Well-Differentiated Liver Cancers Reveal the Potential Link Between ACE2 Dysfunction and Metabolic Breakdown.

Lise Desquilles

INSERM, INRAE, Univ Rennes, Nutrition Metabolisms and Cancer, Rennes, France

Luis Cano

INSERM, INRAE, Univ Rennes, Nutrition Metabolisms and Cancer, Rennes, France

Gevorg Ghukasyan

Univ Rennes, CNRS, INSERM, UMS Biosit, Core Facility H2P2, F-35000, France

Clémence Landreau

INSERM, INRAE, Univ Rennes, Nutrition Metabolisms and Cancer, Rennes, France

Anne Corlu

INSERM, INRAE, Univ Rennes, Nutrition Metabolisms and Cancer, Rennes, France

Bruno Clément

INSERM, INRAE, Univ Rennes, Nutrition Metabolisms and Cancer, Rennes, France

Bruno Turlin

INSERM, INRAE, Univ Rennes, Nutrition Metabolisms and Cancer, Rennes, France

Romain Désert

INSERM, INRAE, Univ Rennes, Nutrition Metabolisms and Cancer, Rennes, France

Orlando Musso (✉ orlando.musso@univ-rennes1.fr)

INSERM, INRAE, Univ Rennes, Nutrition Metabolisms and Cancer, Rennes, France

Research Article

Keywords: liver metabolism, DNA methylation, DC-SIGNR, MRP2, CLEC4M, TMPRSS2, DPP4, SARS-CoV-2, hepatocellular carcinoma, multiplex immunohistochemistry, confocal slide scanning, CTNNB1, tumor transcriptomics, tumor classifications, functional expression networks, weighted gene correlation network analysis, tissue microarrays, the cancer genome atlas, Desert's hepatocellular carcinoma classification, periportal, perivenous, stem,

Posted Date: August 20th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-797837/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Version of Record: A version of this preprint was published at Scientific Reports on February 3rd, 2022.

See the published version at <https://doi.org/10.1038/s41598-021-03710-0>.

Abstract

Angiotensin-converting enzyme 2 (*ACE2*) is the receptor of the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) causing Coronavirus disease 2019 (COVID-19). Transmembrane serine protease 2 (*TMPRSS2*) is a coreceptor. Abnormal hepatic function in COVID-19 suggests specific or bystander liver disease. Because liver cancer cells express the *ACE2* viral receptor, they are widely used as models of SARS-CoV-2 infection *in vitro*. Therefore, the purpose of this study was to analyze *ACE2* and *TMPRSS2* expression and localization in human liver cancers and in non-tumor livers. We studied *ACE2* and *TMPRSS2* in two transcriptomic datasets totaling 1503 liver cancers and 47 non-tumor samples and in tissue microarrays including 41 HCCs and five non-tumor samples by multiplex high-resolution confocal immunohistochemistry and quantitative image analysis. In liver cancers, we detected *ACE2* and *TMPRSS2* at the biliary pole of tumor hepatocytes. In non-tumor livers, we identified *ACE2* in hepatocyte's bile canaliculi, biliary epithelium, sinusoidal and capillary endothelial cells. Tumors carrying mutated β -catenin showed *ACE2* DNA hypomethylation and higher mRNA and protein expression, consistently with predicted β -catenin response sites in the *ACE2* promoter. Finally, *ACE2* and *TMPRSS2* co-expression network highlighted hepatocyte-specific functions, oxidative stress and inflammation, suggesting a link between inflammation, *ACE2* dysfunction and metabolic breakdown.

Introduction

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) causes the Coronavirus disease 2019 (COVID-19)¹⁻³. SARS-CoV-2 is an enveloped RNA betacoronavirus phylogenetically similar to SARS-CoV and the Middle East Respiratory Syndrome Coronavirus (MERS-CoV). Although ~80% of SARS-CoV-2-infected individuals develop asymptomatic self-limited upper airway forms of COVID-19, approximately 15% need hospitalization and 5% develop severe disease with acute respiratory distress syndrome, immune dysregulation and a “cytokine storm” with disseminated intravascular coagulation^{1,3,4}. Approximately 50% of hospitalized patients present comorbidities: hypertension, type-2 diabetes and coronary heart disease^{1-3,5,6}.

The baseline prevalence of chronic liver disease in COVID-19 was about three percent in two meta-analyses assembling 3301 patients^{7,8}, but the mean prevalence of COVID-19 related liver dysfunction was about 20% (range 14-53%)^{1,6-9}. Liver dysfunction correlates with the extent of pulmonary lesions¹⁰. Patients with hepatocyte-type dysfunction at admission are at higher risk of progressing to severe COVID-19¹¹. After admission, antiviral drugs such as lopinavir and ritonavir are risk factors for liver damage¹¹. Autopsies suggested that SARS-CoV-2 infects and replicates within hepatocytes in severe COVID-19 cases¹²⁻¹⁴ and SARS-CoV-2 RNA has been detected in the respiratory epithelium, kidney, liver, heart, brain, blood and stools in severe COVID-19 cases^{15,16}. In addition, crown-like viral particles with complete envelope and typical spikes have been observed in lung, kidney^{16,17} and in hepatocytes¹⁴ suggesting multiorgan targeting of COVID-19.

Cell entry of SARS-CoV and SARS-CoV-2 depends on the binding of the viral spike (S) protein to the Angiotensin-converting enzyme 2 (ACE2) and on the serine protease TMPRSS2 for S protein fusion^{18,19}. The rate of SARS-CoV-2 infection correlates with cell surface ACE2 expression^{18,19}. Importantly, Huh-7^{18,20} human hepatocellular carcinoma (HCC) and HepG2 hepatoblastoma²⁰ cells are widely used models of SARS-CoV-2 infection because they express cell surface ACE2 and can be infected at high titers.

As liver cancer cells are *in vitro* models for the study of SARS-CoV-2 entry and cytopathic effects, we extensively studied mRNA, protein expression and localization of the viral receptors ACE2 and TMPRSS2 in human HCCs. This characterization will be useful to design *in vitro* studies on virus entry, replication and metabolic alterations in liver cells.

Results

Predominant expression of ACE2 and TMPRSS2 in well-differentiated HCCs

We first searched for relationships of *ACE2* and *TMPRSS2* mRNA expression levels with HCC aggressiveness. To this end, overall and disease-free survival analyses were carried out in the TCGA dataset (n= 370). After applying the exclusion criteria described in Supplementary Fig. 1A, the survival dataset consisted of 256 patients. In consistency with previous reports²¹⁻²³, higher *ACE2* and *TMPRSS2* mRNA expression were associated with better overall and disease-free survival (Supplementary Fig. 1B and 2A). *ACE2* and *TMPRSS2* mRNAs were also detected in 47 non-tumor livers in the TCGA dataset. Whereas *ACE2* mRNA expression was 1.7-fold higher in non-tumors livers than in HCCs, *TMPRSS2* expression did not differ between both groups (Supplementary Fig. 2B).

