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Conflict of interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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Reply to: "Are Hedgehog and Wnt/β-catenin pathways involved in hepatitis C virus-mediated EMT?"

Persistent activation of Hedgehog or Wnt/β-catenin pathways is not involved in EMT mediated by HCV NS5A protein

To the Editor:

We thank Conti *et al.* for the interesting discussion of our recent paper. We agree that it is of major interest to delineate the molecular mechanisms leading from HCV protein NS5A to Twist2 induction, which we have shown to trigger EMT in hepatocytes. Several plausible effectors of NS5A could be involved in this process, including the Wnt/β-catenin and the Hedgehog (HH) pathways.

Interestingly, at least three different HCV proteins have been reported to trigger EMT in hepatocytes: some isotypes of core [1], E1/E2 [2] and, as reported by us, NS5A [3]. While the end results of these processes appear quite similar, the different viral proteins employ distinct mechanisms of EMT induction, with the notable involvement of TGF-β signalling for the core and envelope proteins but not for NS5A-mediated EMT. Moreover, they probably operate with different kinetics following their expression (discussed in [3]). The very fact that HCV has evolved several mechanisms to trigger EMT strongly suggests that the process is important for viral physiology and/or spread. It also means that the mechanisms ascribed to NS5A-mediated induction of EMT are expected to be present in experimental models that include the whole complement of viral proteins, while the reverse is not necessarily true. Following this line of argument, we note that the increased level of expression of the components of the Hedgehog pathway and their impact on HCV replication were observed in the model based on JFH1 infection of HuH7.5 cells [4]. We have obtained comparable results in the infection of human primary hepatocytes (PHH) (Fig. 1A), thus confirming the results of Choi *et al.* in this experimental setting. To our knowledge, there are no reports of effects of NS5A on HH expression or signalling. Our data suggest that increased synthesis of components of the Hedgehog pathway is not required for NS5A-mediated EMT, since expression of Sonic Hedgehog, Gli1 or Patched is not altered in cells that undergo this process under the influence of NS5A [3], the BMEL-NS5A (Fig. 1B).

The situation is a little different for the Wnt/β-catenin pathway. NS5A activates β-catenin signalling through interactions with the catalytic subunit of PI(3)K [5] or directly [6], leading in both cases to accumulation and nuclear translocation of β-catenin and transcription of its target genes, such as axin2, glutamine synthase or c-myc. This scheme has recently been elegantly confirmed in several cellular and *in vivo* contexts by Higgs and colleagues [7]. In addition to this non-transcriptional regulation of β-catenin signalling, HCV core protein has been reported to induce transcription of several Wnt ligands, their receptors (Frizzled) and co-receptors (LRP5) in hepatoma cells [8,9]. In contrast to these reports and the interesting data of Conti *et al.*, we see no long-term alteration of wnt5 expression in the JFH1-infected PHH. Interestingly, expression of LRP5 is high in these cells and transcription of axin2, one of β-catenin target genes, is increased. These results suggest that the pathway is persistently

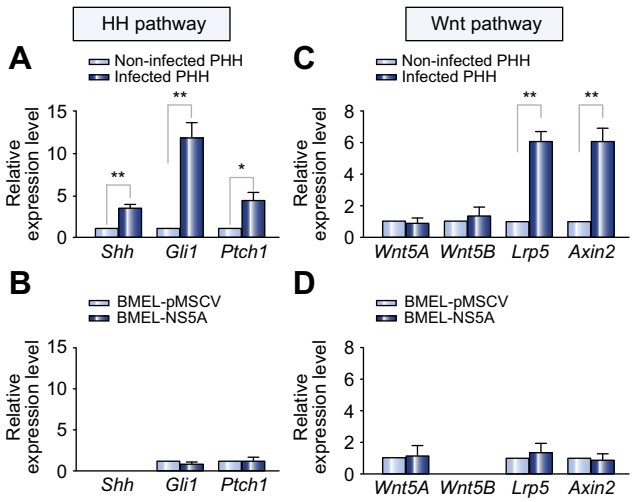

Fig. 1. Activation of HH and Wnt pathways by HCV does not account for NS5A-induced EMT. All experimental conditions were described in [3]. (A) Control and HCV-infected PHH (see Fig. 3D in Akkari *et al.* [3]) were collected three weeks after the infection and analysed by RT-qPCR to evaluate mRNA levels of components of HH and Wnt pathways as well as *Axin2*, a Wnt signalling target gene. Means ± SEM of three samples from one of three independent experiments are shown. (B) Expression of HH and Wnt pathways components and a Wnt target (*Axin2*) was analysed in BMEL-pMSCV and BMEL-NS5A cell lines. RT-qPCR quantification of mRNA was normalised to *HPRT* level and was arbitrarily set as 1 in control cells. *Shh* and *Wnt5b* mRNAs were below the level of detection in BMEL cells. Means and values from two independent experiments quantified in triplicate are shown. Statistical analysis was performed by unpaired Student's *t* test. $^{*}p$ <0.05, $^{**}p$ <0.01.

activated up to at least three weeks following HCV infection of PHH (Fig. 1C). However, although we cannot exclude an early transient activation of Wnt/β-catenin signalling in BMEL expressing NS5A, we found no evidence either of increased expression of components of this pathway or, more significantly, of sustained transcriptional activation of axin2 in BMEL-NS5A cells undergoing EMT (Fig. 1D). Thus, our results suggest that neither Hedgehog nor β-catenin signalling is required for NS5A-mediated EMT. It remains an open question whether these pathways participate in EMT induction orchestrated by other HCV proteins.

Conflict of interest

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Effect of albumin on survival in septic cirrhotic patients other than spontaneous bacterial peritonitis. The question remains open

To the Editor:
We read the manuscript by Guevara *et al.* with interest [1]. The authors evaluated the effect of albumin administration on the 3-month survival in cirrhotic patients hospitalized for infections other than spontaneous bacterial peritonitis (SBP). After randomization, 54 and 56 patients received respectively antibiotics alone, and both antibiotics plus intravenous albumin (1.5 g/kg the first day and 1 g/kg at day 3). The authors concluded that albumin administration with antibiotics showed a potential survival benefit in per-protocol analysis as compared with the control group, and that such a beneficial effect was probably due to the improvement in effective arterial blood volume reflected by the improvement in renal function. However, we feel that their conclusions may require closer examination.

First, this hopeful conclusion contrasts with the absence of a 3-month survival benefit when the analysis was performed on