The phosphoribosylaminoimidazole carboxylase, phosphoribosylaminoimidazole succinocarboxamide synthetase, PAICS, is differentially expressed following infection by the coronavirus family.

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We mined published and public microarray datasets¹⁻⁶ to discover conserved host cell machinery transcriptionally modulated by the coronavirus family. We found significant differential expression and transcriptional repression of a gene utilized during *de novo* purine synthesis, PAICS, following infection of human cells with the Middle East Respiratory Syndrome (MERS) coronavirus *in vitro*, in the lungs of mice following infection with the Severe Acute Respiratory Syndrome (SARS) coronavirus 1 (SARS-CoV-1), in the blood and lungs of ferrets following intranasal infection with SARS-CoV-1, but not in human cells infected with the human coronavirus HCoV-229E. These data indicate transcriptional modulation of *de novo* purine synthesis pathways by viruses of the coronavirus family or during coronavirus infection.

Keywords: SARS-CoV-1, SARS-CoV-2, MERS-CoV, HCoV-229E, coronavirus, host-pathogen interactions, systems biology of viral infection, targeted therapeutics in 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 host cells and tissues 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 PAICS following infection by human coronaviruses MERS-CoV and SARS-CoV-1.

Methods

We used datasets GSE100509¹, GSE56677², GSE59185³, GSE68820⁴ and GSE225815 and GSE89167⁶ 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 Calu-3 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 was utilized. GSE59185 was generated using Agilent-028005 SurePrint G3 Mouse GE

1 8x60K Microarray technology with $n=3$ lung tissue from mock-infected BALB/c mice and
2 $n=3$ lung tissue from SARS-CoV-1-infected BALB/c mice, both at 2 days post-infection;
3 platform GPL13912 was utilized. GSE68820 was generated using Agilent-014868
4 Whole Mouse Genome Microarray 4x44K G4122F technology with $n=5$ for lung tissue
5 from mock-infected C57BL6/NJ mice and $n=5$ for lung tissue from SARS-CoV-1-infected
6 C57BL6/NJ mice, both at 2 days post-infection; platform GPL7202 was utilized.
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8 GSE22581 was generated using Affymetrix Canine Genome 2.0 Array technology with
9 $n=3$ each for ferret lung on day 0, day 1 and day 2 post-infection through the intranasal
10 route with SARS-CoV-1; platform GPL3738 was utilized. GSE89167 was generated
11 using 039494 SurePrint G3 Human GE v2 8x60K Microarray 039381 technology with
12 $n=2$ HuH-7 cells and $n=2$ HCoV-229E infected HuH-7 cells; platform GPL16699 was
13 utilized. The Benjamini and Hochberg method of p -value adjustment was used for
14 ranking of differential expression but raw p -values were used for assessment of
15 statistical significance of global differential expression. Log-transformation of data was
16 auto-detected, and the NCBI generated category of platform annotation was used.
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19 A statistical test was performed to evaluate the significance of difference in
20
21 PAICS mRNA expression levels in Calu-3 2B4 cells with MERS-CoV infection at 3
22 hours, 7 hours, 12 hours, 18 hours and 24 hours post-infection as compared to Calu-3
23 2B4 cells at baseline (0 hours) using a one-way ANOVA with Dunnett's multiple
24 comparisons test. Only p -values less than 0.05 were considered statistically significant.
25
26 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.

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

We identified the gene encoding the phosphoribosylaminoimidazole carboxylase, phosphoribosylaminoimidazole succinocarboxamide synthetase, PAICS, 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, PAICS ranked 4055 out of 34127 total transcripts (Table 1), or more significant differential expression than 88.1% of the measured transcriptome. Differential expression of PAICS in MERS-CoV-infected primary human microvascular endothelial cells was statistically significant (Table 1; $p=5.34E-17$).

We queried a second microarray dataset² to determine if differential expression of PAICS could be observed following infection of a human lung adenocarcinoma cell line (Calu-3 2B4) with a separate isolate of MERS-CoV, MERS-CoV London (Eng1). Again, PAICS was among the genes most differentially expressed following infection of human cells with a MERS-CoV (Table 2); here, even more markedly so. When sorting each of the transcripts measured by microarray based on significance of change in expression between uninfected and MERS-CoV London infected Calu-3 cells, PAICS ranked 15 out of 28653 total transcripts (Table 2). Differential expression of PAICS in

1 MERS-CoV-London-infected Calu-3 cells was statistically significant (Table 2;
2 $p=3.32E-15$).

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4
5 PAICS is differentially expressed in the lungs of both BALB/c and C57BL6/NJ mice
6 infected with SARS-CoV-1.

