1 **ATP5D is differentially expressed following infection by the coronavirus family.**

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6 We mined published and public microarray datasets¹⁻⁶ to discover conserved host cell
7 machinery transcriptionally modulated by the coronavirus family. We found significant
8 differential expression and transcriptional repression of the gene encoding the
9 mitochondrial F₁ ATP synthase subunit δ , ATP5D, following infection of human cells with
10 the Middle East Respiratory Syndrome (MERS) coronavirus *in vitro*. ATP5D was
11 differentially expressed, to a lesser extent, in the lungs of BALB/c mice infected with the
12 Severe Acute Respiratory Syndrome (SARS) coronavirus 1 (SARS-CoV-1) but not in
13 human cells infected with the human coronavirus HCoV-229E. These data document
14 unique differential expression of a mitochondrial ATP synthase subunit by viruses of the
15 coronavirus family.
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26 Keywords: SARS-CoV-1, SARS-CoV-2, MERS-CoV, HCoV-229E, coronavirus, host-
27 pathogen interactions, systems biology of viral infection, targeted therapeutics in
28 coronavirus infection.

Nearly 20,000,000 people have been infected with the novel human coronavirus SARS-CoV-2 in less than one year^{7,8}. Deciphering the host transcriptional response is important to understanding host-pathogen interactions. We mined published and public microarray datasets¹⁻⁶ to identify genes most differentially expressed in the host following infection with a series of human coronaviruses: the Middle East Respiratory Syndrome (MERS) coronavirus (MERS-CoV), the Severe Acute Respiratory Syndrome (SARS) coronavirus 1 (SARS-CoV-1), and the human coronavirus 229E (HCoV-229E). This integrated systems-level transcriptome analysis revealed transcriptional modulation of the ATP synthase subunit δ (ATP5D) following infection by human coronaviruses MERS-CoV and SARS-CoV.

Methods

We used datasets GSE100509¹, GSE56677², GSE89167³, GSE59185⁴, GSE68820⁵ and GSE22581⁶ for this analysis of gene expression changes following coronavirus infections. GSE100509 was generated using Agilent-026652 Whole Human Genome Microarray 4x44K v2 technology with $n=5$ each for primary human microvascular endothelial cells infected with MERS-CoV at 0 hours, 12 hours, 24 hours, 36 hours and 48 hours; platform GPL13497 was utilized. GSE56677 was generated using Agilent-039494 SurePrint G3 Human GE v2 8x60K Microarray 039381 technology with $n=3$ each for CALU3 2B4 cells infected with MERS-CoV London (Eng1): at 0 hours, 3 hours, 7 hours, 12 hours, 18 hours and 24 hours post-infection; platform GPL17077

1 was utilized. GSE89167 was generated using 039494 SurePrint G3 Human GE v2
2 8x60K Microarray 039381 technology with $n=2$ HuH-7 cells and $n=2$ HCoV-229E
3 infected HuH-7 cells; platform GPL16699 was utilized. GSE59185 was generated using
4 Agilent-028005 SurePrint G3 Mouse GE 8x60K Microarray technology with $n=3$ lung
5 tissue from mock-infected BALB/c mice and $n=3$ lung tissue from SARS-CoV-1-infected
6 BALB/c mice, both at 2 days post-infection; platform GPL13912 was utilized.
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8 GSE68820 was generated using Agilent-014868 Whole Mouse Genome Microarray
9 4x44K G4122F technology with $n=5$ for lung tissue from mock-infected C57BL6/NJ mice
10 and $n=5$ for lung tissue from SARS-CoV-1-infected C57BL6/NJ mice, both at 2 days
11 post-infection; platform GPL7202 was utilized. GSE22581 was generated using
12 Affymetrix Canine Genome 2.0 Array technology with $n=3$ each for ferret lung on day 0,
13 day 1 and day 2 post-infection through the intranasal route with SARS-CoV-1; platform
14 GPL3738 was utilized. The Benjamini and Hochberg method of p -value adjustment was
15 used for ranking of differential expression but raw p -values were used for assessment of
16 statistical significance of global differential expression. Log-transformation of data was
17 auto-detected, and the NCBI generated category of platform annotation was used.
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22 A statistical test was performed to evaluate the significance of difference in
23 ATP5D mRNA expression levels in CALU3 2B4 cells with MERS-CoV infection at 3
24 hours, 7 hours, 12 hours, 18 hours and 24 hours post-infection as compared to CALU3
25 2B4 cells at baseline (0 hours) using a one-way ANOVA with Dunnett's multiple
26 comparisons test. Only p -values less than 0.05 were considered statistically significant.
27 We used PRISM for all statistical analyses (Version 8.4.0)(455).
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Results

We mined six independent microarray datasets¹⁻⁶ to understand at the systems level and in an unbiased fashion the transcriptional response to coronavirus infection.