In HCCs, the expression of genes associated with relatively good prognosis is frequently preserved in well-differentiated tumors because they maintain the phenotype of adult hepatocytes^{24,25}. To verify if this applied to *ACE2* and *TMPRSS2*, we measured their expression levels in HCCs classified according to their likeness to normal hepatocytes. Thus classified, HCCs are divided into two major classes, namely “low-proliferation” and “high-proliferation”²⁵. Four HCC subclasses result from the interaction of, on one hand, the metabolic phenotype and, on the other hand, the proliferation/differentiation ratios of tumor cells^{24,26}. Hence, in the “low-proliferation” class of well-to-moderately differentiated HCCs, the periportal-type (PP) and perivenous-type (PV) subclasses refer to the preservation of the metabolic zonation phenotypes of normal hepatocytes^{24,26}. At the opposite end of the spectrum, in the “high-proliferation” class of moderately-to-poorly differentiated HCCs, the extracellular-matrix-type (ECM) and STEM-type subclasses, refer to tumors developing an important stromal and vascular support and expressing cancer stem cell markers^{24,26}. In this context, we found higher *ACE2* mRNA expression in perivenous-type HCCs than in the other subclasses (Fig. 1A; Supplementary Fig. 2C) in both the Désert’s cDNA microarray meta-dataset (1133 HCCs)²⁶ (Fig. 1A) and in the TCGA dataset (370 HCCs) (Supplementary Fig. 2C). Of note, *ACE2* mRNA expression was higher in HCCs expressing previously validated β -catenin pathway

activation signatures^{26,27} (Fig. 1B) and in HCCs carrying sequenced β -catenin (*CTNNB1*) activating mutations (Supplementary Fig. 2C). In turn, *TMPRSS2* was expressed at higher levels in periportal-type HCCs (Supplementary Fig. 2C, D), which we previously demonstrated to carry wild-type *CTNNB1*²⁶. The MERS-CoV receptor, *DPP4*, was also related to β -catenin pathway activation (Supplementary Fig. 2E-G). *ACE2* DNA was hypomethylated in tumors carrying *CTNNB1* mutations (Fig. 1C), which is consistent with transcriptional activation of *ACE2*. By contrast, *TMPRSS2* was hyper-methylated in HCCs carrying *CTNNB1* mutations (Supplementary Fig. 3A; Supplementary Table 2), which is consistent with transcriptional repression. Neither *ACE2* nor *DPP4* mRNA expression were related *TERT* and *TP53* gene mutations, which are the two most frequent ones in HCCs (Supplementary Fig. 3C, D). In turn, *TMPRSS2* mRNA levels were higher in HCCs carrying wild-type *TP53* (Supplementary Fig. 3D), which is consistent with the above-described higher expression of *TMPRSS2* in the well-differentiated, non-proliferative class of HCCs, where *TP53* gene mutations are rare^{26,28}. Indeed, *TP53* mutations are most frequent in poorly-differentiated HCCs (S2²⁹; G3³⁰), showing an aggressive, cancer stem cell phenotype^{26,31}.

In line with the higher *ACE2* and *DPP4* mRNA expression in HCCs showing increased β -catenin pathway activation and *CTNNB1* activating mutations, *in silico* analysis of 5,000 base pairs upstream of the transcription start sites of *ACE2* and *DPP4* DNAs revealed putative T-cell factor 4/LEF-1 consensus transcription factor binding sites, respectively (Fig. 1D; Supplementary Fig. 4A, B).

***ACE2* is immunodetected in moderately-to-well-differentiated HCCs preserving hepatocyte polarization and carrying *CTNNB1* mutations.**

In consistency with the above findings, immunohistochemistry followed by quantitative image analysis in tissue microarray of 41 HCCs revealed that the expression of *ACE2* and of the marker of β -catenin pathway activation *GLUL* (glutamine synthetase) were correlated (Spearman's $R = 0.42$; $p = 0.00001$). Both *ACE2* and *GLUL* were 3.6 folds higher in HCCs carrying activating *CTNNB1* mutations than in tumors carrying wild-type *CTNNB1* (Fig. 1, E-H).

HCCs with mutated *CTNNB1* are frequently cholestatic and contain pseudo-glandular structures with bile plugs, where hepatocytes preserve the basal and apical poles³¹. Co-immunolabeling for *ACE2* and *ABCC2* (a.k.a. MRP2, a marker of the biliary pole in hepatocytes) in HCCs, detected *ACE2* at the biliary pole and within the lumen of pseudo-glandular tumor structures and in slit-like bile canaliculi (Fig. 2, A,B). By contrast, *ACE2* was not detected at the basal hepatocyte pole, which was highlighted by the capillary endothelial cell marker *CD34* (Fig. 2C). As expected, the antibodies used detected high levels of *ACE2* in the apical intestinal epithelium, Bowman's capsule and convoluted tubes in the kidney; in turn, *TMPRSS2* was detected in the apical compartment of the epithelial lining of prostatic glands (Supplementary Fig. 5). In HCCs, *TMPRSS2* was also detected at the apical pole of pseudo-glandular formations or within slit-like trabecular structures and in cell-cell borders (Supplementary Fig. 6). Neither *ACE2* (Supplementary Fig. 6) nor *TMPRSS2* (not shown) were detected in myofibroblasts.

In five non-tumor samples from patients undergoing resection of colon cancer metastases and with minimal inflammatory changes in the liver, ACE2 was co-detected with the sinusoidal endothelial cell marker CLEC4M (a.k.a. DC-SIGNR) (Fig. 3, A, C, E) and the bile canaliculi marker ABCC2 (a.k.a. MRP2) (Fig. 3, B, D, F). The presence of ACE2 within bile canaliculi is consistent with the facts that the extracellular part of ACE2 can be cleaved off the cell surface by ADAM17, thus shedding functional ACE2 fragments³²; and that ACE2 is an abundant component of the normal human bile proteome³³. Also, ACE2 was co-detected with CD34 in capillary vessels of the periportal vascular plexus (Fig. 3G). By contrast, ACE2 was not detected in CD68-positive sinusoidal cells (Kupffer cells, Fig. 3H) or ACTA2-positive vascular smooth muscle cells or myofibroblasts (Fig. 3I,J).

