latter were monitored for quantity and morphological changes starting 4 h after infection till 36 h after infection. The untreated BHK cell culture formed a monolayer at 24 h of growth and the cells showed similar morphology during the whole check-up period (4 h till 36 h): well structured cells with clear cell borders, basophile cytoplasm, fine reticular chromatin and prominent nucleoli (Fig. 4A). The cell amount per 100 μm^2

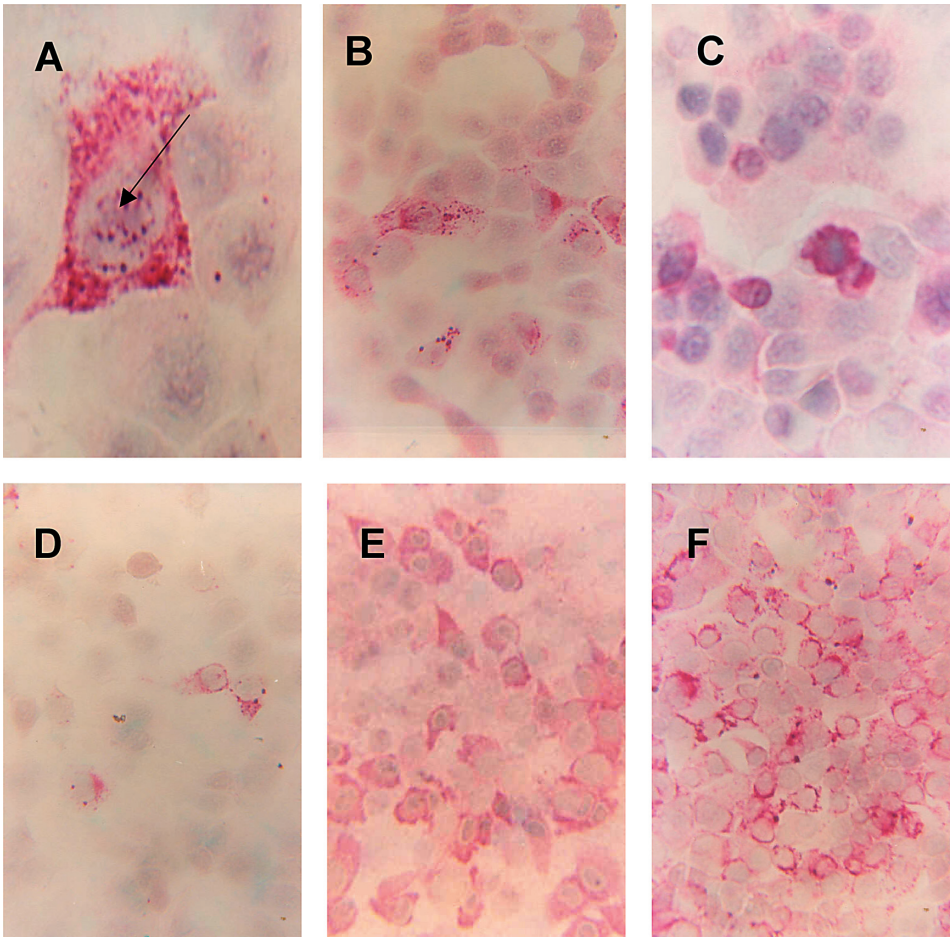

Fig. 3. Immunocytochemical detection of HBV core protein in cell cultures of BHK-21 cells that were infected with SFV1/HBV core variants and SFV1/HBV core wild type. HBcAg was detected after reaction with monoclonal antibody 10C-6. Del 8 (A) shows both nuclear (arrow) and cytoplasmic staining. The pictures show BHK-21 cells expressing the following HBV core variants: A, Del 8 (magnification × 270); B, Del 8 + 2 (× 108); C, Del 17 (× 108); D, Del 9 + 12 (× 108); E, Del 30 (× 108); F, wild type (× 108).

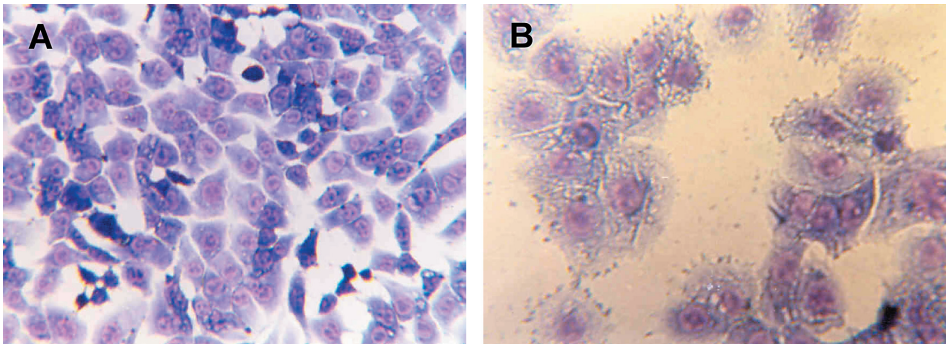

Fig. 4. The cell morphology after infection with recombinant viral particles of del 8 (B) shows signs of degeneration. The control (A) shows uninfected BHK cells (panoptic staining with Leischman-azure II-eosine). Magnification × 108.