ATP5D is differentially expressed in primary human cells and in a human cell line following infection with MERS-CoV.

We identified the gene encoding the mitochondrial ATP synthase subunit delta, ATP5D, as among the genes most differentially expressed following infection of primary human microvascular endothelial cells with MERS-CoV¹. When sorting each of the transcripts measured by microarray based on significance of change in expression between uninfected and MERS-CoV infected cells, ATP5D ranked 4231 out of 34127 total transcripts (Table 1), or more significant differential expression than 87.6% of the measured transcriptome. Differential expression of ATP5D in MERS-CoV-infected primary human microvascular endothelial cells was statistically significant (Table 1; $p=8.08E-17$).

We queried a second microarray dataset² to determine if differential expression of ATP5D could be observed following infection of a human cell line (CALU3 2B4) with a separate isolate of MERS-CoV, MERS-CoV London (Eng1). Again, ATP5D was among the genes most differentially expressed following infection of human cells with a MERS-CoV (Table 2); here, even more markedly. When sorting each of the transcripts measured by microarray based on significance of change in expression between uninfected and MERS-CoV London infected CALU3 cells, ATP5D ranked 103 out of 28653 total transcripts (Table 2). Differential expression of ATP5D in MERS-CoV-

1 London-infected CALU3 cells was statistically significant (Table 2; $p=1.54E-12$).

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4 ATP5D is differentially expressed in the lungs of BALB/c but not C57BL6/NJ mice
5 infected with SARS-CoV-1.

6 We next queried microarray data³⁻⁴ generated from the lungs of mice infected
7 with SARS-CoV-1 to determine if differential expression of ATP5D could be observed
8 following infection with a different coronavirus type and whether this could be observed
9 *in vivo*. ATP5D was differentially expressed in the mouse lung following infection of
10 BALB/c mice with SARS-CoV-1 (Table 3). When sorting each of the transcripts
11 expressed in the lung measured by microarray based on significance of change in
12 expression between mock-infected and SARS-CoV-1-infected mice, ATP5D ranked
13 3267 out of 62976 total transcripts (Table 3), or differential expression more significant
14 than 94.8% of the measured transcriptome. Differential expression of ATP5D in the
15 lungs of SARS-CoV-1-infected BALB/c mice was statistically significant (Table 3;
16 $p=4.04E-04$).

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18 We also queried microarray data⁴ generated from the lungs of a different strain of
19 mice infected with SARS-CoV-1. While differential expression of ATP5D in the lungs of
20 SARS-CoV-1-infected C57BL6/NJ mice was deemed statistically significant (Table 4;
21 $p=5.24E-04$), ATP5D was not differentially expressed in the lungs following infection of
22 C57BL6/NJ mice with SARS-CoV-1; when sorting each of the transcripts measured by
23 microarray expressed in the lung based on significance of change in expression
24 between mock-infected and SARS-CoV-1 infected C57BL6/NJ mice, ATP5D ranked
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1 11707 out of 29649 total transcripts (Table 4), or change in ATP5D expression more
2 significant than only 60.5% of the measured transcriptome.
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5 ATP5D is differentially expressed in the lungs of ferrets infected with SARS-CoV-1.

6 We next queried another microarray dataset⁵ to determine if *in vivo* differential
7 expression of ATP5D could be observed in a separate small animal model of SARS-
8 CoV-1 infection. ATP5D was differentially expressed in the ferret lung following
9 intranasal infection with SARS-CoV-1, albeit significantly less so than in the lungs of
10 BALB/c mice. When sorting each of the transcripts expressed in the ferret lung
11 measured by microarray based on change in expression between mock-infected and
12 infected ferrets, ATP5D ranked 9996 out of 43035 total transcripts (Table 5), or
13 differential expression more significant than 76.8% of the measured transcriptome.
14 Differential expression of ATP5D in the ferret lung following SARS-CoV-1 was
15 statistically significant (Table 5; $p=5.8E-03$).
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19 ATP5D was also differentially expressed in the blood of ferrets following
20 intranasal infection with SARS-CoV-1 (Table 6). When sorting each of the transcripts
21 expressed in the blood of ferrets infected with SARS-CoV-1 measured by microarray
22 based on change in expression between mock-infected and infected ferrets, ATP5D
23 ranked 3282 out of 43035 total transcripts (Table 6). Differential expression of ATP5D in
24 the blood of SARS-CoV-1-infected ferrets trended towards statistical significance (Table
25 6; $p=5.34E-02$).
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ATP5D is differentially expressed in a human cell line following infection with the human coronavirus HCoV-229E.

