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Genomic and transcriptomic profiles in chronic hepatitis B infection

Akademisk avhandling

Som för avläggande av medicine doktorsexamen vid Sahlgrenska akademin, Göteborgs universitet kommer att offentligen försvaras i föreläsningssalen, Guldhedsgatan 10a, 26 maj 2023, klockan 13:00

av **Johan Ringlander**

Fakultetsopponent:

Anders Widell, docent

Lunds Universitet, Sverige

Avhandlingen baseras på följande delarbeten

I. **Ringlander J**, Skoglund C, Prakash K, Andersson ME, Larsson SB, Tang KW, Rydell GE, Abrahamsson S, Castedal M, Norder H, Hellstrand K, Lindh M. Deep sequencing of liver explant transcriptomes reveals extensive expression from integrated hepatitis B virus DNA. *J Viral Hepat* 2020;27(11): 1162-1170.

II. **Ringlander J**, Strömberg LG, Stenbäck JB, Andersson ME, Abrahamsson S, Larsson SB, Rydell GE, Lindh M. Enrichment reveals extensive integration of hepatitis B virus in hepatitis delta infected patients. In manuscript.

III. **Ringlander J**, Malmström S, Eilard A, Strömberg LG, Stenbäck JB, Andersson ME, Larsson SB, Kann M, Nilsson S, Hellstrand K, Rydell GE, Lindh M. Genomic distribution of DNA/RNA virions in hepatitis B patient sera remains stable for years. In manuscript.

IV. **Ringlander J**, Andersson ME, Prakash K, Larsson SB, Lindh M. Deep sequencing of hepatitis B virus using Ion Torrent fusion primer method. *J Virol Methods* 2022;299: 114315.

V. **Ringlander J**, Stenbäck JB, Schmidt D, Gustafsson J, Norberg P, Andersson ME, Lindh M. Efficient and accurate whole-genome sequencing of hepatitis B virus using Nanopore. In manuscript.

SAHLGRENSKA AKADEMIN
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Genomic and transcriptomic profiles in chronic hepatitis B infection

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Abstract

Chronic infection with hepatitis B virus (HBV) affects >250 million people globally and is the most common cause of hepatocellular carcinoma and liver cirrhosis worldwide. Approximately one million deaths each year are attributed to HBV infection. The virus is adapted to the human host and has developed mechanisms to evade immunity. For example, integration of HBV DNA into the chromosomal DNA of hepatocytes yields the formation of HBV surface antigen (HBsAg). HBsAg derived from integrated HBV DNA is not required for virus replication but may facilitate viral persistence by dampening antiviral immunity. In paper I we utilized deep sequencing to show that HBV integrations, with ensuing production of HBsAg, are much more common than previously appreciated. We also found that integrated HBV DNA may facilitate co-infection with the hepatitis delta virus (HDV), which propagates only in the presence of HBsAg. In paper II we show that samples from patients lacking HBV replication still carried HBV DNA integrations along with HDV RNA, implying that HDV replication may utilize integration-derived HBsAg. Accordingly, we observed that HBsAg levels in blood correlated with the number of HBV integrations in liver tissue samples from HDV-infected patients. By combining sequencing methods and digital PCR we provide evidence to support that the HBV particle often contains both degraded RNA as well as incomplete HBV DNA genomes (paper III). The thesis additionally includes deep sequencing-based methods for HBV whole genome sequencing, which generated highly accurate consensus sequences much faster and less costly compared to Sanger sequencing (paper IV and V). In conclusion, we show that HBV integrations are highly expressed and contribute to HBsAg levels in blood, may support HDV replication and that novel deep sequencing methods may be valuable in routine diagnostics.

Keywords: hepatitis B virus, hepatitis delta virus, integration, deep sequencing, cirrhosis, HCC

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