DR. SILVIA SOOKOIAN (Orcid ID : 0000-0001-5929-5470)

Article type : Letter to the Editor

Handling editor: Luca Valenti

SARS-CoV-2 virus and liver expression of host receptors: Putative mechanisms of liver involvement in COVID-19

Short running title: COVID-19 and liver

Carlos J Pirola [1,2*] , Silvia Sookoian [1,3*],

[1] University of Buenos Aires, School of Medicine, Institute of Medical Research A Lanari, Ciudad Autónoma de Buenos Aires, Argentina.

[2] National Scientific and Technical Research Council (CONICET)−University of Buenos Aires, Institute of Medical Research (IDIM), Department of Molecular Genetics and Biology of Complex Diseases, Ciudad Autónoma de Buenos Aires, Argentina.

[3] National Scientific and Technical Research Council (CONICET)−University of Buenos Aires, Institute of Medical Research (IDIM), Department of Clinical and Molecular Hepatology, Ciudad Autónoma de Buenos Aires, Argentina.

[*] Co-corresponding authorship

Correspondence: Co-Corresponding Authors

Carlos J. Pirola, Ph.D. and Silvia Sookoian, M.D., Ph.D.

Instituto de Investigaciones Médicas, IDIM-CONICET

Combatientes de Malvinas 3150, CABA-1427, Argentina

E-mail: pirola.carlos@conicet.gov.ar and ssookoian@intramed.net

Phone: 54-11-52873905

SS and CJP should be considered joint senior authors

Financial support: This study was partially supported by grants PICT 2014-1816, PICT 2015-0551, and PICT 2016-0135 (Agencia Nacional de Promoción Científica y Tecnológica, FONCyT), CONICET Proyectos Unidades Ejecutoras 2017, PUE 0055.

Declaration of interests: None

Electronic word count: 423**; number of figures:** 1

Keywords: COVID-19; SARS-CoV-2; liver; single-cell transcriptomics; ACE2; TMPRSS2; FURIN; host.

To the Editor,

Zhang et al. showed that COVID-19 affected patients' present liver biochemistry abnormalities, including elevation of aminotransferases, gamma-glutamyl transferase, and alkaline phosphatase [1]. Hence, several possible clinical scenarios in the setting of liver diseases have been postulated. First, patients with chronic liver disease may be more vulnerable to the severe clinical consequences of COVID-19, including oxygen desaturation and hypoxemia due to severe pneumonia or the cytokine storm [1;2]. Second, liver biochemistry abnormalities are the consequence of drug toxicity.

There is a third potential but poorly explored clinical scenario, which is the possibility that the novel 2019 coronavirus, also known as SARS-CoV-2, may directly or indirectly cause liver injury. In fact, SARS-CoV2 viral load in the stool, which has been detected in about 48% of patients even in stool collected after respiratory samples tested negative [3], is likely to be associated with portal venous viremia.

We assessed the gene expression levels of SARS-CoV2-interacting host receptors in the liver tissue and their distribution across cell types according to single-cell transcriptomic experiments retrieved from the Single Cell Portal. We focused on angiotensin-converting enzyme 2 (*ACE2*), transmembrane serine protease 2 (*TMPRSS2*), and paired basic amino acid cleaving enzyme (*FURIN*) gene expression levels. Our analysis shows that the three human host receptors are expressed in the liver tissue; however, expression levels extensively vary across cell types. *ACE2* presents the highest expression levels in cholangiocytes, followed by hepatocytes (**Figure 1C**). *TMPRSS2* is expressed in cholangiocytes, hepatocytes, periportal liver sinusoidal endothelial cells, erytroid cells, and in a much lesser extent in non-inflammatory macrophages and alpha-beta T cells (**Figure 1D**). *FURIN* shows expression levels across all cell types, from hepatocytes to all populations of liver resident cells (**Figure 1E**).

Together, these findings support the possibility that SARS-CoV-2 may cause direct liver injury by viral cytopathic effect (directly by lysis and/or by inducing necrotic/apoptotic effect/s). Furthermore, the expression pattern in cell clusters associated with numerous active immune pathways, for example, inflammatory macrophages, natural killer cells, plasma cells, mature B cells, and cells of the liver endothelial microenvironment, opens the possibility of SARS-CoV-2 - immune-mediated liver damage.

Not surprisingly, reports from the past 2003-SARS (severe acute respiratory syndrome) epidemic showed not only liver impairment in up to 60% of the patients but also confirmed the presence of

SARS-coronavirus by RT-PCR in liver biopsies presenting mild to moderate lobular inflammation and apoptosis [4].

In conclusion, to understand the pathogenesis of SARS-CoV-2 –related liver disease, additional research must be guaranteed, including the search for evidence of viral replication in hepatocytes and liver histology characterization.

Author contributions: SS: study concept and design; data acquisition; data analysis and interpretation; manuscript drafting; securing funding. CJP: study concept and design; data acquisition; data analysis and interpretation; manuscript drafting; securing funding.

