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Scientific Article

Deficient Radiation Transcription Response in COVID-19 Patients



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Abstract

Purpose: The ongoing SARS-CoV-2 pandemic has resulted in over 6.3 million deaths and 560 million COVID-19 cases worldwide. Clinical management of hospitalized patients is complex due to the heterogeneous course of COVID-19. Low-dose radiation therapy is known to dampen localized chronic inflammation and has been suggested to be used to reduce lung inflammation in patients with COVID-19. However, it is unknown whether SARS-CoV-2 alters the radiation response and associated radiation exposure related risk.

Methods and Materials: We generated gene expression profiles from circulating leukocytes of hospitalized patients with COVID-19 and healthy donors.

Results: The *p53* signaling pathway was found to be dysregulated, with mRNA levels of *p53*, *ATM*, and *CHK2* being lower in patients with COVID-19. Several key *p53* target genes involved in cell cycle arrest, apoptosis, and *p53* feedback inhibition were upregulated in patients with COVID-19 while other *p53* target genes were downregulated. This dysregulation has functional consequences as the transcription of *p53*-dependant genes (*CCNG1*, *GADD45A*, *DDB2*, *SESN1*, *FDXR*, *APOBEC*) was reduced 24 hours after x-ray exposure ex vivo to both low (100 mGy) or high (2 Gy) doses.

Conclusions: SARS-CoV-2 infection affects a DNA damage response that may modify radiation-induced health risks in exposed patients with COVID-19.

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Nanopore sequencing yielded the aggregated .fastq files for each healthy donor and patient with COVID-19. We have uploaded the raw .fastq files and gene count tables for patients with COVID-19 and healthy donors to the Sequence Read Archive (SRA) database. Please use the reference PRJNA927243 to access the deposited data within the SRA database. The SRA records are accessible at: <https://www.ncbi.nlm.nih.gov/sra/PRJNA927243>.

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Introduction

As of July 2022, the World Health Organization reported over 560 million confirmed cases of COVID-19 and 6.3 million deaths. The course and clinical outcomes of patients with COVID-19 are highly heterogeneous.¹ While most individuals eventually mount a protective immune response and recover, others can develop progressive and life-threatening bilateral pneumonia and acute respiratory distress syndrome.¹ The SARS-CoV-2 virus binds to the angiotensin-converting enzyme-2 receptor² and enters alveolar cells in the lungs. The rapid virus replication in the alveolar epithelium triggers a strong dysregulated host immune response,^{3,4} which in a subset of patients culminates in a cytokine storm syndrome which may confer respiratory distress syndrome and death. This cytokine storm results from the massive activation of *NF- κ B*-dependent inflammatory signaling pathways³⁻⁵ in hematopoietic cells.

Exposure of inflamed tissues to single or repeated low/moderate doses of ionizing radiation is known to be efficient in treating chronic osteomuscular inflammatory conditions such as arthritis.^{6,7} Low-dose radiation therapy (LD-RT) treatment has been shown to modulate immune cell activation and reduce inflammation for extended periods. In light of historical data on the use of LD-RT to treat various forms of pneumonia,⁸ exposure of the lungs to low/moderate radiation dose(s) has been discussed as a way to mitigate inflammation and eventually prevent the development of the cytokine storm in patients with COVID-19.⁹ Despite controversy on this use of LD-RT (see, for example, Schaeue and McBride and Rodel et al),^{10,11} several clinical trials are underway (see Yu et al for a recent review of clinical trials).¹² This topic, therefore, requires further investigation to identify patients who would benefit from LD-RT application while considering the potential risk for radiation-induced cancer and noncancer disease.^{13,14} Most importantly, the question of an eventual modulation of the patients' radiation response during COVID-19 infection has not been addressed.

In this study, we performed transcriptional profiling of peripheral blood cells by third-generation nanopore sequencing to obtain a comprehensive view of gene expression dysregulation in patients with COVID-19. We were specifically interested in pathways activated in response to radiation and the eventual functional consequences of their potential dysregulation because many patients with COVID-19 are likely to receive low doses of ionizing radiation for both diagnostic and therapeutic purposes, with potential doses up to 1 Gy for LD-RT. In

addition, patients with COVID-19 may have to be exposed to high-dose radiation during cancer radiation therapy (RT). Our results show that the transcription of numerous genes involved in the response to radiation-induced DNA damage is strongly downregulated. The resulting reduced low expression levels of key damage response genes will have functional consequences, as the response was observed in blood cells of patients with COVID-19 following low- and high-dose ex vivo irradiation. Most importantly, the transcription of essential genes controlling the DNA damage response checkpoint activation in response to DNA double-strand breaks (*ATM*, *CHK2*, *p53*) is strongly diminished. These results might significantly affect current and proposed COVID-19 diagnostic procedures and treatment, both LD-RT and cancer RT, and the radiation-induced estimation of health risk in these patients.

Methods and Materials

Samples

Venous blood from 10 patients with COVID-19 (5 male and 5 female) was sourced through Tissue Solutions Ltd. COVID-19 samples were obtained from hospitals in the United Kingdom according to the United Kingdom National Health Service Research Ethics Committee (REC) approved protocols (no. REC 19/WS/0164) and with approved consent forms for molecular and genetic analysis. Venous blood from 10 healthy donors (5 male and 5 female) was taken at the Radiation, Chemical and Environmental Hazards Directorate, United Kingdom Health Security Agency (Chilton, United Kingdom) with informed consent and the ethical approval of the West Midlands - Solihull REC (no. REC 14/WM/1182). Patients with COVID-19 and healthy donor details are provided in Table E1.

White blood cell and peripheral blood mononuclear cell isolation

White blood cells (WBCs) were isolated from approximately 3 to 4 mL whole blood samples by a density gradient centrifugation using HetaSep (STEMCell Technologies, Vancouver, Canada). Peripheral blood mononuclear cells (PBMCs) were isolated from approximately 3 to 4 mL whole blood samples by a density gradient centrifugation using Lymphoprep (STEMCell Technologies). RNA was extracted from WBC and PBMC

samples using the miRNAeasy Mini kit (Qiagen, Manchester, United Kingdom). The quantity of isolated RNA was determined by spectrophotometry with an ND-1000 NanoDrop (Thermo Fisher Scientific, Waltham, MA), and quality was assessed using a Tapestation 220 (Agilent Technologies, Santa Clara, CA).