***ACE2* and *TMPRSS2* co-expression network highlight metabolic functions typical of normal hepatocytes.**

By Weighted Gene Correlation Network Analysis³⁴ comparing periportal-type (n= 342) and perivenous-type (n=225) HCCs within the Désert's dataset (n= 1133), we found that *ACE2* and *TMPRSS2* were related through interacting metabolic functions (Fig. 4A), including detoxification, amino acid catabolism, lipid metabolism, fatty acid oxidation and catabolism of organic compounds, which is probably related to the carboxypeptidase functions of ACE2, involved in the degradation of inflammatory polypeptides^{35,36} (Fig. 4B). These findings are in line with the above-described expression of *ACE2* and *TMPRSS2* in well-to-moderately differentiated HCCs preserving the metabolic programs of adult hepatocytes. Next, we specifically investigated the *ACE2* co-expression network in HCCs and analyzed associated gene functions. Not surprisingly, *ACE2* was associated with hepatocyte-specific functions such as amino acid metabolism, xenobiotic detoxification, fatty acid uptake and oxidation. High *ACE2* expression was associated with low expression levels of oxidative stress markers and cytokine-mediated inflammatory signals (Supplementary Fig. 7A,B). These findings raise the hypothesis of a link between inflammation, ACE2 dysfunction and metabolic breakdown that may be relevant to the pathogenesis of COVID-19.

Discussion

In this work, we show that ACE2 and TMPRSS2 mRNA and protein are detected preferentially in well-to-moderately differentiated HCCs. In particular, ACE2 is detected at the biliary pole in tumors preserving hepatocyte polarization. By contrast, in non-tumor livers, ACE2 is detected not only in the epithelial lining of bile ducts and within hepatocyte's bile canaliculi, but also in the CLEC4M-positive sinusoidal endothelium and the CD34-positive capillary vessels of the periportal plexus. TMPRSS2, in turn, is not detected in normal liver, but is present at the biliary pole in well-to-moderately differentiated HCCs, which is consistent with the functional association of ACE2 and TMPRSS2 through periportal and perivenous metabolic networks. Of note, high-resolution 500-nm optical sections revealed ACE2 within hepatocyte's bile canaliculi in histologically normal liver. This finding agrees with the high content of N-glycosylated, lectin-binding ACE2 in the normal human bile proteome³³ and with the role of ACE2 in amino acid uptake in the gut and in the regulation of the splanchnic blood flow³⁵.

In HCCs carrying activating β -catenin(*CTNNB1*) mutations, *ACE2* DNA is hypomethylated, and *ACE2* mRNA expression upregulated. Conversely, *TMPRSS2* mRNA is lower in HCCs carrying mutated *CTNNB1* and its DNA hypermethylated. This finding agrees with the fact that only *ACE2* (and *DPP4*, but not *TMPRSS2*) contain TCF/LEF-1 binding sites that are predicted to respond to β -catenin transcriptional activation. Neither *ACE2*, nor *DPP4* or *TMPRSS2* are associated to *TERT* or *TP53* gene mutations. On the contrary, HCCs containing mutated *TP53* show relatively lower levels of *TMPRSS2* (Supplementary Fig. 3D). This association does not necessarily imply a direct regulation of *TMPRSS2* by *TP53*, but may be coincidental. In fact, well-differentiated, periportal-type HCCs express the highest levels of *TMPRSS2* and, as we previously showed, have the lowest prevalence of *TP53* mutations²⁶. Nonetheless, the relationships of *ACE2* and *TMPRSS2* with histological, transcriptomic and genomic HCC subclasses converge to demonstrate that well-to-moderately differentiated tumors express the highest levels of this SARS-CoV-2 receptor and co-receptor couple.

The above findings agree with further observations in this work: the *ACE2* co-expression network included liver-enriched genes involved in hepatocyte-specific functions, such as amino acid metabolism (*GNMT*), xenobiotic detoxification (*CYP3A4*), fatty acid uptake, bile acid reconjugation (*SLC27A5*), enterohepatic circulation of bile acids and cholesterol homeostasis (*SLC10A1*). *ACE2* was also co-expressed with *SIRT1* and *CD36* and the fatty acid oxidation enzymes *ACADL*, *EHHADH*, *ACADM* and *ECI2*, and alternative glucose metabolism pathways (*CRYL1*), as well as enzymes involved in gluconeogenesis (*PCK2*). This network is in line with the role of *ACE2* as a regulator of fatty acid uptake in hepatocytes through the angiotensin₁₋₇-MAS1-SIRTUIN1-CD36 axis³⁷. Indeed, *ACE2* knock-out mice show low levels of peripheral lipid uptake, but high hepatocyte uptake of triglycerides, resulting in steatosis, lipid peroxidation, oxidative stress and inflammation³⁷. In turn, the induction of oxidative stress by *ACE2* deficiency may be related to its effects on glucose and lipid metabolism because the *ACE2*/Ang₁₋₇/MAS axis increases glucose uptake, decreasing insulin resistance. Thus, *ACE2* knock-out mice exhibit high oxidative stress, whereas exposure to Ang₁₋₇ reduces oxidative stress in liver cells through insulin signaling³⁸. Taken together, this body of evidence matches the metabolic landscape of HCCs carrying activating *CTNNB1* mutations. These tumors oxidize fatty acids as a source of energy to feed the oxidative phosphorylation pathway³⁹, but resist to oxidative stress⁴⁰ and suppress inflammation⁴¹.

ACE2 expression was related to gene functions that smother inflammation. Indeed, *ACE2* was related to low levels of the inflammation-related transcripts *BIRC3*, *MSN*, *MMP9*, *LTB*, *TNFRSF4*, *IL4R*, *CCL20* and *IL32*, among others (Supplementary Table 3). *ACE2* exerts anti-inflammatory effects through carboxyterminal cleavage of vasoactive polypeptides, such as Apelin-13, which induces vasodilation, and des-arg9-bradykinin, a neutrophil chemoattractant⁴². In turn, deletion of *ACE2* leads to tissue injury and oxidative stress, through upregulation of the *AT1* receptor and inflammatory cytokines like *IL1 β* , *IL6*, *IL17*, *RANTES*, *ICAM1*, *TNF α* , *TNFRSF1A* and downregulation of the anti-inflammatory cytokine *IL10*⁴³. This picture is surprisingly similar to that seen in the spectrum going from immune dysregulation to macrophage activation syndrome in moderate-to-severe COVID-19^{4,44}.

The anti-inflammatory role of ACE2 goes well with its up-regulation in response to liver injury, such as bile-duct ligation⁴⁵ and with the evidence that ACE2 produced by hepatocytes limits fibrogenesis through degradation of AngII to Ang₁₋₇³⁵. In human liver cirrhosis, ACE2 protein is increased by >90 folds in hepatocytes. *In vitro*, hypoxia increases ACE2 activity in hepatocytes, enhancing acetylcholine-induced vasodilatation³⁶. As hypoxia activates Wnt/ β -catenin signaling, it would be interesting to investigate whether ACE2 expression in perivenous hepatocytes depends on β -catenin^{32,46}.

