The MAX dimerization protein MXD1 is differentially expressed and transcriptionally induced in multiple models of coronavirus infection.

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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 independent public (3) and published (4, 5) datasets, containing transcriptome data from infection models of the human coronavirus 229E, severe acute respiratory syndrome (SARS) coronavirus and Middle East (MERS) coronavirus to discover genes that are differentially expressed in coronaviruses and identify potential therapeutic targets and host cell vulnerabilities. We identified MXD1 as differentially expressed genes following coronavirus infection *in vivo* and *in vitro*, conserved across HCoV, MERS-CoV, and SARS-CoV. MXD1 may be involved in the cellular response to COVID19 infection.

Keywords: MXD1, coronavirus, SARS coronavirus, MERS coronavirus, human coronavirus, HCoV, systems biology of viral infection, COVID19.

Viruses are classified according to a system known as the “Baltimore” classification of viruses (6) wherein the characteristics of the viral genome, including the nature of its genome - whether it is plus or minus strand, also known as positive-sense or negative-sense, whether it is single-stranded or double-stranded, and whether it is composed of RNA or DNA - is used to group viruses. Coronaviruses are single-stranded, plus-strand RNA viruses that contain an envelope surrounding their viral particle (7). Their genome is largest of all RNA viruses, ranging from 27 to 33 kb in size (7), and they obtain their name from the crown-like appearance of the viral particle imparted by the structure of the large-surface glycoprotein (8). The coronaviridae family includes seven viruses capable of infecting humans. These include the severe acute respiratory distress syndrome, or SARS coronavirus (9), the Middle East respiratory syndrome coronavirus, or MERS coronavirus (10), the human coronaviruses (HCoV) 229E, OC43, HKU and NL63 (11-13), and the novel human coronavirus now known as COVID19 (14, 15). The World Health Organization reported 209,839 cases of COVID19 and 8778 deaths from COVID19 infection world-wide as of March 19, 2020 (16). 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 coronavirus *in vitro* and *in vivo* using published or public datasets (3-5). In multiple datasets containing transcriptome information from cells and mice infected with three different coronaviruses, we identified interleukin 6 (IL6) and the interleukin 1 receptor antagonist (IL1RN) as among the genes most differentially expressed following coronavirus infection. Importantly, MXD1 was both transcriptionally induced after infection with SARS-CoV, MERS-CoV, and HCoV 229E. MXD1 is a transcriptional target of the host cell gene expression program following infection with coronaviruses.

Methods

We used datasets GSE100509 (3), GSE89167 (4), and GSE59185 (5) 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 MXD1 in BALB/c mice using a two-tailed, unpaired t-test with Welch's correction. A statistical test was performed to evaluate the significance of difference between mRNA expression levels of MXD1 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 MXD1 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 three independent microarray datasets, public (3) or published (4, 5) containing transcriptome data from models of coronavirus infection in mice and in human cells. Across experimental models, we identified MXD1 as differentially expressed following coronavirus infection.

MXD1 is differentially expressed in the lungs of BALB/c mice infected with SARS coronavirus.

We also identified MXD1 as differentially expressed in the lungs of BALB/c mice infected with SARS-CoV when comparing infected mice and mock-infected mice (Table 2). When sorting all of the transcripts expressed in the mouse lung based on change in expression with SARS-CoV infection and with mock infection, MXD1 ranked 102 out of 62976 transcripts, respectively. Differential expression of MXD1 in BALB/c mice following infection with SARS-CoV was statistically significant (Table 1; $p=2.37E-08$).

MXD1 is transcriptionally induced in the lungs following infection of BALB/c mice with SARS coronavirus.

We also extracted exact mRNA expression values for MXD1 from the lungs of BALB/c mice infected with SARS coronavirus or mock-infected in order to compare expression levels of MXD1 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 MXD1 in the lungs of BALB/c mice with and without SARS-CoV infection was statistically significant. MXD1 were expressed at significantly higher levels in the lungs of BALB/c mice at 2 days post-infection with SARS-CoV as compared to mock-infected mice at 2 days (Figure 1; $p<0.0001$). We calculated a 1.27-fold increase in MXD1 expression in the lungs of BALB/c mice infected with SARS coronavirus relative to the lungs of mock-infected mice (Table 1).