Whole blood irradiation ex vivo

One-half-mL whole blood samples from 10 healthy donors and 8 patients with COVID-19 (due to an issue with the x-ray system on the day, it was not possible to irradiate patients' 8 and 9 blood samples) were collected and exposed to x-ray doses of 0.1 Gy and 2 Gy at a dose rate of 0.5 Gy per minute. Irradiations were performed at room temperature using an A.G.O. HS x-ray system (Aldermaston, Reading, UK; output 13 mA, 250 kV peak, 0.5 Gy/min). After irradiation, blood samples were placed in an incubator for 24 hours at 37°C in a humidified 5% CO₂ atmosphere. After 24 hours, the blood was mixed with 1 mL of RNA (Thermo Fisher Scientific) and stored at 80°C until processed for RNA extraction. RNA was extracted using a RiboPure™ Blood RNA Purification Kit (Thermo Fisher Scientific).

nCounter analysis

Samples were analyzed by the nCounter Analysis System (NanoString Technologies Inc, Seattle, WA) according to the manufacturers' guidelines. The samples were run using 100 ng of PBMCs RNA per sample on the Human Inflammation V2 panel, which consists of 249 genes with the additional COVID-19 Panel Plus Beta, which includes probes targeting 8 open reading frames of the SARS-CoV-2 virus, plus 1 probe each for the human and mouse genes encoding the viral receptor.

Sequencing

Nanopore sequencing was performed using a MinION sequencer (Oxford Nanopore Technologies, UK) with the libraries prepared using the SQK-PCS109 kit according to the instructions. cDNA was prepared from 50 ng of whole blood RNA using strand-switching and variable-length nucleotide sequence primers. After the cDNA synthesis, selective polymerase chain reaction (PCR) amplification was performed for full-length transcripts before specific adapters were added to start the sequencing. The samples were run in SpotON flow cells in a MinION sequencer.

Reverse transcription

According to the manufacturer's protocol, reverse transcription reactions were performed with 350 ng RNA using a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA). Cycling conditions were as follows: 25°C for 10 minutes, 37°C for 120 minutes, and 85°C for 5 minutes.

TaqMan multiplex quantitative PCR

Real-time PCR was performed using a Rotor-Gene Q (Qiagen, Manchester, UK). Reactions were run in triplicate with primer and probe sets for target genes at 300 nM each and 2.5 µL cDNA in 30 µL reaction volume (PerfeCTa MultiPlex qPCR SuperMix; Quanta BioSciences, Beverly, MA). 3',6-Carboxyfluorescein/Black Hole Quencher 1, 6-hexachlorofluorescein (HEX)/Black Hole Quencher 1, Texas Red/Black Hole Quencher 2, CY5/Black Hole Quencher 3, Atto 680/Black Hole Quencher 3, and Atto 390/Deep Dark Quencher 1 (Eurogentec Ltd, Fawley, UK) were used as fluorochrome reporters for the hydrolysis probes analyzed in multiplexed reactions. Primer designs are listed in Table E2 and references therein.¹⁵⁻¹⁷ Cycling parameters were 45 cycles of 2 minutes at 95°C (10 seconds at 95°C and 60 seconds at 60°C). Data were collected and analyzed using Rotor-Gene Q Series software. Gene target cycle threshold values were normalized to internal control (*HPRT*). Cycle threshold values were converted to transcript quantity using standard curves obtained by serial dilution of PCR-amplified DNA fragments of each gene and run in each reaction. The linear dynamic range of the standard curves covering 6 orders of magnitude (serial dilution from 3.2×10^{-4} to 8.2×10^{-10}) gave PCR efficiencies between 93% and 103% for each gene with $R^2 > .998$.

Bioinformatics analysis

Raw sequencing data preprocessing

We used the differential gene expression (DGE) and differential transcript usage pipelines for the bioinformatics data analysis (<https://github.com/nanoporetech/pipeline-transcriptome-de>). This pipeline uses snakemake, minimap2, salmon, edgeR, DEXSeq, and stageR packages. We input the patients with COVID-19 and healthy donors fastq files generated with the MinION sequencer, reference, and annotation files (GRCh38.93 for a human cDNA) in the DGE differential transcript usage pipeline. Subsequently, we used the output of edgeR, which contains the DGE results for the mapped gene set in tsv format, to build the volcano plots and pathway enrichment analysis.

Sequencing data visualization

We built a Volcano plot: 1 plot showing all differentially expressed genes filtered by the preselected gene panel corresponding to the Human Inflammation V2 panel, Nanostring Technologies (false discovery rate [FDR] < 0.05 for both). The genes with FDR < 0.05 were considered significant. We filtered the upregulated and downregulated genes by the log₂ fold change (log₂FC) thresholds [-1; 1].

Pathway enrichment analysis

We used the Kyoto Encyclopedia of Genes and Genomes *p53* signaling pathway as a reference to analyze differentially expressed genes (matched by EntrezID) involved in the radiation response in COVID-19 blood.

Multiplex real-time polymerase PCR statistical analysis

We performed the statistical analyses for multiplex real-time (MQRT)-PCR using Minitab software (State College, PA). Data are presented as means ± standard error of the mean. Comparisons were analyzed by an unpaired *t* test (Student *t* test). Statistical analyses were performed in log-transformed data. A significance of *P* ≤ .05 and *P* ≤ .01 was applied to all statistical tests performed.

Results

We aimed to evaluate whether the expression of genes involved in response to radiation-induced DNA damage and repair was affected following SARS-CoV-2 infection. To achieve this without any a priori identity of putatively affected genes, we used Nanopore technology to sequence cDNA libraries prepared from whole blood RNA obtained from patients with COVID-19 and healthy donors. This approach allows the identification and quantification of full-length transcripts. Comparing transcripts obtained from patients with COVID-19 and healthy donors allows the identification of differentially expressed, up- and downregulated genes.

