Hepatitis B virus replication and integration

Akademisk avhandling
som för avläggande av medicine doktorsexamen vid Sahlgrenska akademin vid Göteborgs universitet kommer att offentligen försvaras i Mikrobiologens föreläsningssal, vån 3, Guldhedsatan 10A, Göteborg

Fredagen den 2 maj 2014 kl. 13.00

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Avhandlingen baseras på följande arbeten:


III. Larsson SB, Malmström S, Hannoun C, Norkrans G, Lindh M. Reduced serum levels of hepatitis B virus DNA and HBsAg by suppression of cccDNA and pgRNA but not of S-RNA. Submitted.


Göteborg 2014

UNIVERSITY OF GOTHENBURG
Hepatitis B virus replication and integration

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ABSTRACT

Chronic infection with hepatitis B virus (HBV) affects 240 million people worldwide and may cause liver disease including hepatocellular carcinoma (HCC). Initially patients have high levels of HBV DNA in their blood, no liver disease and express the e antigen (HBeAg). At some point an immune response is mounted and the viral load decreases with several log10 copies/ml, they lose HBeAg and severe liver damage may follow if the virus is not cleared efficiently. Meanwhile, the circulating levels of the surface antigen (HBsAg), not bound to viral particles, remain high. In the liver, the viral DNA might integrate in the genome of hepatocytes. This has been proposed as a mechanism potentially promoting cancer development.

The aims of this thesis were to investigate the mechanisms behind the great decline in HBV DNA at loss of HBeAg while the HBsAg levels remain relatively stable, to evaluate the utility of quantification of HBsAg in serum as a marker for liver damage, and to assess the extent of integrated HBV DNA in liver biopsies.

The main methods used in this thesis are various types of polymerase chain reaction (PCR). The material was blood samples and liver biopsies from chronic carriers of HBV. Viral load in the liver, both DNA and RNA, was quantified by real-time PCR and integrated HBV DNA sequences were identified using Alu-PCR.

Serum levels of HBV DNA and HBsAg correlated with intrahepatic levels of covalently closed circular DNA (cccDNA), the template for new viral particles and antigens. By comparing viral load between patients positive or negative for HBeAg it was found that the 3-5 log10 decline of HBV DNA at HBeAg seroconversion mainly is explained by decrease in cccDNA and reduced transcriptional efficiency of pregenomic RNA (pgRNA), the template for virus DNA. However, retention of viral particles and decreased half-life of virions seem to have an additional impact.

By comparing results of serum levels of HBsAg and histological examination of liver biopsies it was concluded that a cut-off of <3.0 log10 IU/ml of HBsAg and <4.0 log10 copies/ml could identify patients with low liver damage with a specificity of 96%.

With Alu-PCR integrated sequences were detected in 36 of 48 liver biopsies examined. In total 45 integrated sequences were analysed from 32 different patients. Integration of HBV DNA was thus a very common event in the chronic HBV carriers.

In summary, this study contributes to the understanding of the replication and integration of the hepatitis B virus.

Keywords: hepatitis B virus, HBV DNA, HBsAg, replication, integration

http://hdl.handle.net/2077/34851