This body of matching data suggests that ACE2 may regulate energy metabolism, protecting against oxidative stress and inflammation. As both *TMPRSS2* and *ACE2* are functionally connected through metabolic pathways in the liver, it may be reasonable to hypothesize that ACE2 depletion resulting from SARS-CoV-2 infection¹⁸ may contribute to metabolic dysfunction and inflammation. Our results point to the relevance of further studies on the signaling pathways and pathological contexts leading to cell surface *ACE2* availability and to the possible impact of viral infection on the disruption of the *ACE2* metabolic network.

Materials And Methods

Patients, samples and datasets

Human hepatocellular carcinoma (HCC): immunohistochemistry was performed on a tissue microarray composed of 41 HCC cases and two histologically normal liver controls, for which the exon 3 of β -catenin (*CTNNB1*) had been Sanger-sequenced, as we previously described²⁶. In addition, three formalin-fixed, paraffin-embedded non-tumor liver tissue blocks from patients undergoing resection of liver metastases from extra-hepatic cancers were added to the immunohistochemistry analysis. Tissue samples and patient data were collected and anonymized after obtaining written informed consent from the patients. De-identification was performed in accordance with the Health Insurance Portability and Accountability Act (HIPAA) Privacy Rule. The study was approved by Inserm's Institutional Advisory Board (approval number 19-630). Research involving human research participants has been performed in accordance with the Declaration of Helsinki. All experiments were performed in accordance with relevant guidelines and regulations. Two different immunohistochemical methods were used to detect primary antibodies: peroxidase-labeled secondary antibodies, revealed by a chromogenic substrate or multiplex immunofluorescence followed by analysis with a confocal slide scanner. The Désert's microarray meta-dataset composed of 1133 human HCCs was previously described²⁶. In this dataset, HCCs expressing a β -catenin-activated transcriptomic program were identified with a 5-gene signature composed of *GLUL*, *LGR5*, *ODAM*, *VNN1* and *HAL*, which predicts activating *CTNNB1* mutations with high sensitivity (0.86-0.91) and specificity (0.83-1.0)²⁶. β -catenin pathway activation was confirmed with an independent 23-gene signature²⁷. The publicly available TCGA dataset was composed of 370 HCCs and 47 matching non-tumor samples. RNA sequencing, mutation and methylation data were extracted as described²⁶, using the *TCGAbiolinks* R package. Raw mRNA expression was median-normalized (*DESeq* R package). DNA methylation was quantile-normalized (*preprocessCore* R package); probes with B-value over 0.8 or

under 0.2 were respectively considered as hypermethylated or hypomethylated. The R-package *Limma* was used to identify differential methylation.

See *Supplementary Information* for a detailed description of immunohistochemical methods and statistical analyses and Supplementary Table 1 for primary, secondary antibodies, fluorochromes used, epitope unmasking and incubation conditions.

Abbreviations

ACE2, Angiotensin-converting enzyme 2; Ang, Angiotensin; CLEC4M, C-Type Lectin Domain Family 4 Member M; DPP4, Dipeptidyl Peptidase 4; COVID-19, Coronavirus disease 2019; *CTNNB1*, β -catenin; HCC, Hepatocellular carcinoma; LSECs, MERS-CoV, Middle East Respiratory Syndrome Coronavirus; SARS-CoV-2, Severe Acute Respiratory Syndrome Coronavirus 2; TCGA, The Cancer Genome Atlas; TMPRSS2, Transmembrane serine protease 2.

Declarations

Acknowledgements: We thank Caroline Aninat, LatifaBousarghin, Pierre-Jean Ferron and Olivier Loréal for insightful discussions; Michèle Le Guennec, Patricia Jouas, Adina Pascu and Thomas Poussou for logistics and administrative support.

Financial support: Inserm; Univ Rennes 1; Ministère de l'Enseignement Supérieur; Institut National du Cancer, grant # INCA_12688; Ligue Nationale Contre le Cancer 2018, Comités d'Ille-et-Vilaine et Vendée.

Data availability: Datasets analyzed in this study are available at The Cancer Genome Atlas or at the Gene Expression Omnibus databases. Accession numbers and standard, publicly available R packages used are provided in the main text and supplementary files. The procedures applied for merging nine publicly available transcriptomic datasets into a meta-dataset totaling 1133 hepatocellular carcinomas have been previously published²⁶. A filtered, normalized and batch-effect-corrected user-friendly version of this meta-dataset is available from the corresponding author upon reasonable request.

Contributors: Study design, OM, LD, LC, AC, BC; meta-datasets merge and pretreatment, LD, RD; database search, LD, LC, CL, OM; anatomic pathology analysis, BT, LC, OM; immunohistochemistry, GG; statistics, LD, LC, OM; image analysis, LC, OM; data analysis, LD, LC, OM; manuscript preparation, LD, LC, OM; manuscript editing, all authors.

Competing interests: None

References

1. Guan, W.-j. *et al.* Clinical Characteristics of Coronavirus Disease 2019 in China. *New England Journal of Medicine* **382**, 1708-1720, doi:10.1056/NEJMoa2002032 (2020).