To validate this approach, as SARS-CoV-2 infection induces a strong regulation of the expression of many genes involved in various immune processes,^{3,18-20} we first compared in healthy controls and patients with COVID-19 the expression of a set of 249 genes coding for factors involved in inflammation in healthy controls and patients with COVID-19 by nCounter analysis and Nanopore sequencing (Fig. E1). Thus, the results obtained from both methods are largely similar, confirming that Nanopore sequencing can be used to identify genes differentially expressed in patients with COVID-19 and healthy donors. Additionally, the heatmap of differentially expressed genes in nonexposed to ionising radiation. COVID-19-positive patients versus the healthy controls

(Fig. E2) shows the clear separation in gene expression between these 2 groups, suggesting the effect of COVID-19 infection is substantial, notwithstanding the heterogeneous character of the COVID-19 group.

Ionizing radiation exposure induces a cellular response coordinated by the *ATM*-dependent, DNA-damage response (DDR), which encompasses stabilizing and activating the *p53* tumor suppressor protein. Activated *p53* then regulates the transcription of numerous target genes coding for proteins involved in processes such as cell cycle arrest, DNA damage repair, cell survival, and cell death. Hence, *p53* activation plays a central role in cell fate following radiation exposure. Therefore, we compared the expression level of the genes belonging to the “*p53* signaling pathway” as defined in the Kyoto Encyclopedia of Genes and Genomes in patients with COVID-19 and healthy controls.

The transcription of numerous *p53* target genes included in this pathway is dysregulated in patients with COVID-19, with log₂FC ranging from -0.5 to -1.3 for downregulated genes and 0.5 to 3.1 for upregulated genes (Fig. 1, Table E3). All downstream functional pathways are affected: cell cycle control and cellular senescence, apoptosis, DNA repair and damage prevention, inhibition of angiogenesis and metastasis, inhibition of insulin-like growth factor-1/*mTOR*, exosome-mediated secretion, and *p53* negative feedback. The upregulation of several genes involved in *p53* feedback inhibition is surprising with most of the modulated *p53* target genes being more abundantly transcribed in patients with COVID-19. However, there is no coordination of the transcription of *p53* target genes, with a few genes being less transcribed. Even more surprising, we found that the levels of *ATM*, *CHK2*, and *p53* transcripts were lower in patients with COVID-19 compared with healthy controls (log₂FC values, -0.67, -0.50, and -0.97 for *ATM*, *CHK2*, and *p53*, respectively, with *P* < .01 and FDR < 0.05 for all; Fig. 2, Table E3). Thus, the genes coding the proteins responsible for *p53* activation in response to DNA damage, including the *TP53* gene itself, are coordinately downregulated in the blood of patients with COVID-19.

To validate and extend these findings, we compared the expression of a series of genes regulated by *p53* in WBCs and PBMCs purified from patients with COVID-19 and healthy donors. This series included *CDKN1A*, *GADD45A*, and *PMAIP1/NOXA*, belonging to the *p53* signaling networks, and 3 additional genes, *CCNG1*, *PHPT1*, and *MYC*. All were found to be upregulated (Fig. 1) in patients with COVID-19. MQRT-PCR results confirmed a higher level of *CDKN1A* and *GADD45A* transcripts in the WBCs and PBMCs of patients with COVID-19, whereas the level of *NOXA* was significantly higher only in PBMCs. The expression of *CCNG1*, *PHPT1*, and *MYC* was also dysregulated with different patterns: *CCNG1* was increased only in PBMCs, *PHPT1* was reduced in both WBCs and PBMCs, and *MYC* was significantly reduced

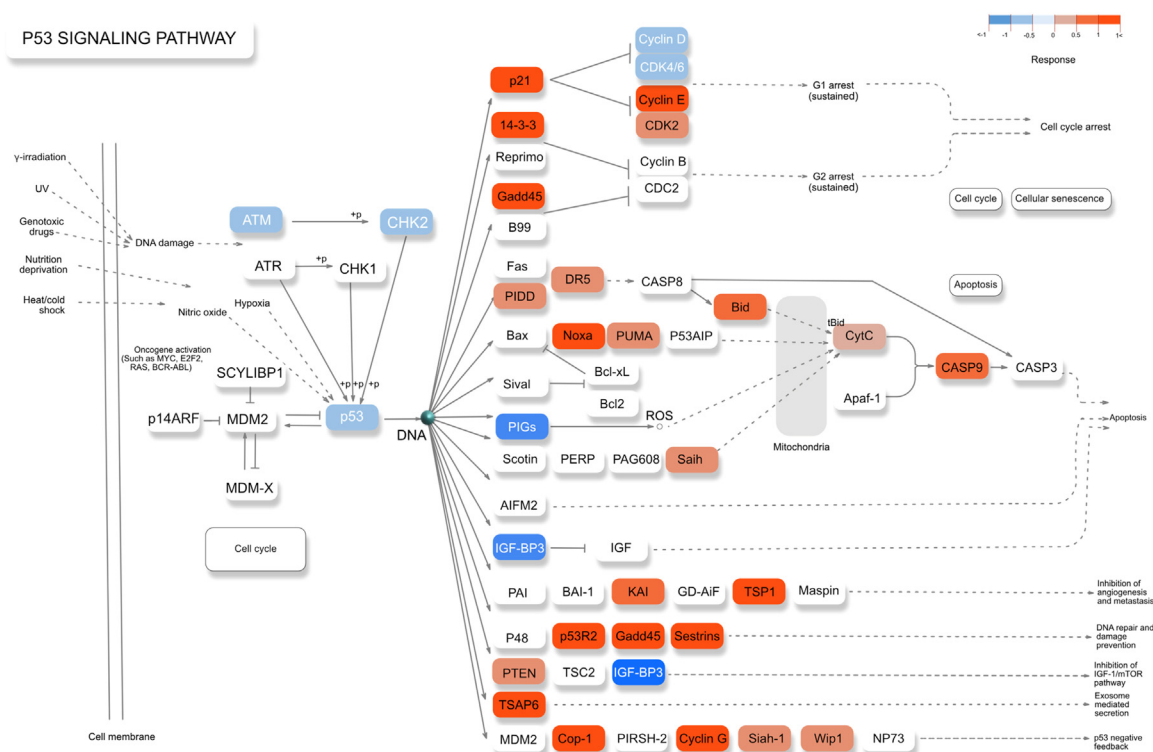


Figure 1 Expression of genes belonging to the *p53* signaling Kyoto Encyclopedia of Genes and Genomes pathway in patients with COVID-19. Colors indicate up (red) and down (blue) regulation of the genes as determined by Nanopore sequencing. The intensity of the color indicates the extent of regulation, as indicated on the scale of color versus log₂ fold change value.

