Coronaviruses induce the expression of the peptide antigen transporter TAP1.

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Coronavirus infection is an emerging public health threat in the United States and worldwide (1). We mined published microarray data to perform systems-level analysis of host cell transcription following infection with multiple coronavirus types in order to identify therapeutic targets and host cell vulnerabilities (2, 3). We identified the antigen peptide transporter TAP1 as differentially expressed *in vitro* in a human cell line following infection with human coronavirus 229E and *in vivo* in the lungs of mice following infection with the severe acute respiratory distress syndrome SARS coronavirus. While TAP1 functions in the transport of major histocompatibility (MHC) class I-associated peptides displayed to CD8 cytotoxic T-cells, TAP1 also functions in the transport of MHC-I receptors themselves (4). One criteria natural killer cells use to lyse cells is the absence of surface MHC-I (5), and hepatitis C virus up-regulates TAP1 to increase surface expression of MHC-I, inhibit NK-cell cytotoxicity and promote infection (6). We propose coronaviruses similarly up-regulate TAP1 to impair natural killer cell-mediated cytotoxicity responses and avoid recognition by NK cells.

Keywords: TAP1, coronavirus, SARS coronavirus, HCoV coronavirus, human coronavirus, COVID19, systems biology of viral infection, virus host-cell interactions.

1 Coronaviruses are a family of enveloped single-stranded positive-sense RNA viruses
2 (7), seven of which are capable of infecting humans (8-13). The COVID19 infection pandemic
3 that is currently a public health problem in the United States and worldwide is caused by a
4 novel coronavirus, the severe respiratory distress syndrome coronavirus 2, or SARS-CoV-2
5 (13). The death toll in the United States from SARS-CoV-2 has surpassed 4,000 and globally
6 the death toll has reached 46,000 (14). There are no FDA-approved treatments for SARS-
7 CoV-2 infection (15).

8
9 To facilitate discovery for the identification of therapeutic targets in coronavirus
10 infection, we mined published transcriptome data from *in vitro* and *in vivo* models of
11 coronavirus infection (2, 3). We identified the peptide antigen transporter TAP1 as differentially
12 expressed in human cells *in vitro* and in the lungs of mice *in vivo* after infection with
13 coronaviruses. TAP1 functions in the presentation of antigen peptides by facilitating the
14 transport of peptide fragments, antigen, to Class I major histocompatibility (MHC) receptors the
15 cell surface of antigen-presenting cells (4). In addition to peptide antigen, TAP1 facilitates cell-
16 surface expression of MHC-I receptors themselves (4). We found that both HCoV 229E and
17 SARS-CoV induce the expression of TAP1, and we propose the function of this is to avoid
18 recognition by natural killer (NK) cells, which use MHC-I expression to discriminate between
19 self and non-self during recognition of virally-infected cells (5). As TAP1 induction was
20 observed in human cells and in mice, in non-antigen presenting cells, and conserved between
21 two types of coronavirus, we suggest that TAP1 induction may be a property of the SARS-
22 CoV-2 virus in COVID19 infection as well. This remains to be determined.

23 24 25 **Methods**

26 We used datasets GSE59185 (2) and GSE89167 (3) for this systems-level differential
27 gene expression analysis of coronavirus infections in conjunction with GEO2R. GSE8916 was
28 generated using Agilent-039494 SurePrint G3 Human GE v2 8x60K Microarray technology.

1 GSE59185 was generated using Agilent-028005 SurePrint G3 Mouse GE 8x60K Microarray
2 technology.

3 The Benjamin and Hochberg method of p-value adjustment was used for ranking of
4 differential expression but raw p-values were used for assessment of statistical significance of
5 global differential expression. Log-transformation of data was auto-detected, and the NCBI
6 generated category of platform annotation was used.
7

8 A statistical test was performed to evaluate the significance of difference between mRNA
9 expression levels of TAP1 in BALB/c mice with coronavirus or mock infection, and in Huh-7 cells
10 with and without coronavirus infection, using a two-tailed, unpaired t-test with Welch's
11 correction. Only p-values less than 0.05 were considered statistically significant. We used
12 PRISM for all statistical analyses (Version 8.4.0)(455).
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14

15 Results

16 We mined published transcriptome data from *in vitro* and *in vivo* coronavirus infection
17 models to identify genes differentially expressed after coronavirus infection for therapeutic target
18 discovery (2, 3).
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21 TAP1 is differentially expressed in the human liver cell line Huh-7 when comparing cells infected
22 with human coronavirus 229E and uninfected cells.
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24 We identified the peptide antigen transporter TAP1 as differentially expressed following
25 infection of the human liver cell line Huh-7 with human coronavirus 229E when compared to
26 uninfected Huh-7 cells. When sorting all of the transcripts produced in the cell based on change
27 in expression between infected and uninfected cells, TAP1 ranked 241 out of 62976 total
28 transcripts (Table 1). Differential expression of TAP1 following HCoV 229E infection was
statistically significant (Table 1; $p=0.0008966$).

1 We extended our studies to an *in vivo* model of coronavirus infection of the mouse
2 respiratory tract to evaluate if this phenomena could be observed following infection with a
3 different coronavirus type in a model that more accurately replicates infection dynamics.
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5
6 TAP1 is differentially expressed in the mouse lung when comparing mice infected with SARS-
7 CoV and mock-infected mice.

8 Again, we identified the peptide antigen transporter TAP1 as differentially expressed in
9 the mouse lung following infection of mice with SARS-CoV when compared to the lungs of
10 mock-infected mice. When sorting all of the transcripts produced in the mouse lung based on
11 change in expression between SARS-CoV infected and mock-infected mice, TAP1 ranked 141
12 out of 62976 total transcripts (Table 2). Differential expression of TAP1 following SARS-CoV
13 infection was statistically significant (Table 2; $p=4.22E-08$).
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16
17 TAP1 expression is transcriptionally induced in a human liver cell line following infection with
18 human coronavirus 229E.

