



Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.



SARS-CoV-2 suppresses mRNA expression of selenoproteins associated with ferroptosis, endoplasmic reticulum stress and DNA synthesis

Yijun Wang^{a,1}, Jinbao Huang^{a,1}, Yong Sun^b, David Stubbs^c, Jun He^b, Weiwei Li^b, Fuming Wang^a, Zhirong Liu^b, Jan A. Ruzicka^d, Ethan Will Taylor^c, Margaret P. Rayman^e, Xiaochun Wan^{a,**}, Jinsong Zhang^{a,*}

^a The State Key Laboratory of Tea Plant Biology and Utilization, School of Tea & Food Science, Anhui Agricultural University, Hefei, China

^b Public Health Research Institute of Anhui Province, Anhui Provincial Center for Disease Control and Prevention, Hefei, China

^c Department of Chemistry and Biochemistry, University of North Carolina at Greensboro, Greensboro, NC, USA

^d Department of Basic Pharmaceutical Sciences, Fred C. Wilson School of Pharmacy, High Point University, One University Parkway, High Point, NC, USA

^e Department of Nutritional Sciences, Faculty of Health and Medical Sciences, University of Surrey, Guildford, United Kingdom

ARTICLE INFO

Handling Editor: Dr. Jose Luis Domingo

Keywords:

Selenium
Selenoprotein
mRNA expression
SARS-CoV-2
COVID-19

ABSTRACT

Higher selenium status has been shown to improve the clinical outcome of infections caused by a range of evolutionally diverse viruses, including SARS-CoV-2. However, the impact of SARS-CoV-2 on host-cell selenoproteins remains elusive. The present study investigated the influence of SARS-CoV-2 on expression of selenoprotein mRNAs in Vero cells. SARS-CoV-2 triggered an inflammatory response as evidenced by increased *IL-6* expression. Of the 25 selenoproteins, SARS-CoV-2 significantly suppressed mRNA expression of ferroptosis-associated GPX4, DNA synthesis-related TXNRD3 and endoplasmic reticulum-resident SELENOF, SELENOK, SELENOM and SELENOS. Computational analysis has predicted an antisense interaction between SARS-CoV-2 and TXNRD3 mRNA, which is translated with high efficiency in the lung. Here, we confirmed the predicted SARS-CoV-2/TXNRD3 antisense interaction *in vitro* using DNA oligonucleotides, providing a plausible mechanism for the observed mRNA knockdown. Inhibition of TXNRD3 decreases DNA synthesis which is thereby likely to increase the ribonucleotide pool for RNA synthesis and, accordingly, RNA virus production. The present findings provide evidence for a direct inhibitory effect of SARS-CoV-2 replication on the expression of a specific set of selenoprotein mRNAs, which merits further investigation in the light of established evidence for correlations between dietary selenium status and the outcome of SARS-CoV-2 infection.

1. Introduction

The world is undergoing a pandemic of Coronavirus Disease-2019 (COVID-19) caused by infection with severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). Selenium (Se), an essential micronutrient, is the only trace element to be specified in the genetic code; 25 genes encode selenoproteins that have a selenocysteine residue at their active center (Rayman, 2012). Many selenoproteins are implicated in the anti-oxidant, anti-inflammatory and anti-viral actions of Se (Beck et al., 2004; Steinbrenner et al., 2015). Se has been found to be a significant factor affecting the incidence and severity of a number of viral diseases in both animals and humans (Beck et al., 2004; Steinbrenner

et al., 2015). RNA viruses such as coxsackievirus B3 and influenza A/Bangkok/1/79 (H3N2) mutated into more virulent strains in a Se-deficient host (Beck et al., 2004; Steinbrenner et al., 2015). Se supplementation or higher Se status has been shown to improve the clinical outcome of infections caused by a range of evolutionally diverse viruses (Beck et al., 2004; Steinbrenner et al., 2015).

Se status is mainly affected by dietary Se intake and a link between Se status and the prognosis of COVID-19 patients in China has been identified; when the cure rate (%) in cities outside Hubei province was plotted against population hair-Se concentration – a surrogate for Se intake from foods – a significant, positive linear association was shown with a Pearson *r* of 0.85 ($p < 0.0001$) (Zhang et al., 2020a). Supporting

* Corresponding author.

** Corresponding author.

E-mail addresses: xcwan@ahau.edu.cn (X. Wan), zjs@ahau.edu.cn, jszhang@ustc.edu.cn (J. Zhang).

¹ These authors contributed equally.

<https://doi.org/10.1016/j.fct.2021.112286>

Received 9 April 2021; Received in revised form 13 May 2021; Accepted 15 May 2021

Available online 21 May 2021

0278-6915/© 2021 Elsevier Ltd. All rights reserved.

that finding, a German study found that serum Se and SELENOP concentrations in COVID-19 patients were significantly lower than in healthy controls and were significantly higher in samples from COVID-19 survivors than from non-survivors (Moghaddam et al., 2020). Furthermore, a Korean study showed that the percentage of COVID-19 patients that were Se deficient increased with COVID-19 severity (Im et al., 2020). Relevant to these observations, Ebselen, an organo-selenium compound [designed as a mimic for glutathione peroxidase-1 (GPX1)], was found to have the strongest inhibitory activity against the SARS-CoV-2 main protease in the current drug arsenal of 10,000 compounds; this protease mediates the life cycle of the virus and is a well-recognized target for inhibition (Jin et al., 2020). These new findings, together with published evidence on other viruses (Beck et al., 2004; Steinbrenner et al., 2015), suggest that Se compounds or Se-rich foods may be used to achieve both prophylaxis and therapy in COVID-19. The possible mechanisms by which Se protects against COVID-19 have been discussed in several recent reviews (Zhang et al., 2020b; Hiffler and Rakotoambinina, 2020; Bermano et al., 2020). However, whether SARS-CoV-2 also affects the expression of host selenoproteins is currently unknown. Hence, in the current study, we investigated the potential impact of SARS-CoV-2 infection at the mRNA level on a model host *in vitro* system, African Green Monkey kidney cells (Vero cells), which express the same 25 known selenoproteins as are found in humans.

