Gene Expression Profiling of Endoplasmic Reticulum Stress in Hepatitis C Virus-Containing Cells Treated with an Inhibitor of Protein Disulfide Isomerases

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Supporting Information

ABSTRACT: Protein disulfide isomerases (PDIs) catalyze disulfide bond formation between protein cysteine residues during protein folding in the endoplasmic reticulum (ER) lumen and are essential for maintaining ER homeostasis. The life cycle of the hepatitis C virus (HCV) is closely associated with the ER. Synthesis and maturation of HCV proteins occur in the ER membrane and are mediated by multiple host cell factors that include also PDI. Here, we present a study investigating the effect of PDI inhibition on Huh7 human hepatoma cells harboring an HCV subgenomic replicon using the abscisic acid-derived PDI inhibitor origamicin. Transcriptional profiling shows that origamicin changed the expression levels of genes involved in the oxidative and ER stress responses and the unfolded protein response, as indicated by the upregulation of antioxidant enzymes and chaperone proteins, the downregulation of cell-cycle proteins, and induction of apoptosis-associated genes. Our data suggest that origamicin negatively impacts HCV replication by causing an imbalance in cellular homeostasis and induction of stress responses. These insights suggest that inhibition of PDIs by low-molecular-weight inhibitors could be a promising approach to the discovery of novel antiviral compounds.

INTRODUCTION

A cell is a spatially limited environment of tightly regulated biological processes that ensure cell viability under varying external conditions. Cells respond to stress conditions by activating various counter measures, a prominent example being the endoplasmic reticulum (ER) stress response.1,2 The ER plays a key role in the proteosynthesis and folding of secretory and transmembrane proteins.3 Perturbation of ER homeostasis impairs these processes and leads to accumulation of misfolded and unfolded proteins in the ER lumen,1,2 triggering the unfolded protein response (UPR), which includes the upregulation of molecular chaperones and the protein degradation machinery.4 Another well-known example of a cellular stress condition is oxidative stress, which usually results from the increased concentration of reactive oxygen species (ROS).5,6 Examples of ROS are hydrogen peroxide (H₂O₂), hypochlorous acid (HOCl), or superoxide anion (O₂⁻). ROS cause oxidative stress by oxidatively modifying proteins, impair their function, and induce protein unfolding and aggregation.5,7,8 ROS also play an important role in several physiological processes provided their location and activity are within a narrow range and the overall cellular redox homeostasis is not disrupted.7 For instance, hydrogen peroxide can act as a regulator of cell proliferation, signal transduction, apoptosis, and is involved in the formation of disulfide bonds in proteins.6,9−11

The protein disulfide bond formation in the ER is mediated by sequential action of oxidoreduction 1 (Ero1α and Ero1β) and protein disulfide isomerase (PDI).11−13 The cascade is initiated by catalytic transfer of electrons from molecular oxygen to two cysteines in Ero1 that form a disulfide bond.14,15 The oxidized Ero1 interacts with PDI and catalyzes formation of a disulfide bond in PDI while being reduced. Finally, oxidized PDI is primed to catalyze the oxidation of a cysteine...
pair in a client protein, creating either a de novo disulfide bond or rearranging a pre-existing one.  

Although neither the X-ray nor the nuclear magnetic resonance structure of full-length human PDI has been determined, structural information derived from crystal structures of PDI fragments and PDI functional homologs has been obtained and suggests that PDI has a horseshoe shape consisting of four thioredoxin domains and a linker region termed \( \alpha' \). These structural domains are arranged in the order \( \alpha, \beta, \beta', \alpha' \), and \( \alpha' \) thereby creating the overall architecture of PDI. Both the \( \alpha \) and \( \alpha' \) thioredoxin domains have an active site that include a pair of cysteines within a \(-CXXC-\) motif. The \( \beta \) and \( \beta' \) domains, however, lack catalytic activity. The linker connecting the domains \( \beta' \) and \( \alpha' \) provides structural flexibility and modulates access to substrate proteins.  

The catalytically active cysteine pairs present in the \( \alpha \) and \( \alpha' \) domains represent the molecular basis of PDI’s redox activity. Notably, PDI also possesses an additional chaperone activity. Apparently, this chaperone activity functions independently of the redox activity, however, recent experiments cast doubt on this assumption. The chaperone activity of PDI plays a role in protein folding and acts to avoid protein misfolding and protein aggregation. The PDI chaperone function is important for cell survival under conditions of elevated temperature or oxidative stress. In summary, PDI plays a crucial role in oxidative protein folding and ER homeostasis because of its dual role as an oxidoreductase and molecular chaperone.  