19 Next, we obtained exact expression values for TAP1 mRNA in cells infected with HCoV
20 229E to compare TAP1 expression with and without coronavirus infection. TAP1 was expressed
21 at higher levels in Huh-7 cells following infection with HCoV 229E when compared to uninfected
22 Huh-7 cells, and this difference was statistically significant (Figure 1; $p=0.0150$). We calculated
23 a 1.77 fold change in TAP1 expression between HCoV 229E infected and uninfected HuH-7
24 cells (Table 1).
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1 TAP1 expression is transcriptionally induced in the lungs of mice following infection with SARS
2 coronavirus.

3 We also obtained exact expression values for TAP1 mRNA in the lungs of mice infected
4 with SARS-CoV to compare TAP1 expression with coronavirus infection and with mock-infection
5 at 2 days post-infection. TAP1 was expressed at higher levels in the mouse lung following
6 infection with SARS-CoV when compared to the lungs of mock-infected mice at 2 days post-
7 infection, and this difference was statistically significant (Figure 2; $p < 0.0001$). We calculated a
8 1.37 fold change in TAP1 expression between the lungs of SARS-CoV infected mice and the
9 lungs of mock-infected mice at 2 days post-infection (Table 1).
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13 Thus, we identified TAP1 as among the genes whose expression changed most
14 significantly following coronavirus infection of a human cell line and in the mouse lung, and that
15 TAP1 was expressed at significantly higher levels in both HuH-7 cells and in the mouse lung
16 following coronavirus. Importantly, TAP1 induction following coronavirus infection was
17 conserved between two different types of coronavirus infection: HCoV 229E and SARS-CoV.
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20 **Discussion**

21 We found that in human cells and in the lungs of mice (3, 2), coronavirus infection lead
22 to the transcriptional induction of the peptide antigen transporter TAP1. TAP1 was among the
23 genes whose expression changed most significantly when comparing the transcriptomes of a
24 human liver cell line with and without human coronavirus 229E infection, and between the
25 lungs of mice infected with SARS-CoV and mock-infected mice. Thus, TAP1 induction is
26 conserved across species, is found *in vitro* and *in vivo*, and is observed in cells that are not
27 inherently considered antigen presenting cells. TAP1 functions to facilitate presentation of
28 antigen by class I MHC receptors to cytotoxic T-cells (4).

1 MHC-I receptor/peptide complexes on the cell surface interact with cognate T-cell
2 receptors on CD8+ cytotoxic T cells to activate the cytolytic function of CD8+ T-cells (16).
3 However, we found that coronavirus infection lead to increased expression of TAP1, and this
4 was found in non-antigen presenting cells *in vitro*, meaning coronavirus induction of TAP1
5 expression was independent of the ability of a cell to present antigen and cell autonomous.
6 Moreover, TAP1 induction by a virus to facilitate a CD8+ cytolytic response against itself would
7 be counterproductive from the standpoint of the pathogen. Another type of potent immune
8 cell, the natural killer (NK) cell, uses expression of MHC-I on the cell surface to discriminate
9 between self and non-self, considering cells that express MHC-I as “self” (5). Indeed, the core
10 protein from hepatitis C virus (HCV) induces expression of TAP1 in cells infected with HCV (6).
11 This has two major effects: increased expression of MHC-I on the surface of cells infected with
12 HCV, and decreased cytolytic responses against these cells infected with HCV by NK cells (6).
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14 We suggest that, similar to hepatitis C virus, coronavirus induces expression of TAP1 to
15 promote surface expression of MHC-I and avoid recognition by NK cells and subsequent NK-
16 cell derived anti-viral responses. We found that human coronavirus 229E and SARS
17 coronavirus infection leads to transcriptional induction of TAP1. TAP1 induction to prevent NK-
18 cell cytolytic responses may be a conserved feature of coronaviruses, shared by the novel
19 coronavirus SARS-CoV-2. This remains to be determined.
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Rank	ID	p-value	t	B	Fold change	Gene	Gene name
241	33175/ NM_000593	0.0008966	6.23	-0.078804	1.77	TAP1	transporter 1, ATP-binding cassette, sub-family B (MDR/TAP)

Table 1: TAP1 is differentially expressed in the human liver cell line Huh-7 when comparing cells infected with human coronavirus 229E and uninfected cells.

Rank of differential expression, transcript ID, p-value of differential expression, t, a moderated t-statistic, B, the log-odds of differential expression between groups compared, the gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	Fold change	Gene	Gene name
141	21803/ NM_013683	4.22E-08	2.25E+01	9.54981	1.37	Tap1	transporter 1, ATP-binding cassette, sub-family B (MDR/TAP)

Table 2: TAP1 is differentially expressed in the mouse lung when comparing mice infected with SARS-CoV and mock-infected mice.

Rank of differential expression, transcript ID, p-value of differential expression, t, a moderated t-statistic, B, the log-odds of differential expression between groups compared, the gene and gene name are listed in this chart.

Figure 1: TAP1 expression is transcriptionally induced in a human liver cell line following infection with human coronavirus 229E.

The expression levels of TAP1 with and without HCoV 229E infection is graphically displayed with mean value marked and p-value, the result of a statistical test evaluating significance of change in expression between groups, listed.

Figure 2: TAP1 expression is transcriptionally induced in the lungs of mice following infection with SARS coronavirus.

The expression levels of TAP1 in the lungs of mice infected with SARS-CoV and in the lungs of mock-infected mice is graphically displayed with mean value marked and p-value, the result of a statistical test evaluating significance of change in expression between groups, listed.