2. Materials and methods

2.1. Cells, virus and viral inoculation

Vero cells were obtained from American Type Culture Collection (ATCC) and maintained in Dulbecco's Modified Eagle's media (Corning, USA) supplemented with 10% fetal bovine serum (Gibco, Invitrogen), 2 mM L-glutamine, 100 U/ml penicillin and 0.1 mg/ml streptomycin at 37 °C in a humidified atmosphere of 5% CO₂. Patient-derived SARS-CoV-2 (SZ005) was isolated by the Anhui Provincial Center for Disease Control and Prevention (Anhui, China). The viral titer was determined by 50% tissue culture infective dose (TCID₅₀) according to the cytopathic effect by use of the Karber method. All the infection experiments were performed in a biosafety level-3 (BSL-3) laboratory. Vero cells were seeded on 6-well plates with a density of 1×10^6 cells/well and infected with 20 TCID₅₀ virus at 37 °C.

2.2. RNA extraction and quantitative real-time RT-PCR (qRT-PCR)

After viral infection for 48 h, the cell culture was subjected to virus inactivation treatment and then divided into two replicates. One (100 µL) was used for viral RNA isolation on an automatic nucleic acid extraction workstation (TANBead, Taiwan) according to the manufacturer's instructions. Reverse transcription was performed with TaqMan Fast Virus 1-Step Master Mix (ThermoFisher, Catalog Numbers

4444432). Briefly, 2 µL viral RNA was used as template for one-step quantitative PCR. The full-length S gene of SARS-CoV-2 was synthesized and cloned into pcDNA3.1 as positive control plasmid. Serial dilutions of the positive control (1000–1,000,000,000 copies per µL) were used to establish a standard curve for determining the initial starting amount of the target template in experimental samples. The primers and probe used for quantitative PCR were: SB-F: GGCTGTTTAA-TAGGGGCTGAAC, SB-R: ACCAAGTGACATAGTGTAGGCA, SB probe: 5' FAM-AGACTAATTCTCCTCGGCGGCACG-BHQ.

The rest of the cell suspension was used for the RNA extraction of host cells. Total RNA was extracted using an RNeasy mini kit (Qiagen Inc., Valencia, CA) and the reverse transcription reaction was conducted using a Goscript™ Reverse Transcription System kit (Promega, Madison, WI). The RNA quality was confirmed by spectrophotometry and electrophoresis. A Power SYBR® Green PCR Master Mix kit (Life Technologies, Warrington, UK) was employed to conduct the qRT-PCR on an ABI QuantStudio 7Pro system. The expression level of a target gene mRNA was normalized to the mRNA level of glyceraldehyde 3-phosphate dehydrogenase (GAPDH). The amount of the target gene expression was calculated by the $2^{-\Delta\Delta CT}$ method. The sequences of the genes involved in the present study were obtained from Genbank (www.ncbi.nlm.nih.gov/Genbank), and the sequences of primers used are listed in Supplementary Table 1.

2.3. Activities of GPX and thioredoxin reductase (TXNRD)

GPX and TXNRD activities were measured according to the methods described (Sun et al., 2014; Wang et al., 2014). One unit of GPX or TXNRD was defined as 1 µmol of NADPH oxidized per minute and the resulting GPX or TXNRD activity was presented as mU/mg protein.

2.4. Gel shift assay for SARS-CoV-2/TXNRD3 antisense interaction

The antisense pairing between regions of SARS-CoV-2 and human TXNRD3 that were previously identified computationally (20) spanned the following sequence regions of SARS-CoV-2 (NC_045512): 17824–17848 and TXNRD3 (NM_052883.2): 1322–1347. In order to assure adequate ethidium bromide labelling of the single strands, which requires some internal secondary structure, oligos based on those sequences were extended by 10 bases of the native sequences at both the 5' and 3' ends. This extension also served as a way to replicate the behavior of the antisense interaction when the regions of interest are each part of a larger native sequence context. Thus, the oligos used in the gel shift assay were bases 17814–17858 of NC_045512 (coronavirus oligo C) and bases 1312–1357 of NM_052883.2 (TXNRD3 oligo T). A randomly shuffled version of viral sequence C was generated and labeled as R in Fig. 4. Oligos were ordered from Integrated DNA Technologies, Inc., Coralville, IA.

The lyophilized DNA was dissolved in PBS to create 1 µM stock solutions which were stored at 4 °C. One microliter of each sample (T, C or

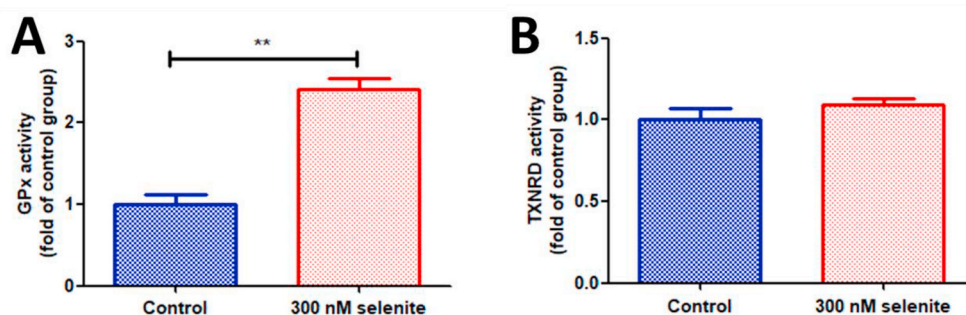


Fig. 1. Influence of sodium selenite on GPX and TXNRD activities in Vero cells. (A) GPX activity. (B) TXNRD activity. Data are presented as mean \pm SEM (n = 3). Compared to control, **p < 0.01. Basal activities of GPX and TXNRD were 23 and 0.3 mU/mg protein, respectively.