only in PBMCs (Fig. 1). These results largely validate our sequencing results and confirm that different *p53* target genes, which are usually coordinately upregulated in blood cells after radiation exposure, are both up- and downregulated in blood cells of patients with COVID-19. Importantly, we also confirmed by MQRT-PCR the reduced level of *ATM*, *CHK2*, and *TP53* mRNA in WBCs and PBMCs of patients with COVID-19 (Fig. 3).

Together, *ATM*, *CHK2*, and *p53* control a significant component of the response to radiation. We next examined whether the differences in expression observed in blood cells of patients with COVID-19 are maintained after exposure to low-dose (0.1 Gy) and high-dose (2 Gy) radiation. The amount of *ATM* (Fig. 4A) and *p53* (Fig. 4C) transcripts remained lower in irradiated whole blood of patients with COVID-19. In contrast, the level of *CHK2* mRNA was no longer different between the 2 groups of donors, suggesting that *CHK2* expression is differentially regulated in patients with COVID-19 and healthy donor blood after 24 hours of culture and a low- or high-dose exposure.

We also analyzed the expression of a collection of genes proposed as biomarkers of radiation exposure in these samples. The radiation response is altered in blood cells of patients with COVID-19 (Figs. 5, 6). The

expression of *CCNG1*, *DDB2*, and *PHPT1* genes, on the one hand, and of *GADD45A*, *PUMA*, *SESNI*, *NOXA*, and *MYC* genes, on the other, are only induced in healthy donors but not in patients with COVID-19 in response to low- and high-dose exposure, respectively. Furthermore, for *FDXR*, *DDB2*, *PHPT1*, and *APOBEC* genes, which are induced in both groups of donors after exposure to 2 Gy, the induction level is significantly lower in COVID-19 samples compared with healthy controls. For *FDXR*, this lower induction is already visible after exposure to 0.1 Gy (Fig. 6). Therefore, the radiation response elicited by both low- and high-dose exposure is deficient in patients with COVID-19.

Discussion

We compared the expression of genes coding for proteins involved in response to radiation in blood cells of patients with COVID-19 and healthy donors and their induction 24 hours after exposure to low- and high-dose radiation ex vivo. Given the central role of the *p53* tumor suppressor protein in this process,²¹ we focused on the group of genes included in the *ATM/CHK2/p53* signaling pathway.

