WP1.10. Targeting interaction of HBV core, precore and X proteins with host cell machineries (Pichová, Weber, Cvačka)

Research aims

- To elucidate interactions of HBc, HBx, and HBe with cellular factors.
- To identify the pathways that are affected or manipulated in the host cells by HBc, HBx and HBe.
- To investigate the structure of complexes of viral and cellular proteins.
- To design novel types of inhibitors targeting the disruption of host-viral protein complexes

Research plan and methodology

Despite the intensive research of HBV, little is known about the regulations of molecular mechanisms of HBV replication, cccDNA formation and degradation, about reactivation of HBV, and modulation of the virus-host interactions. HBV core, HBx, and HBV precore proteins play an important role in these processes. Therefore, we plan to target interactions of these proteins with host cell machineries.

Interactions of HBV core protein with cellular proteins

Using MS analyses we have identified core-interacting proteins in hepatocytes. Among the identified proteins we have found proteins involved in: (i) ubiquitin proteasome degradation, (ii) epigenetic regulation of transcription, (iii) post-translational modifications e.g. arginine methylation, and (iv) carcinogenesis.

i) Proteins involved in ubiquitin proteasome pathway. The host ubiquitin proteasome pathway has already been suggested to play important role in HBV replication, maturation and virus production. We plan to study interaction of HBc with F-box-only protein 3 (FBXO3) and ubiquitin conjugating enzyme E2 (UBE2O) and their effect on HBV transcription, replication and virus release. Both proteins belong to the group of ubiquitin ligases. While FBXO3 is a substrate recognition component of the SCF (SKP1-CUL1-F-box protein)-type E3 ubiquitin ligase complex mediating poly-ubiquitination of target proteins, UBE2O predominantly mediates protein mono-ubiquitination.

ii) Proteins involved in epigenetic modifications and control of transcription. There is an increasing evidence that HBV replication is regulated by epigenetic mechanisms and microRNA (reviewed in[1]). The proteins (NP1L1, BCORL-1, and RUVBL1/RUVBL2), which were detected in our MS analysis, play a role in various aspects of transcriptional control and epigenetics modifications. Here we will investigate their interaction with HBc. Furthermore, we will investigate epigenetic HBc modifications (ubiquitination, methylation, phosphorylation, etc.) by mass spectrometry techniques.

iii) Proteins involved in arginine methylation. Our preliminary data suggest that protein arginine methyltransferase 5 (PRMT5) may play a negative role in HBV replication. The PRMT5 is the major enzyme responsible for mono- and symmetric dimethylation of arginine residues. PRMT5 is found in complex with the WD-repeat protein MEP50. PRMT5 is a protein of many functions (reviewed in2). Our goal will be to characterize the mechanism of PRMT5-mediated inhibition of HBV replication, and identify the cellular PRMT involved in the methylation of HBc.

iv) Proteins involved in host defense pathway. NRDP1 (neuregulin receptor degradation protein 1) acts as E3 ubiquitin-protein ligase and regulates the degradation of target proteins. It can promote TRIF-dependent production of type I interferon and inhibits infection with the vesicular stomatitis virus. Furthermore, NRDP1 was shown to promote activation of TBK1 and IRF3. We will analyze interactions of HBc with E3 ubiquitin-protein ligase (NRDP1)

Interactions of HBx with cellular proteins

Multiple cellular proteins participating in transcription, signal transduction, cell cycle progression, apoptosis, protein degradation pathways, and genetic stability have been shown to interact with HBx protein[2]. In this project, we will analyse the interaction of HBx with proteins involved in ubiquitin-proteasome degradation, epigenetic control, and host defence.

i) Ubiquitin-proteasome pathway. Recently the formation of multimeric complex HBx- DDB1-Smc5/6 was shown to be critical for regulation of HBV transcription[3]. The damaged DNA binding protein (DDB1) is an adaptor of Cullin-RING ubiquitin-E3 (CLR4E3) ligase complex, which targets specific proteins for degradation by proteasome. HBx redirects the function of CRL4 to target the host restriction Smc5/6 complex, which is bound to cccDNA and silences transcription, for degradation. Little is known about critical protein-protein interactions within this complex[4]. Therefore, we will investigate interactions between individual components of SMC5/6 complex with HBx (and its regions), and with the DDB1 protein.