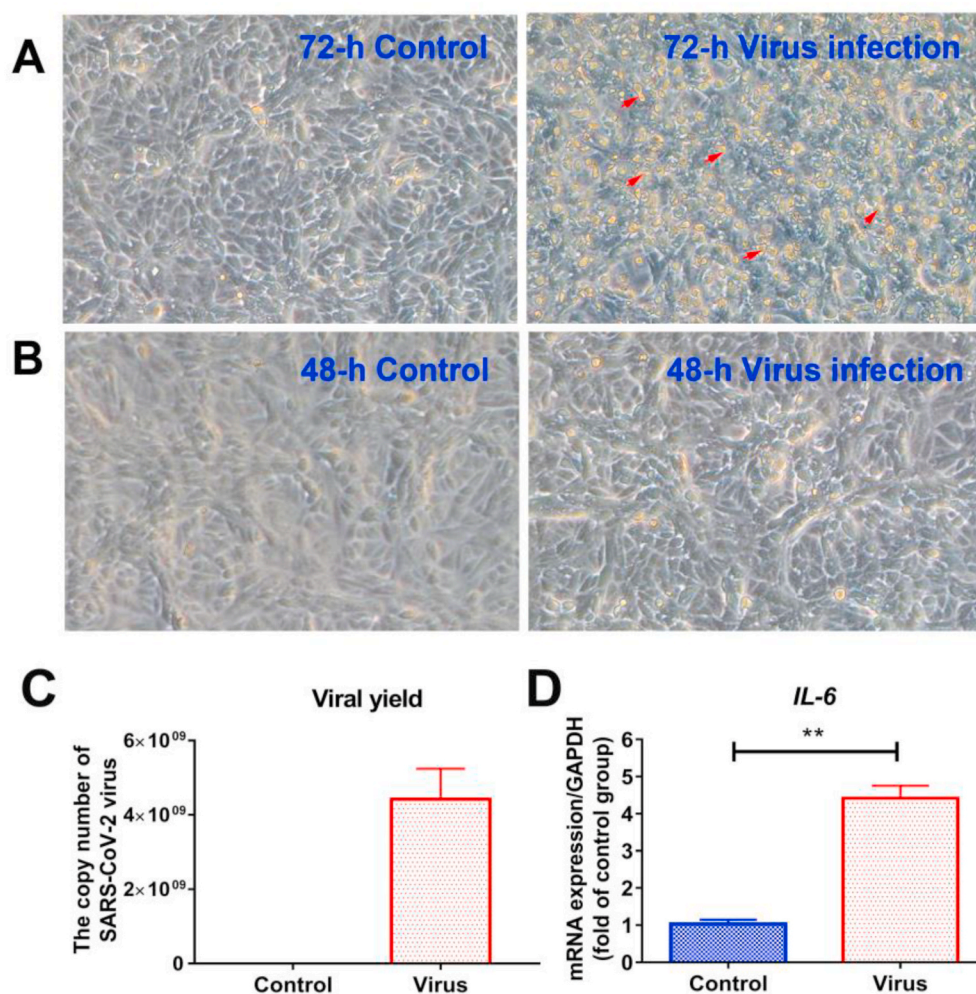


Fig. 2. Responses of host cells to SARS-CoV-2 infection. Vero cells were infected with SARS-CoV-2 at 20-fold TCID₅₀. A, micrographs of control and virus-infected cells (72-h incubation); the yellow bubbles marked by red arrows represent dead cells; B, micrographs of control and virus-infected cells (48-h incubation). Following a 48-h incubation, viral copy numbers and abundance of mRNAs encoding IL-6 were quantified by quantitative real time polymerase chain reaction. C, viral copy number; D, gene expression. Data are expressed as mean ± SEM (n = 6), statistical differences were examined by the Mann Whitney test (**p < 0.01). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

R) was further diluted in PBS, to a total volume of 10 µL (at 100 nM). Oligos, either singly or in pairs of equal volumes of T + C or T + R, were incubated at room temperature for 1 h. The tubes were then heated at 66 °C for 15 min, and then cooled at room temperature for 40 min. Along with 2 µL of loading dye, the DNA was placed into the lanes, using 10 µL of either the single strands or pair mixtures, giving about 1 pmol of total DNA per well. The 5% TBE agarose gel, with ethidium bromide, was run at 65v for 4 h while the set up was chilled in ice water. The gel was then analyzed and imaged with a BIO-RAD Gel Doc™ XR+ Molecular Imager.

2.5. Statistical analysis

Data are expressed as the mean ± standard error of the mean (n = 6 or 3), and analyzed using the IBM SPSS Statistics 22.0 (IBM, Armonk, NY). The Mann Whitney test was used to assess the difference between two groups. Significant levels of p < 0.05 and p < 0.01 were set for all tests.

3. Results

3.1. Cell model for selenoprotein gene assessment

Vero cells, as tool cells for investigating SARS-CoV-2, were maintained in Dulbecco's Modified Eagle's media supplemented with 10% fetal bovine serum. It has been shown that 10% fetal bovine serum does not provide sufficient Se for full biosynthesis of selenoproteins (Verma et al., 2008); we thus firstly evaluated Se status in Vero cells. Following

supplementation of sodium selenite at 300 nM for 48 h, GPX activity increased by 2.4-fold (Fig. 1A), which is consistent with the report by Verma and colleagues (Verma et al., 2008) in which Vero cells were used for virus infection. However, TXNRD activity remained unchanged (Fig. 1B). These results suggest that the Vero cells were cultured under suboptimal-Se condition. Given the worldwide prevalence of SARS-CoV-2 on selenoprotein genes in the Vero cells without Se treatment.

Vero cells were infected with SARS-CoV-2 at 20-fold TCID₅₀. Pilot experiments showed that significant cytopathology occurred after 72-h incubation (Fig. 2A). Following a 48-h incubation, which did not cause morphological alteration of the cells (Fig. 2B), SARS-CoV-2 viral copy number in the cultured cells reached 4.4×10^9 (Fig. 2C); mRNA concentration of IL-6 was significantly up-regulated, i.e. by 4.3-fold (Fig. 2D). IL-6 is positively correlated with COVID-19 severity (Gubernatorova et al., 2020) and can affect the selenoenzyme, GPX, in an isozyme-specific manner (Martitz et al., 2015). Specifically, GPX1 mRNA expression remained unaffected while that of GPX4 decreased in IL-6-treated human hepatocytes (Martitz et al., 2015). Taken together, a 48-h infection of Vero cells by SARS-CoV-2 appears to be an optimal time point for examining gene expression of selenoproteins, since (i) the virus had initiated an inflammatory response as evidenced by IL-6, (ii) a link between IL-6 and selenoprotein levels has been established, and (iii) a causal relationship between potential alterations of selenoproteins and cell damage could be distinguished. Therefore, we next evaluated gene expression of selenoproteins at this time point.

