1 **The DNA Damage Inducible Transcript DDIT3 is differentially expressed and**
2 **transcriptionally induced in models of coronavirus infection.**

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7 The coronavirus COVID19 pandemic is an emerging biosafety threat to the nation and the
8 world (1). There are no treatments approved for coronavirus infection in humans (2) and there
9 is a lack of information available regarding the basic transcriptional behavior of human cells
10 and mammalian tissues following coronavirus infection. We mined two independent datasets
11 (3, 4), public (3) and published (4) containing transcriptome data from infection models of the
12 Middle East respiratory syndrome (MERS) coronavirus and human coronavirus (HCoV) to
13 discover genes that are differentially expressed in coronaviruses and identify potential
14 therapeutic targets and host cell vulnerabilities. We identified the DNA Damage Inducible
15 Transcript DDIT3 as a conserved differentially expressed gene following coronavirus infection.
16 DDIT3 may be involved in the cellular response to COVID19 infection.

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27 **Keywords:** DDIT3, coronavirus, MERS coronavirus, human coronavirus, HCoV, systems biology
28 of viral infection, COVID19.

Viruses are classified according to a system known as the “Baltimore” classification of viruses (5) wherein the characteristics of the viral genome, including whether it is plus or minus strand, also known as positive-sense or negative-sense, whether the genome is single-stranded or double-stranded, whether their genome is composed of RNA or DNA, and whether or not they use a reverse transcriptase to replicate are used to group viruses. Coronaviruses are plus-strand RNA viruses that contain an envelope surrounding their viral particle (6). Their genome is the largest of all RNA viruses, ranging from 27 to 33 kb in size (7). They obtain their name from the crown-like appearance of the viral particle imparted by the structure of the large-surface glycoprotein (7). The coronaviridae family includes seven viruses capable of infecting humans, including the severe acute respiratory distress syndrome, or SARS coronavirus (8), the Middle East respiratory syndrome coronavirus, or MERS coronavirus (9), the human coronaviruses (HCoV) 229E, OC43, HKU and NL63 (10-12), and the novel human coronavirus now known as COVID19 (13, 14). As of March 19, 2020, the World Health Organization reported 209,839 cases of COVID19 and 8778 deaths from COVID19 infection world-wide (15). There are no FDA-approved treatments for human coronavirus infection.

We used a systems-level approach to identify the genes whose expression changes most significantly following infection with multiple types of coronavirus, including MERS-CoV and HCoV 229E using two independent datasets (3, 4). From these datasets were mined transcriptome data following coronavirus infection in human cell culture and in primary human endothelial cells. Across both of these datasets, we identified DDIT3 as among the genes most differentially expressed following coronavirus infection. DDIT3 represents a transcriptional target of the host cell gene expression program following infection with coronaviruses in human cells.

Methods

We used datasets GSE100509 (3) and GSE89167 (4) for this systems-level differential gene expression analysis of coronavirus infections in conjunction with GEO2R.

The Benjamin and Hochberg method of p-value adjustment was used for ranking of differential expression but raw p-values were used for assessment of statistical significance of global differential expression. Log-transformation of data was auto-detected, and the NCBI generated category of platform annotation was used.

A statistical test was performed to evaluate the significance of difference between mRNA expression levels of DDIT3 in HuH-7 cells with and without HCoV 229E infection using a two-tailed, unpaired t-test. A statistical test was performed to evaluate the significance of difference between mRNA expression levels of DDIT3 in human primary microvascular endothelial cells using a one-way ANOVA with multiple comparisons, all compared to baseline infection at 0 hours. Only p-values less than 0.05 were considered statistically significant. We used PRISM for all statistical analyses (Version 8.4.0)(455).

Results

We mined two independent microarray datasets, public (3) and published (4) containing transcriptome data from models of coronavirus infection in primary human cells and cell culture. Across experimental models, we identified the DDIT3 as differentially expressed following coronavirus infection.

DDIT3 is differentially expressed in primary human microvascular endothelial cells when comparing cells infected with wild-type Middle East respiratory syndrome coronavirus (MERS-CoV), icMERS-CoV EMC2012 and uninfected cells.

We identified DDIT3 as differentially expressed following infection of primary human microvascular endothelial cells with wild-type Middle East respiratory syndrome coronavirus (MERS-CoV), icMERS-CoV EMC2012 when compared to non-infected cells (Table 1) (3). When

1 sorting all of the transcripts expressed in human microvascular endothelial cells measured by
2 microarray based on change in expression with and without infection, DDIT3 ranked 91 out of
3 34127 transcripts. Differential expression of DDIT3 in primary human microvascular endothelial
4 cells following infection with MERS-CoV was statistically significant (Table 1; $p=0.00019859$).

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6 DDIT3 is differentially expressed in the human cell line HuH-7 when comparing cells with
7 human coronavirus 229E and uninfected cells.

8 We also identified DDIT3 as differentially expressed in the human cell line HuH-7 when
9 comparing cells with human coronavirus 229E with uninfected cells (Table 2) (4). When sorting
10 all of the transcripts expressed in human HuH-7 cells measured by microarray based on
11 change in expression with and without infection with human coronavirus 229E, DDIT3 ranked
12 **15** out of 62976 transcripts. Differential expression of DDIT3 in HuH-7 following infection with
13 HCoV 229E was statistically significant (Table 2; $p=$ **0.00002468**).