2. Bhatraju, P. K. *et al.* Covid-19 in Critically Ill Patients in the Seattle Region – Case Series. *New England Journal of Medicine*, doi:10.1056/NEJMoa2004500 (2020).
3. Grasselli, G. *et al.* Baseline Characteristics and Outcomes of 1591 Patients Infected With SARS-CoV-2 Admitted to ICUs of the Lombardy Region, Italy. *JAMA***323**, 1574-1581, doi:10.1001/jama.2020.5394 (2020).
4. Giamarellos-Bourboulis, E. J. *et al.* Complex Immune Dysregulation in COVID-19 Patients with Severe Respiratory Failure. *Cell Host Microbe*, doi:10.1016/j.chom.2020.04.009 (2020).
5. Zhou, F. *et al.* Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet***395**, 1054-1062, doi:10.1016/S0140-6736(20)30566-3 (2020).
6. Wu, C. *et al.* Risk Factors Associated With Acute Respiratory Distress Syndrome and Death in Patients With Coronavirus Disease 2019 Pneumonia in Wuhan, China. *JAMA Internal Medicine*, doi:10.1001/jamainternmed.2020.0994 (2020).
7. Mantovani, A., Beatrice, G. & Dalbeni, A. Coronavirus disease 2019 and prevalence of chronic liver disease: A meta-analysis. *Liver International***n/a**, doi:10.1111/liv.14465.
8. Mao, R. *et al.* Manifestations and prognosis of gastrointestinal and liver involvement in patients with COVID-19: a systematic review and meta-analysis. *Lancet Gastroenterol Hepatol*, doi:10.1016/S2468-1253(20)30126-6 (2020).
9. Zhang, C., Shi, L. & Wang, F. S. Liver injury in COVID-19: management and challenges. *Lancet Gastroenterol Hepatol***5**, 428-430, doi:10.1016/S2468-1253(20)30057-1 (2020).
10. Xie, H. *et al.* Clinical characteristics of non-ICU hospitalized patients with coronavirus disease 2019 and liver injury: A retrospective study. *Liver International***n/a**, doi:10.1111/liv.14449.
11. Cai, Q. *et al.* COVID-19: Abnormal liver function tests. *Journal of hepatology*, doi:10.1016/j.jhep.2020.04.006 (2020).
12. Xu, Z. *et al.* Pathological findings of COVID-19 associated with acute respiratory distress syndrome. *Lancet Respir Med***8**, 420-422, doi:10.1016/S2213-2600(20)30076-X (2020).
13. Tian, S. *et al.* Pathological study of the 2019 novel coronavirus disease (COVID-19) through postmortem core biopsies. *Modern Pathology*, doi:10.1038/s41379-020-0536-x (2020).
14. Wang, Y. *et al.* SARS-CoV-2 infection of the liver directly contributes to hepatic impairment in patients with COVID-19. *Journal of hepatology*, doi:10.1016/j.jhep.2020.05.002 (2020).
15. Chen, W. *et al.* Detectable 2019-nCoV viral RNA in blood is a strong indicator for the further clinical severity. *Emerging Microbes & Infections***9**, 469-473, doi:10.1080/22221751.2020.1732837 (2020).
16. Puelles, V. G. *et al.* Multiorgan and Renal Tropism of SARS-CoV-2. *N Engl J Med*, doi:10.1056/NEJMc2011400 (2020).
17. Su, H. *et al.* Renal histopathological analysis of 26 postmortem findings of patients with COVID-19 in China. *Kidney Int*, doi:10.1016/j.kint.2020.04.003 (2020).

18. Hoffmann, M. *et al.* SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. *Cell***181**, 271-280 e278, doi:10.1016/j.cell.2020.02.052 (2020).
19. Zhou, P. *et al.* A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature***579**, 270-273, doi:10.1038/s41586-020-2012-7 (2020).
20. Nie, J. *et al.* Establishment and validation of a pseudovirus neutralization assay for SARS-CoV-2. *Emerging Microbes & Infections***9**, 680-686, doi:10.1080/22221751.2020.1743767 (2020).
21. Ye, G. *et al.* The association of renin-angiotensin system genes with the progression of hepatocellular carcinoma. *Biochem Biophys Res Commun***459**, 18-23 (2015).
22. Dai, Y. J. *et al.* A profiling analysis on the receptor ACE2 expression reveals the potential risk of different type of cancers vulnerable to SARS-CoV-2 infection. *Ann Transl Med***8**, 61 (2020).
23. Fu, J. *et al.* Expressions and significances of the angiotensin-converting enzyme 2 gene, the receptor of SARS-CoV-2 for COVID-19. *Mol Biol Rep*, 1-10, doi:10.1007/s11033-020-05478-4 (2020).
24. Desert, R., Nieto, N. & Musso, O. Dimensions of hepatocellular carcinoma phenotypic diversity. *World journal of gastroenterology***24**, 4536-4547, doi:10.3748/wjg.v24.i40.4536 (2018).
25. Zucman-Rossi, J., Villanueva, A., Nault, J. C. & Llovet, J. M. Genetic Landscape and Biomarkers of Hepatocellular Carcinoma. *Gastroenterology***149**, 1226-1239 e1224, doi:10.1053/j.gastro.2015.05.061 (2015).
26. Desert, R. *et al.* Human hepatocellular carcinomas with a periportal phenotype have the lowest potential for early recurrence after curative resection. *Hepatology***66**, 1502-1518, doi:10.1002/hep.29254 (2017).
27. Abitbol, S. *et al.* AXIN deficiency in human and mouse hepatocytes induces hepatocellular carcinoma in the absence of beta-catenin activation. *Journal of hepatology***68**, 1203-1213, doi:10.1016/j.jhep.2017.12.018 (2018).
28. Guichard, C. *et al.* Integrated analysis of somatic mutations and focal copy-number changes identifies key genes and pathways in hepatocellular carcinoma. *Nature genetics***44**, 694-698, doi:10.1038/ng.2256 (2012).
29. Hoshida, Y. *et al.* Integrative transcriptome analysis reveals common molecular subclasses of human hepatocellular carcinoma. *Cancer research***69**, 7385-7392, doi:10.1158/0008-5472.CAN-09-1089 (2009).
30. Boyault, S. *et al.* Transcriptome classification of HCC is related to gene alterations and to new therapeutic targets. *Hepatology***45**, 42-52, doi:10.1002/hep.21467 (2007).
31. Calderaro, J. *et al.* Histological subtypes of hepatocellular carcinoma are related to gene mutations and molecular tumour classification. *Journal of hepatology***67**, 727-738, doi:10.1016/j.jhep.2017.05.014 (2017).
32. Joshi, S., Wollenzien, H., Leclerc, E. & Jarajapu, Y. P. Hypoxic regulation of angiotensin-converting enzyme 2 and Mas receptor in human CD34+ cells. *J Cell Physiol***234**, 20420-20431, doi:10.1002/jcp.28643 (2019).