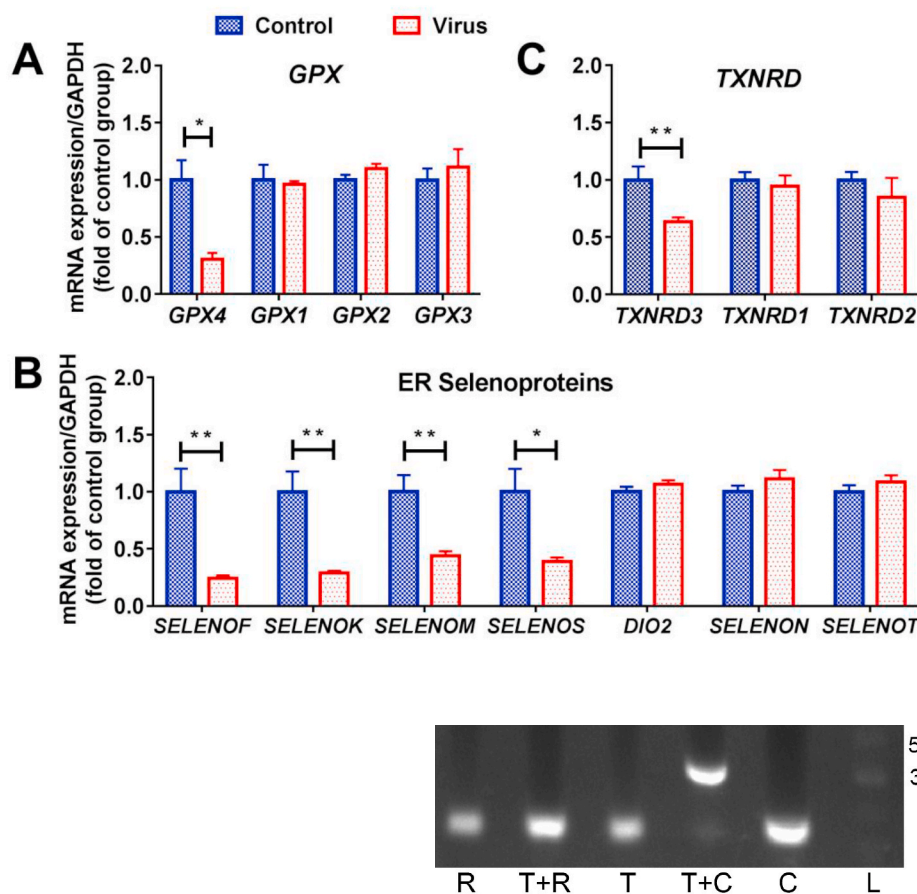


Fig. 3. Gene expression of GPX and TXNRD families and ER-resident selenoproteins. Vero cells were infected with SARS-CoV-2 at 20-fold TCID₅₀. Following a 48-h incubation, the abundance of mRNAs encoding selenoproteins were quantified by quantitative real time polymerase chain reaction. A, genes of GPX family; B, genes of TXNRD family; C, genes of ER-resident selenoproteins. Data are expressed as mean \pm SEM (n = 6), statistical differences were examined by the Mann Whitney test (*p < 0.05 and **p < 0.01).

Fig. 4. Antisense interaction between regions of SARS-CoV-2 and TXNRD3 mRNAs shown by gel shift assay using cDNA oligos. Lane labels correspond to oligos for the complementary single stranded regions of the TXNRD3 (T) and coronavirus (C) sequences identified previously (20), a randomly shuffled version of the viral sequence (R), and a dsDNA sequence ladder at right (L). As detailed in *Methods*, after incubation, lanes were loaded with either a single oligo (each of about 45 bases) or an approximately equimolar pair (T + C or T + R). All the single sequence lanes and the T + R lane migrate as single stranded, resulting in their uniform vertical placement. Only the paired lane of T + C migrates more slowly, because the strands hybridize and move as a duplex, falling between the 35bp and 50bp dsDNA size markers. The results show target-specific *in vitro* hybridization of the predicted SARS-CoV-2/TXNRD3 antisense pair.

3.2. Impact on GPX family

The selenocysteine-containing GPX family is the best characterized among the selenoproteins. It includes: cytoplasmic GPX1; GPX2, primarily found in the epithelium of the gastrointestinal tract; circulating GPX3 (mainly secreted by the kidney); GPX4, localized in the cytoplasmic, mitochondrial and nuclear compartments; and olfactory epithelium GPX6. All GPXs use glutathione as a cofactor, but GPX4 is unique in the GPX family in that it protects against iron-dependent ferroptotic cell death (Alim et al., 2019; Conrad and Proneth, 2020). We found that SARS-CoV-2 did not alter mRNA concentrations of GPX1, GPX2 and GPX3 but significantly down-regulated that of GPX4 by 69.4% (Fig. 3A). As expected, GPX6 was not detected.

3.3. Impact on endoplasmic reticulum (ER)-resident selenoproteins

Seven selenoproteins including SELENOF, SELENOM, SELENOK, SELENOS, SELENON, SELENOT and iodothyronine deiodinase 2 (DIO2) are residents of the ER (Shchedrina et al., 2010). SELENOF and its distant homologue, SELENOM, possess redox-active motifs, thus they regulate redox homeostasis, catalyze the reduction or rearrangement of disulfide bonds in ER-localized proteins and facilitate ER protein-folding (Labunskyy et al., 2014). SELENOK, along with SELENOS, promotes

ER-associated degradation (ERAD) of errant proteins by recruiting cytosolic valosin-containing protein to increase translocation of misfolded proteins from the ER lumen to the cytosol (Labunskyy et al., 2014). Another three ER-resident selenoproteins, namely SELENON, SELENOT and DIO2 have not been found to be involved with either ER protein-folding or ERAD (Labunskyy et al., 2014). Intriguingly, SARS-CoV-2 infection targeted ER-resident selenoproteins that have known functions of ER protein-folding or ERAD, as shown by significant reduction of mRNA concentrations of SELENOF, SELENOM, SELENOK and SELENOS, i.e. by 75.9%, 56.2%, 71.3% and 61.1%, respectively. On the other hand, mRNA concentrations of SELENON, SELENOT and DIO2 that have no documented functions in ER protein-folding or ERAD were not significantly influenced by SARS-CoV-2 infection (Fig. 3B).

