MAP3K8 (COT/Tpl-2) is differentially expressed and transcriptionally repressed in models of coronavirus infection.

Shahan Mamoor¹

¹Thomas Jefferson School of Law

San Diego, CA 92101

mamoorsk@tjssl.edu

The coronavirus COVID19 pandemic is an emerging biosafety threat to the nation and the world (1). There are no treatments approved for coronavirus infection in humans (2) and there is a lack of information available regarding the basic transcriptional behavior of human cells and mammalian tissues following coronavirus infection. We mined three independent datasets (3-5), public (3) and published (4, 5) containing transcriptome data from infection models of the Middle East respiratory syndrome (MERS) coronavirus, human coronavirus (HCoV) and SARS coronavirus to discover genes that are differentially expressed in coronaviruses and identify potential therapeutic targets and host cell vulnerabilities. We identified MAP3K8, also known as COT and Tpl-2 (6-8) as a conserved differentially expressed gene following coronavirus infection in primary human cells, in a human cell line, and in the lungs of infected ferrets. MAP3K8 expression dramatically decreased within 12-24 hours of coronavirus infection, both in the lungs of ferrets infected with SARS coronavirus and in primary human microvascular endothelial cells infected with MERS coronavirus. MAP3K8 may be involved in the cellular response to COVID19 infection or a host cell vulnerability, exploited by coronaviruses.

Keywords: MAP3K8, COT, Tpl-2, coronavirus, MERS coronavirus, human coronavirus, HCoV, SARS coronavirus, systems biology of viral infection, COVID19.

Viruses are classified according to a system known as the “Baltimore” classification of viruses (9) 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 (10). Their genome is largest of all RNA viruses, ranging from 27 to 33 kb in size (11). They obtain their name from the crown-like appearance of the viral particle imparted by the structure of the large-surface glycoprotein (11). The coronaviridae family includes seven viruses capable of infecting humans, including the severe acute respiratory distress syndrome, or SARS coronavirus (12), the Middle East respiratory syndrome coronavirus, or MERS coronavirus (13), the human coronaviruses (HCoV) 229E, OC43, HKU and NL63 (14-16), and the novel human coronavirus now known as COVID19 (17, 18). As of March 19, 2020, the World Health Organization reported 209,839 cases of COVID19 and 8778 deaths from COVID19 infection world-wide (19). 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 three independent datasets (3-5). From these datasets we mined transcriptome data following coronavirus infection in human cell culture, in primary human endothelial cells, and in the lungs of infected ferrets. Across both of these datasets, we identified the serine/threonine kinase MAP3K8, also known as COT and Tpl-2 as among the genes most differentially expressed following coronavirus infection. MAP3K8 represents a transcriptional target of the host cell gene expression program following infection with coronaviruses in human cells.

Methods

We used datasets GSE100509 (3), GSE89167 (4) and GSE22581 (5) for this systems-level differential gene expression analysis of coronavirus infections in conjunction with GEO2R.

1 GSE100509 was generated using Agilent-026652 Whole Human Genome Microarray 4x44K
2 technology. GSE89167 was generated using Agilent-039494 SurePrint G3 Human GE v2
3 8x60K Microarray technology. GSE22581 was generated using Affymetrix Canine Genome 2.0
4 Array technology.

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

9
10 A statistical test was performed to evaluate the significance of difference between mRNA
11 expression levels of MAP3K8 in HuH-7 cells with and without HCoV 229E infection using a two-
12 tailed, unpaired t-test. A statistical test was performed to evaluate the significance of difference
13 between mRNA expression levels of MAP3K8 in human primary microvascular endothelial cells
14 using a one-way ANOVA with multiple comparisons, all compared to baseline infection at 0
15 hours. A statistical test was performed to evaluate the significance of difference between mRNA
16 expression levels of MAP3K8 in uninfected ferret lung at day 0, infected ferret lung at day 1, and
17 infected ferret lung at day 2 using a one-way ANOVA with multiple comparisons, all compared to
18 uninfected lungs at day 0. Only p-values less than 0.05 were considered statistically significant.
19 We used PRISM for all statistical analyses (Version 8.4.0)(455).
20
21

22 **Results**

23 We mined three independent microarray datasets, public (3) and published (4, 5)
24 containing transcriptome data from models of coronavirus infection in primary human cells, cell
25 culture, and the respiratory system of ferrets. Across experimental models, we identified
26 MAP3K8 as differentially expressed following coronavirus infection.
27

28 MAP3K8 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.

1 We identified MAP3K8 as differentially expressed following infection of primary human
2 microvascular endothelial cells with wild-type Middle East respiratory syndrome coronavirus
3 (MERS-CoV), icMERS-CoV EMC2012 when compared to non-infected cells (Table 1) (3). When
4 sorting all of the transcripts expressed in human microvascular endothelial cells measured by
5 microarray based on change in expression with and without infection, MAP3K8 ranked 70 out
6 of 34127 transcripts. Differential expression of MAP3K8 in primary human microvascular
7 endothelial cells following infection with MERS-CoV was statistically significant (Table 1;
8 $p=7.3E-27$).

