CHARLES UNIVERSITY

L-17
DISCOVERY OF NEW ANTIVIRAL DEFENSE MECHANISMS

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Chronic infection with hepatitis B virus (HBV) is a major public health problem that affects approximately 250 million people worldwide. Treatment of chronic hepatitis B with nucleot(s)ide analogues inhibits the formation of new infectious viral particles but does not eliminate stable covalently closed circular DNA (cccDNA) in hepatocytes, and pegylated interferon α (IFN-α) monotherapy leads to functional cure in less than 8% of people with chronic hepatitis B. To target the mechanisms by which HBV escapes antiviral IFN-α effects, we propose (i) to study the interplay between promyelocytic leukemia protein nuclear bodies (PML-NBs) and HBV in the cytoplasm and nucleus of infected hepatocytes[1], (ii) to identify and characterize interactions of the HBV core protein (HBc) with host proteins and to inhibit virus replication by targeting this mechanism[2], and (iii) to investigate the effects of microenvironment of HBV-infected hepatocyte on plasmacytoid dendritic cells (pDCs), which are important cellular component of the innate immunity system and producers of IFN-α[3]. Our goal is to uncover the antiviral effect of PML NB in the cytoplasm, as well as that of the IFN-α-induced PML NBs on the conversion of HBc-mediated rcDNA to cccDNA and the role of histone, such as H3.3, loading and cccDNA chromatinization and transcription. The early steps of HBV infection were followed by confocal microscopy combining 3D-fluorescence in situ hybridization (FISH) with immunostaining to visualize the location of HBV DNA in the cytoplasm and in the nucleus. Investigation of the regulation of HBV transcription by the histone variant H3.3 and its chaperones HIRA and DAXX/ATRX, and the dynamics of viral chromatin in an antiviral state could allow the discovery of new therapeutic targets. Another approach to achieve functional HBV cure is to target the interface between viral and cellular proteins of HBV cccDNA. We are using proximity-dependent biotinylation proteomic technique (BioID2) to identify novel proteins interacting with HBc and cccDNA. The goal is to identify host proteins that affect viral replication and select new target candidates for possible therapeutic intervention. We used the in vitro high-throughput screening assay to identify small compounds that modulate the respective protein-protein interactions. Among the HBc BioID interactome hits (P<0.01), we characterized some in more details, such as CCDC88A (GIV, GIRDIN) protein, an interacting partner of EGFR and coreceptor of HBV entry; HBV restriction SMC5-SMC6 complex localization factor 2 (SLF2); and the exon-junction complex (EJC) recycling factor PYM1, which binds to the capsid of flavivirus and elicits antiviral activity against these viruses. In addition, we aim to validate whether small molecules modulating NRF1 (nuclear factor erythroid 2-related factor 2; NFE2L1) pathway, responsible for proteasome synthesis and heat shock protein expression, discovered in our group affect HBV replication[4]. Finally, our goal is to target and counteract the inhibitory mechanism by which the microenvironment of HBV-infected hepatocytes suppress production of IFN-α by pDCs. Our aim is to decipher which component of conditioned medium (CM), including infectious HBV particles, subviral particles (SVPs), HBV proteins or extracellular vesicles (EVs), or alternatively a close cell-to-cell contact of HBV-infected hepatocytes co-cultured with pDCs, is responsible for the inhibitory effect[3]. We investigate the effect of the neutral sphingomyelinase-2 inhibitor GW4869, which blocks the export of EVs and HBV particles from hepatocytes on the restoration of the activity of pDC exposed to HBV-infected hepatocytes. We also study a possible inhibitory effect of microRNA, namely the miR-122, miR-146a, and miR155, present as a cargo in EVs from HBV-infected hepatocytes, transferred into pDCs. Collectively, we propose to study mechanisms of HBV escape from intrinsic and innate immunity for discovery of new antiviral therapeutic targets to develop drugs for the treatment of chronically infected HBV patients.

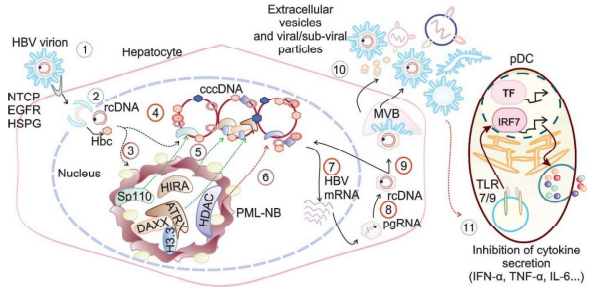

Fig. 1. **Overview of research questions directed towards early steps of HBV replication and interaction with pDCs.**

Acknowledgement
This work was supported by the project National Institute of Virology and Bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) – Funded by the European Union – Next Generation EU.

REFERENCES

1. Cohen C., Corpet A., Roubille S., Maroui MA., Poccardi N., Rousseau A., Kleijwegt C., Binda O., Texier P., Sawtell N., Labetoulle M., Lomonte P.: PLoS Pathog. *14*, e1007313 (2018).
2. Langerová H., Lubyová B., Zábranský A., Hubálek M., Glendová K., Aillot L., Hodek J., Strunin D., Janovec V., Hirsch I., Weber J.: Cells. *9*, 2547 (2020).
3. Janovec V., Hodek J., Clarova K., Hofman T., Dostalik P., Fronek J., Chlupac J., Chaperot L., Durand S., Baumert T.F., Pichova I., Lubyova B., Hirsch I., Weber J.: Sci. Rep. *10*,12767 (2020).
4. Fassmannová D., Sedlák F., Sedláček J., Špička I., Grantz Šašková K.: Cancers *12*(5), 1065 (2020).