We then extended our studies to determine whether infection of human cells with a coronavirus could lead to differential expression MXD1 as seen in the lungs of mice. We analyzed a dataset containing transcriptome data following infection of a human liver cell line, Huh-7 with human coronavirus 229E, and following infection of human primary cells of the microvascular endothelial type with will-type Middle East respiratory syndrome (MERS)-CoV.

MXD1 is differentially expressed after infection of a human cell line with human coronavirus HCoV 229E.

Transcriptome analysis of a second microarray dataset revealed that MXD1 was also among the genes most differentially expressed following infection of a human liver cell line, Huh-7 with human coronavirus 229E when compared to uninfected Huh-7 cells. When sorting all of the transcripts produced in Huh-7 cells after infection with HCoV 229E based on change in expression between infected and uninfected cells, MXD1 ranked 197 out of 62976 total transcripts detected and measured by microarray. Differential expression of MXD1 in the human liver cell line Huh-7 following infection with HCoV 229E was statistically significant (Table 2; $p=0.00067829$).

MXD1 is differentially expressed in human primary microvascular endothelial cells following infection with wild-type MERS-CoV.

Transcriptome analysis of a third microarray dataset revealed that MXD1 was among the genes most differentially expressed following infection of primary human cells (microvascular endothelial) following infection with Middle East Respiratory Syndrome (MERS) coronavirus when compared to uninfected primary human microvascular endothelial cells. When sorting all of the transcripts produced in primary microvascular endothelial cells after infection with MERS-CoV based on change in expression between infected and uninfected cells, MXD1 ranked 32 out of 34127 total transcripts detected and measured by microarray. Differential expression of MXD1 in primary microvascular endothelial cells infected with MERS-CoV was statistically significant (Table 3; $p=8.52E-29$).

MXD1 is transcriptionally induced in the Huh7 cells following infection with HCoV 229E human coronavirus.

We also extracted exact mRNA expression values for MXD1 from the human liver cell line, Huh-7 after infection with human coronavirus 229E when compared to uninfected Huh-7 cells in order to compare expression levels of MXD1 between these two groups. We also

performed a statistical test to evaluate whether the difference in expression of MXD1 in Huh-7 cells after infection with human coronavirus 229E infection was statistically significant. MXD1 was expressed at significantly higher levels in Huh-7 cells after infection with human coronavirus 229E as compared to uninfected cells (Figure 2; $p=0.0306$). We calculated a 1.41-fold increase in MXD1 expression in Huh-7 cells after infection with human coronavirus 229E as compared to uninfected cells (Table 2).

MXD1 is transcriptionally induced in human primary microvascular endothelial cells following infection with wild-type MERS-CoV.

We also extracted exact mRNA expression values for MXD1 from human primary microvascular endothelial cells following infection with wild-type MERS-CoV and uninfected cells in order to compare expression levels of MXD1 between these two groups. We also performed a statistical test to evaluate whether the difference in expression of MXD1 in human primary microvascular endothelial cells following infection with wild-type MERS-CoV as compared to uninfected cells was statistically significant. MXD1 was expressed at significantly higher levels in human primary microvascular endothelial cells-infection with MERS-CoV as compared to cells at 0 hours of infection, at each time point measured: 12 hours, 24 hours, 36 hours, and 48 hours post-infection (Figure 3; $p<0.0001$ for all comparisons). We calculated a fold increase of 1.16 at 12 hours, 1.41 at 24 hours, 1.39 at 36 hours, and 1.24 at 48 hours, of MXD1 in human primary microvascular endothelial cells after infection with MERS-CoV, relative to cells infected at 0 hours (Table 3).

Thus, we found that the MAX dimerization protein MXD1 was among the genes whose expression changed most significantly, out of the entire transcriptome, after infection with SARS coronavirus in the lungs of mice, MERS coronavirus in human primary endothelial cells, and human coronavirus 229E in a human liver cell line. In each case, MXD1 expression was significant induced after coronavirus infection.

Discussion

COVID19 is a newly discovered coronavirus that infects humans and whose spread has lead to a global pandemic (15, 16) 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 (3-5) containing transcriptome data from *in vitro* and *in vivo* infection models of coronavirus - BALB/c mice infected with SARS coronavirus, the Huh-7 cell line infected with human coronavirus 229E, and human primary microvascular endothelial cells infected with MERS-CoV. Across each experimental model, we identified MXD1 as one of the genes whose expression changed most significantly following infection with a coronavirus. In all experimental models analyzed, MXD1 expression significantly increased after infection with a coronavirus.