In this study, we investigated how PDI inhibition affects replication of the hepatitis C virus (HCV), a member of the hepacivirus genus. The positive-sense single-stranded RNA replication of the hepatitis C virus (HCV), a member of the hepacivirus genus. The positive-sense single-stranded RNA replication of the hepatitis C virus (HCV), a member of the hepacivirus genus. The positive-sense single-stranded RNA replication of the hepatitis C virus (HCV), a member of the hepacivirus genus. The positive-sense single-stranded RNA replication of the hepatitis C virus (HCV), a member of the hepacivirus genus. The positive-sense single-stranded RNA replication of the hepatitis C virus (HCV), a member of the hepacivirus genus. The positive-sense single-stranded RNA replication of the hepatitis C virus (HCV), a member of the hepacivirus genus. The positive-sense single-stranded RNA replication of the hepatitis C virus (HCV), a member of the hepacivirus genus. The positive-sense single-stranded RNA replication of the hepatitis C virus (HCV), a member of the hepacivirus genus.

![Figure 1](https://example.com/figure1.png)  

**Figure 1.** Study design. Chemical structures of (a) (+)-S-ABA (top) and origmaticin (bottom). (b) Schematic representation of the subgenomic HCV replicon (pFK-I389neo/luc/NS3-3’/5.1) used in this study. (c) Overview of the experimental workflow.

The volcano plots indicate that only a few genes showed statistically significant expression changes at the lower concentration, whereas the higher concentration cyclohexenone ring (Figure 1A, bottom), allowing bio-orthogonal labeling. We investigated the effect of origmaticin treatment on global mRNA levels by using a hepatocyte cell line (Huh7) harboring the HCV subgenomic replicon pFK-I389neo/luc/NS3-3’/5.1 (Figure 1B). This replicon system, termed E9 cells, is widely used to study the replication of HCV in a cell culture model. We treated the replicon cells with two concentrations of origmaticin (i.e., mock, 25 and 50 μM) for 24 h. To confirm that PDI inhibition impairs HCV replication in our model system, we also tested the effect of two well-described PDI inhibitors, 16F16 and securinine (Supporting Information, Figure S1), which showed similar antiviral activity, further supporting a role for PDI in the viral life cycle. After the treatment, we extracted the total RNA and performed an Affymetrix microarray RNA quantification to identify genes with changed mRNA expression level. The in silico analysis of the expression profile also included pathway and gene ontology (GO) analyses, and was subsequently validated by quantitative real-time polymerase chain reaction (qRT-PCR) on selected differentially expressed mRNAs. An overview of the experimental workflow is depicted in Figure 1C.

**Origamicin Induces Changes in the mRNA Profile in HCV Replicon Cells.** We performed an Affymetrix microarray hybridization experiment to identify the gene expression profile of origmaticin-treated Huh7 cells harboring the HCV subgenomic replicon. After the data preprocessing, we performed a hierarchical cluster analysis as well as a principal component analysis (PCA) to determine the overall similarity among mRNA profiles under the tested conditions (Supporting Information, Figure S2). We found that the mRNA profile of cells treated with 25 μM origmaticin, which corresponds to the origmaticin HCV replicon IC\(_{50}\) value, was similar to that of the mock-treated cell. However, when Huh7 cells were treated with 50 μM origmaticin concentration, which was reported to have a strong inhibitory effect on the HCV replicons, we observed pronounced changes in the mRNA profile. Volcano plots, which relate the fold change of gene expression to the \( p \)-value of the corresponding change, nicely illustrate this observation (Supporting Information, Figure S3). The volcano plots indicate that only a few genes showed statistically significant expression changes at the lower origmaticin concentration, whereas the higher concentration.
caused expression changes in a substantially larger number of genes.

In order to further study the origamicin-induced changes in mRNA levels, we removed low-intensity mRNA signals from the data set and also excluded mRNAs showing an expression change of less than two, and finally performed correction for the false discovery rate (FDR) using the Benjamin-Hochberg procedure. We found that 25 μM origamicin induced the differential expression of 165 genes of which 88 were upregulated, whereas the treatment with 50 μM origamicin increased the number of differentially expressed genes (DEGs) more than 13-fold to 2196 with a ratio of upregulated versus downregulated of nearly 2:1 (Figure 2A). Interestingly, almost all DEGs at the lower concentration of 25 μM origamicin are part of the DEGs at 50 μM origamicin (Figure 2B). A heat map of all the 157 DEGs shared between both treatment conditions revealed similarities in the gene expression pattern. In addition, the expression changes of these selected DEGs showed the same pattern as the hierarchical cluster analysis and the PCA of the global gene expression.

**Origamicin Affects Cellular Redox Homeostasis and Stress Responses.** We focused on the upregulated genes in the subsequent analysis. The lists of the top 25 upregulated genes in the 25 μM origamicin treatment (Table 1) and the ones in the 50 μM origamicin treatment (Table 2) show a similar pattern of upregulated genes.