10 MAP3K8 is differentially expressed in the human cell line HuH-7 when comparing cells with
11 human coronavirus 229E and uninfected cells.

12 We also identified MAP3K8 as differentially expressed in the human cell line HuH-7
13 when comparing cells with human coronavirus 229E with uninfected cells (Table 2) (4). When
14 sorting all of the transcripts expressed in human HuH-7 cells measured by microarray based
15 on change in expression with and without infection with human coronavirus 229E, MAP3K8
16 ranked 151 out of 62976 transcripts. Differential expression of MAP3K8 in HuH-7 following
17 infection with HCoV 229E was statistically significant (Table 2; $p=0.0005105$).

19 MAP3K8 is differentially expressed in the lungs of ferrets infected with SARS coronavirus when
20 comparing lung tissue from infected ferrets and lung tissue from uninfected ferrets.

21 We also identified MAP3K8 as differentially expressed in the ferret lung in a ferret model
22 of coronavirus infection *in vivo*. When comparing the lungs of ferrets infected with SARS
23 coronavirus and the lungs of uninfected ferrets, MAP3K8 was among the genes whose
24 expression changed most significantly (Table 3) (5). When sorting all of the transcripts
25 expressed in the ferret lung measured by microarray based on change in expression with and
26 without infection with SARS coronavirus, MAP3K8 ranked 108 out of 43035 transcripts.
27 Differential expression of MAP3K8 in ferret lung following infection with SARS coronavirus was
28 statistically significant (Table 3; $p=5.78E-08$).

1 MAP3K8 is transcriptionally repressed following infection of primary human microvascular
2 endothelial cells with wild-type Middle East respiratory syndrome coronavirus (MERS-CoV),
3 icMERS-CoV EMC2012.

4 We extracted exact mRNA expression values for MAP3K8 from primary human
5 microvascular endothelial cells infection with wild-type Middle East respiratory syndrome
6 coronavirus (MERS-CoV), icMERS-CoV, and from uninfected primary human microvascular
7 endothelial cells in order to compare expression levels of MAP3K8 between these two groups
8 rather than relative to the rest of the transcriptome as assessed in differential gene expression
9 analysis. This dataset contained transcriptome information from infection of primary human
10 microvascular endothelial cells at 12 hours, 24 hours, 36 hours and 48 hours post-infection at
11 compared to baseline (0 hours). We also performed a statistical test to evaluate whether the
12 difference in expression of MAP3K8 in primary human microvascular endothelial cells infection
13 with and without MERS-CoV infection was statistically significant. MAP3K8 was expressed at
14 significantly lower levels at 12 hours, 24 hours, 36 hours, and 48 hours post-infection with
15 MERS-CoV as compared to cells infected at baseline (0 hours). MAP3K8 expression
16 decreased dramatically from 0 to 12 hours, and remained significantly below the baseline level
17 of expression (0 hours) (Figure 1; $p < 0.0001$ for all comparisons relative to baseline (0 hours)).
18

19
20 MAP3K8 is transcriptionally induced in the human cell line HuH-7 following infection with
21 human coronavirus 229E.

22 We also extracted exact mRNA expression values for MAP3K8 from HuH-7 cells
23 following infection with human coronavirus 229E and from uninfected HuH-7 cells in order to
24 compare expression levels of MAP3K8 between these two groups rather than relative to the
25 rest of the transcriptome as assessed in differential gene expression analysis. We also
26 performed a statistical test to evaluate whether the difference in expression of MAP3K8 in
27 HuH-7 cells following infection with human coronavirus 229E as compared to uninfected
28 HuH-7 cells was statistically significant. In contrast to the decrease in expression seen in

primary human endothelial cells, MAP3K8 was expressed at significantly higher levels in HuH-7 cells following infection with HCoV 229E (Figure 2; $p=0.0228$).

The expression of MAP3K8 significantly decreases in the ferret lung following infection of ferrets with SARS coronavirus.

