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Letter to the Editor

Is SARS-CoV-2 an oncogenic virus?

Dear Editor

Recently, in this journal, Wu et al. (1) and Gao et al. (2) have both indicated that host genetic variation related to COVID-19 might be associated to endometrial cancer. We here add evidence from gene expression analysis supporting that the connection of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and cancer could be more general, in line with several other viral infections that represent serious risks for carcinogenesis in humans. The SARS-CoV-2 has developed similar strategies to Epstein-Barr virus (EBV) and hepatitis B virus (HSV1) to control p53 by hijacking the protein *via* virus antigens, and ultimately leading to its degradation (3, 4). Specifically, the Nsp2 viral protein of the SARS-CoV-2 interacts with the prohibitin 1 and 2 (PHB1, PHB2) that are primarily located in the mitochondrion and play an essential role in maintaining mitochondrial DNA activity. Their depletion triggers a chain of cell responses that lead to a leakage of reactive oxygen species (ROS) to the nucleus and oxidative damage, that ultimately provokes the impairment of the transactivation of p53-dependent genes. In addition, the Nsp3 SARS-CoV-2 protein binds and activates the RING finger and CHY zinc finger domain-cotainin protein 1 (RCHY1) and E3 ubiquitin ligase, promoting p53 degradation (5). Therefore, SARS-CoV-2 has the ability to trigger external and internal apoptotic pathways of the host cells, facilitating its spread. Impairment of p53 could be seen as a strategy of the virus to take advantage of the cell pathways controlled by this protein for its own benefit during acute phase of infection, therefore evading host immune response and facilitating its replication (3). In this context, a reduced expression of p53 during the acute phase of infection is also a biomarker of severe disease.

Although it has not been demonstrated yet, it has been hypothesized that a long-term inhibition of p53 by the SARS-CoV-2 could be carcinogenic. The onco-suppressive protein p53 is a key player within the apoptotic signaling pathway and regulates the expression of about 500 target genes; therefore, it plays a role in cell cycle arrest, cell aging, cell death, etc. (6). We examine three gene expression datasets to demonstrate that p53 is downregulated during acute SARS-CoV-2 infection and long coronavirus-disease 19 (COVID-19); a long-term reduction of p53 could be interpreted as a risk factor in carcinogenesis.

We analyzed *TP53* gene expression in blood from COVID-19 patients stratified by severity as well as healthy controls using the RNAseq data from Jackson et al. (7) and *n*-Counter (overlapping) data from Gómez-Carballa et al. (8) (n = 65 and n =30, respectively). Processing of raw data was carried out as in the original articles. In addition, gene expression data of long-COVID-19 patients and healthy controls (n = 44) from another RNAseq convalescence study (9) (12-, 16-, and 24-weeks post infection) were also analyzed. For the three studies, severity was determined during the acute phase of the infection. Data normalization was undertaken as in (7). Wilcoxon test was used to assess statistical significance between groups, and Spearman test for the computation of the correlation indices (r) and *P*-values. We also evaluated whether the overall expression of a p53 related pathways is up- or down-regulated using the Fast Approximation to ROAST Gene Set Test with Mean Aggregated Set Statistics (*fry*; https://f1000research.com/slides/5–2605) by selecting Gene Ontology (GO) processes including the term 'p53' in the description as well as *TP53* gene within the gene-set. We carried out two independent comparisons: (*i*) severe patients *vs*. non severe patients (including healthy controls) from the acute COVID-19 cohort, and (*ii*) 24 w.p.i severe/critical patients *vs*. healthy controls from the long-COVID-19 cohort. We used *edgeR* package (https://bioconductor.org/packages/release/bioc/html/edgeR.html) to process raw count data for the *fry* analysis.

The data show that *TP53* is downregulated in patients with the highest WHO severity scores in both the RNAseq (Fig. 1A) and the *n*-Counter datasets (Fig. 1B). These differences are statistically significant when compared against controls and mild patients. In addition, we have also observed that *TP53* gene expression is negatively correlated with the length of symptoms until sample collection only in severe patients (Fig. 1A and 1B).

We further re-analyzed *TP53* blood gene expression data available in a follow-up study of long-COVID-19 patients (9) also stratified by severity (in the acute phase) and sampled at different timepoints, namely, 12-, 16- and 24-weeks post-infection (w.p.i) (Gene Expression Omnibus acc. n°: GSE169687). Mild / moderate patients showed statistically significant downregulation of *TP53* expression when compared to healthy controls at 16 w.p.i, and a reactivation towards normal values at 24 w.p.i (Fig. 1C). However, in severe / critical patients *TP53* is progressively downregulated to at least 24 w.p.i (statistically significant when compared to controls) with no evidence of recovery to the *TP53* expression level observed in controls (Fig. 1D).

In addition, the data indicate that the downregulation of *TP53* has a significant impact on a number of its interacting genes. Thus, there are a total of eight pathways (GO terms) related to *TP53* that are significantly up- and down-regulated in acute severe patients and in 24 w.p.i long-COVID-19 severe / critical patients when compared to non-severe (including healthy controls) and a healthy control group, respectively (Table S1). The affected pathways are related to e.g., apoptosis, DNA damage response and signal transduction (Table S1).