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15 DDIT3 is transcriptionally induced following infection of primary human microvascular
16 endothelial cells with wild-type Middle East respiratory syndrome coronavirus (MERS-CoV),
17 icMERS-CoV EMC2012.

18 We extracted exact mRNA expression values for DDIT3 from primary human
19 microvascular endothelial cells infection with wild-type Middle East respiratory syndrome
20 coronavirus (MERS-CoV), icMERS-CoV, and from uninfected primary human microvascular
21 endothelial cells in order to compare expression levels of DDIT3 between these two groups
22 rather than relative to the rest of the transcriptome as assessed in differential gene expression
23 analysis. This dataset contained transcriptome information from infection of primary human
24 microvascular endothelial cells at 12 hours, 24 hours, 36 hours and 48 hours post-infection at
25 compared to baseline (0 hours). We also performed a statistical test to evaluate whether the
26 difference in expression of DDIT3 in primary human microvascular endothelial cells infection
27 with and without MERS-CoV infection was statistically significant, DDIT3 was expressed at
28 significantly higher levels higher levels at 12 hours, 24 hours, 36 hours, and 48 hours post-

infection with MERS-CoV as compared to cells infected at baseline (0 hours) (Figure 1; $p < 0.0001$ for all comparisons).

DDIT3 is transcriptionally induced in the human cell line HuH-7 following infection with human coronavirus 229E.

We also extracted exact mRNA expression values for DDIT3 from HuH-7 cells following infection with human coronavirus 229E and from uninfected HuH-7 cells in order to compare expression levels of DDIT3 between these two groups rather than relative to the rest of the transcriptome as assessed in differential gene expression analysis. We also performed a statistical test to evaluate whether the difference in expression of DDIT3 in HuH-7 cells following infection with human coronavirus 229E as compared to uninfected HuH-7 cells was statistically significant. DDIT3 was expressed at significantly higher levels in HuH-7 cells following infection with HCoV 229E (Figure 2; $p = 0.0133$).

Discussion

COVID19 is a newly discovered coronavirus that infects humans and whose spread has lead to a global pandemic (19) with zero available therapeutic strategies. To facilitate target discovery and to further understanding of the basic transcriptional program of cells and tissues following coronavirus infection, we mined two independent microarray datasets containing transcriptome data of human cells infected with MERS-CoV and HCoV 229E, in primary human cells and in human cell culture. Across both experimental models, we identified DDIT3 as one of the genes whose expression changed most significantly following infection with a coronavirus. In both experimental models analyzed, DDIT3 expression significantly increased after infection with coronavirus.

DDIT3, also known as CHOP (C/EBP homologous protein) or GADD153 (F1 arrest and DNA damage 153), is a basic leucine zipper transcription factor belonging to the C/EBP family. DDIT has roles in the cellular response to stress with roles in the ER stress response and DNA

1 damage signaling (16-23). DDIT3 possesses both nuclear and cytoplasmic localizations, with
2 nuclear DDIT3 affecting genes involved in the cell cycle and cytoplasmic DDIT3 affecting genes
3 involved in cell migration (16). DDIT3 has also been implicated in lineage commitment and cell
4 fate decisions in hematopoiesis (24).

5 DDIT3 expression should be evaluated in the cells and tissues of patients infected with
6 the novel coronavirus COVID19 to assess whether COVID19 infection similarly leads to
7 induction of DDIT3 as seen after infection with MERS-CoV and HCoV 229E. Modulation of
8 DDIT3 may potentially represent a therapeutic strategy in coronavirus infection broadly and
9 specifically in COVID19 infection.
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Rank	ID	p-value	F	Gene	Gene name
30	A_23_P21134 NM_004083	7.37E-29	1820.78	DDIT3	DNA-damage- inducible transcript 3

Table 1: DDIT3 is differentially expressed in primary human microvascular endothelial cells when comparing cells infected with wild-type Middle East respiratory syndrome coronavirus (MERS-CoV), icMERS-CoV EMC2012 and non-infected cells.

The rank of differential expression globally, the probe/transcript ID, the p-value of differential expression globally, F, a statistic used when more than two groups are compared in differential gene expression analysis (rather than the moderated t-statistic “t”), the gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	Gene	Gene name
91	6414/ NM_004083	0.00019859	8.28	1.140452	DDIT3	DNA-damage-inducible transcript 3

Table 2: DDIT3 is differentially expressed in the human cell line HuH-7 when comparing cells with human coronavirus-229E and non-infected cells.

The rank of differential expression globally, the probe/transcript ID, the p-value of differential expression globally, the moderated t-statistic “t”, the gene and gene name are listed in this chart.

Figure 1: DDIT3 is transcriptionally induced following infection of primary human microvascular endothelial cells with wild-type Middle East respiratory syndrome coronavirus (MERS-CoV), icMERS-CoV.

Expression of DDIT3 at the mRNA level is graphically represented with the mean value marked and the p-value listed for each comparison relative to baseline infection at 0 hours.

Figure 2: DDIT3 is transcriptionally induced in the human cell line HuH-7 following infection with human coronavirus 229E.

Expression of DDIT3 at the mRNA level is graphically represented with the mean value marked and the p-value listed, the result of a test evaluating the statistical significance of difference in expression of DDIT3 with and without HCoV infection.