Interestingly, we noticed the enrichment of several genes that are involved in cellular stress responses, for example, PTGR1, HMOX1, and S100P. PTGR1 acts as an antioxidant enzyme and HMOX1 is a well-established component of the oxidative stress response. S100P was described as upregulated during the ER stress response and as being involved in apoptosis. To verify our initial conclusions, we performed pathway analysis and GO classification analysis on the identified DEGs. However, we were not able to find a clear pattern among the upregulated genes for the 25 μM origamicin treatment. As described above, the general mild effect of this treatment condition is likely to have only a small effect on the gene expression profile. The absence of an obvious pattern among the downregulated genes at this treatment condition supports this assumption (Supporting Information, Table S1). By contrast, pathway analysis of the identified upregulated genes in the 50 μM origamicin treatment regimen indicated that these genes are all involved in the UPR and in ER-related pathways (Figure 3A). Furthermore, GO classification analysis on the high concentration treatment regime shows an accumulation of genes involved in the ER and oxidative stress response, the unfolding protein response, and apoptosis-related processes (Figure 3B). Interestingly, a majority of all genes downregulated by 50 μM origamicin (Supporting Information, Table S2) are part of the cell cycle and cell division pathways (Supporting Information, Figure S4). This observation is in agreement with previous reports of initiation of cell cycle arrest in the G1 phase mediated by the protein kinase R (PKR)-like endoplasmic reticulum kinase (PERK) receptor. The cell cycle arrest is caused by the phosphorylation of eIF2, which also causes translational attenuation to prevent the ER from further protein loading, consistent with the changes in mRNA levels that we observe related to cell cycle arrest.

**Experimental Validation of the in Silico Analysis by qRT-PCR.** We validated our computational analysis on selected candidate genes that were previously identified as upregulated in both treatment conditions and showed a broad range of expression changes (Supporting Information, Table S3).
Therefore, we repeated the treatment of E9 cells with 25 μM origamicin, extracted the RNA, and performed qRT-PCR quantification of mRNAs for selected candidate genes: PTGR1, AKR1C1, HMOX1, BLVRB, S100P, CSTA, G6PD, CBR1, and NQO1. All of these genes showed significant upregulation as expected (Figure 4A). Moreover, the magnitude of the expression change was in agreement with our microarray data indicated by a Pearson’s product-moment correlation coefficient of $r = 0.81$ (Figure 4B).

| Table 1. Top 25 Genes Upregulated Upon Treatment with 25 μM Origamicin |
|---------------------------------------------------------------|
| HGNC gene title | gene symbol | log2 FC | adj. p val |
|-----------------|------------|---------|------------|
| lactate dehydrogenase B | LDHB | 4.1614 | 0.0007 |
| prostaglandin reductase 1 | PTGR1 | 3.8695 | 0.0008 |
| TIMP metallopeptidase inhibitor 4 | TIMP4 | 2.6268 | 0.0020 |
| heme oxygenase 1 | HMOX1 | 2.4266 | 0.0014 |
| secreted phosphoprotein 1 | SPP1 | 2.1407 | 0.0020 |
| PTGR1 | 1.9536 | 0.0014 |
| AKR1C1 | 1.9984 | 0.0071 |
| S100 calcium binding protein P | S100P | 1.9409 | 0.0005 |
| AKR1C1 | 1.8081 | 0.0020 |
| biliverdin reductase B | BLVRB | 1.7900 | 0.0008 |
| microtubule-associated protein 1A | MAP1A | 1.6942 | 0.0015 |
| tumor necrosis factor superfamily member 9 | TNFSF9 | 1.6447 | 0.0092 |
| coagulation factor II-thrombin receptor like 2 | F2RL2 | 1.6105 | 0.0133 |
| complement C1s | C1S | 1.6080 | 0.0080 |
| cell cycle progression 1 | CCG1 | 1.5958 | 0.0016 |
| small integral membrane protein 14 | SMIM14 | 1.5908 | 0.0016 |
| neuronal cell adhesion molecule | NRCAM | 1.5658 | 0.0096 |
| asparagine synthetase (glutamine-hydrolyzing) | ASNS | 1.5463 | 0.0007 |
| G protein subunit α L | GNAL | 1.5241 | 0.0025 |
| kynureninase | KNU | 1.4894 | 0.0122 |
| uncharacterized LOC1053377348 | LOC1053377348 | 1.4676 | 0.0118 |
| cystatin A | CSTA | 1.4530 | 0.0042 |
| nuclear protein 1, transcriptional regulator | NUPR1 | 1.4346 | 0.0196 |

