Doctoral thesis “Chronic viral hepatitis and human lipid and carbohydrate metabolism”

Author: Liviu Sorin Enache

PhD advisors: prof. Minodora Dobreanu, prof. Patrice André

ABSTRACT

Hepatitis B virus (HBV) infection is tightly linked with hepatic fuel metabolism. HBV replication depends on the activity of several liver-enriched nuclear receptors and transcription factors, such as PPARα, HNF4α, and FoxO1, involved in the metabolic adaptive response to fasting. Thus, viral replication is augmented by nutrient deprivation and by energy stress in cellular models, as well as by fasting, in mouse models. PGC-1α, a major regulator of the metabolic adaptive response to fasting, is involved in the enhancement of HBV transcription, through its interaction with multiple transcription factors. The bile acids receptor, FXRα (NR1H4), who is able to activate the Core promoter of HBV, can be coactivated by PGC-1α. Another important player in the metabolic adaptation to energy deprivation is the protein deacetylase SIRT1. When active, hepatic SIRT1 is able to deacetylate and activate both PGC-1α and FXRα. In view of these data, we hypothesized that SIRT1 may cooperate with PGC-1α and FXRα in order to increase the transcriptional activity of HBV. In the first part of our work, we studied the role of the cooperation between these three metabolic factors in viral replication. We thus identified a metabolic subnetwork, composed of FXRα, PGC-1α and SIRT1, that enhances the transcriptional activity of HBV.

We showed that SIRT1 increases the activity of the Core promoter by acting on other factors, including FXRα. We also observed that the deacetylase function of SIRT1 was necessary for the enhancement of FXRα effect on the HBV Core promoter. Another target of SIRT1, known for its coactivating effect on FXRα, is PGC-1α. Through a series of overexpression and silencing experiments, we showed that not only the coactivation of FXRα by PGC-1α was enahanced by SIRT1, but also the presence of PGC-1α was needed for the effect of SIRT1 on the activation of the HBV Core promoter by FXRα. These data suggest that FXRα, PGC-1α and SIRT1 cooperate in the modulation of the transcriptional activity of Core promoter. We then confirmed our initial observations and showed that the activation of SIRT1/PGC-1α/FXRα axis induces HBV RNA transcription in cell lines of liver or extrahepatic origin. These results enforce the ideea that HBV replication could be modulated by the nutritional status.

Previous reports from studies conducted *in vitro* and in animal models suggested that HBV transcription is controlled in the same manner as gluconeogenic genes. Our next hypothesis was that, in humans, HBV replication could have diurnal fluctuations according to fasting and refeeding periods. The aim of our second study was to assess whether plasma HBV viral load shows significant variations along the nichthemeron in chronically HBV-infected patients with active viral replication. We observed the existence of a circadian cycle of HBV infection in humans, which underlines the rôle of nutrient availability in the modulation of HBV replication, previously predicted by experimental models.

Circulating nucleic acids are promissing biomarkers in many fields of human pathology. Although cellular factors such as nuclear receptors and transcription factors linked to lipid and carbohydrate metabolism are involved in several pathogenic aspects of chronic hepatitis B, their corresponding RNA sequences have not been assessed as potential biomarkers. We thus attempted to detect the mRNA sequences of several metabolic factors in plasma samples of healthy individuals and chronic hepatitis B patients.

We successfully detected more than 30 plasma mRNA sequences corresponding to enzymes, transporters, nuclear receptors and transcription factors involved in the synthesis and oxidation of fatty acids, synthesis, transport and excretion of cholesterol, and in energy sensing and expenditure. The circadian variation and the multiple correlations in the expression patterns of these plasma transcripts are similar to those previously described in cells both *in vitro* and *in vivo*. Moreover, we found significant differences in the plasma mRNA profiles of HBV carriers compared with healthy controls, similar to those found in experimental models of infection. Similarities with previous experimental findings include overexpression of CPT1A and genes involved in cholesterol biosynthesis (SREBF2, FDPS), and an increase in the level of liver-specific transcripts (ALB, HP). Our results suggest that circulating mRNAs reflect, at least in part, the transcriptional status of the organism or certain organs, providing a “virtual biopsy”.

Further research is warranted to shed new light on the complex relationship between HBV life cycle and host lipid-carbohydrate-fuel metabolism and may lead to the identification of both actionable targets in antiviral therapy, and putative biomarkers in chronic hepatitis B.

Keywords: hepatitis B virus, nuclear receptors, farnesoid X receptor, PPAR gamma coactivator 1a, sirtuin 1, circadian cycle, circulating nucleic acids, energy sensors, viral replication.

Laboratories:

INSERM U1111, 21 Ave. Tony Garnier, 69365 Lyon Cedex 07, FR.

Univ. Med & Pharm Tg. Mures; SCJU, Central Med. Lab., 50 Str. Gh. Marinescu, Tirgu Mures, RO.