Nature Reviews Gastroenterology & Hepatology **11**, 704 (2014); published online 21 October 2014; doi:10.1038/nrgastro.2014.179

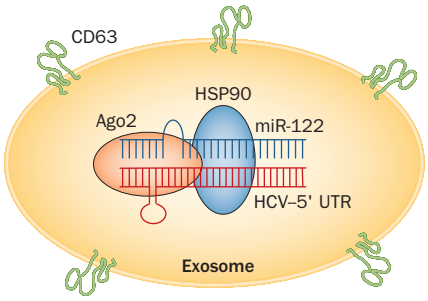
HEPATITIS

Exosomal route of HCV transmission exists in patients

A new study published in *PLoS Pathogens* has demonstrated that patients infected with HCV secrete exosomes containing replication competent HCV RNA, which can transmit infection to primary human hepatocytes. In addition, HCV RNA was shown to associate with three proteins known to enhance HCV replication: microRNA (miR-)122; argonaute 2 (Ago2); and heat shock protein (HSP) 90.

HCV transmission via exosomes has been identified previously *in vitro*, highlighting why HCV therapies such as neutralizing antibodies are not 100% effective. Bukong *et al.* have now expanded our insight into this alternative route of HCV transmission.

"The critical technical challenge was to isolate 'pure' exosomes that had no free HCV or lipo-viral protein contamination," says corresponding author Gyongyi Szabo. The method they developed involved serial filtration, precipitation and CD63 (a marker of exosomes) immunomagnetic selection to isolate 'pure' exosomes. Using this technique, exosomes from the serum of patients infected with HCV who were either treatment naive or nonresponders were shown to have higher levels of Ago2 and HSP90 than uninfected individuals. Furthermore, RNA chromatin-immunoprecipitation analysis of exosomes from HCV-infected patients demonstrated higher levels of Ago2 bound to miR-122 and HCV RNA than in healthy controls.



HCV-infected exosome. Image produced in consultation with G. Szabo.

As some HCV therapies are not effective against exosomal transmission of viral RNA, the authors investigated alternative therapeutic solutions. When miR-122 or HSP90 were inhibited in HCV-infected exosomes from Huh 7.5 cells, exosomal HCV transmission into uninfected cells was lower compared with untreated exosomes.

Disrupting the endocytosis pathway by altering the pH in early endosomes was the next avenue for evaluation in the study. The pH of the early endosomes was neutralized using an H^+-ATPase inhibitor and a PPI. Lowering the pH prevented entry of exosomes and subsequent HCV infection in Huh 7.5 cells.

Future work will study how Ago2–miR-122–HSP90 associates with HCV RNA, is packaged up and gets released in exosomes. In addition, the clinical relevance of how this route is affected by new antiviral drugs will be assessed.

Gillian Patman

Original article Bukong, T. N. *et al.* Exosomes from hepatitis C infected patients transmit HCV infection and contain replication competent viral RNA in complex with Ago2–miR122–HSP90. *PLoS Pathog.* doi:10.1371/journal.ppat.1004424