| Table 2. Top 25 Genes Upregulated upon Treatment with 50 μM Origamicin |
|---------------------------------------------------------------|
| HGNC gene title | gene symbol | log2 FC | adj. p val |
|-----------------|------------|---------|------------|
| prostaglandin reductase 1 | PTGR1 | 5.9905 | 0.0000 |
| family with sequence similarity 129 member A | FAM129A | 5.0422 | 0.0002 |
| TIMP metallopeptidase inhibitor 4 | TIMP4 | 5.0177 | 0.0000 |
| heme oxygenase 1 | HMOX1 | 4.7228 | 0.0000 |
| nuclear protein 1, transcriptional regulator | NUPR1 | 4.5687 | 0.0000 |
| lactate dehydrogenase B | LDHB | 4.5585 | 0.0000 |
| PTGR1 | 4.3559 | 0.0000 |
| AKR1C1 | 3.9627 | 0.0000 |
| S100P | 3.8402 | 0.0000 |
| CSTA | 3.7008 | 0.0002 |
| HMOX1 | 3.5927 | 0.0001 |
| BLVRB | 3.3751 | 0.0001 |
| MAP1A | 3.2877 | 0.0000 |
| TNFSF9 | 3.2408 | 0.0000 |
| S100P | 3.1793 | 0.0002 |
| OSGIN1 | 3.1367 | 0.0001 |
| PRNP | 3.1717 | 0.0000 |
| SLC16A14 | 3.1883 | 0.0000 |
| BLVRB | 3.1650 | 0.0001 |
| GDF15 | 3.1372 | 0.0001 |
| PABPC1L | 3.1283 | 0.0001 |
| SEL1L family member 3 | SEL1L3 | 3.0400 | 0.0001 |
| MALAT1 | 2.8536 | 0.0001 |
| NRCAM | 2.7128 | 0.0003 |
| ASNS | 2.5277 | 0.0001 |
| GNAL | 2.5933 | 0.0001 |
| KNU | 2.5909 | 0.0009 |
| LOC1053377348 | 2.5584 | 0.0008 |
| CSTA | 2.4961 | 0.0008 |
| NUPR1 | 2.4592 | 0.0000 |
| MALAT1 | 2.3856 | 0.0001 |
| CTH | 2.3128 | 0.0003 |
| ATIP | 2.2843 | 0.0000 |
| AKR1C2 | 2.2607 | 0.0000 |
| SPX | 2.1717 | 0.0000 |
| MALAT1 | 2.0539 | 0.0001 |

It should be noted that several of these selected genes (e.g. HMOX1, S100P, CSTA) are known for their inhibitory role in HCV replication.
Here, we present new evidence for PDI inhibition by origamicin, a relatively uncharacterized inhibitor of PDIs, originally derived from the plant hormone ABA.32 Unlike ABA, this synthetic small molecule inhibits the activity of PDI.32 This indicates that the introduced chemical modifications converted ABA into a new type of PDI inhibitor.

To our knowledge, this is the first study focusing on the effect of PDI inhibition on cellular metabolism using comparative gene expression analysis. We identified a large number of DEGs upon origamicin treatment. Remarkably, all upregulated genes among these DEGs are linked to protein unfolding response, redox homeostasis, and ER stress. As PDI is directly involved in oxidative protein folding in the ER, we conclude that exposure of cells to this PDI inhibitor impairs protein folding in the ER lumen and induces ER stress (Figure 5). The cellular counter measure for ER stress is the UPR, which can induce apoptosis as a long-term response.51 The immediate response, however, is the PERK-mediated translation attenuation and cell cycle arrest as well as upregulation of proteins involved in assisting protein folding, that is, chaperones.52 A common example of such a chaperone in the context of ER stress is HSPAS (also referred to as BiP or GRP78).53,54 We were not able to detect upregulation of HSPA5 in our study; however, it has been shown that hepatocytes Hsp40 and Hsp70 are potent modulators of the UPR.53 This is in agreement with our finding that several HSP40 or HSP70 chaperones are significantly upregulated (e.g., Hsp40B4, Hsp40B9, Hsp70A1, Hsp70B1).

HCV replication critically depends on optimal metabolic conditions that include an efficiently working protein biosynthesis machinery.56 In addition, HCV is heavily dependent on a proper ER environment because of the location of HCV’s viral factories in the ER and the dependency on the ER for viral egress.57,58 Hence, it is expected that perturbation of the ER homeostasis through PDI inhibition causes disruption of the HCV life cycle but the exact mechanism has to be elucidated in further studies.

Moreover, the formation of protein disulfide bonds in the glycoproteins E1 and E2 is a prerequisite for the assembly of infectious viral particles.31,59 Consequently, the failure of the disulfide bond formation system, mainly constituted by PDIs, might substantially affect the production of infectious viral particles. We are aware of the limitations of a subgenomic replicon system lacking these envelope proteins and, hence, HCV replicon models are not a suitable choice to study viral assembly and egress. Further research using infectious virus is needed to determine the antiviral effect of PDI inhibition on HCV’s life cycle. There are other (flavi)viruses—such as the dengue virus or Zika virus—similar to HCV are also strongly dependent on ER homeostasis and PDI activity.60 Thus, they are interesting targets for further investigating the impact of PDI inhibition on viral life cycles.

**CONCLUSIONS**

Our findings have implications for the role of PDI in the life cycle of HCV and potentially for other members of the flaviviridae family. We conclude that chemical modification of naturally occurring molecules is a promising approach to provide new antivirals and novel drugs for other diseases, which are desperately needed.
Tissue Culture. Huh-7 human hepatoma cells, a kind gift from Dr. Lubica Supekova (The Scripps Research Institute, La Jolla, CA), harboring the HCV subgenomic replicon plasmid pFK-1389neo/luc/NS3-3′/5.1, obtained as previously described,33 were grown in Dulbecco’s modified Eagle medium (Gibco-Invitrogen, Burlington, ON, Canada) supplemented with 100 nmol nonessential amino acids/L (Gibco, Burlington, ON, Canada), 50 U penicillin/mL, 50 μg/mL streptomycin, 10% fetal bovine serum (Cansera International, Rexdale, ON; PAA Laboratories, Etobicoke, ON, Canada), and 250 μg/mL G418 Geneticin (Gibco, Burlington, ON, Canada). The pFK-1389neo/luc/NS3-3′/5.1 plasmid that contains HCV subgenomic replicons and expresses HCV nonstructural proteins (NS3 to NS5B) from the encephalomyocarditis virus internal ribosome entry site was utilized as previously described.32,60,61 Cells were either mock-treated with methanol or treated with origamicin (provided by Suzanne Abrams) with a final concentration of 25 or 50 μM, respectively, for 24 h and then harvested.