33. Kristiansen, T. Z. *et al.* A proteomic analysis of human bile. *Mol Cell Proteomics***3**, 715-728, doi:10.1074/mcp.M400015-MCP200 (2004).
34. Langfelder, P. & Horvath, S. WGCNA: an R package for weighted correlation network analysis. *BMC Bioinformatics***9**, 559, doi:10.1186/1471-2105-9-559 (2008).
35. Osterreicher, C. H. *et al.* Angiotensin-converting-enzyme 2 inhibits liver fibrosis in mice. *Hepatology***50**, 929-938, doi:10.1002/hep.23104 (2009).
36. Paizis, G. *et al.* Chronic liver injury in rats and humans upregulates the novel enzyme angiotensin converting enzyme 2. *Gut***54**, 1790-1796, doi:10.1136/gut.2004.062398 (2005).
37. Nunes-Souza, V. *et al.* CD36/Sirtuin 1 Axis Impairment Contributes to Hepatic Steatosis in ACE2-Deficient Mice. *Oxid Med Cell Longev***2016**, 6487509, doi:10.1155/2016/6487509 (2016).
38. Cao, X., Yang, F. Y., Xin, Z., Xie, R. R. & Yang, J. K. The ACE2/Ang-(1-7)/Mas axis can inhibit hepatic insulin resistance. *Mol Cell Endocrinol***393**, 30-38, doi:10.1016/j.mce.2014.05.024 (2014).
39. Senni, N. *et al.* beta-catenin-activated hepatocellular carcinomas are addicted to fatty acids. *Gut*, doi:10.1136/gutjnl-2017-315448 (2018).
40. Charawi, S. *et al.* LKB1 signaling is activated in CTNNB1-mutated HCC and positively regulates β -catenin-dependent CTNNB1-mutated HCC. *The Journal of Pathology***247**, 435-443, doi:10.1002/path.5202 (2019).
41. Sia, D. *et al.* Identification of an Immune-specific Class of Hepatocellular Carcinoma, Based on Molecular Features. *Gastroenterology***153**, 812-826, doi:10.1053/j.gastro.2017.06.007 (2017).
42. Arendse, L. B. *et al.* Novel Therapeutic Approaches Targeting the Renin-Angiotensin System and Associated Peptides in Hypertension and Heart Failure. *Pharmacol Rev***71**, 539-570, doi:10.1124/pr.118.017129 (2019).
43. Jin, H.-Y. *et al.* Deletion of angiotensin-converting enzyme 2 exacerbates renal inflammation and injury in apolipoprotein E-deficient mice through modulation of the nephrin and TNF- α -TNFRSF1A signaling. *Journal of Translational Medicine***13**, 255, doi:10.1186/s12967-015-0616-8 (2015).
44. Pedersen, S. F. & Ho, Y.-C. SARS-CoV-2: a storm is raging. *The Journal of Clinical Investigation***130**, 2202-2205, doi:10.1172/jci137647 (2020).
45. Paizis, G. *et al.* Up-regulation of components of the renin-angiotensin system in the bile duct-ligated rat liver. *Gastroenterology***123**, 1667-1676, doi:10.1053/gast.2002.36561 (2002).
46. Zhan, L. *et al.* Hypoxic postconditioning activates the Wnt/ β -catenin pathway and protects against transient global cerebral ischemia through Dkk1 Inhibition and GSK-3 β inactivation. *The FASEB Journal***33**, 9291-9307, doi:10.1096/fj.201802633R (2019).
47. Shannon, P. *et al.* Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res***13**, 2498-2504, doi:10.1101/gr.1239303 (2003).
48. Zhou, Y. *et al.* Metascape provides a biologist-oriented resource for the analysis of systems-level datasets. *Nat Commun***10**, 1523, doi:10.1038/s41467-019-09234-6 (2019).

Figures

Figure 1

High *ACE2* levels in well-differentiated, non-proliferative HCCs, with mutated β -catenin (*CTNNB1*) and hypomethylated DNA. (a and b): *ACE2* mRNA expression in the Désert's meta-dataset of 1133 HCCs²⁶. (a) HCC subclasses: PP, periportal-type; PV, perivenous-type; ECM, extracellular-matrix-type and STEM, stem-cell-type HCCs. (b) *ACE2* mRNA expression according to β -catenin activation levels, assessed as

described²⁶. (c) ACE2 DNA is hypomethylated in HCCs carrying mutated CTNNB1 (TCGA dataset). (d) TCF/LEF-1 binding sites, responding to β -catenin transcriptional activation in the proximal ACE2 DNA sequence upstream the transcription start site, according to the PROMO program (TRANSFAC database). (e and f) Immunohistochemical detection (brown signal) of ACE2 (e) and GLUL (glutamine synthetase, f) in an HCC tissue microarray (TMA). Slides were slightly counterstained with hematoxylin (blue). Three 1-mm in diameter spots were punched from each formalin-fixed paraffin-embedded routine liver tissue block (n= 41 HCCs; 2 normal liver controls). Digital slides were acquired with a 20X objective. (g) ACE2 and GLUL immunohistochemical signal scoring in HCCs carrying mutated (MUT) versus wild-type (WT) CTNNB1. To consider intratumor heterogeneity, TMA scores are reported for the available individual tissue spots; their number is indicated for each bar in g. (h) Higher magnification from the indicated spots in e and f.

Figure 2

ACE2 is detected in the biliary pole of moderately-to-well-differentiated HCCs with trabecular or pseudo-glandular patterns. Combined immunoperoxidase and immunofluorescence analysis of a tissue microarray containing 41 HCCs and two normal liver controls, spotted in triplicates. Representative

images are shown. Immunoperoxidase signal (brown) is counterstained with hematoxylin (blue). ACE2 appears in red by immunofluorescence, other markers in green and nuclei in blue (DAPI staining). (a, b) ACE2 colocalizes with the apical hepatocyte marker ABCC2 (a.k.a. MRP2) (arrows). (c) Tumor capillary vessels are marked with CD34. Confocal digital images were acquired with a 40X objective. Images are Z-stacks of four 500 nm focusing steps.

Figure 3

ACE2 is detected in sinusoidal endothelial cells, bile ducts and capillaries of the periportal plexus in non-tumor livers. Co-immunostaining for ACE2 and the indicated markers in three non-tumor livers from patients undergoing resection of colon cancer metastasis. Representative images are shown. ACE2 appears in red by immunofluorescence, other markers in green and nuclei in blue (DAPI staining). (a, b) Low power views (X5 magnification) show ACE2 in a bile duct (BD) and in a zone with mild inflammatory infiltration (arrow) and dystrophic bile ducts (BDs). The sinusoidal endothelial cell marker CLEC4M (a.k.a. DC-SIGNR) and the bile canaliculi marker ABCC2 (a.k.a. MRP2) show lobular staining at this magnification. Pt, portal tract. (c, d) At X30 magnification, ACE is detected in sinusoidal endothelial cells, which can be identified by comparing CLEC4M (c) and ABCC2 (d) staining. Digital images were acquired with a microscope scanner and a X40 objective and exported at the indicated magnifications (Nanozoomer, Hamamatsu Photonics). (e, f) Confocal scans of whole tissue sections as Z-stacks of 4 X 500 nm focusing steps at X40 (e) and X143 (f). ACE2 is detected in CLEC4M-positive sinusoidal endothelial cells (e) and within ABCC2-positive bile canaliculi (f). ACE2-positive bile ducts (BDs) are seen in (e). (g) At 28X magnification, ACE2 is sparsely detected in CD34-positive endothelial cells of the periportal vascular plexus (arrows). (h) At 24X magnification, no evidence of ACE2 detection in CD68-positive Kupffer cells. (i, j) No evidence of ACE2 detection in vascular smooth muscle (i, 10X magnification) or in ACTA2 (a.k.a alpha smooth muscle actin)-positive cells within portal tract stroma (j, 20X magnification). ACE2-positive cells within the portal tract are ACTA2 negative (arrows).

