Annals of Hepatology 30 (2025) 101746

Contents lists available at ScienceDirect

Annals of Hepatology

journal homepage: www.elsevier.es/annalsofhepatology

Letters to the editor

Differentially expressed genes in Huh7 cells during co-culture with LX2 cells demonstrating dynamics of intercellular communication

Dear Editor:

We were very interested in the letter regarding our article that was received. Initially, we appreciate that Junxi Liu and Boda Zhou paid significant interest in our work [1]. As the title of our article suggests, we found that the hepatitis C Virus (HCV) proteins can regulate fibrosis-related genes on hepatocyte cells Huh7 during co-culture with hepatic stellate cells (HSCs) LX2, we not only found fibrosis-inducing genes due to the expression of the NS5A and Core proteins of HCV, but we also observed that during different co-culture times with LX2, Huh7 cells also expressed anti-fibrotic genes. Honestly, it was surprising to observe this type of regulation; however, the molecular mechanisms that can regulate viruses through the expression of their proteins have always been characterized as being complex. So far, and as mentioned by Junxi Liu and Boda Zhou, previous studies have examined the impact of HCV proteins only on the activation of hepatic stellate cells. "However, we would also like to highlight some important points to consider: 1) Relatively few studies analyze these types of mechanisms in co-culture systems. 2) Analyzing the dynamics of intercellular communication over different times is crucial. 3) It is also necessary to analyze the expression of all viral proteins and their impact on this type of regulation. 4) Emphasis is needed on the molecular differences in the phenotypes of quiescent and inactive HSCs. 5) Consider the dual effect of HCV proteins in the induction/blocking of apoptosis.

We believe there is a need to evolve the way these types of studies are conducted, where it has been shown that intercellular communication within the hepatic parenchyma is important to understanding such a complex disease. Establishing co-culture systems, organoids, or any technique that allows working with different cell lineages is crucial to further understanding how this regulation occurs. The activation of HSCs occurs to repair damaged tissue, and this process must be regulated to achieve that objective [2]. HSCs transition from a quiescent state to an activated state but can revert to an inactive state, where the molecular markers and inactivation processes differ from quiescence [3,4].

On the other hand, it is also important for us to emphasize that this dual effect we observe in regulating fibrosis-associated genes is similar to what has been previously observed with different HCV proteins and their effect on the induction or blocking of programmed cell death. This depends on different infection times and/or the nature of the viral protein (particularly NS5A) with different levels of protein phosphorylation that define its nuclear or cytoplasmic localization. Furthermore, this type of regulation of programmed cell death mechanisms might also impact the regulation of mechanisms associated with fibrosis and HSC activation, as all these processes occur simultaneously during the viral infection.

In our study, we observed that HCV Core expression in Huh7 co-cultured with LX2 shows up-regulation of pro-fibrotic genes at 48 h, and after 72 h, we observed downregulation of these genes, and with NS5A, we observed up-regulation of some anti-fibrotic genes at 48 h of co-culture in Huh7 cells (Fig. 1). These results were consistent with regulating activation biomarker expression in LX2 cells. However, it is important to consider molecular inactivation processes to determine whether the LX2 cells are being inactivated or if those that remained quiescent predominated.

It is important to note the limitations of our study, including the co-culture system using transwell inserts with 0.3 μm pores, which excludes the exchange of smaller molecules, and the qPCR array is limited to genes related to fibrosis processes. It would be useful to conduct transcriptomic and proteomic assays to thoroughly analyze all the signaling pathways that might be involved. Additionally, we observed this type of regulation with the expression of a single viral protein per system it is important to evaluate this type of regulation in a system where diverse HCV viral protein co-expression exists.

For these reasons, we cannot yet conclude that the therapy can be directed towards this type of anti-fibrotic regulation induced by HCV, as mentioned by Junxi Liu and Boda Zhou. What we propose is to go further to expand the understanding of all the molecular mechanisms

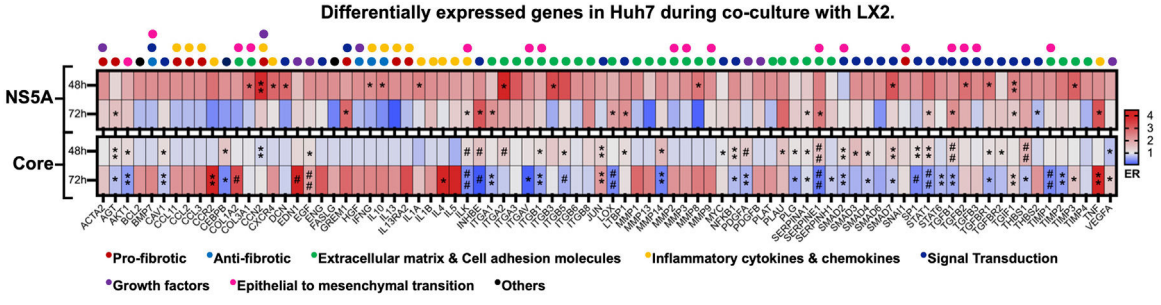

Fig. 1. Differentially expressed genes in Huh7 cells during co-culture with LX2 cells. Huh7 cells were transfected with NS5A and Core HCV plasmids for 24 h and were co-cultured with LX2 for 48 and 72 h, then 84 fibrosis-related gene expressions were evaluated in Huh7 cells by the RT2 Profiler human fibrosis PCR Array. The figure shows a heat map of the relative expression of fibrosis-related genes evaluated. The superior dots indicated the molecular pathway associated with each gene. Multiple student T-test, ## = $p < 0.0001$, # = $p < 0.001$, ** = $p < 0.01$, * = $p < 0.05$.

https://doi.org/10.1016/j.aohep.2024.101746

that different HCV proteins can regulate to verify that the HCV proteins are capable of downregulating hepatic fibrosis at certain pathways.

Finally, we appreciate Junxi Liu and Boda Zhou's interest in considering this type of regulation, which is new and relevant to this kind of disease. We reiterate the need to consider intercellular communication to more accurately assess these hepatic diseases. We also agree with Junxi Liu and Boda Zhou that these types of studies are necessary for investigating the complex interactions between HCV proteins and host cells and may serve to identify new therapeutic targets that offer more precise and effective treatment options for patients with chronic HCV infection [5].

Declaration of interest

None

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