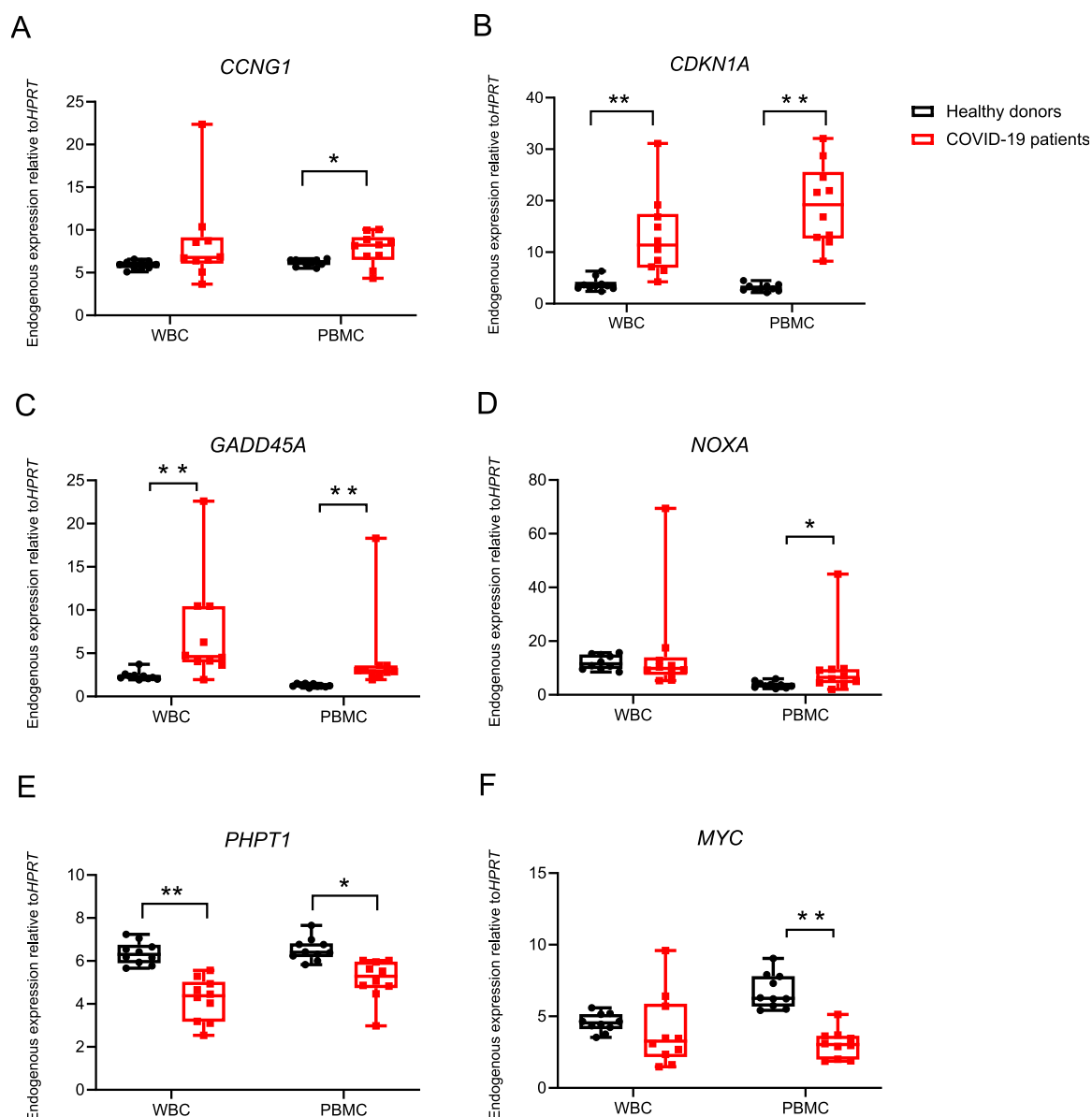


Figure 2 Multiplex real-time polymerase chain reaction expression of (A) *CCNG1*, (B) *CDKN1A*, (C) *GADD45A*, (D) *NOXA*, (E) *PHPT1*, and (F) *MYC* in white blood cells (WBC) and peripheral blood mononuclear cells (PBMC) of healthy donors (black circles) and patients with COVID-19 (red squares). The box plot shows the expression of genes in 10 healthy donors and 10 patients with COVID-19, normalized to the expression levels of *HPRT*. Statistical analysis was performed in log-transformed data. *Significantly different (*t* test; $P \leq .05$). **Significantly different (*t* test; $P \leq .001$).

We used third generation Nanopore sequencing to determine basal level of expression of these genes in whole blood. To ascertain whether this experimental approach is suitable for comparing gene expression between patients and healthy donors, we compared Nanopore sequencing results with the expression of a set of inflammatory genes regulated in patients with COVID-19 by nCounter analysis. The results were highly similar, irrespective of whether the genes were up- or downregulated (Fig. E1). This comparison, therefore, validated our technical choice for analyzing

the expression of genes of interest at the steady state in patients with COVID-19.

The *ATM/CHK2/p53* signaling pathway includes *p53* upstream activators, downstream effectors, and downstream regulators. The transcription of a large group of genes coding for *p53* downstream effectors was found upregulated in patients with COVID-19 (Fig. 1). This group includes genes coding for proteins involved in all effector functions of *p53* activation (eg, *CDKN1A*, *GADD45*, *NOXA*, *BID*, *PTEN*; Fig. 1), including *p53* feedback inhibition (eg, *SIAH1*, *COP1*, *Wip1*; Fig. 1). However,

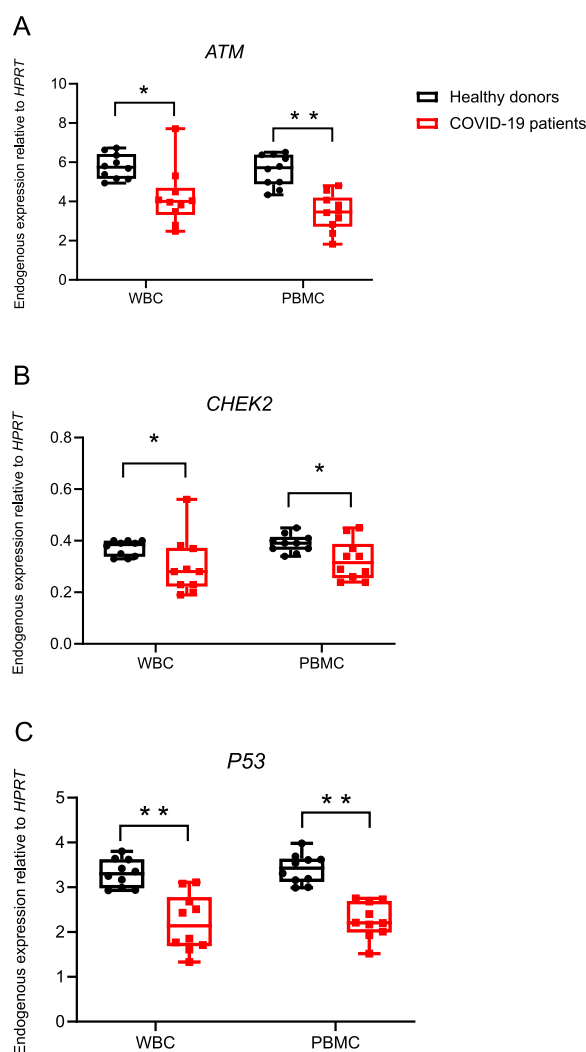


Figure 3 Multiplex real-time polymerase chain reaction expression of (A) *ATM*, (B) *CHEK2*, and (C) *p53* in white blood cells (WBC) and peripheral blood mononuclear cells (PBMC) of healthy donors (black circles) and patients with COVID-19 (red squares). The box plot shows the expression of genes in 10 healthy donors and 10 patients with COVID-19, normalized to the expression levels of *HPRT*. Statistical analysis was performed in log-transformed data. *Significantly different (t test; $P \leq .05$). **Significantly different (t test; $P \leq .001$).

the expression of a smaller group of genes coding for factors also involved in some of the same functions was downregulated in the same samples. These results are surprising on 2 accounts. First, we observe opposite regulation of genes coding for different proteins involved in the same effector functions (cell cycle, apoptosis, inhibition of insulin-like growth factor-1 pathway). Second, we also observe upregulation of the expression of genes involved in suppressing *p53*-mediated transcription while many *p53* target genes are upregulated. Thus, since opposite