gradually increased during the check-up period (Table 1). Cell morphology changes appear already 4 h after the infection: significantly increased basophily of cytoplasm, especially of the perinuclear cytoplasm, of most cells in the case of wt HBc and much rarely in Del 8. The above features could be regarded as a morphological manifestation of an increased ribonucleoprotein content. Evidence of increased intracellular formation of complexes between proteins and nucleic acids in the cytoplasm of wt HBc-infected cells was revealed also by staining of cells with fluorescent probe ANS together with EB (blue fluorescence if nucleic acids are complexed with proteins, data are not presented). The staining properties could be regarded as morphological manifestation of HBc accumulation/concentration on ribosomes in rough ER. As shown by the specific Mabs, the perinuclear cytoplasm is the main place where HBc is densely packed during the early period (4 h) after infection.

Besides the above observations, morphological signs of degeneration also were revealed even 4 h after infection in a small proportion of cells and in almost all cells 36 h after infection. Signs of degeneration such as pale nucleoli, not well defined cell borders, vacuolated cytoplasm, and, in most cases, reduced basophily of the cytoplasm were seen after infection with core deletion variants, as well as with the wt HBc and pSFV. At the same time, the nuclei showed well-structured chromatin without any signs of apoptosis (as shown by nuclear staining with Hoechst 33258 bis benzidine). Cell number per 100 μm^2 gradually diminished after infection with SFV alone as well as with wt HBc or the deletion variants (Table 1). Thus, no specific cytopathic effects were found apart from the effects typical for SFV infection.

Discussion

In the present work we studied the subcellular localisation of internally deleted HBV core proteins (CID). These HBV variants were originally derived from the serum of renal transplant recipients. An association with a severe course of the disease leading to liver cirrhosis and end stage liver disease was shown.

After infection of BHK cells with recombinant SFV particles encoding the wt and deleted HBc genes, HBc protein was detected in all cases in the cytoplasm of infected

cells by immunostaining with an anti-HBc Mab. The wt HBc protein showed a strong cytoplasmic staining, whereas cytoplasmic staining of the HBc deletion variants was much weaker. We have recently shown that HBc proteins with CID can be efficiently synthesised in an eukaryotic expression system *in vitro*, independently on the deletion size; *in vivo* however they are rapidly degraded by intracellular degradation pathways (in preparation). The weaker immunospecific cytoplasmic staining as well as the lower proportion of cells expressing HBc genes with CID, may reflect the effects of the ubiquitin-proteasome machinery in the degradation of misfolded HBc proteins.

Only deletion variants of HBc protein, but not undeleted wt HBc protein showed nuclear as well as cytoplasmic localisation. We suggest that the decisive factors for subcellular localisation are the ability of the expressed wt HBc protein for self assembly and the stability of the assembled HBc capsids. As the diameter of HBc particles exceeds the functional diameter of the nucleopores (Dworetzky, Feldherr 1988), HBc particles can not be transported through the nuclear membrane (Guidotti et al. 1994) and dissociation of the HBc capsids is required for the uptake of HBc protein into the nucleus. Full length wt HBc protein, synthesised in BHK cells is stable and assembles into HBc particles, as demonstrated by electron microscopy (not shown). The observed HBc deletions appear mostly in a region that hardly tolerates mutations (Koschel et al. 1999), probably because it is mostly α-helical (Wynne et al. 1999), and deletions lead to a deformation and at least partial unfolding of the HBc protein.