REFERENCES

(1) Zhang Y, Zheng L, Liu L, Zhao M, Xiao J, Zhao Q. Liver impairment in COVID-19 patients: a retrospective analysis of 115 cases from a single center in Wuhan city, China. *Liver Int* 2020. doi: 10.1111/liv.14455. [Epub ahead of print]

(2) Zhang C, Shi L, Wang FS. Liver injury in COVID-19: management and challenges. *Lancet Gastroenterol Hepatol* 2020 (5):428-430.

(3) Cheung KS, Hung IF, Chan PP et al. Gastrointestinal Manifestations of SARS-CoV-2 Infection and Virus Load in Fecal Samples from the Hong Kong Cohort and Systematic Review and Meta-analysis. *Gastroenterology* 2020. doi: 10.1053/j.gastro.2020.03.065. [Epub ahead of print]

(4) Chau TN, Lee KC, Yao H et al. SARS-associated viral hepatitis caused by a novel coronavirus: report of three cases. *Hepatology* 2004;39:302-310.

(5) MacParland SA, Liu JC, Ma XZ et al. Single cell RNA sequencing of human liver reveals distinct intrahepatic macrophage populations. *Nat Commun* 2018;9:4383.

Figure 1

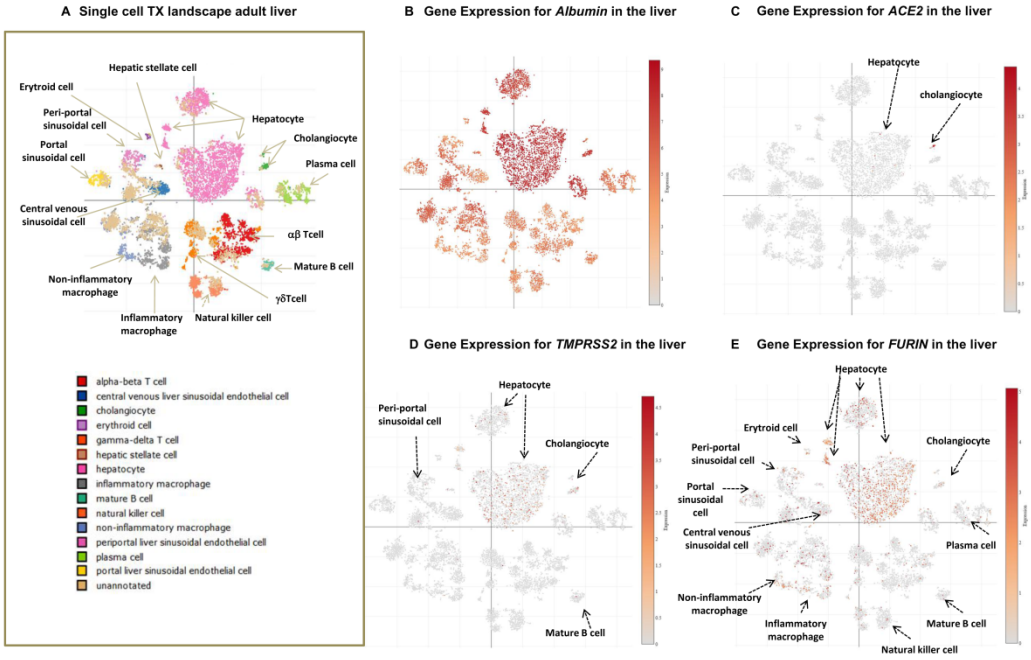
Liver gene expression profiling across cell types of host receptors implicated in SARS-CoV-2 infection

Profiling of gene expression was retrieved from the Single Cell Portal available at https://singlecell.broadinstitute.org/single_cell. The analysis was focused on the adult liver dataset from the Human Cell Atlas March 2020 Release, a collection of 23 human single-cell datasets. The human liver cellular landscape analysis by single cell RNA-seq is based on the study of MacParland et al. [5]. Human liver tissue was obtained from livers procured from deceased donors deemed acceptable for liver transplantation.

A. Annotation of liver whole transcriptome involved 15 clusters, including hepatocytes, alpha-beta T cells, central liver sinusoidal endothelial cells, cholangiocytes, erythroid cells, gamma-delta T cells, hepatic stellate cells, inflammatory macrophage, mature B cells, natural killer cells, non-inflammatory macrophages, periportal liver sinusoidal endothelial cells, plasma cells, portal liver sinusoidal endothelial cells, and unannotated cells (**A**).

B. To illustrate the pattern and magnitude of differential gene expression levels at different cells in the liver, we assessed the pattern of gene expression of albumin (*ALB*)- the most abundant protein in human blood that is highly expressed in the liver.

C-E. Exploration of *ACE2*, *TMPRSS,* and *FURIN* expression in the liver.



liv_14500_f1.tif