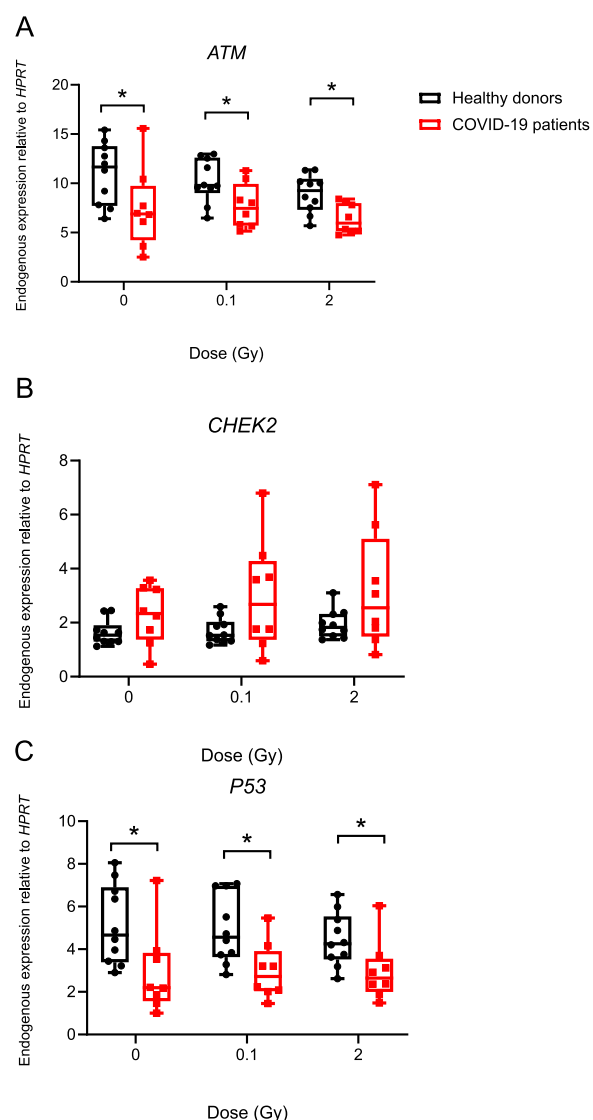


Figure 4 Multiplex real-time polymerase chain reaction expression of (A) *ATM*, (B) *CHEK2*, and (C) *p53* in whole blood of healthy donors (black circles) and patients with COVID-19 (red squares) 24 hours after ex vivo irradiation with 0 Gy, 0.1 Gy, and 2 Gy. The box plot shows the expression of genes in 10 healthy donors and 8 patients with COVID-19, normalized to the expression levels of *HPRT*. Statistical analysis was performed in log-transformed data. *Significantly different (t test; $P \leq .05$). **Significantly different (t test; $P \leq .001$).

effector functions of *p53* are induced simultaneously, the coordination of *p53* transcriptional activity appears defective in blood cells of patients with COVID-19. This dysregulation is also observed when we analyze by quantitative PCR the expression of additional *p53*-regulated genes not included in the *ATM/CHEK2/p53* pathway: whereas expression of *CCNG1* is upregulated, expression of *MYC*