Total RNA Preparation for Microarray Experiments. Total RNA was prepared using the RNeasy Mini kit (Qiagen, Mississauga, Ontario) as per the manufacturer’s protocols. The RNA was quantified using the NanoDrop ND-1000 spectrophotometer, and the quality of the RNA was verified using the RNA 6000 Pico LabChip kit (Agilent Technologies, Mississauga, Ontario).

Gene Expression Analysis. Cellular RNA samples were hybridized to high-density oligonucleotide arrays (Affymetrix GeneChip HG-U133 Plus2). Primary image analysis was performed by using GeneChip version 3.1 (Affymetrix, Santa Clara, CA). The data sets were processed as previously described62 and subsequently converted to log, transformed data. A first analysis for DEGs (log, fold change cut-off > 2) was performed using a two-sided t-test for unpaired data with equal variances (p-value cut-off < 0.05). The R packages “genefilter” and “limma” were used to further analyze and filter the data set.63,64 Genes with expression values lower than the mean in at least three microarray samples were deemed “absent” and excluded from the subsequent analysis. DEGs were determined using the empirical Bayes approach in combination with multiple hypothesis testing based on the methods of Benjamini and Hochberg.36 An adjusted FDR p-value of <0.05 was set as the threshold for significance. Genes with expression values lower than the mean in at least three microarray samples were deemed “absent” and excluded from the subsequent analysis. DEGs were determined using the empirical Bayes approach in combination with multiple hypothesis testing based on the methods of Benjamini and Hochberg.36 An adjusted FDR p-value of <0.05 was set as the threshold for significance. Genes were annotated using the Affymetrix annotation files (HG-U133_Plus_2; release 36) in conjunction with GeneAnnot 2.2.65 Pathway and GO analyses were performed using the ToppGene Suite.66 Affymetrix raw data are provided in the Supporting Information.

Luciferase Reporter Assay and Total Protein Quantification. Huh-7 cells stably expressing HCV subgenomic replicons were treated with PDI inhibitors (i.e., 16F16 and securinine, Sigma-Aldrich) for 24 h as described previously.32 Briefly, cells were washed twice in PBS and lysed with cell culture lysis buffer (Promega, Madison, WI). Luciferase assay substrate was added, and expression of luciferase was determined by bioluminescence measurement on an Lmax luminometer ( Molecular Devices Corporation, Sunnyvale, CA). Total protein concentration was determined by BioRad DC protein assay (Bio-Rad, Mississauga, Canada) according to the manufacturer’s protocol. EC50 values were...
determined by plotting luminescence activity against the concentration values and fit using a four-parameter equation.

Quantitative Real-Time Polymerase Chain Reaction. RNA isolation and quantification was performed as described above. The RNA integrity was confirmed by electrophoresis on 1.0% agarose gel in 1× TBE (Ambion). Reverse transcription of 500 ng of total RNA using the Superscript II RT kit (Invitrogen) according to the manufacturer’s protocols was performed on a T100 thermalycler (Bio-Rad). Quantitative PCR was subsequently performed on a CFx thermal cycler (Bio-Rad) using iQ SYBR Green Supermix (Bio-Rad), as per the manufacturer’s protocol. Primer sequences are listed in the Supporting Information, Table S4. Fold changes in expression relative to mock-treated samples were calculated with the 2−ΔΔCt method using 18S rRNA levels for normalization. Data are presented as the mean of replicates. Statistical significance was evaluated using unpaired two-tailed Student’s t-test. Correlation analysis was performed using Pearson’s product-moment correlation coefficient.

Statistical Analysis. We used the auxiliary R packages “gplots”, “plotrix”, “ggplot2”, and “Venn diagram”7,72 and functions such as cor.test() for data analysis and drawing figures.

ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.8b02676.

HCV replication inhibition by PDI inhibitors, clustering of microarray data, volcano plots of gene expression, GO and pathway analyses, top 25 genes downregulated genes, selected genes, oligonucleotides used in this study (PDF)

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Notes
The authors declare no competing financial interest.

ACKNOWLEDGMENTS
This work was supported by a grant from the Canadian Institutes of Health Research (CIHR) and Natural Sciences and Engineering Research Council (NSERC) of Canada. D.O. was supported by a postdoctoral fellowship from the CIHR. The authors would like to thank Prof. Dr. Suzanne Abrams for providing origamin and Sylvie Bélanger for technical assistance.