The diagram illustrates a network of gene-gene and gene-protein interactions. The central node, IMPRSS2, is a gene (pink) that acts as a hub, connecting to a large number of other genes and proteins. The peripheral nodes include genes like PXMP2, INSIG1, XDH, HMGL1, SHMT1, AFM, PCCB, PHYH, AZGP1, SLC27A5, MPDZ, ACSM3, SLC46A3, SLC44A, NAT2, AASS, CYP3A4, ALLU11L1, EXPH5, ECM2, HSD17B4, MTHFD1, RUNDC3B, SLC10A1, F13B, PGRMC1, SAA4, EPHX1, HPR, GPT, OGDHL, ALDH8A1, FDX1, C4BPA, METTL7A, BPHL, RAB17, GSTK1, EPHX2, CYP4A11, EC12, GBA3, FAH, IRF6, APOF, SOD1, MLYCD, F9, BAAT, CYP2J2, UGP2, MASP1, CDHR5, AKR7A3, RORC, GNMT, CYP2C8, INHBC, C8G, SUOX, FBP1, ECHDC2, ANXA10, CCL16, GADD45A, PNPO, SYBU, PINK1, AADAC, MASP2, HAO2, CFHR2, RDH16, CPT2, CYP4F2, FMO3, AQP9, DAO, GHR, C6, ENTDP5, REP52, TST, SLC22A1, MTHFS, DHRS3, KLBK1, C4BPA, METTL7A, BPHL, RAB17, GSTK1, EPHX2, CYP4A11, EC12, GBA3, FAH, IRF6, APOF, SOD1, MLYCD, F9, BAAT, CYP2J2, UGP2, MASP1, CDHR5, AKR7A3, RORC, GNMT, CYP2C8, INHBC, C8G, SUOX, FBP1, ECHDC2, ANXA10, CCL16, GADD45A, PNPO, SYBU, PINK1, AADAC, MASP2, HAO2, CFHR2, RDH16, CPT2, CYP4F2, FMO3, AQP9, DAO, GHR, C6, ENTDP5, REP52, TST, SLC22A1, MTHFS, DHRS3, KLBK1, C4BPA, METTL7A, BPHL, RAB17, GSTK1, EPHX2, CYP4A11, EC12, GBA3, FAH, IRF6, APOF, SOD1, MLYCD, F9, BAAT, CYP2J2, UGP2, MASP1, CDHR5, AKR7A3, RORC, GNMT, CYP2C8, INHBC, C8G, SUOX, FBP1, ECHDC2, ANXA10, CCL16, GADD45A, PNPO, SYBU, PINK1, AADAC, MASP2, HAO2, CFHR2, RDH16, CPT2, CYP4F2, FMO3, AQP9, DAO, GHR, C6, ENTDP5, REP52, TST, SLC22A1, MTHFS, DHRS3, KLBK1, C4BPA, METTL7A, BPHL, RAB17, GSTK1, EPHX2, CYP4A11, EC12, GBA3, FAH, IRF6, APOF, SOD1, MLYCD, F9, BAAT, CYP2J2, UGP2, MASP1, CDHR5, AKR7A3, RORC, GNMT, CYP2C8, INHBC, C8G, SUOX, FBP1, ECHDC2, ANXA10, CCL16, GADD45A, PNPO, SYBU, PINK1, AADAC, MASP2, HAO2, CFHR2, RDH16, CPT2, CYP4F2, FMO3, AQP9, DAO, GHR, C6, ENTDP5, REP52, TST, SLC22A1, MTHFS, DHRS3, KLBK1, C4BPA, METTL7A, BPHL, RAB17, GSTK1, EPHX2, CYP4A11, EC12, GBA3, FAH, IRF6, APOF, SOD1, MLYCD, F9, BAAT, CYP2J2, UGP2, MASP1, CDHR5, AKR7A3, RORC, GNMT, CYP2C8, INHBC, C8G, SUOX, FBP1, ECHDC2, ANXA10, CCL16, GADD45A, PNPO, SYBU, PINK1, AADAC, MASP2, HAO2, CFHR2, RDH16, CPT2, CYP4F2, FMO3, AQP9, DAO, GHR, C6, ENTDP5, REP52, TST, SLC22A1, MTHFS, DHRS3, KLBK1, C4BPA, METTL7A, BPHL, RAB17, GSTK1, EPHX2, CYP4A11, EC12, GBA3, FAH, IRF6, APOF, SOD1, MLYCD, F9, BAAT, CYP2J2, UGP2, MASP1, CDHR5, AKR7A3, RORC, GNMT, CYP2C8, INHBC, C8G, SUOX, FBP1, ECHDC2, ANXA10, CCL16, GADD45A, PNPO, SYBU, PINK1, AADAC, MASP2, HAO2, CFHR2, RDH16, CPT2, CYP4F2, FMO3, AQP9, DAO, GHR, C6, ENTDP5, REP52, TST, SLC22A1, MTHFS, DHRS3, KLBK1, C4BPA, METTL7A, BPHL, RAB17, GSTK1, EPHX2, CYP4A11, EC12, GBA3, FAH, IRF6, APOF, SOD1, MLYCD, F9, BAAT, CYP2J2, UGP2, MASP1, CDHR5, AKR7A3, RORC, GNMT, CYP2C8, INHBC, C8G, SUOX, FBP1, ECHDC2, ANXA10, CCL16, GADD45A, PNPO, SYBU, PINK1, AADAC, MASP2, HAO2, CFHR2, RDH16, CPT2, CYP4F2, FMO3, AQP9, DAO, GHR, C6, ENTDP5, REP52, TST, SLC22A1, MTHFS, DHRS3, KLBK1, C4BPA, METTL7A, BPHL, RAB17, GSTK1, EPHX2, CYP4A11, EC12, GBA3, FAH, IRF6, APOF, SOD1, MLYCD, F9, BAAT, CYP2J2, UGP2, MASP1, CDHR5, AKR7A3, RORC, GNMT, CYP2C8, INHBC, C8G, SUOX, FBP1, ECHDC2, ANXA10, CCL16, GADD45A, PNPO, SYBU, PINK1, AADAC, MASP2, HAO2, CFHR2, RDH16, CPT2, CYP4F2, FMO3, AQP9, DAO, GHR, C6, ENTDP5, REP52, TST, SLC22A1, MTHFS, DHRS3, KLBK1, C4BPA, METTL7A, BPHL, RAB17, GSTK1, EPHX2, CYP4A11, EC12, GBA3, FAH, IRF6, APOF, SOD1, MLYCD, F9, BAAT, CYP2J2, UGP2, MASP1, CDHR5, AKR7A3, RORC, GNMT, CYP2C8, INHBC, C8G, SUOX, FBP1, ECHDC2, ANXA10, CCL16, GADD45A, PNPO, SYBU, PINK1, AADAC, MASP2, HAO2, CFHR2, RDH16, CPT2, CYP4F2, FMO3, AQP9, DAO, GHR, C6, ENTDP5, REP52, TST, SLC22A1, MTHFS, DHRS3, KLBK1, C4BPA, METTL7A, BPHL, RAB17