We also extracted exact mRNA expression values for MAP3K8 in the ferret lung following infection of ferrets with SARS coronavirus, in order to compare expression levels of MAP3K8 between these two groups rather than relative to the rest of the transcriptome as assessed in differential gene expression analysis. We compared expression of MAP3K8 at baseline (0 hours) in the lungs of uninfected ferrets, and in the lungs of ferrets on days 1 and 2 after infection. We also performed a statistical test to evaluate whether the difference in expression of MAP3K8 in the ferret lung with and without SARS infection was statistically significant. MAP3K8 was expressed at significantly lower levels on days 1 and 2 post-infection with SARS-CoV as compared to the uninfected ferret lung at baseline (0 hours). MAP3K8 expression decreased dramatically from 0 to 24 hours (Figure 3; $p<0.0001$). Expression of MAP3K8 in the ferret lung increased to a significant extent from 24 to 48 hours post-infection (Figure 3; $p=0.0004$) but remained significantly below the baseline level of expression at 48 hours (Figure 3; $p=0.0022$).

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 three 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, and an *in vivo* model of SARS coronavirus infection of the ferret lung. Across each experimental model, we identified MAP3K8 as one of the genes whose expression changed most significantly following infection with a coronavirus. In two of three

analyzed, the serine/threonine kinase MAP3K8 expression significantly decreased after infection with coronavirus (MERS-CoV and SARS-CoV). In cell culture, MAP3K8 expression increased after infection with coronavirus (HCoV 229E).

MAP3K8 is a serine threonine kinase in the mitogen-activated protein kinase (MAPK) pathway important for transducing cellular signals from the cell membrane to the nucleus. MAP3K8 can activate the nuclear factor of activated T-cells, NFAT, and plays an important role in activation of T-cells and secretion of IL-2 through NFAT-mediate gene expression (6-8). MAP3K8 $-/-$ macrophages are compromised in their ability to produce tumor necrosis factor alpha (TNF α) in response to TLR engagement (20). In a mouse model of *Listeria monocytogenes* infection, MAP3K8 $-/-$ mice rapidly succumb to intravenous *Listeria* infection, manifest increased pathogen burden and both macrophages and dendritic cells from MAP3K8 $-/-$ are defective in their ability to produce the pro-inflammatory cytokine IL-1 β (20).

Interestingly, pharmacological inhibition of MAP3K8 results in a loss of cytotoxic T-cell effector functions in human but not mouse CD8 $^{+}$ cytotoxic T-lymphocytes (CTLs), and MAP3K8 small molecule inhibition blocks interferon gamma (IFN γ) and TNF α production by CTLs (21). This suggests a potential role for MAP3K8 inhibition by coronaviruses in the disabling of host adaptive effector function, specifically of cytotoxic T-cell responses.

We found that MAP3K8 was differentially expressed in a human cell line following infection with human coronavirus, in primary human cells after infection with Middle East respiratory syndrome coronavirus, and in the ferret lung after infection with SARS coronavirus. MAP3K8 expression should be evaluated in the cells and tissues of patients infected with the novel coronavirus COVID19 to assess whether COVID19 infection similarly leads to repression of MAP3K8 mRNA levels as seen after infection with MERS-CoV and SARS coronavirus. Modulation of MAP3K8 expression may represent a therapeutic strategy in coronavirus infection broadly and specifically in COVID19 infection.

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Rank	ID	p-value	F	Gene	Gene name
70	A_33_P3246505/ AB209539	7.3E-27	1228.3572	MAP3K8	mitogen-activated protein kinase kinase kinase 8

Table 1: MAP3K8 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
151	46653/ NM_005204	0.0005105	6.94	0.39723	MAP3K8	mitogen-activated protein kinase kinase kinase 8

Table 2: MAP3K8 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.

Rank	ID	p-value	F	Gene	Gene name
108	CfaAffx. 6888.1.S1_at	5.78E-08	3.21E+02	MAP3K8	mitogen-activated protein kinase kinase kinase 8

Table 3: MAP3K8 is differentially expressed in the lungs of ferrets infected with SARS coronavirus when comparing the lungs of ferrets infected with SARS and the lungs of uninfected ferrets.

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.

Figure 1: MAP3K8 is transcriptionally repressed following infection of primary human microvascular endothelial cells with wild-type Middle East respiratory syndrome coronavirus (MERS-CoV).

Expression of MAP3K8 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: MAP3K8 is transcriptionally induced in the human cell line HuH-7 following infection with human coronavirus 229E.

Expression of MAP3K8 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 MAP3K8 with and without HCoV infection.

Figure 3: The expression of MAP3K8 in the lungs of ferrets significantly decreases within hours of infection with SARS coronavirus.

Expression of MAP3K8 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 MAP3K8 in ferret lung with and without SARS infection.