We show convergent evidence from three different transcriptomic datasets and techniques that represent a molecular proof of concept that p53 may be acutely and persistently reduced after severe SARS-CoV-2 infection. A persistent reduction of the p53 tumor suppression functions, as might be the case in long-COVID-19 se-

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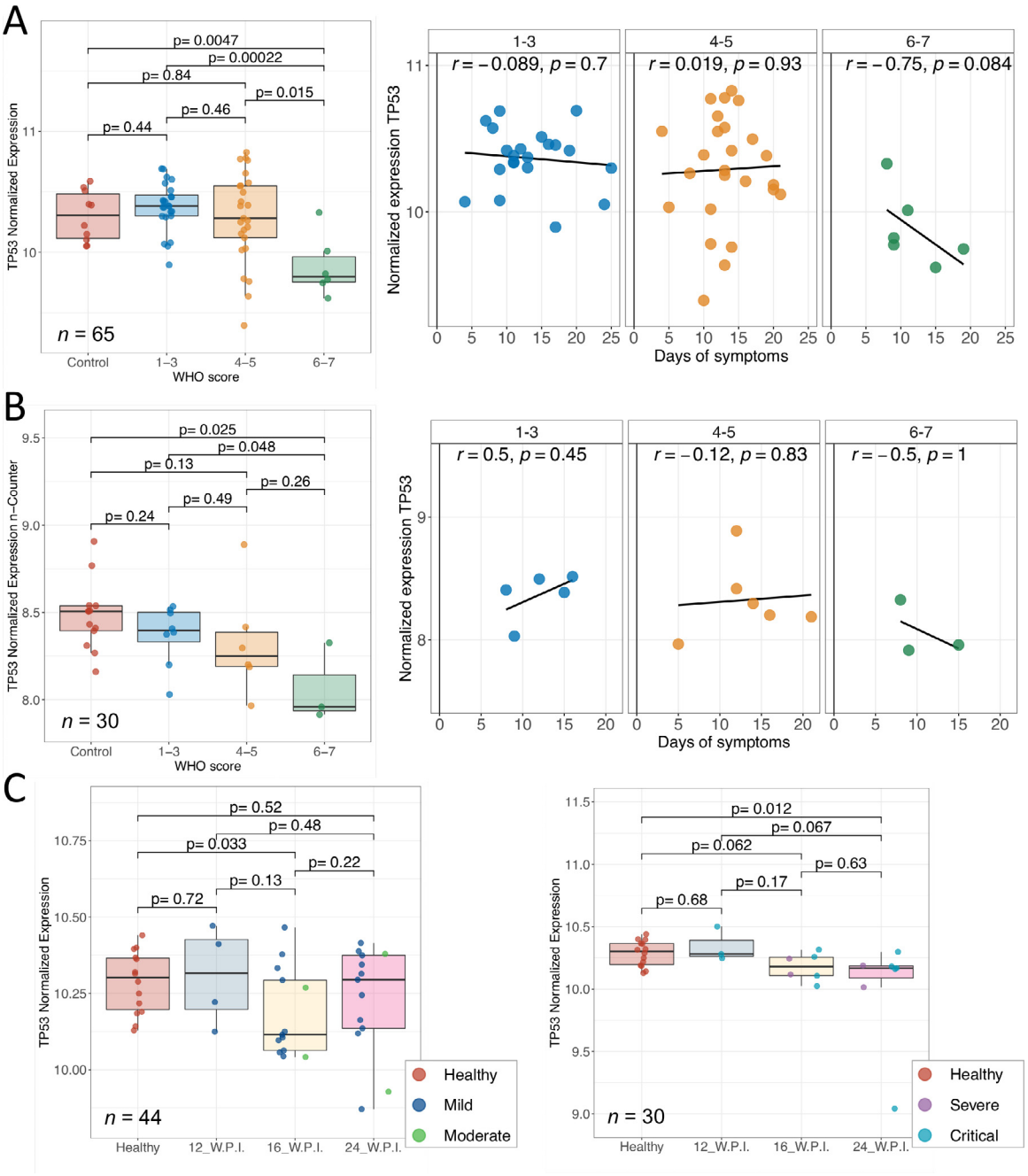

Fig. 1. (A) *TP53* gene expression in blood samples from COVID-19 patients and healthy controls from the RNAseq dataset (7) and stratified by WHO severity score (left). Correlation between days of symptoms to sample collection and *TP53* expression in the RNAseq dataset (right). (B) *TP53* gene expression in blood samples from COVID-19 patients and healthy controls from the *n*-Counter dataset (8) and stratified by WHO severity score (left); patients from this cohort partially overlap with those in the RNAseq dataset. Correlation between days of symptoms to sample collection and *TP53* expression in the *n*-Counter dataset (right). Asymptomatic patients were not included in the correlation analyses (r = spearman correlation coefficient). (C) *TP53* gene expression in blood samples from mild/moderate (left) and severe/critical (right) long-COVID-19 patients as well as healthy controls from Ryan et al. (9) collected at different timepoints post infection (w.p.*i* = weeks post infection); here we used the authors' scores of severity (9).

vere patients, may constitute a risk factor for oncogenesis comparable to pathogenic mutations in *TP53*. Such long-term reduction of p53 might trigger cancer onset or contribute to worsen the course of patients with an ongoing tumoral process (1, 2). Future efforts should target larger cohorts and follow-up time, and assess additional types of samples, including lung tissue. A causal relationship between SAR-CoV-2 and cancer has not been demonstrated but, if confirmed, it would have enormous impact on public health.

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Declaration of Competing Interest

The authors report no conflict of interest.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2022.08.005.

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