, GSTK1, EPHX2, CYP4A11, EC12, GBA3, FAH, IRF6, APOF, SOD1, MLYCD, F9, BAAT, CYP2J2, UGP2, MASP1, CDHR5, AKR7A3, RORC, GNMT, CYP2C8, INHBC, C8G, SUOX, FBP1, ECHDC2, ANXA10, CCL16, GADD45A, PNPO, SYBU, PINK1, AADAC, MASP2, HAO2, CFHR2, RDH16, CPT2, CYP4F2, FMO3, AQP9, DAO, GHR, C6, ENTDP5, REP52, TST, SLC22A1, MTHFS, DHRS3, KLBK1, C4BPA, METTL7A, BPHL, RAB17, GSTK1, EPHX2, CYP4A11, EC12, GBA3, FAH, IRF6, APOF, SOD1, MLYCD, F9, BAAT, CYP2J2, UGP2, MASP1, CDHR5, AKR7A3, RORC, GNMT, CYP2C8, INHBC, C8G, SUOX, FBP1, ECHDC2, ANXA10, CCL16, GADD45A, PNPO, SYBU, PINK1, AADAC, MASP2, HAO2, CFHR2, RDH16, CPT2, CYP4F2, FMO3, AQP9, DAO, GHR, C6, ENTDP5, REP52, TST, SLC22A1, MTHFS, DHRS3, KLBK1, C4BPA, METTL7A, BPHL, RAB17, GSTK1, EPHX2, CYP4A11, EC12, GBA3, FAH, IRF6, APOF, SOD1, MLYCD, F9, BAAT, CYP2J2, UGP2, MASP1, CDHR5, AKR7A3, RORC, GNMT, CYP2C8, INHBC, C8G, SUOX, FBP1, ECHDC2, ANXA10, CCL16, GADD45A, PNPO, SYBU, PINK1, AADAC, MASP2, HAO2, CFHR2, RDH16, CPT2, CYP4F2, FMO3, AQP9, DAO, GHR, C6, ENTDP5, REP52, TST, SLC22A1, MTHFS, DHRS3, KLBK1, C4BPA, METTL7A, BPHL, RAB17, GSTK1, EPHX2, CYP4A11, EC12, GBA3, FAH, IRF6, APOF, SOD1, MLYCD, F9, BAAT, CYP2J2, UGP2, MASP1, CDHR5, AKR7A3, RORC, GNMT, CYP2C8, INHBC, C8G, SUOX, FBP1, ECHDC2, ANXA10, CCL16, GADD45A, PNPO, SYBU, PINK1, AADAC, MASP2, HAO2, CFHR2, RDH16, CPT2, CYP4F2, FMO3, AQP9, DAO, GHR, C6, ENTDP5, REP52, TST, SLC22A1, MTHFS, DHRS3, KLBK1, C4BPA, METTL7A, BPHL, RAB17, GSTK1, EPHX2, CYP4A11, EC12, GBA3, FAH, IRF6, APOF, SOD1, MLYCD, F9, BAAT, CYP2J2, UGP2, MASP1, CDHR5, AKR7A3, RORC, GNMT, CYP2C8, INHBC, C8G, SUOX, FBP1, ECHDC2, ANXA10, CCL16, GADD45A, PNPO, SYBU, PINK1, AADAC, MASP2, HAO2, CFHR2, RDH16, CPT2, CYP4F2, FMO3, AQP9, DAO, GHR, C6, ENTDP5, REP52, TST, SLC22A1, MTHFS, DHRS3, KLBK1, C4BPA, METTL7A, BPHL, RAB17, GSTK1, EPHX2, CYP4A11, EC12, GBA3, FAH, IRF6, APOF, SOD1, MLYCD, F9, BAAT, CYP2J2, UGP2, MASP1, CDHR5, AKR7A3, RORC, GNMT, CYP2C8, INHBC, C8G, SUOX, FBP1, ECHDC2, ANXA10, CCL16, GADD45A, PNPO, SYBU, PINK1, AADAC, MASP2, HAO2, CFHR2, RDH16, CPT2, CYP4F2, FMO3, AQP9, DAO, GHR, C6, ENTDP5, REP52, TST, SLC22A1, MTHFS, DHRS3, KLBK1, C4BPA, METTL7A, BPHL, RAB17, GSTK1, EPHX2, CYP4A11, EC12, GBA3, FAH, IRF6, APOF, SOD1, MLYCD, F9, BAAT, CYP2J2, UGP2, MASP1, CDHR5, AKR7A3, RORC, GNMT, CYP2C8, INHBC, C8G, SUOX, FBP1, ECHDC2, ANXA10, CCL16, GADD45A, PNPO, SYBU, PINK1, AADAC, MASP2, HAO2, CFHR2, RDH16, CPT2, CYP4F2, FMO3, AQP9, DAO, GHR, C6, ENTDP5, REP52, TST, SLC22A1, MTHFS, DHRS3, KLBK1, C4BPA, METTL7A, BPHL, RAB17, GSTK1, EPHX2, CYP4A11, EC12, GBA3, FAH, IRF6, APOF, SOD1, MLYCD, F9, BAAT, CYP2J2, UGP2, MASP1, CDHR5, AKR7A3, RORC, GNMT, CYP2C8, INHBC, C8G, SUOX, FBP1, ECHDC2, ANXA10, CCL16, GADD45A, PNPO, SYBU, PINK1, AADAC, MASP2

Page 17/18

with red borders and black links; genes co-expressed with both ACE2 and TMPRSS2, light-blue nodes with red borders and blue links. Link thickness is proportional to the correlation coefficient between a given node and ACE2 or TMPRSS2. (b) Functional analysis and gene ontology enrichment of the gene co-expression network shown in b are visualized with Express Analysis from Metascape⁴⁸. Gene ontology (GO) terms, families and P values are indicated.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Desquillesetal.Supplementarydata.docx](#)
- [DesquillesetalSuppTable1.pdf](#)
- [DesquillesetalSuppTable2.pdf](#)
- [DesquillesetalSuppTable3.xlsx](#)
- [DesquillesetalSuppTable4.pdf](#)
- [DesquillesetalSuppTable5.pdf](#)
- [SuppFigure1Desquillesetal.pdf](#)
- [SuppFigure2Desquillesetal.pdf](#)
- [SuppFigure3Desquillesetal.pdf](#)
- [SuppFigure4Desquillesetal.pdf](#)
- [SuppFigure5Desquillesetal.pdf](#)
- [SuppFigure6Desquillesetal.pdf](#)
- [SuppFigure7Desquillesetal.pdf](#)