ROLE OF HEPATITIS B VIRUS SPLICED VARIANTS IN LIVER PATHOGENESIS

Synopsis/Abstract (max 800 words)

Despite an efficient prophylactic vaccine, chronic hepatitis B (CHB) remains a major heath burden worldwide and increases the likelihood of developing severe liver diseases, including fibrosis, cirrhosis and hepatocellular carcinoma (HCC). Current standard-of-care treatments, albeit efficient in maintaining the infection under control, cannot completely eliminate the hepatitis B virus (HBV) and treated patients remain at high risk of developing severe liver disease.

Viral persistence is linked to the inability to clear the viral minichromosome, namely the covalently close circular (ccc)DNA, which associates with host and viral factors to adopt a highly stable chromatinised episomal structure in the nucleus of infected hepatocytes. cccDNA serves as the template for the transcription of six viral mRNAs by the host RNA polymerase II, including the pregenomic (pg)RNA. The latter is retro-transcribed and encapsidated to generate new infectious viral particles. Similar to host transcripts, pgRNA undergoes alternative splicing generating twenty spliced variants (SpVs) identified so far in the serum of CHB patients or in cell culture. Strikingly, several studies pointed out that the repertoire and the proportion of HBV SpVs evolve along the natural history of the disease. In particular, their proportion increased one to three years before the onset of HCC, strongly suggesting that **circulating HBV SpVs could represent a biomarker of liver pathogenesis**. At least some HBV SpVs encode viral proteins involved in HBV-induced liver disease and in viral replication. However, by which mechanisms they are acting still remains elusive for the majority of them. Furthermore, the precise mechanisms regulating this process are also poorly understood.

We recently identified DDX5 and DDX17 as master regulators of HBV RNA metabolism. In particular, these two functionally redundant RNA helicases regulate HBV RNA splicing by preventing the recognition of a specific splicing donor site. Consequently, their silencing in HepG2-NTCP cells and primary human hepatocytes (PHH) increased the proportion of the related HBV SpVs, including SP17 that was the mostly induced. We performed polysome fractionation and transfection assays to demonstrate that SP17 encodes a novel viral protein in HepG2-NTCP cells. RNA-seq experiments in hepatocytes expressing SP17 indicated that SP17 activated the Focal Adhesion Kinase (FAK) pathway, involved in active hepatocyte proliferation and in the activation of Hepatic Stellate cells (HSCs) via the increased production of CXCL8. Interestingly, analysis of published transcriptomic databases revealed that **SP17-activated genes in vitro were more expressed in CHB patients while SP17-repressed genes in vitro were down-regulated in CHB.** Moreover, we and other groups demonstrated that the expression of DDX5 and DDX17 was lower in CHB patients and that low DDX5 expression, and thus theoretically high SP17 levels, were a prognostic factor for HBV-induced HCC development. Altogether, our observations strongly suggest that SP17 could participate to the development of the HBV-induced liver disease.

This proposal will thus aim to **(1)** further characterise the functions of this novel SP17 viral protein in HBV-induced liver pathogenesis both *in vitro* and *in vivo* and **(2)** determine whether circulating HBV SpVs represent a non-invasive biomarker of the different stages of the CHB disease.

(1) The functional role of SP17 will be firstly investigated in *in vitro* cellular models with an initial focus on the FAK pathway. HepG2-NTCP cells expressing SP17 will be treated with GSK2256098, a selective FAK inhibitor currently in Phase II clinical trial against adenocarcinoma, to assess its role in SP17-induced hepatocyte phenotype. In parallel, SP17 functions in hepatocytes will be addressed in liver-humanised mice that will be infected with an adenoviral vector allowing the hepatocyte-specific expression of SP17 and subjected to hepatic insults such as CCl_4 or denitrosamine (DEN). Onset of fibrosis and HCC will be

assessed by a combination of molecular biology (qPCR, Nanostring nCounter available in the host laboratory) and immunohistochemistry (collagen staining) approaches. As in *in vitro* models, the contribution of FAK will be determined by treating these mice with the selective FAK inhibitor. Finally, spatial transcriptomics will be performed on liver biopsies obtained from a cohort of CHB patients belonging to the biobank of the host laboratory using the Nanostring CosMx technology on selected SP17-positive regions.

(2) The host laboratory manages a cohort of more than 2000 CHB patients covering all the stages of the disease from which 20 paired biopsies and serum are available. The expression of HBV SpVs in both the biopsies and serum will be followed by our HBV-specific 5'RACE-PCR approach and correlated with intrahepatic gene expression profiling assessed by RNA-seq and cytokine levels assessed by Isoplexis technology. The usage of HBV SpVs as biomarkers will be finally validated in other cohorts, including the French national HEPAT-B cohort and cohorts of patients at end-stage liver disease in collaboration with the University of Padova and University of Leipzig.

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Proposed collaboration within ArchiFun network (not mandatory at this stage): University of Padova and University of Leipzig. Our team has already on-going collaborations with the groups directed by Prof. Thomas Berg (Leipzig) and Prof. Francesco Paolo Russo (Padova).

Proposed list of secondments (not mandatory, but recommended if known already):
On-going collaborations with Dr. C Bourgeois at LBMC in Lyon (experts in RNA splicing), Dr J Rieusset at CARMEN, Lyon (oxidative stress and liver pathogenesis), Dr. H. Strick-Marchand at Institut Pasteur in Paris (liver humanised model) will represent an opportunity for the PhD fellow to visit other laboratories and be trained in specific technical aereas.

Main ArchiFun theme involved:

- [x] Host-pathogen interactions;
- [ ] Mechanisms of bacterial resistance and cancer onsets;
- [ ] Neurodegenerative and autoimmune diseases;
- [x] Translational research in prevalent diseases;
- [ ] Physiology and ecology;
- [ ] Neurosciences and cognition.