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8 We next queried microarray data³⁻⁴ generated from the lungs of mice infected
9 with SARS-CoV-1 to determine if differential expression of PAICS could be observed
10 following infection with a different coronavirus and whether this could be observed *in*
11 *vivo*. PAICS was differentially expressed in the mouse lung following infection of BALB/
12 c mice with SARS-CoV-1 (Table 3). When sorting each of the transcripts expressed in
13 the lung measured by microarray based on significance of change in expression
14 between mock-infected and SARS-CoV-1-infected mice, PAICS ranked 2998 out of
15 62976 total transcripts (Table 3), or differential expression more significant than 95.2%
16 of the measured transcriptome. Differential expression of PAICS in the lungs of SARS-
17 CoV-1-infected BALB/c mice was statistically significant (Table 3; $p=3.07E-04$).

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20 We also queried microarray data⁴ generated from the lungs of a different strain of
21 mice infected with SARS-CoV-1. PAICS was differentially expressed in the lungs
22 following infection of C57BL6/NJ mice with SARS-CoV-1, but substantially less so than
23 in the lungs of BALB/c mice. When sorting each of the transcripts measured by
24 microarray expressed in the lung based on significance of change in expression
25 between mock-infected and SARS-CoV-1 infected C57BL6/NJ mice, PAICS ranked
26 8711 out of 29649 total transcripts (Table 4), or change in PAICS expression more
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1 significant than 70.6% of the measured transcriptome. Differential expression of PAICS
2 in the lungs of SARS-CoV-1-infected C57BL6/NJ mice was statistically significant (Table
3 4; $p=6.87E-05$).

6 PAICS is differentially expressed in the lungs of ferrets infected with SARS-CoV-1.

7
8 We next queried another microarray dataset⁵ to determine if *in vivo* differential
9 expression of PAICS could be observed in a separate small animal model of SARS-
10 CoV-1 infection. We found that PAICS was also differentially expressed in the ferret
11 lung following intranasal infection with SARS-CoV-1. When sorting each of the
12 transcripts expressed in the ferret lung measured by microarray based on change in
13 expression between mock-infected and infected ferrets, PAICS ranked 5922 out of
14 43035 total transcripts (Table 5), or differential expression more significant than 86.2%
15 of the measured transcriptome. Differential expression of PAICS in the ferret lung
16 following SARS-CoV-1 was statistically significant (Table 5; $p=1.4E-03$).

19 PAICS 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 SARS-CoV-1 ferrets based on change in expression between
22 mock-infected and infected ferrets, PAICS ranked 664 out of 43035 total transcripts, or
23 differential expression more significant than 98.5% of the measured transcriptome
24 (Table 6). Differential expression of PAICS in the blood of SARS-CoV-1-infected ferrets
25 was statistically significant (Table 6; $p=1.01E-02$).

1 PAICS is differentially expressed in a human cell line following infection with the human
2 coronavirus HCoV-229E.

3
4 Finally, we queried a sixth microarray dataset⁶ to determine if PAICS differential
5 expression could be observed following infection with a third human coronavirus, the
6 human coronavirus HCoV-229E. We found that differential expression of PAICS
7 was not a completely conserved feature of infection with the human coronaviruses, as
8 infection of the HuH-7 hepatocellular carcinoma cell line with HCoV-229E did not result
9 in differential expression of PAICS *in vitro* (Table 7). When sorting each of the
10 transcripts measured by microarray based on change in expression between uninfected
11 and HCoV-229E-infected HuH-7 cells, PAICS ranked 38521 out of 62976 total
12 transcripts (Table 7), or differential expression more statistically significant than 38.8%
13 of genes. Differential expression of PAICS in HCoV-229E-infected Huh-7 cells was not
14 statistically significant (Table 7; $p=0.5562389$).
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18 Transcriptional repression of PAICS in human cells following infection with MERS-CoV
19 London (Eng1).

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21 We assessed the kinetics of PAICS transcription in Calu-3 cells following
22 infection with an isolate of MERS-CoV, MERS-CoV London (Eng1). While PAICS levels
23 initially increased, within 24 hours, we observed a substantial and significant decrease
24 in PAICS mRNA levels as compared to baseline (Figure 1). At 3 hours post-infection,
25 increased PAICS mRNA levels could be observed (Figure 1; $p=0.0065$), with a mean
26 fold increase of 1.0154 ± 0.0020 in PAICS mRNA levels (Table 1). At 7 hours post-
27 infection, PAICS mRNA levels remained significantly higher at 7 hours as compared to
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baseline (0 hours) (Figure 1; $p=0.0041$), with a mean fold increase of 1.0164 ± 0.0059 in PAICS. From 7 to 24 hours, however, PAICS levels decreased precipitously, with significantly lower PAICS mRNA levels at 18 hours (Figure 1; $p<0.0001$) and 24 hours (Figure 1; $p<0.0001$); we calculated mean fold changes in PAICS mRNA levels of 0.9910 ± 0.0047 at 12 hours (Figure 1; $p=0.1234$), 0.9112 ± 0.0056 at 18 hours, and 0.7953 ± 0.0055 at 24 hours (Table 1).

Thus, by mining published and public microarray data we found here differential expression of PAICS in human cells following infection with two types of MERS-CoV, in the lungs of BALB/c and C57BL6/NJ mice infected with SARS-CoV-1, and in the blood and lungs of ferrets following intranasal infection with SARS-CoV-1, but not in a human cell line following infection with HCoV-229E. PAICS levels were significantly decreased within 7 hours of infection with a MERS-CoV isolate *in vitro*.