3.4. Impact on the TXNRD family and ten additional selenoproteins

The TXNRD family contains the ubiquitous cytoplasmic/nuclear TXNRD1 and mitochondrial TXNRD2, as well as TXNRD3 (the thioredoxin/glutathione reductase with glutaredoxin activity), which in contrast to TXNRD1 and 2 contains an N-terminal glutaredoxin domain (Sun et al., 2005). TXNRD3 is predominately expressed in the testis; however, pulmonary TXNRD3 protein levels are also high according to the Human Protein Atlas (Uhlen et al., 2015). Thioredoxin serves as an

Table 1

Gene expression of twenty-five selenoproteins except GPX and TXNRD families and ER-resident selenoproteins.

Selenoprotein	Control	Virus	p
DIO1	1.00 ± 0.05	1.00 ± 0.05	>0.05
DIO3	1.00 ± 0.07	1.11 ± 0.15	>0.05
MSRB1	1.00 ± 0.39	0.24 ± 0.03	>0.05
SELENOH	1.00 ± 0.10	0.81 ± 0.04	>0.05
SELENOI	ND	ND	–
SELENOO	1.00 ± 0.20	1.03 ± 0.18	>0.05
SELENOP	1.00 ± 0.04	1.06 ± 0.05	>0.05
SELENOV	1.00 ± 0.02	1.22 ± 0.10	>0.05
SELENOW	1.00 ± 0.06	0.82 ± 0.03	>0.05
SEPHS2	1.00 ± 0.03	1.24 ± 0.05	>0.05

Following a 48-h incubation, the abundance of mRNAs encoding selenoproteins were quantified by quantitative real time polymerase chain reaction. Data are expressed as mean ± SEM (n = 6), statistical differences were examined by the Mann Whitney test. ND, undetected.

electron donor for ribonucleotide reductase which catalyzes the conversion of ribonucleotides to deoxyribonucleotides for DNA synthesis (Arner and Holmgren, 2006). Inhibition of TXNRD decreases DNA synthesis and thereby increases the ribonucleotide pool for RNA synthesis; thus, RNA viruses may target TXNRD for efficient reproduction. According to computational analysis, SARS-CoV-2 has the potential for antisense-mediated knockdown of *TXNRD3* but not *TXNRD1* or *TXNRD2* (Taylor, 2020). Consistent with the computational prediction, our study found that SARS-CoV-2 did not lower mRNA concentration of *TXNRD1* or *TXNRD2* but significantly lowered mRNA concentration of *TXNRD3*, by 36.9% (Fig. 3C). Notably, however, SARS-CoV-2 did not affect mRNA concentrations of the remaining ten selenoproteins, including DIO1 (iodothyronine deiodinase 1), DIO3 (iodothyronine deiodinase 3), MSRB1 (methionine-R-sulfoxide reductase), SELENOH, SELENOI, SELENOO, SELENOP, SELENOV, SELENOW and SEPHS2 (selenophosphate synthetase 2) (Table 1).

3.5. Molecular mechanism underlying *TXNRD3* knockdown

Potential antisense interactions between viral mRNAs and isoforms of TXNRD were first reported for HIV-1 and Ebola virus in 2016 (Taylor et al., 2016), and most recently, for Zika and other RNA viruses including SARS-CoV-2, as recently reviewed with graphical representations (Taylor, 2020). Such interactions have the potential to inhibit protein synthesis, either with or without knockdown or degradation of the targeted host mRNA (Taylor et al., 2016). Using a gel mobility shift assay and synthetic oligonucleotides, we assessed a potential antisense interaction between SARS-CoV-2 and a region of human *TXNRD3* that was identified computationally, corresponding to the predicted antisense pair (Taylor, 2020). Fig. 4 shows that DNA oligos corresponding to those specific regions of SARS-CoV-2 and *TXNRD3* mRNA hybridized quantitatively to form a single slower moving band corresponding to the expected duplex DNA. This interaction was sequence specific, because a negative control consisting of a randomly shuffled SARS-CoV-2 oligo of identical composition still moved as a single band in the presence of the *TXNRD3* oligo.

4. Discussion

Of the 25 mammalian selenoproteins, our findings show that SARS-CoV-2 reduced mRNA concentrations of six selenoproteins in Vero cells at the onset of the inflammatory response but prior to perceived cell damage. Some of these selenoproteins may be implicated in the pathogenesis of COVID-19 and SARS-CoV-2 may target some of these selenoproteins to promote the viral life cycle, as discussed below. It should be noted that data from mRNA steady-state concentrations are not equivalent to gene expression. The world of selenoproteins, in particular, is complicated by a number of levels at which final protein

expression can be regulated, including transcription, translational efficacy, post-translational modifications, etc. Therefore, given our lack of protein data, much of the subsequent discussion represents our extrapolation of what may be happening.

SARS-CoV-2 increases the production of mitochondrial reactive oxygen species (ROS) which in turn accelerate SARS-CoV-2 replication (Codo et al., 2020). Treatment by antioxidants such as mitoquinol and N-acetylcysteine substantially inhibits SARS-CoV-2 replication (Codo et al., 2020). Of the five members of the GPX family, only GPX4 is located in the mitochondria, thus SARS-CoV-2 may benefit more from targeting GPX4 than other family members. GPX4 specifically protects against ferroptotic cell death which normally occurs in cells of the immune system. Leukopenia in COVID-19 patients may be associated with SARS-CoV-2 induced ferroptosis (Cuadrado et al., 2020). The current findings suggest that SARS-CoV-2 may have induced ferroptosis in leukocytes that was associated with SARS-CoV-2 suppression of *GPX4*.