ii) Epigenetic control mediated by arginine methylation. Our preliminary data show that MEP50, the co-activator of the protein arginine methyltransferase PRMT5 also interacts with HBx protein. We plan to analyze the mutual binding and the higher complex forming abilities of both HBV proteins HBc and HBx with methylosome PRMT5-MEP50. Moreover, also protein methyltransferase PRMT1 was reported to be a binding partner of HBx and a negative regulator of HBV transcription[5]. The binding of HBx to PRMT1 might benefit viral replication by relieving the inhibitory activity of PRMT1 on HBV transcription and represents an attractive target to study.

iii) Host defense pathway. We will analyze the interaction between HBx and NRDP1, which we also identified as a binding partner of HBc. Cao at al. recently reported that HBx protein interacts with NRDP1 and decreases its stability, which results in the up-regulation of ErbB3 and promotion of HCC, associated with chronic HBV infection[6].

In particular, we will verify HBc and HBx interactions with cellular partners in HepG2-NTCP cells via co-immunoprecipitation and pull-down assays. We will determine their effect on HBV infection/replication by overexpression of cellular proteins as well as by downregulation of expression using the CRISPR/Cas9 system. The interactions will be further characterized by determining the minimal binding region of particular interacting proteins. Therefore, series of N- and C-terminal truncations will be employed in co-immunoprecipitation experiments and the identified regions will be further studied by mutational analyses. Postranslation modification of proteins will be analyzed by MS techniques in collaboration with J. Cvačka using a new high-resolution mass spectrometer, which is a part of the proposed capital investment of this project.

To unravel the structural basis of HBc and HBx interactions, we will clone, express, and purify its individual regions as well as the studied host proteins or their particular domains. Expression of Smc5/6 derived domains will be performed in E. coli according to procedure described by Roy et al.[7] DDB1 protein, MEP50, NRDP1 and/or their truncated variants will be expressed in insect cells. These expression systems are well established in our laboratory. Purification procedures will be performed using newly purchased FPLC systems. The more detailed analysis of the studied interactions will be characterized using SPR, AlphaScreen, and NMR titration experiments in collaboration with Václav Veverka. The structures of selected complexes will be solved using X ray in collaboration with Pavlína Řezáčová. In cases, where the increased dynamics of studied proteins will prevent crystallization, the protein-protein complexes will be characterized using NMR spectroscopy. Molecular modelling will be used to pinpoint potential structural motifs using solved crystal structures of HBc and HBx with ligands in collaboration with Jiří Vondrášek.

HBV Precore maturation and involvement of cellular proteins in precore localization

We will analyze the intracellular pathway of precore protein maturation and mechanisms influencing the distribution of precore protein processing products into individual cellular compartments. We will identify the interacting cellular partners, verify the interaction, and then, based on determination of structural motifs responsible for protein–protein interactions, we will select potential therapeutic targets and design new inhibitors.

In particular, we will perform subcellular fractionation experiments with metabolically labelled cells to study intracellular pathways involved in HBe localization. These experiments will be supported by co-localization studies using indirect immunofluorescence staining visualized by newly purchased confocal microscope. Since HBe contains a disulfide bridge crucial for its structure, we will investigate the maturation of the protein in dependence on the redox conditions and we will look for cooperating oxidoreductases involved in the folding process. We also plan to focus on identification of cellular

interacting partners playing a role in the targeting of HBe to the individual cellular compartments using co-immunoprecipitation assays followed by mass spectroscopy analyses. To study the effect of the precore protein on cellular gene expression, we will take advantage of the inducible expression in stably transfected hepatocytes (HepaRG-TR-E, Tet-on) to analyze changes in the transcriptome profile using next generation sequencing. Selected genes and their products will be further characterized with relation to the chronic state of HBV infection and carcinogenesis.

We further plan to design inhibitors targeting the disruption of selected host-viral protein complexes. During the last decade, it has become increasingly clear, that at least in certain cases, small molecules can act as quite effective protein-protein interaction inhibitors (PPIIs). This is attributed to the fact that most PPIs involve so-called "hot spots," relatively small parts of the interface that are essential for high-affinity binding[8]. Therefore, we will screen IOCB library of small molecular compounds for their ability to inhibit tested interactions using a homogeneous time resolved fluorescence resonance energy transfer (TR-FRET) assay. For core protein interactions, we will also test known HBc allosteric modulators such as heteroaryldihydropyrimidines, phenylpropenamides and sulfanilamides. The promising compounds will be further characterized and tested for inhibition of HBV infection in HepG2-NTCP cells. The compounds will be synthetized by Pavel Majer's and Ulrich Jahn's groups. This WP will also extensively use new FPLC and preparative LC apparatuses, which are proposed capital investments of this project.