Finally, we queried a sixth microarray dataset⁶ to determine if ATP5D differential expression could be observed following infection with a third human coronavirus, the human coronavirus HCoV-229E. We found that differential expression of ATP5D was not a conserved feature of infection with the human coronaviruses, as infection of the Huh-7 cell line following infection with HCoV-229E *in vitro* did not result in differential expression of ATP5D (Table 7). When sorting each of the transcripts measured by microarray based on change in expression between uninfected and HCoV-229E-infected HuH-7 cells, ATP5D ranked 21197 out of 62976 total transcripts (Table 7). Differential expression of ATP5D in HCoV-229E-infected Huh-7 cells was not statistically significant (Table 7; $p=0.2731897$).

Rapid transcriptional repression of ATP5D in human cells following infection with MERS-CoV London.

We assessed the kinetics of ATP5D transcription in CALU3 cells following infection with MERS-CoV London. At 3 hours post-infection, decreased ATP5D mRNA levels could already be observed (Figure 1; $p=0.0789$), with a mean fold decrease of 0.9873 ± 0.0093 in ATP5D mRNA levels (Table 1). By 7 hours post-infection, ATP5D levels continued to decrease and ATP5D mRNA levels were significantly lower at 7 hours as compared to baseline (0 hours) (Figure 1; $p=0.0001$), with a mean fold decrease of 0.9679 ± 0.0031 in ATP5D mRNA levels (Table 1). From 12 to 24 hours, ATP5D levels continued to progressively decrease, with significantly lower ATP5D

mRNA levels at 12 hours (Figure 1; $p<0.0001$), 18 hours (Figure 1; $p<0.0001$), and 24 hours (Figure 1; $p<0.0001$); we calculated mean fold decreases in ATP5D mRNA levels of 0.9483 ± 0.0088 at 12 hours, 0.8616 ± 0.0051 at 18 hours, and 0.8483 ± 0.0013 at 24 hours (Table 1).

Thus, by mining published and public microarray data we found here differential expression of ATP5D in human cells following infection with two types of MERS-CoV, and to a lesser extent, in the lungs of BALB/c mice and ferrets following infection with SARS-CoV-1, but not in a human cell line following infection with HCoV-229E. ATP5D levels were significantly decreased within 7 hours of infection with MERS-CoV London *in vitro*.

Discussion

A major fraction of the energy created in cells is produced by the F_1F_0 ATP synthase, found on the inner membrane of the mitochondria^{9,10}. ATP synthesis by the F_1F_0 ATP synthase involves a proton gradient which provides a driving force to propel rotation of the F_0 motor^{9,10}. Proton flux across the inner membrane provides rotational power for ATP synthesis by driving rotation of a structure known as the c ring, which then transmits this force to catalytic sites on the F_1 portion, inducing conformational change and resulting in ATP synthesis from ADP and phosphate^{9,10}. ATP5D is the delta (δ) subunit of the mitochondrial F_1 complex ATP synthase. The δ subunit (ATP5D) can be found in the water-soluble portion of ATP synthase, the F_1 region, consisting of subunits $\alpha_3\beta_3\gamma_1\delta_1\epsilon_1$ ¹⁰.

1 A study utilizing a yeast two-hybrid approach for identification of interaction
2 partners for the platelet cell surface thrombopoietin (TPO) receptor c-Mpl demonstrated
3 an interaction between the cytoplasmic domain of Mpl and ATP5D¹¹; TPO stimulation
4 resulted in abrogation of ATP5D binding to Mpl¹¹. Localization of ATP5D adjacent to the
5 plasma membrane was described in this study¹¹. A study performing comparative
6 proteomic analysis of spinal cord from 10 patients with amyotrophic lateral sclerosis
7 (ALS) and 10 controls found significantly decreased ATP5D protein in the anterior horn
8 of the spinal cord of patients with ALS¹². This study also described ATP5D localization
9 at the plasma membrane, at synapses¹². The δ subunit of sweet potato root F₁ bears
10 homology to the oligomycin sensitivity-conferring subunit of bovine F₁¹³.