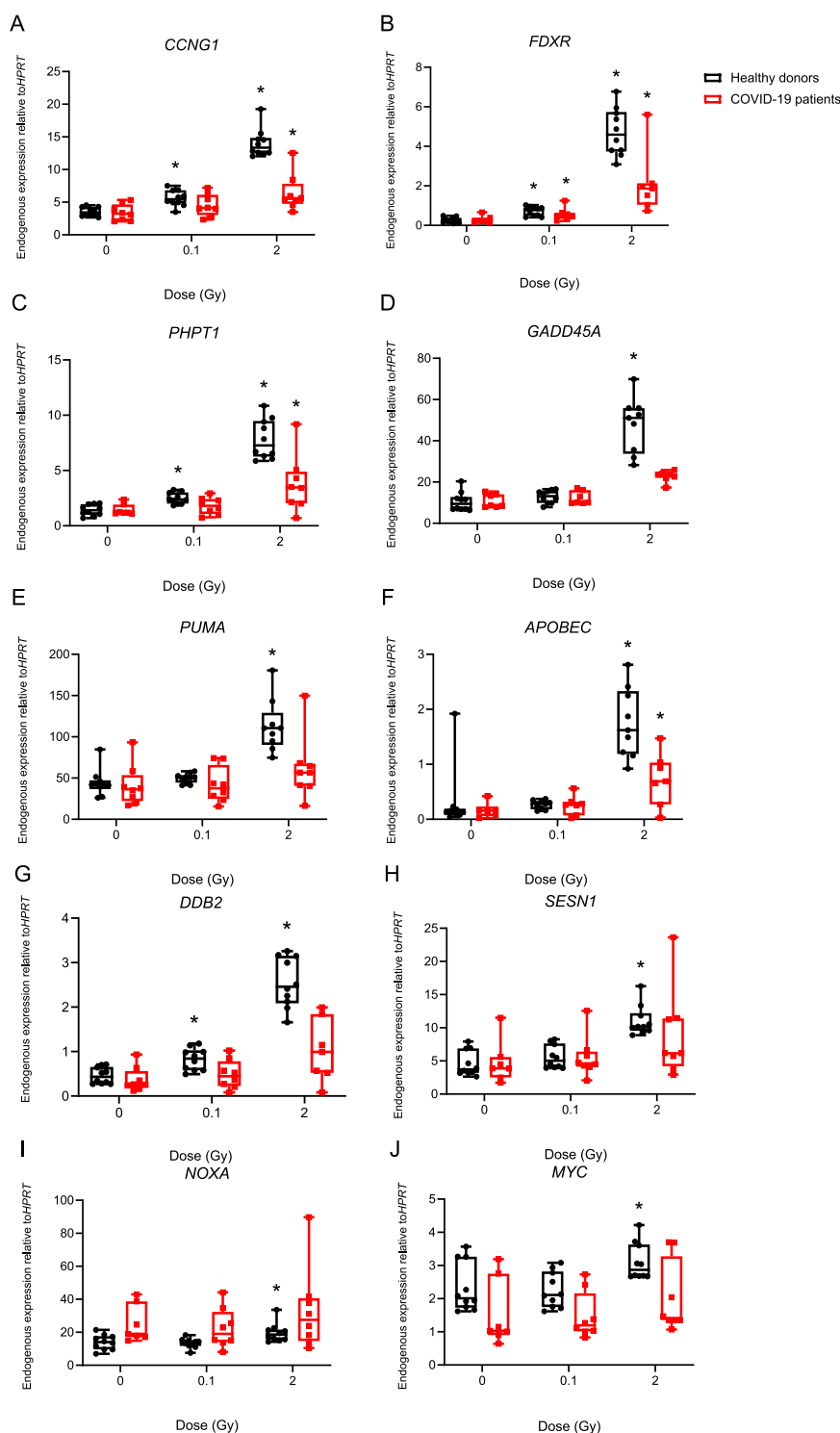


Figure 5 Multiplex real-time polymerase chain reaction expression of (A) *CCNG1*, (B) *FDXR*, (C) *PHPT1*, (D) *GADD45A*, (E) *PUMA*, (F) *APOBEC*, (G) *DDB2*, (H) *SESNI*, (I) *NOXA*, and (J) *MYC* in whole blood of healthy donors (black circles) and patients with COVID-19 (red squares) 24 hours after ex vivo irradiation with 0 Gy, 0.1 Gy, and 2 Gy. The box plot shows the expression of genes in 10 healthy donors and 7 to 8 patients with COVID-19, normalized to the expression levels of *HPRT*. Statistical analysis of expression in irradiated versus nonirradiated samples of healthy donors and patients with COVID-19 was performed in log-transformed data. *Significantly different (t test; $P \leq .05$) compared with the respective (healthy donor or COVID-19) nonirradiated sample level.

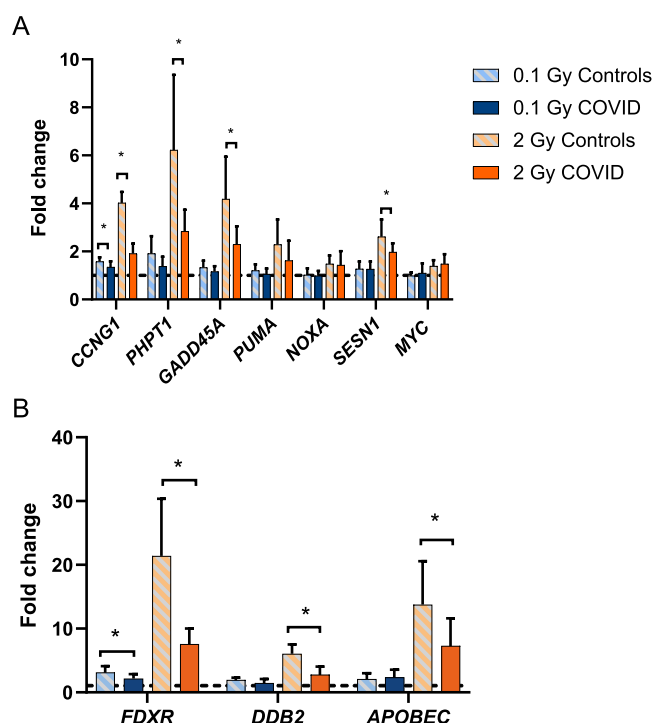


Figure 6 Induction of gene expression by low- and high-dose radiation in irradiated healthy donors and blood cells of patients with COVID-19.

and *PHPT1* is downregulated in PBMCs of patients with COVID-19 (Fig. 2).

However, the most surprising finding is the downregulation of *TP53* and its upstream activators *ATM* and *CHK2* in patients with COVID-19 while the transcription of most *p53* target genes is increased. Activation of the *ATM*-dependent DDR response culminating with *p53* activation is usually regulated by phosphorylation.²² Considering the decreased expression of *TP53*, *ATM*, and *CHK2* genes, we would have expected a lower expression (compared with healthy donors) of most of the *p53* target genes.

This unexpected pattern of *p53*-target gene expression regulation is probably best explained by expression mechanisms at least partially independent of *p53*. Indeed, gene expression results from the interplay of various transcription factors and coactivators at promoters and enhancers. It is, for example, well known that there is cross-talk between *p53* and *NF-κB*, 1 of the major transcription factors involved in the transcriptional regulation of immune responses.^{23–28} Hence, *p53* is necessary for some *NF-κB* targets expression, while in contrast, *NF-κB* is needed for the *p53* transactivation of others. Importantly, depending on the target genes considered, interactions between these transcription factors can result in induction or repression of expression.^{25,27} For a subset of target genes, the activity of *p53* or *NF-κB* is dependent on their competition for binding to the CREB-binding protein/p300

transcriptional coactivators.^{23,28} Several genes belonging to the *p53* signaling pathway are found in lists of *NF-κB* targets compiled over the years,^{29–31} including *ATM* and *TP53*. As SARS-CoV-2 infection results in strong systemic activation of innate and adaptive immunity, blood cells from patients with COVID-19 are in a highly inflammatory environment. The regulated expression of inflammatory genes (Fig. E1) suggests that the *NF-κB* signaling pathways are activated in these cells. The increased expression of genes coding for different *NF-κB* subunits (*NFKB2*; log₂FC = 1.14; *RelB*; log₂FC = 1.72) in patients with COVID-19 further suggests that these pathways are selectively activated. The resulting transcriptional landscape in blood cells of patients with COVID-19 is therefore quite different from that of healthy controls, and *NF-κB* transcriptional activity most likely contributes to the divergent modulation of *p53* target genes expression and the reduced levels of *p53*, *ATM*, and *CHK2* mRNA.