[1] Zhang, X.; Hou, J.; Lu, M., Regulation of hepatitis B virus replication by epigenetic mechanisms and microRNAs. Front Genet 2013, 4, 202.

[2] Stopa, N.; Krebs, J. E.; Shechter, D., The PRMT5 arginine methyltransferase: many roles in development, cancer and beyond. Cell Mol. Life Sci 2015, 72 (11), 2041-2059.

[3] Decorsiere, A.; Mueller, H.; van Breugel, P. C.; Abdul, F.; Gerossier, L.; Beran, R. K.; Livingston, C. M.; Niu, C.; Fletcher, S. P.; Hantz, O.; Strubin, M., Hepatitis B virus X protein identifies the Smc5/6 complex as a host restriction factor. Nature 2016, 531 (7594), 386-389.

[4] Li, T.; Robert, E. I.; van Breugel, P. C.; Strubin, M.; Zheng, N., A promiscuous alpha-helical motif anchors viral hijackers and substrate receptors to the CUL4-DDB1 ubiquitin ligase machinery. Nat. Struct. Mol. Biol 2010, 17 (1), 105-111.

[5] Benhenda, S.; Ducroux, A.; Riviere, L.; Sobhian, B.; Ward, M. D.; Dion, S.; Hantz, O.; Protzer, U.; Michel, M. L.; Benkirane, M.; Semmes, O. J.; Buendia, M. A.; Neuveut, C., Methyltransferase PRMT1 is a binding partner of HBx and a negative regulator of hepatitis B virus transcription. J Virol 2013, 87 (8), 4360-4371.

[6] Cao, K.; Gong, H.; Qiu, Z.; Wen, Q.; Zhang, B.; Tang, T.; Zhou, X.; Cao, T.; Wang, B.; Shi, H.; Wang, R., Hepatitis B virus X protein reduces the stability of Nrdp1 to up-regulate ErbB3 in hepatocellular carcinoma cells. Tumour. Biol 2016.

[7] Roy, M. A.; Dhanaraman, T.; D'Amours, D., The Smc5-Smc6 heterodimer associates with DNA through several independent binding domains. Sci. Rep 2015, 5, 9797.

[8] Jubb, H.; Higueruelo, A. P.; Winter, A.; Blundell, T. L., Structural biology and drug discovery for protein-protein interactions. Trends Pharmacol. Sci 2012, 33 (5), 241-248

Research schedule

Interactions of HBV core protein with cellular proteins

2017-2022

- Identification, verification and characterization of the host proteins interacting with HBc.
- Analysis of upregulation and downregulation of HBc-interacting partners on HBV replication.
- Characterization of HBc modifications and their influence on its epigenetic function in the HBV life cycle.
- Identification and verification of HBc-interaction domains.

2021-2022

- Testing the known HBc modulators and IOCB library of small molecular compounds.
- Analysis and optimization of hits.

Interactions of HBx with cellular proteins

2017-2022

- Verification and characterization (mutational analysis) of the HBx-host proteins interactions in cells
- Identification of the HBx minimal binding regions required for the interaction.
- Optimization of the screening procedures and initial testing of the IOCB library of small molecular compounds for potential inhibitors.

2019-2022

- Determination of the structural basis for the studied interactions by X-ray or NMR spectroscopy
- Screening for inhibitors of HBx-host protein interactions - identification of the leading compounds for further modifications and development.

HBV Precore maturation and involvement of cellular protein in precore localization

2017-2022

- Mapping the intracellular pathway of the HBe, looking for new interacting partners, validation of the interactions.

2019-2022

- Transcriptome sequencing and gene expression analysis.

2020-2022

- Structural characterization of protein-protein interactions between precore and individual cellular proteins, molecular modelling and design of potential inhibitors.

Publications and patents

Publications (Jimp)

	Jimp
2017	0
2018	2
2019	2
2020	2
2021	3
2022	3
Total	12

Journal of Virology
Virology
Journal of Molecular Biology
Journal of Biological Chemistry

Cooperation with foreign institutions

- Cancer Research Center – CRCL, Lyon University, France
- University of Heidelberg, Department of Molecular Virology, Germany
- Gilead Sciences, USA, analysis of HBV protein interactions with cellular proteins for development of specific inhibitors