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Title of the thesis: "Molecular Characterization of Envelope Protein Variants of Hepatitis B Virus"

Keywords: Hepatitis B Virus (HBV), HBV mutants, Large Hepatitis B Surface Protein (LHBS), Middle Hepatitis B Virus Surface protein (MHBS), Small Hepatitis B virus Surface Protein (SHBS), replicative intermediates, cccDNA, pgRNA

Abstract

Hepatitis B disease caused by Hepatitis B virus could pave way for different types of liver manifestations ranging from acute to chronic, cirrhosis to decompensated liver ultimately leading to hepatocellular carcinoma. HBV is an enveloped virus with partially double-stranded DNA and overlapping reading frames. The genetic material is present inside nucleocapsid which is further engulfed by a lipid bilayer membrane that incorporates the three envelope proteins. There are three types of envelope proteins Large, Middle and Small envelope proteins (LHBS, MHBS, SHBS). HBV envelope proteins have a diverse role in HBV life cycle beginning from the infection of hepatocytes by virions to the maturation and assembly of the virions. Hepatitis B surface antigen (HBsAg) is the principal serological marker for the diagnosis of HBV infection.

Due to the lack of proofreading functions of HBV polymerase enzyme, the viral genome is prone to variations or mutations in its genome.

The thesis aimed to study the clinically relevant envelope protein mutants/variants in respect to the viral life cycle to help us understand the strategies used by the mutant virus in its infection, morphogenesis and assembly. Many clones of varying lengths of replication competent HBV genome have been established to study the replication cycle of HBV individually. However, no study has been undertaken to analyze the role of size of HBV genome affecting the expression and secretion of viral proteins. This study was used to determine the antigenic production and secretion at the very beginning of replicative cycle when cells were transfected with replication competent viral genomes of varying lengths. More than full length HBV construct, by virtue of being capable of undergoing transcription without the synthesis of cccDNA intermediate appeared to be better system for studying viral life cycle in *in vitro* culture system as compared to the full-length construct.

Clinically relevant envelope protein mutations were generated with the help of site-directed mutagenesis and were propagated in bacterial and mammalian cell culture system. Secretion of HBsAg and HBeAg was determined through ELISA. Similarly, intracellular HBsAg levels were also found out. Moreover, various replicative intermediates were isolated from clinically relevant mutations of envelope proteins and were studied. Intracellular associated core DNA was isolated from the transfected cells along with the nuclear cccDNA levels in each of the mutant. Further, RNA was isolated from the transfected cells, cDNA synthesis was done and the pgRNA levels in all the variants were found out semi-quantitatively.

Based on the results of all the replicative intermediates (core-associated DNA, cccDNA and pgRNA), LHBS appears to be the most important envelope protein in carrying viral life cycle at the level of replicative intermediates as well as morphogenesis and maturation of viral particles.

Changes in LHBS when compared with changes in MHBS and WT, cause drastic increase in HBsAg production as well as secretion, which is a hallmark of the phenotypic expression of viral life cycle. In the absence of LHBS, maturation and morphogenesis of infectious virions get reduced. Consequently, more and more molecules of HBsAg are likely to remain unoccupied resulting into more and more secretion and retention of HBsAg. For most of the mutations (both, SHBS as well mutations of MHBS and LHBS) analyzed in the present study, phenotypic variation in viral life cycle as represented by HBsAg and HBeAg secretion in culture supernatants and by cytosolic retention of HBsAg are apparently mediated by proportionately varying amount of viral replicative intermediates i.e. viral core particle associated DNA, nuclear cccDNA and encapsidated RNA. Levels of replicative intermediates (nuclear associated cccDNA, pgRNA and viral core associated DNA) for specific types mutations may not necessarily appear in agreement with the replication phenotypes i.e. secretion levels of HBsAg and HBeAg in cell culture media and production of HBsAg in cytosolic fragment. This correlation may vary with different types of mutants of envelope proteins depending on the purpose and reasons for which virus gets its genome mutated. For instance, mutations arising due to long term therapy with nucleos(t)ide analogues among chronic Hepatitis B patients or mutations needed by the virus in order to reduce its infectious virion production but still maintaining the replication of its genome efficiently.