The Stimulator of Interferon Genes (STING) is pivotal in initiating innate immune responses against viral infection. *GPX4* is required for facilitating STING activation (Jia et al., 2020). SARS-CoV-2 may exploit evasion of the immune system by suppressing *GPX4*. *GPX4* is a so-called “housekeeping” gene, ranking high in the hierarchy of selenoprotein expression, whereas *GPX1* is ranked much lower and is less likely to be expressed at low Se status (Sunde and Raines, 2011). While moderate Se deficiency causes a significant reduction in *GPX1* concentration, *GPX4* concentration is not much reduced because *GPX4* is high in the hierarchy of selenoprotein expression. Current results suggest that SARS-CoV-2 infection in Se-deficient individuals may synergistically destroy GPX defenses resulting in severe oxidative stress capable of causing viral mutation to more virulent strains (Seale et al., 2020). In addition to nutritional Se deficiency, either severe hypoxia or a pronounced rise in IL-6, two hallmarks of severe COVID-19, suppresses hepatic *SELENOV* expression (Martitz et al., 2015; Becker et al., 2014) resulting in reduced circulating *SELENOV* and impaired Se delivery to other tissues. Indeed, serum *SELENOV* concentration has been found to be inversely associated with COVID-19 severity (Moghaddam et al., 2020). Thus, SARS-CoV-2 infection may cause both tissue depletion of Se and transcriptional inactivation of *GPX4*, thereby synergistically destroying GPX defenses.

Coronavirus replication induces ER stress and the unfolded protein response in infected cells (Sureda et al., 2020; Chan et al., 2006; Versteeg et al., 2007; Liao et al., 2013). A link between coronavirus infection and ER-resident selenoproteins has not previously been documented, although ER-resident selenoproteins are known to participate in maintaining ER homeostasis (Shchedrina et al., 2010). Of the seven ER-resident selenoproteins, we found that the mRNA steady-state levels of four selenoproteins were decreased by SARS-CoV-2. We know that impaired *SELENOF* and *SELENOV* increase protein misfolding, causing ER stress, whereas impaired *SELENOK* and *SELENOI* attenuate the ERAD of misfolded proteins (Labunskyy et al., 2014). Concomitant down-regulation of *SELENOF*, *SELENOV*, *SELENOK* and *SELENOI* provoked by SARS-CoV-2 infection is likely to result in increased concentration of misfolded proteins in the ER and profound ER stress. Of all the ER-resident selenoproteins, we found *SELENOF* expression to be the most affected by SARS-CoV-2 infection (75.9% decrease). This is interesting given a recent report that *SELENOF* may be targeted for proteolysis by the SARS-CoV-2 main protease M^{pro} , because the *SELENOF* protein contains a sequence (TVLQ/AVSA) that is almost identical to a known viral M^{pro} cleavage site (TVLQ/AVGA) (Taylor and Radding, 2020). This dual targeting of *SELENOF* by SARS-CoV-2 at both the mRNA and protein levels suggests that disruption of *SELENOF* function may be particularly important for SARS-CoV-2 replication. Moreover, *SELENOF* belongs to the group of stress-related selenoproteins that is subject to regulation by dietary Se. In addition, a polymorphic site at nucleotide position 1125 (A/G) located in the SECIS element of *SELENOF* affects the efficiency of selenocysteine incorporation into *SELENOF* in a Se-dependent fashion (Hu et al., 2001; Kumaraswamy et al., 2002).

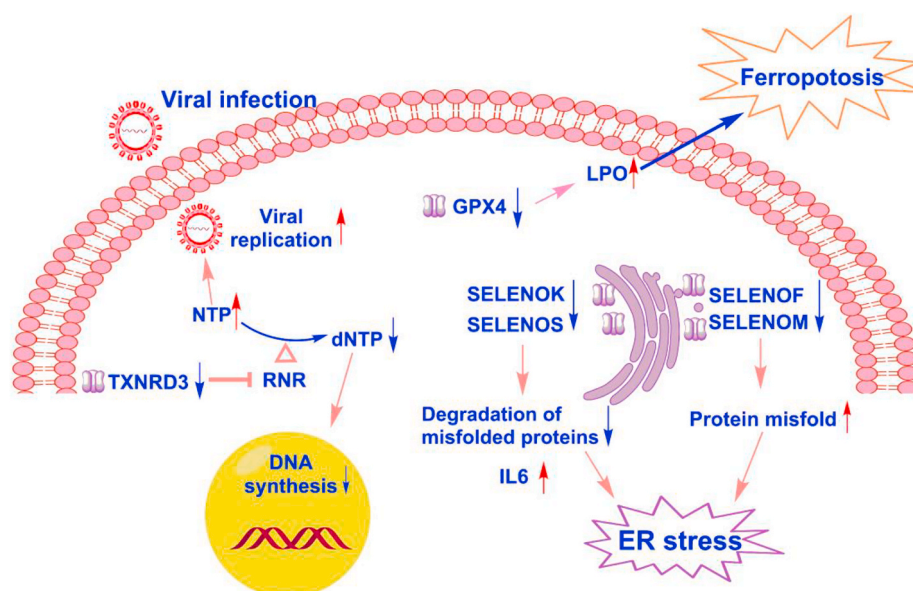


Fig. 5. Possible consequences of SARS-CoV-2 induced down-regulation of selenoprotein genes. LPO, lipid peroxidation; NTP, nucleoside triphosphate; dNTP, deoxyribonucleoside triphosphate; RNR, ribonucleotide reductase.

The A1125 variant is prevalent in African Americans (Hu et al., 2001); a large UK cohort study found that blacks were at significantly higher risk of death from COVID-19 than those of white ethnicity, even after adjustment for other factors (Williamson et al., 2020). These lines of evidence suggest that SELENOF may be deeply involved in pathogenesis of COVID-19. A direct mechanistic link between reduced expression of SELENOF and increased production of inflammatory cytokines has been well documented (Curran et al., 2005). Carriers of the A-allele of the SELENOS -105G/A promoter polymorphism (rs28665122) have high levels of inflammatory cytokines (Curran et al., 2005). Hence down-regulation of SELENOS induced by SARS-CoV-2 may promote an inflammatory response.