The question remains whether these results reflect the natural situation where HBc wt and its variants coexist in the cell. In this case, the CID HBc might be stabilised in mixed HBc particles composed of wt and deleted HBc protein. The ability of HBc proteins with CID to form mixed HBc particles was shown for the 17 amino acid deletion variant del 17 in *E. coli* expression (Preikschat et al. 2000). In the yeast two hybrid system, the del 17 variant showed weak interaction with protein of the same variant type and intermediate interaction with wt HBc; only variant del 8 showed strong interaction with both wt and analogous del 8 protein. All other variants however showed no interaction with wt and in the case of del 8+2 and del 9+12 only weak interaction with protein of the same type (R. Krenzer, MD thesis). Therefore, in the natural environment in the infected hepatocytes, formation of mixed HBc particles seems not very likely, at least in the case of variants del 8+2 and del 30. This means that HBc proteins with deletions are not part of the HBc capsids and can be translocated through the nuclear pore complex. In the case of the variant del 8, it is possible that the HBc variant is included into mixed HBc particles, but due to its variant folding it can be assumed that an unknown amount of it is left unassembled.

When BHK cells are infected with recombinant SFV particles encoding the HBV pregenome, wt HBc protein is present within the nucleus. This is not necessarily a contradiction. During the natural course of infection the dissociation of HBc particles has to occur when the HBV genome is imported into the nucleus (Kann et al. 1997). Kann et al. (1994, 1999) suggested that phosphorylation of HBc protein (Liao, Ou 1995) serves as a maturation signal that is concomitant with DNA synthesis and induces a conformational change within the HBc protein in a way that the carboxyterminal nuclear localisation signal becomes exposed and can serve as a signal for nuclear targeting. Only phosphorylated HBc particles bind to the nuclear pore complex (Kann et al. 1999). As binding of nucleic acid stabilises the HBc capsids (Birnbaum, Nassal 1990), phosphorylation renders HBc

particles less stable and favours dissociation. We suppose that HBc protein that is derived from an SFV-encoded HBV pregenome is assembly competent and encapsidates HBV pregenomic RNA, which appears in the SFV system as subgenomic RNA, and serves also as mRNA for the synthesis of HBV polymerase. After encapsidation of pregenomic HBV RNA alone with HBV polymerase, DNA synthesis proceeds, which leads eventually to the dissociation of HBc capsids during the nuclear import of the HBV genome. Due to this dissociation, HBc protein is detectable within the nucleus.

The function of HBc protein within the nucleus is not very clear. It does not seem to be necessary for the import of the HBV genome (Kann et al. 1997). Liao and Ou (1995) speculate that it may bind to supercoiled DNA and thereby suppress viral mRNA transcription as a step in establishing a persistent infection.

Our results do not support the hypothesis that nuclear localisation of HBc protein coincides with milder forms of hepatitis. All but variant del 9+12 were isolated from patients with liver cirrhosis and ESLD. As we observed nuclear localisation for HBc proteins with CID for variants for which an association with ESLD was shown, it is tempting to speculate that their nuclear localisation might play a role in pathogenesis. Several mechanisms might be involved. HBc protein inhibits the expression of the beta interferon (IFN) gene (Whitten et al. 1991) and could be instrumental in the establishment of viral infection and inhibition of the CTL response. Recently it has been shown that the HBc gene suppresses the tumour suppressor gene p53 (Kwon, Rho 2003). Furthermore it downregulates the human MxA protein, an IFN-inducible GTPase with antiviral activity, both by affecting the IFN-stimulated response elements and by direct interaction with the MxA promoter (Fernandez et al. 2003). Therefore the detailed elucidation of the putative role of HBc and its variants with CID mutations in the HBV pathogenesis should be a subject of further study.

Acknowledgements

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Iekšējo HBV *core* proteīna delēcijvariantu lokalizācija šūnās

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Kopsavilkums

Hepatīta B vīrusa (HBV) variantus ar iekšējām HBV *core* (HBc) proteīna delēcijām (CID) izolēja no pacientiem ar nieru transplantātu un galēji smagu aknu bojājumu. Darba mērķis bija noteikt šo HBc variantu lokalizāciju inficētā šūnā. Izmantoja kāmju nieru šūnu kultūru BHK-21 C13. Kultūras infekcija ar rekombinantiem Semliki meža vīrusiem, kas kodē kā delēcijas saturošus, tā arī nedeletētos savvaļas tipa HBc gēnus, izraisīja HBc proteīna parādīšanos šūnu kodolā tikai HBc delēcijvariantu, bet ne savvaļas HBc gadījumā. Visticamāk, ir izmainīta HBc delēcijvariantu telpiskās struktūras izveidošanās, jeb foldings, un tas izraisa to nestabilitāti šūnās. Šādi HBc delēcijvarianti nespēj pašasociēties par pilnvērtīgām HBc kapsīdām, atrodas šūnā neasociētā veidā un tāpēc ir spējīgi pārvarēt kodola membrānu un nokļūt šūnas kodolā. Mēs uzskatām ka šādu HBc delēcijvariantu klātbūtne šūnu kodolā var būt saistīta ar HBV patoģenitāti.