We also tested the activity of the *ATM/CHK2/p53* pathway ex vivo in irradiated blood cells.³² Radiation-induced DNA double-strand break activation of the *ATM/CHK2/p53* pathway induces the transcription of genes including *CCNG1*, *FDXR*, *APOBEC*, *DDB2*, *GADD45*, *PUMA*, *SESN1*, *NOXA*, and *MYC* analyzed here. *CCNG1*, *FDXR*, *PHPT1*, *APOBEC*, and *DDB2*, but not *GADD45*, *PUMA*, *SESN1*, *NOXA*, and *MYC*, are induced in blood from patients with COVID-19 exposed ex vivo to 2 Gy. Such dysfunctional regulation of genes is

specific to patients with COVID-19 and, to our knowledge, has not been previously observed in blood/PBMCs exposed in vitro or in patients treated by RT. Induction at 0.1 Gy is detectable in blood cells of patients with COVID-19 only for *FDXR* but not for *CCNG1*, *PHPT1*, or *DDB2*, likely because *FDXR* is the most responsive gene and more easily detected at low doses. Overall, the results presented in Figures 5 and 6 clearly show that the radiation-induced activation of the pathway is abnormally weak, similar to that reported for Ataxia Telangiectasia patients, who carry *ATM* mutations, and Li-Fraumeni syndrome patients who are heterozygous for *p53*.³² The effect seems even more pronounced in COVID-19 than in patients with Ataxia Telangiectasia, for whom the difference with healthy controls is clear at 2 hours postexposure but less pronounced at 24 hours.³³

Viral infection or viral proteins have been found to impair several aspects of the initial events of the DDR. Epstein-Barr virus *BZLF1* protein, for example, impairs accumulation of *RNF8* and *53BP1* proteins at double-strand break sites in irradiated cells, resulting in impaired DNA repair.³⁴ *HTLV* tax proteins can also attenuate *ATM* activity, leading to a reduced formation of *MDC1* foci after radiation exposure.³⁵ Cytomegalovirus replication in cells induces an *ATM*-dependent checkpoint response, but this response is deficient because several DDR proteins, including *ATM* and *CHK2*, relocalize to the cytosol on viral particles.³⁶ In the same line, radiation-induced expression of several *p53*-dependent genes has been found to be modulated in blood cells exposed at the same time to lipopolysaccharide, a bacterial cell wall component eliciting a strong inflammatory reaction.^{37,38,39} Interestingly, lipopolysaccharide-activation of inflammatory signaling pathways could enhance or reduce the effects of radiation on the expression of different genes. Thus, microorganisms or microorganism products can disrupt the expression and/or function of DDR components in different cellular models. Our results demonstrate that SARS-CoV-2 infection can affect profoundly the radiation response at the cellular level in leukocytes.

We show that the expression of a collection of genes involved in the radiation response is dysregulated in patients with COVID-19, and, most probably as a direct consequence, the ex-vivo radiation response of blood cells of patients with COVID-19 is deficient/suboptimal. We do not know the duration after COVID-19 recovery of these effects, and importantly, if they persist in patients suffering from “long COVID.” Our results were obtained in a small group of severely ill, aged patients. Our findings, of course, need to be consolidated in larger, more diverse groups of patients, including younger subjects, with age-matched healthy control donors and a better stratification of clinical parameters to determine whether they are also detectable in asymptomatic, mild, and long-term patients with COVID-19 and the duration of their

persistence after recovery. It is somewhat difficult at this point to appreciate the consequences of these observations for radiation-induced adverse effects in vivo. However, these issues are essential to evaluate as it is likely that a sizable fraction of patients who recently recovered from COVID-19 or are still affected by a form of long COVID will receive significant radiation doses for cancer RT. COVID-19-associated pulmonary damage, observed even in recovered patients, is suggested to be an additional comorbidity for radiation-induced injury after thoracic exposure.⁴⁰ The mechanistic impairment of the radiation response observed in this study may contribute to this comorbidity. Our findings of a decrease in *TP53* transcriptional level raises concerns about radiation toxicity risks. In head and neck tumors associated with human papillomavirus infection, human papillomavirus-induced proteasomal degradation of *p53* confers higher radiosensitivity.⁴¹ Thus, viral products can affect tissue radiosensitivity. Although we only have information on *TP53* mRNA level in patients with COVID-19, SARS-CoV-2 infection might also result in higher radiosensitivity due to a lower level of *p53* protein in blood cells and potentially other tissues. This potential modulation of radiation sensitivity could affect the clinical outcome of LR-RT applied to patients with COVID-19. Further studies are required to determine the duration and consequences of the dysregulated expression of the *p53* signaling pathway genes reported here to provide safer management of cancer patients.

We were conscious that patients with COVID-19 were older than healthy donors (Table E1) as we could not age-match donors in our cohort of healthy donors obtained exclusively from the working population. As the transcription of *p53* and some of its target genes has been shown to decrease during replicative senescence,⁴² age is a potential confounding factor in our study, and *p53* expression is indeed lower in healthy controls >54 years of age (Fig. E3). However, importantly, we have not observed any decrease in the radiation response of *p53* transcriptional target genes in healthy donors aged >54 years (Fig. E4). Although *PUMA* and *NOXA* responses are different at 0.1 Gy, all other genes and, importantly, all genes after 2 Gy exposure, are not significantly differentially expressed. Hence, age does not affect the radiation response, at least in this study, confirming that the deficient radiation response identified in patients with COVID-19 is associated with SARS-CoV-2 infection rather than age. We also note that the outliers seen in Figure 3 panels are different donors in the different panels and are never the youngest patient (45 years old).

Conclusion

Finally, the use of LD-RT to treat patients with COVID-19 is debated in light of the increased possibility

of radiation-induced risk of cancer or noncancer disease death.^{13,14} These risks were calculated for a “normal” background and response. We clearly show that the response is abnormal, necessitating the accuracy of these probability estimations, which should therefore be revisited. In this context, a limited survey of clinicians identified several barriers to the use of LD-RT in patients with COVID-19, including the “potential to do harm to the patient, including difficulty in predicting harm and lack of existing data to inform quantification of risks”.⁴³ If the dysregulated radiation response stems from the lower level of *ATM*, *CHK2*, and *p53* expression, one could envisage bedside sequencing before irradiation to inform the decision. Nanopore sequencing was proposed to provide a robust radiation-induced transcriptomic signature to identify biomarkers of radiation exposure.¹⁵ This pipeline could be adapted to develop a quick and high-throughput platform to measure the activity of the *ATM/CHK2/p53* pathway in hospitalized patients with COVID-19 requiring RT to minimize the potential health risk associated with radiation exposure in the context of a deficient radiation response.

Supplementary materials

Supplementary material associated with this article can be found in the online version at [doi:10.1016/j.adro.2023.101215](https://doi.org/10.1016/j.adro.2023.101215).

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