Structural and functional characterization of HEV ORF3 protein by NMR Spectroscopy

Danai Moschidi*[2,1], Elian Dupré[1,2], Emmanuelle Boll[1,2], François-Xavier Cantrelle[2,1], Zoe Lens[1,2], and Xavier Hanoulle[1,2]

[2]Univ. Lille, Inserm, CHU Lille, Institut Pasteur de Lille, U1167 – Univ. Lille, Inserm, CHU Lille, Institut Pasteur de Lille, U1167 - RID-AGE - Risk Factors and Molecular Determinants of Aging-Related Diseases, F-59000 Lille, France – 1, rue du professeur Calmette BP 245 F-59019 LILLE Cedex, France

[1]CNRS ERL9002 Lille – CNRS ERL9002 - BSI - Integrative Structural Biology, F-59000 Lille, France – 50 avenue du Halley BP 70478 F-59658 Villeneuve d'Ascq Cédex, France

Résumé

Hepatitis E Virus (HEV) is the most common cause of acute viral hepatitis worldwide with over 22 million infections and around 44,000 deaths recorded annually. In developing countries, HEV is transmitted via the fecal-oral route through contaminated drinking water, whereas in developed countries, the transmission occurs by consumption of uncooked or undercooked meat from infected animals. There is no specific treatment for HEV infection, apart from a vaccine which is available only in China. Therefore, HEV represents a major public health problem which is constantly growing around the world. HEV causes liver infection with broad range of clinical manifestations ranging from asymptomatic cases to acute liver failure patients. HEV is small, icosahedral virus with 27 to 34nm diameter that belongs to *Hepeviridae* family. It is a quasi-enveloped virus with non-enveloped virions found in the faeces and bile and enveloped virions by host-cell-derived membranes found in bloodstream. It contains a ~7.2kb positive-sense, single-stranded RNA genome. The viral genome contains three open reading frames: ORF1, ORF2 and ORF3. ORF1 encodes a non-structural polyprotein that includes multiple functional domains responsible for the replication of the viral genome. ORF2 encodes the viral capsid protein that assembles to make the viral particles. ORF3 encodes a small regulatory multifunctional protein which is poorly characterized. Previous studies have shown that ORF3 is involved in the release of the infectious viral particles and interacts with other virus and host proteins inside the cell. For this process, ORF3 interacts with Tsg101 protein from ESCRT-I machinery. It is also reported ORF3 is associated with the intracellular membranes and the plasma membrane. There are two different proposed modes of its membrane anchoring. The first one is the oligomerization of ORF3 with a transmembrane insertion that forms an ion channel like, a viroporin, and the second one is the membrane-association of ORF3 via palmitoylation of its N-terminal Cysteine-rich region. This study focuses on the better understanding of the role of ORF3 during the HEV life cycle and the mechanism for the release of infectious viral particles. We performed a structural and dynamic characterization of HEV ORF3 using NMR Spectroscopy, and characterized its interaction with human Tsg101 by different biophysical techniques. We also aimed at deciphering its membrane association using Nanodiscs. This information could ultimately be used to design anti-HEV compounds.

*Intervenant