REFERENCES
(1) Ron, D.; Walter, P. Signal integration in the endoplasmic reticulum unfolded protein response. *Nat. Rev. Mol. Cell Biol.* 2007, 8, 519–529.
(2) Zhang, K.; Kaufman, R. J. The unfolded protein response: a stress signaling pathway critical for health and disease. *Neurology* 2006, 66, S102–S109.
(3) Hebert, D. N.; Molinari, M. In and Out of the ER: Protein Folding, Quality Control, Degradation, and Related Human Diseases. *Physiol. Rev.* 2007, 87, 1377–1408.
(4) Gardner, B. M.; Pincus, D.; Gotthardt, K.; Gallagher, C. M.; Walter, P. Endoplasmic reticulum stress sensing in the unfolded protein response. *Cold Spring Harbor Perspect. Biol.* 2013, 5, a013169.
(5) Imlay, J. A. Pathways of oxidative damage. *Annu. Rev. Microbiol.* 2003, 57, 395–418.
(6) Suzuki, Y. J.; Forman, H. J.; Sevanian, A. Oxidants as stimulators of signal transduction. *Free Radical Biol. Med.* 1997, 22, 269–285.
(7) Winter, J.; Ilbert, M.; Graf, P. C. F.; Ossicelli, D.; Jakob, U. Bleach activates a redox-regulated chaperone by oxidative protein unfolding. *Cell* 2008, 135, 691–701.
(8) Malhotra, J. D.; Kaufman, R. J. Endoplasmic reticulum stress and oxidative stress: a vicious cycle or a double-edged sword? *Antioxid. Redox Signaling* 2007, 9, 2277–2294.
(9) Nakamura, H.; Nakamura, K.; Yodoi, J. Redox regulation of cellular activation. *Annu. Rev. Immunol.* 1997, 15, 351–369.
(10) Pierce, G. B.; Parchment, R. E.; Lewellyn, A. L. Hydrogen peroxide as a mediator of programmed cell death in the blastocyst. *Differentiation* 1991, 46, 181–186.
(11) Ali Khan, H.; Mutus, B. Protein disulfide isomerase is a multifunctional protein with multiple physiological roles. *Front. Chem.* 2014, 2, 70.
(12) Sevier, C. S.; Kaiser, C. A. Ero1 and redox homeostasis in the endoplasmic reticulum. *Biochem. Biophys. Acta Mol. Cell Res.* 2008, 1783, 549–556.
(13) Benham, A. M.; van Lith, M.; Sittia, R.; Braakman, I. Ero1-PDI interactions, the response to redox flux and the implications for disulflde bond formation in the mammalian endoplasmic reticulum. *Philos. Trans. R. Soc., B* 2013, 368, 20110403.
(14) Tu, B. P.; Weissman, J. S. The FAD- and O2-dependent reaction cycle of Ero1-mediated oxidative protein folding in the endoplasmic reticulum. *Mol. Cell* 2002, 10, 983–994.
(15) Gross, E.; et al. Generating disulfides enzymatically: reaction products and electron acceptors of the endoplasmic reticulum thiol oxidase Ero1p. *Proc. Natl. Acad. Sci. U.S.A.* 2006, 103, 299–304.
(16) Hatahet, F.; Ruddock, L. W. Protein Disulfide Isomerase: A Critical Evaluation of Its Function in Disulfide Bond Formation. *Antioxid. Redox Signaling* 2009, 11, 2807–2850.
(17) Tian, G.; Xiang, S.; Noiva, R.; Lennarz, W. J.; Schindelin, H. The crystal structure of yeast protein disulfide isomerase suggests cooperativity between its active sites. *Cell* 2006, 124, 61–73.
(18) Tian, G.; et al. The catalytic activity of protein-disulfide isomerase requires a conformationally flexible molecule. *J. Biol. Chem.* 2008, 283, 33630–33640.
(19) Nguyen, V. D.; et al. Alternative Conformations of the x Region of Human Protein Disulfide-Isomerase Module Exposure of the Substrate Binding b’ Domain. *J. Mol. Biol.* 2008, 383, 1144–1155.
(20) Wang, C.; et al. Plasticity of Human Protein Disulfide Isomerase. *J. Biol. Chem.* 2010, 285, 26788–26797.
(21) Darby, N. J.; Creighton, T. E. Functional properties of the individual thio redox-like domains of protein disulfide isomerase. *Biochemistry* 1995, 34, 11725–11735.
(22) Wilson, R.; Lees, J. F.; Bulleid, N. J. Protein disulfide isomerase acts as a molecular chaperone during the assembly of procollagen. *J. Biol. Chem.* 1998, 273, 9637–9643.
(23) Quan, H.; Fan, G.; Wang, C.-c. Independence of the Chaperone Activity of Protein Disulfide Isomerase from Its Thioredoxin-like Active Site. J. Biol. Chem. 1995, 270, 17078–17080.
(24) Wang, C.; et al. Structural insights into the redox-regulated dynamic conformations of human protein disulfide isomerase. Antioxid. Redox Signaling 2013, 19, 36–45.