Some large DNA viruses are equipped with their own ribonucleotide reductase to facilitate DNA synthesis for virus production (Taylor, 2020). Conversely, RNA viruses may attempt to suppress the diversion of ribonucleotides for DNA synthesis in order to enhance RNA synthesis. SARS-CoV-2 targets TXNRD3 by antisense at several sites, with computed interaction energies equivalent to the strongest microRNA interactions (Taylor, 2020). Consistent with that computational finding, our results provide experimental confirmation of the predicted antisense interaction, and show that SARS-CoV-2 significantly reduces mRNA concentration of TXNRD3 but not mRNA concentrations of TXNRD1 and TXNRD2. Pulmonary TXNRD3 protein levels are normally high according to the Human Protein Atlas (Uhlen et al., 2015). Thus, by knockdown of TXNRD3 at the mRNA level, possibly in coordination with proteolysis of TXNRD1 by the SARS-CoV-2 main protease M^{pro} (Taylor and Radding, 2020), SARS-CoV-2 may boost virus production in the lung, the major site of SARS-CoV-2 replication.

It has been documented that higher Se status ameliorates clinical symptoms caused by the infections of evolutionally diverse viruses including SARS-CoV-2 (Beck et al., 2004; Steinbrenner et al., 2015; Zhang et al., 2020a; Moghaddam et al., 2020); however, potential influences of viruses on host-cell selenoproteins have seldom been addressed. The strength of the present study is that it is the first study utilizing a BSL-3 laboratory to characterize the influence of SARS-CoV-2 on mRNA expression of selenoproteins; it has also shown that SARS-CoV-2 can significantly suppress mRNA expression of a number of selenoproteins. The limitations of the present study are that it only assessed selenoprotein mRNA concentrations without further evaluating protein and/or enzymatic activities, owing to the restrictions placed on what is allowed to be done in the BSL-3 laboratory to which we had

access.

In summary, our findings as summarized in Fig. 5 suggest that (i) SARS-CoV-2 suppresses GPX4 gene expression facilitating ferroptosis; (ii) the ER is an organelle that is severely affected by SARS-CoV-2 since SARS-CoV-2 suppressed the expression of SELENOF, SELENOM, SELENOK and SELENOS simultaneously; (iii) SARS-CoV-2 may boost virus production by down-regulating TXNRD3.

CRediT authorship contribution statement

Yijun Wang: performed the experiments, analyzed the data, wrote the manuscript. **Jinbao Huang:** performed the experiments, analyzed the data, wrote the manuscript. **Yong Sun:** performed the experiments. **David Stubbs:** performed the experiments. **Jun He:** performed the experiments. **Weiwei Li:** performed the experiments. **Fuming Wang:** performed the experiments. **Zhirong Liu:** analyzed the data. **Jan A. Ruzicka:** performed the experiments. **Ethan Will Taylor:** conceived and designed the experiments, analyzed the data, wrote the manuscript. **Margaret P. Rayman:** conceived and designed the experiments, wrote the manuscript. **Xiaochun Wan:** conceived and designed the experiments, analyzed the data, wrote the manuscript. **Jinsong Zhang:** conceived and designed the experiments, analyzed the data, wrote the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This study was supported by the Emergency Research Project of Novel Coronavirus Infection of Anhui Province (202004a07020002 and 202004a07020004), National Natural Science Foundation of China (31972459 and 31771971), Key Research and Development Program of Anhui Province (1804b06020367 and 201904b11020038), and by an unrestricted gift from the Dr. Arthur and Bonnie Ennis Foundation, Decatur, IL, to E.W. Taylor. The authors thank Zhuhui Zhang, Meng Wang, and Yinglu Ge (Anhui Provincial Center for Disease Control and Prevention) for their support of this study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.fct.2021.112286>.