14 We found here that the gene encoding the mitochondrial F₁ ATP synthase δ
15 subunit, ATP5D, was among the genes most differentially expressed following infection
16 of human cells with MERS-CoV. ATP5D was also differentially expressed, to a less
17 significant extent, in the lungs of BALB/c mice and ferrets infected with SARS-CoV-1,
18 but not in a human cell line following infection with the human coronavirus HCoV-229E,
19 nor in the lungs of C57BL6/NJ mice infected with SARS-CoV-1. Future studies can
20 determine what role, if any, modulation of ATP5D expression has on coronavirus
21 infection outcomes *in vitro* and *in vivo*.

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Rank	ID	p-value	F	Gene	Gene name
4231	A_23_P5089	8.08E-17	165.4023	ATP5D	ATP synthase, H+ transporting, mitochondrial F1 complex, delta subunit

Table 1: Differential expression of ATP5D in primary human microvascular endothelial cells following infection with MERS-CoV.

The rank of differential expression, probe ID, p-value of global differential expression, F statistic, gene and gene name are listed in this chart.

Rank	ID	p-value	F	FC	Gene	Gene name
103	A_23_P5089	1.54E-12	161.5279	<u>3hr</u> : 0.9873 ± 0.0093 <u>7hr</u> : 0.9679 ± 0.0031 <u>12hr</u> : 0.9483 ± 0.0088 <u>18hr</u> : 0.8616 ± 0.0051 <u>24hr</u> : 0.8483 ± 0.0013	ATP5D	ATP synthase, H+ transporting, mitochondrial F1 complex, delta subunit

Table 2: Differential expression of ATP5D in the CALU3 2B4 human cell line following infection with MERS-CoV London.

The rank of differential expression, probe ID, p-value of global differential expression, F statistic, fold change of ATP5D expression as compared to baseline (0 hours), gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	Gene	Gene name
3267	49187	4.04E-04	-6.07	-0.02679	Atp5d	ATP synthase, H ⁺ transporting, mitochondrial F1 complex, delta subunit

Table 3: Differential expression of ATP5D in the lungs of BALB/c mice following infection with SARS-CoV-1.

The rank of differential expression, probe ID, p-value of global differential expression, F statistic, gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	Gene	Gene name
11707	A_51_P302588	5.24E-04	-4.94219	-1.271008	Atp5d	ATP synthase, H ⁺ transporting, mitochondrial F1 complex, delta subunit

Table 4: ATP5D is not differentially expressed in the lungs of C57BL6/NJ mice following infection with SARS-CoV-1.

The rank of differential expression, probe ID, p-value of global differential expression, t, a moderated t-statistic, B, the log-odds of differential expression between the groups compared (mock-infected and infected mouse lung), gene and gene name are listed in this chart.

Rank	ID	p-value	F	Gene	Gene name
9996	Cfa.4477.1.S1_at	5.8E-03	1.11E+01	ATP5D	ATP synthase, H ⁺ transporting, mitochondrial F1 complex, delta subunit

Table 5: Differential expression of ATP5D in the lungs of ferrets following intranasal infection with SARS-CoV-1.

The rank of differential expression, probe ID, p-value of global differential expression, F statistic, gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	Gene	Gene name
3282	CfaAffx.29864.1.S1_s_at	5.34E-02	-2.348569	-3.7247	ATP5D	ATP synthase, H+ transporting, mitochondrial F1 complex, delta subunit

Table 6: ATP5D is also differentially expressed in the blood of ferrets infected with SARS-CoV-1.

The rank of differential expression, probe ID, p-value of global differential expression, t, a moderated t-statistic, B, the log-odds of differential expression between the groups compared (mock-infected and infected ferret blood), gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	Gene	Gene name
21197	36593	0.2731897	-1.1987951	-4.767	ATP5D	ATP synthase, H ⁺ transporting, mitochondrial F1 complex, delta subunit

Table 7: ATP5D is not differentially expressed in the Huh-7 human cell line following infection with HCoV-229E.

The rank of differential expression, probe ID, p-value of global differential expression, t, a moderated t-statistic, B, the log-odds of differential expression between the groups compared (uninfected and infected Huh-7 cells), gene and gene name are listed in this chart.

Figure 1: Rapid transcriptional repression of ATP5D in human cells following infection with MERS-CoV London.

ATP5D mRNA expression levels are graphically represented from 3 hours to 24 hours with mean mRNA level marked and the result of a statistical test evaluating significance of difference in ATP5D expression as compared to baseline (0 hours), a p-value, listed above each time point.