(25) Ngiam, C.; Jeennes, D. J.; Punt, P. J.; Van Den Houdel, C. A. M. J.; Archer, D. B. Characterization of a Foldase, Protein Disulfide Isomerase A, in the Protein Secretory Pathway of Aspergillus niger. Appl. Environ. Microbiol. 2000, 66, 775–782.
(26) Stapleton, J. T.; Fourg, S.; Muezhoff, A. S.; Buhk, J.; Simmonds, P. The GB viruses: A review and proposed classification of GBV-A, GBV-C (HGV), and GBV-D in genus Pegivirus within the family Flaviviridae. J. Gen. Virol. 2011, 92, 233–246.
(27) Reed, K. E.; Rice, C. M. Overview of hepatitis C virus genome structure, polyprotein processing, and protein properties. Curr. Top. Microbiol. Immunol. 2000, 242, 55–84.
(28) Hsu, N.-Y.; et al. Viral reorganization of the secretory pathway generates distinct organelles for RNA replication. Cell 2010, 141, 799–811.
(29) Brass, V.; Gosert, R.; Morandpour, D. Investigation of the hepatitis C virus replication complex. Methods Mol. Biol. 2009, 510, 195–209.
(30) Dubuisson, J. Hepatitis C virus proteins. World J. Gastroenterol. 2007, 13, 2406.
(31) Dubuisson, J.; Rice, C. M. Hepatitis C virus glycoprotein folding: disulfide bond formation and association with calnexin. J. Virol. 1996, 70, 778–786. https://jvi.asm.org/content/70/4/778.long.
(32) Ralak, B.; et al. A Small-Molecule Probe for Hepatitis C Virus Replication that Blocks Protein Folding. Chem. Biol. 2006, 13, 1051–1060.
(33) Sletten, E. M.; Bertozzi, C. R. Bioorthogonal chemistry: fishing for selectivity in a sea of functionality. Angew. Chem., Int. Ed. 2009, 48, 6974–6998.
(34) Lohmann, V.; et al. Replication of subgenomic hepatitis C virus RNAs in a hepatoma cell line. Science 1999, 285, 110–113.
(35) Hoffstom, B. G.; Kaplan, A.; Letso, R.; Schmid, R. S.; Turmel, G. J.; Lo, D. C.; Stockwell, B. R. Inhibitors of protein disulfide isomerase suppress apoptosis induced by misfolded proteins. Nat. Chem. Biol. 2010, 6, 900–906.
(36) Benjamini, Y.; Hochberg, Y. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. J. Am. Stat. Assoc. 1995, 97, 289–300. http://www.jstor.org/stable/2346101
(37) Na, H.-k.; et al. Tobacco smoking-response genes in blood and buccal cells. Toxicol. Lett. 2015, 232, 429–437.
(38) Poss, K. D.; Toney, S. Heme oxygenase 1 is required for mammalian iron reutilization. Proc. Natl. Acad. Sci. U.S.A. 1997, 94, 10919–10924.
(39) Namba, T.; et al. Up-regulation of S100P expression by non-steroidal anti-inflammatory drugs and its role in anti-tumorigenic insulin action, and type 2 diabetes. Science 2004, 306, 457–461.
(40) Bernales, S.; Papa, F. R.; Walter, P. Intracellular signaling by the unfolded protein response. Annu. Rev. Cell Dev. Biol. 2006, 22, 487–508.
(41) Burczynski, M. E.; Sridhar, G. R.; Palackal, N. T.; Penning, T. M. The Reactive Oxygen Species- and Michael Acceptor-inducible Human Aldo-Keto Reductase AKR1C1 Reduces the α,β-Unsaturated Aldehyde 4-Hydroxy-2-nonenal to 1,4-Dihydroxy-2-nonenal. J. Biol. Chem. 2001, 276, 2890–2897.
(42) Wu, S.; et al. BLVRB redox mutation defines heme degradation in a metabolic pathway of enhanced thrombopoeisis in humans. Blood 2016, 128, 699–709.
(43) Westerink, W. M. A.; Stenson, J. C. R.; Horbach, G. J.; Schoonen, W. G. E. J. The development of RAD51C, Cystatin A, p53 and Nrf2 luciferase-reporter assays in metabolically competent HepG2 cells for the assessment of mechanism-based genotoxicity and of oxidative stress in the early research phase of drug development. Mutat. Res., Genet. Toxicol. Environ. Mutagen. 2010, 696, 21–40.
(44) Ho, H.-y.; Cheng, M.-l.; Chiu, D. T.-y. Glucose-6-phosphate dehydrogenase—from oxidative stress to cellular functions and degenerative diseases. Redox Rep. 2007, 12, 109–118.
(45) Kim, Y. N.; et al. Neuroprotective effects of PEP-1-carbonyl reductase 1 against oxidative-stress-induced ischemic neuronal cell damage. Free Radical Biol. Med. 2014, 69, 181–196.