References

- Alim, I., Caulfield, J.T., Chen, Y., Swarup, V., et al., 2019. Selenium drives a transcriptional adaptive Program to block ferroptosis and treat stroke. *Cell* 177, 1262–1279 e1225.
- Arner, E.S., Holmgren, A., 2006. The thioredoxin system in cancer. *Semin. Canc. Biol.* 16, 420–426.
- Beck, M.A., Handy, J., Levander, O.A., 2004. Host nutritional status: the neglected virulence factor. *Trends Microbiol.* 12, 417–423.
- Becker, N.P., Martitz, J., Renko, K., Stoedter, M., et al., 2014. Hypoxia reduces and redirects selenoprotein biosynthesis. *Metall* 6, 1079–1086.
- Bermano, G., Meplan, C., Mercer, D.K., Hesketh, J.E., 2020. Selenium and viral infection: are there lessons for COVID-19? *Br. J. Nutr.* 1–10.
- Chan, C.P., Siu, K.L., Chin, K.T., Yuen, K.Y., et al., 2006. Modulation of the unfolded protein response by the severe acute respiratory syndrome coronavirus spike protein. *J. Virol.* 80, 9279–9287.
- Codo, A.C., Davanzo, G.G., Monteiro, L.B., de Souza, G.F., et al., 2020. Elevated glucose levels favor SARS-CoV-2 infection and monocyte response through a HIF-1 α /Glycolysis-Dependent Axis. *Cell Metabol.* 32, 437–446 e435.
- Conrad, M., Proneth, B., 2020. Selenium: tracing another essential element of ferroptotic cell death. *Cell Chem Biol* 27, 409–419.
- Cuadrado, A., Pajares, M., Benito, C., Jimenez-Villegas, J., et al., 2020. Can activation of NRF2 Be a strategy against COVID-19? *Trends Pharmacol. Sci.* 41, 598–610.
- Curran, J.E., Jowett, J.B., Elliott, K.S., Gao, Y., et al., 2005. Genetic variation in selenoprotein S influences inflammatory response. *Nat. Genet.* 37, 1234–1241.
- Gubernatorova, E.O., Gorshkova, E.A., Polinova, A.I., Drutskaya, M.S., 2020. IL-6: relevance for immunopathology of SARS-CoV-2. *Cytokine Growth Factor Rev.* 53, 13–24.
- Hiffler, L., Rakotoambinina, B., 2020. Selenium and RNA virus interactions: potential implications for SARS-CoV-2 infection (COVID-19). *Front Nutr* 7, 164.
- Hu, Y.J., Korotkov, K.V., Mehta, R., Hatfield, D.L., et al., 2001. Distribution and functional consequences of nucleotide polymorphisms in the 3'-untranslated region of the human Sep15 gene. *Canc. Res.* 61, 2307–2310.
- Im, J.H., Je, Y.S., Baek, J., Chung, M.H., et al., 2020. Nutritional status of patients with COVID-19. *Int. J. Infect. Dis.* 100, 390–393.
- Jia, M., Qin, D., Zhao, C., Chai, L., et al., 2020. Redox homeostasis maintained by GPX4 facilitates STING activation. *Nat. Immunol.* 21, 727–735.
- Jin, Z., Du, X., Xu, Y., Deng, Y., et al., 2020. Structure of M(pro) from SARS-CoV-2 and discovery of its inhibitors. *Nature* 582, 289–293.
- Kumaraswamy, E., Korotkov, K.V., Diamond, A.M., Gladyshev, V.N., Hatfield, D.L., 2002. Genetic and functional analysis of mammalian Sep15 selenoprotein. *Methods Enzymol.* 347, 187–197.
- Labunskyy, V.M., Hatfield, D.L., Gladyshev, V.N., 2014. Selenoproteins: molecular pathways and physiological roles. *Physiol. Rev.* 94, 739–777.
- Liao, Y., Fung, T.S., Huang, M., Fang, S.G., et al., 2013. Upregulation of CHOP/GADD153 during coronavirus infectious bronchitis virus infection modulates apoptosis by restricting activation of the extracellular signal-regulated kinase pathway. *J. Virol.* 87, 8124–8134.
- Martitz, J., Becker, N.P., Renko, K., Stoedter, M., et al., 2015. Gene-specific regulation of hepatic selenoprotein expression by interleukin-6. *Metall* 7, 1515–1521.
- Moghaddam, A., Heller, R.A., Sun, Q., Seelig, J., et al., 2020. Selenium deficiency is associated with mortality risk from COVID-19. *Nutrients* 12.
- Rayman, M.P., 2012. Selenium and human health. *Lancet* 379, 1256–1268.
- Seale, L.A., Torres, D.J., Berry, M.J., Pitts, M.W., 2020. A role for selenium-dependent GPX1 in SARS-CoV-2 virulence. *Am. J. Clin. Nutr.* 112, 447–448.
- Shchedrina, V.A., Zhang, Y., Labunskyy, V.M., Hatfield, D.L., Gladyshev, V.N., 2010. Structure-function relations, physiological roles, and evolution of mammalian ER-resident selenoproteins. *Antioxidants Redox Signal.* 12, 839–849.
- Steinbrenner, H., Al-Quraishy, S., Dkhil, M.A., Wunderlich, F., Sies, H., 2015. Dietary selenium in adjuvant therapy of viral and bacterial infections. *Adv Nutr* 6, 73–82.
- Sun, Q.A., Su, D., Novoselov, S.V., Carlson, B.A., et al., 2005. Reaction mechanism and regulation of mammalian thioredoxin/glutathione reductase. *Biochemistry* 44, 14528–14537.
- Sun, K., Eriksson, S.E., Tan, Y., Zhang, L., et al., 2014. Serum thioredoxin reductase levels increase in response to chemically induced acute liver injury. *Biochim. Biophys. Acta* 1840, 2105–2111.
- Sunde, R.A., Raines, A.M., 2011. Selenium regulation of the selenoprotein and nonselenoprotein transcriptomes in rodents. *Adv Nutr* 2, 138–150.
- Sureda, A., Alizadeh, J., Nabavi, S.F., Berindan-Neagoe, I., et al., 2020. Endoplasmic reticulum as a potential therapeutic target for covid-19 infection management? *Eur. J. Pharmacol.* 882, 173288.
- Taylor, E.W., 2020. RNA viruses vs. DNA synthesis: a general viral strategy that may contribute to the protective antiviral effects of selenium. *Preprints* 2020060069. <https://doi.org/10.20944/preprints202006.0069.v1>.
- Taylor, E.W., Radding, W., 2020. Understanding selenium and glutathione as antiviral factors in COVID-19: does the viral M(pro) protease target host selenoproteins and glutathione synthesis? *Front. Nutr.* 7, 143.
- Taylor, E.W., Ruzicka, J.A., Premadasa, L., Zhao, L., 2016. Cellular selenoprotein mRNA tethering via antisense interactions with Ebola and HIV-1 mRNAs may impact host selenium biochemistry. *Curr. Top. Med. Chem.* 16, 1530–1535.
- Uhlen, M., Fagerberg, L., Hallstrom, B.M., Lindskog, C., et al., 2015. Proteomics. Tissue-based map of the human proteome. *Science* 347, 1260419.
- Verma, S., Molina, Y., Lo, Y.Y., Cropp, B., et al., 2008. In vitro effects of selenium deficiency on West Nile virus replication and cytopathogenicity. *Virol. J.* 5, 66.
- Versteeg, G.A., van de Nes, P.S., Bredenbeek, P.J., Spaan, W.J., 2007. The coronavirus spike protein induces endoplasmic reticulum stress and upregulation of intracellular chemokine mRNA concentrations. *J. Virol.* 81, 10981–10990.
- Wang, X., Sun, K., Tan, Y., Wu, S., Zhang, J., 2014. Efficacy and safety of selenium nanoparticles administered intraperitoneally for the prevention of growth of cancer cells in the peritoneal cavity. *Free Radical Biol. Med.* 72, 1–10.
- Williamson, E.J., Walker, A.J., Bhaskaran, K., Bacon, S., et al., 2020. Factors associated with COVID-19-related death using OpenSAFELY. *Nature* 584, 430–436.
- Zhang, J., Taylor, E.W., Bennett, K., Saad, R., Rayman, M.P., 2020a. Association between regional selenium status and reported outcome of COVID-19 cases in China. *Am. J. Clin. Nutr.* 111, 1297–1299.
- Zhang, J., Saad, R., Taylor, E.W., Rayman, M.P., 2020b. Selenium and selenoproteins in viral infection with potential relevance to COVID-19. *Redox Biol* 37, 101715.