Discussion

The product of the PAICS gene functions in *de novo* synthesis of the purine nucleotides adenine and guanosine, both of which are synthesized from the metabolite inositol monophosphate. PAICS possesses both 5-aminoimidazole ribonucleotide carboxylase (AIRc) and 4-(*N*-succinylcarboxamine)-5-aminoimidazole ribonucleotide synthetase (SAICARs) activity, acting in a bi-functional manner in two steps of inositol monophosphate synthesis from phosphoribosyl pyrophosphate^{9,10}.

Two humans, siblings, with inborn error of purine metabolism were found to possess a lysine to arginine mutation at position 53 (K53R) at the catalytic site of PAICS, manifesting 10% of PAICS function in fibroblasts as compared to controls, with

heterozygous carriers manifesting 50% PAICS function¹¹. PAICS deficiency resulted in multiple congenital malformations and early neonatal death, demonstrating that severely reduced PAICS function is incompatible with development in humans¹¹. In zebrafish, a recessive mutation of the *paics* gene results in pigmentation defects where xanthophore and iridophore pigments are virtually absent¹². Zebrafish *paics* mutants are microphthalmic; this defect is a result of changes in retinoblast cell cycle during development. Importantly, pigmentation and eye growth phenotypes in *paics* mutant zebrafish can be attributed to deficits in ATP synthesis¹². Specifically, deficiency in the ATP synthesis pathway results in prolongation of the S phase in proliferating retinoblasts, leading to cell cycle exit failure and subsequent microphthalmia¹². A maternal-effect mutant of *paics* demonstrated a specific maternal requirement for *de novo* purine synthesis in development of the vertebrate embryo. Perturbed expression of PAICS in coronavirus infection, *in vitro*, has been reported: at 24 hours post-infection of the Caco-2 intestinal epithelial cell line, PAICS gene expression was up-regulated, and among the genes most differentially expressed following infection¹³.

We found here that the gene encoding the phosphoribosylaminoimidazole carboxylase, phosphoribosylaminoimidazole succinocarboxamide synthetase, PAICS, was among the genes most differentially expressed following infection of human cells with MERS-CoV, in the lungs of mice following infection with SARS-CoV-1, and in the blood and lungs of ferrets following infection with SARS-CoV-1. Future studies can determine what role, if any, modulation of PAICS expression has on coronavirus infection outcomes *in vitro* and *in vivo*.

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14 intestinal epithelial cells with severe acute respiratory syndrome coronavirus. *Cellular*
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Rank	ID	p-value	F	Gene	Gene name
4055	A_24_P200427	5.34E-17	171.5715	PAICS	phosphoribosylaminoimidazole carboxylase, phosphoribosylaminoimidazole succinocarboxamide synthetase

Table 1: Differential expression of PAICS 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
15	A_24_P200427	3.32E-15	369.2699	<u>3hr</u> : 1.0154 ± 0.0020 <u>7hr</u> : 1.0164 ± 0.0059 <u>12hr</u> : 0.9910 ± 0.0047 <u>18hr</u> : 0.9112 ± 0.0056 <u>24hr</u> : 0.7953 ± 0.0055	PAICS	phosphoribosylamino imidazole carboxylase, phosphoribosylamino imidazole succinocarboxamide synthetase

Table 2: Differential expression of PAICS in the Calu-3 2B4 human cell line following infection with MERS-CoV London (Eng1).

The rank of differential expression, probe ID, *p*-value of global differential expression, F statistic, fold change of PAICS 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
2998	42671	3.07E-04	-6.33	0.27373	Paics	phosphoribosylaminoimidazole carboxylase, phosphoribosylaminoribosylamino imidazole, succinocarboxamide synthetase

Table 3: Differential expression of PAICS 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
8711	A_51_P142904	6.87E-05	-6.37063	0.891974	Paics	phosphoribosylaminoimidazole carboxylase, phosphoribosylaminoribosylamino imidazole, succinocarboxamide synthetase

Table 4: PAICS is 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
5922	Cfa.1028.1.A1_s_at	1.4E-03	1.8E+01	PAICS	phosphoribosylaminoimidazole carboxylase, phosphoribosylaminoimidazole succinocarboxamide synthetase

Table 5: Differential expression of PAICS 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
664	Cfa. 17965.1.S1_s_at	1.01E-02	-3.569777	-2.3475	PAICS	phosphoribosylaminoimidazole carboxylase, phosphoribosylaminoimidazole succinocarboxamide synthetase

Table 6: PAICS is 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
38521	41094	0.5562389	-0.6207783	-5.165	PAICS	phosphoribosylaminoimidazole carboxylase, phosphoribosylaminoimidazole succinocarboxamide synthetase

Table 7: PAICS 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: Transcriptional repression of PAICS in human cells following infection with MERS-CoV London (Eng1).

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