(46) Dinkova-Kostova, A. T.; Talalay, P. NAD(P)H:quinone acceptor oxidoreductase 1 (NQO1), a multifunctional antioxidant enzyme and exceptionally versatile cytoprotector. Arch. Biochem. Biophys. 2010, S01, 116–123.
(47) Shan, Y.; Zheng, J.; Lambrecht, R. W.; Bonkovsky, H. L. Reciprocal effects of micro-RNA-122 on expression of heme oxygenase-1 and hepatitis C virus genes in human hepatocytes. Gastroenterology 2007, 133, 1166–1174.
(48) Tani, J.; et al. Ca2+-sensitive proteins regulate HCV virus NSSA-FKBPs/FKBP38 interaction and HCV virus RNA replication. Liver Int. 2013, 33, 1008–1018.
(49) Gerdol, G.; et al. Quantitative Proteomics Identifies Serum Response Factor Binding Protein 1 as a Host Factor for Hepatitis C Virus Entry. Cell Rep. 2015, 12, 864–878.
(50) Sano, R.; Reed, J. C. ER stress-induced cell death mechanisms. Biochim. Biophys. Acta Mol. Cell. Res. 2013, 1833, 3460–3470.
(51) Kaufman, R. J. Stress signaling from the lumen of the endoplasmic reticulum: coordination of gene transcriptional and translational controls. Genes Dev. 1999, 13, 1211–1233.
(52) Hendershot, L. M.; Valentine, V. A.; Lee, A. S.; Morris, S. W.; Shapiro, D. N. Localization of the gene encoding human Bip/GRP78, the endoplasmic reticulum cognate of the HSP70 family, to chromosome 9q34. Genomics 1994, 20, 281–284.
(53) Mayer, M. P.; Bukau, B. Hsp70 chaperones: cellular functions and molecular mechanism. Cell. Mol. Life Sci. 2005, 62, 670–684.
(54) Thacker, S. A.; Robinson, P.; Abel, A.; Tweardy, D. J. Modulation of the unfolded protein response during hepatocyte and cardiomyocyte apoptosis in trauma/hemorrhagic shock. Sci. Rep. 2013, 3, 1187.
(55) He, B. Viruses, endoplasmic reticulum stress, and interferon responses. Cell Death Differ. 2006, 13, 393–403.
(56) Inoue, T.; Tsai, B. How Viruses Use the Endoplasmic Reticulum. Cold Spring Harbor Perspect. Biol. 2013, 5, a013250.
(57) Wahid, A.; et al. Disulphide bonds in hepatitis C virus glycoprotein E1 control the assembly and entry functions of E2 glycoprotein. J. Virol. 2013, 87, 1605–1617.
(58) Blázquez, A. B.; Escobiano-Romo, E.; Merino-Ramos, T.; Saiz, J. C.; Martín-Acebes, M. A. Stress responses in flavivirus-infected cells: Activation of unfolded protein response and autophagy. Front. Microbiol. 2014, 5, 266.
(59) Sagan, S. M.; et al. The influence of cholesterol and lipid metabolism on host cell structure and hepatitis C virus replication. Biochem. Cell Biol. 2006, 84, 67–79.
(60) Ralak, B.; et al. Peroxisome Proliferator-Activated Receptor α Antagonism Inhibits Hepatitis C Virus Replication. Chem. Biol. 2006, 13, 23–30.
(61) Su, A. I.; et al. Nonlinear partial differential equations and applications: Genomic analysis of the host response to hepatitis C virus infection. Proc. Natl. Acad. Sci. U.S.A. 2002, 99, 15669–15674.
(62) Ritchie, M. E.; et al. limma powers differential expression analyses for RNA-seqencing and microarray studies. Nucleic Acids Res. 2015, 43, No. e47.
(63) Gentleman, R.; Carey, V.; Huber, W.; Hahne, F. Genefilter Methods for Filtering Genes From High-throughput Experiments. R Package, version 1.56.0, 2016, DOI: 10.18129/B9.bioc.genefilter.
(64) Ferrari, F.; et al. Novel definition files for human GeneChips based on GeneAnnot. BMC Bioinf. 2007, 8, 446.
(66) Chen, J.; Bardes, E. E.; Aronow, B. J.; Jegga, A. G. ToppGene Suite for gene list enrichment analysis and candidate gene prioritization. Nucleic Acids Res. **2009**, *37*, W305−W311.

(67) Livak, K. J.; Schmittgen, T. D. Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 

\[ 2^{-\Delta\Delta CT} \] 

Method. *Methods* **2001**, *25*, 402−408.

(68) Warnes, G. R.; et al. gplots: Various R programming tools for plotting data. *R Package*, version 2.11.3, 2016.

(69) Lemon, J. Plotrix: a package in the red light district of R. *R News* **2006**, *6*, 8−12.

(70) Wickham, H. *ggplot2: Elegant Graphics for Data Analysis*; Springer-Verlag: New York, 2009.

(71) Chen, H.; Boutros, P. C. VennDiagram: a package for the generation of highly-customizable Venn and Euler diagrams in R. *BMC Bioinf.* **2011**, *12*, 35.