Differential expression of MXD1 is conserved between mice and man following infection with diverse coronaviruses. It was not previously known whether the host cell gene expression program in coronavirus infection was conserved based on the type of coronavirus infection. We have found that the genes induced in the host cell following infection with coronaviruses can be conserved between coronavirus types, as seen here, or differential expression of a host cell gene can be unique to a specific coronavirus, as we have previously found with FBXL8 following infection with MERS-CoV (18).

The MAX dimerization protein, MXD1, along with MAX, is part of a repressive transcriptional factor complex (18). The MXD1/MAX complex functions as a competitor to MYC and thereby antagonizes and inhibits MYC transcriptional activity (18). MYC family transcription factors bind to E-boxes found throughout the genome for transcriptional regulation (19). MXD1 has been reported as important for MYCL signaling in dendritic cells in mice (20). The SIN3-interacting domain, SID, is effective as a transcriptional repressor and inhibits MYC-driven cell proliferation in P493-6 and RAMOS Burkitt's lymphoma cancer cell lines (21). MXD1 transcriptional function has been reported as important in multiple cancer

1 types including pancreatic cancer, gastric cancer, melanoma and breast cancer (22-25). MXD1
2 has also been reported as involved in the differentiation of hematopoietic stem cells through
3 microRNA miR-382-5p (26).

4 We found here that infection with SARS coronavirus leads to induction of MXD1 in the
5 lungs of mice early in infection, at 2 days. We also found that MXD1 was differentially
6 expressed and transcriptionally induced in human cells following infection with human
7 coronavirus 229E and with MERS coronavirus. Differential expression of MXD1 following
8 infection with coronavirus appears to be conserved across coronavirus type and at least to
9 some extent, conserved across species. MXD1 expression should be evaluated in the cells
10 and tissues of patients infected with the novel coronavirus associated with COVID19 infection
11 to assess whether COVID19 infection similarly leads to induction of MXD1 as seen after
12 infection with SARS-CoV and HCoV 229E. Future studies should evaluate gene expression of
13 MXD1 target genes in cells infected with coronaviruses to understand how the Myc gene
14 expression program, and how transcription as a system is perturbed in coronavirus-infected
15 cells. Modulation of MXD1 expression may represent a therapeutic strategy in coronavirus
16 infection broadly and specifically in COVID19 infection.
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Rank	ID	p-value	t	B	Fold change	Gene	Gene name
102	58526 NM_010751	2.37E-08	2.43E+01	10.08018	1.27	Mxd1	MAX dimerization protein 1

Table 1: MXD1 is differentially expressed in the lungs of BALB/c mice infected with SARS Coronavirus when compared to the lungs of mock-infected BALB/c mice.

The rank of differential expression, transcript/probe ID, p-value of global differential expression, t, a moderated t-statistic, B, the log-odds of differential expression between the two groups, fold change of expression, gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	Fold change	Gene	Gene name
197	47070 NM_002357	0.00067829	6.57	0.159843	1.41	MXD1	MAX dimerization protein 1

Table 2: MXD1 is differentially expressed in the human liver cell line Huh-7 after infection with human coronavirus HCoV 229E when compared to uninfected Huh-7 cells.

The rank of differential expression, transcript/probe ID, p-value of global differential expression, t, a moderated t-statistic, B, the log-odds of differential expression between the two groups, fold change of expression, gene and gene name are listed in this chart.

Rank	ID	p-value	F	Fold change	Gene	Gene name
32	A_24_P379750 NM_002357	8.52E-29	1798.2688	1.16: 12 hr 1.41: 24 hr 1.39 36 hr 1.24: 48 hr	MXD1	MAX dimerization protein 1

Table 3: MXD1 is differentially expressed in primary human microvascular endothelial cells after infection with Middle East respiratory syndrome coronavirus MERS-CoV when compared to uninfected cells.

The rank of differential expression, transcript/probe ID, p-value of global differential expression, t, a moderated t-statistic, B, the log-odds of differential expression between the two groups, fold change of expression, gene and gene name are listed in this chart.

Figure 1: MXD1 is transcriptionally induced in the lungs of BALB/c mice infected with SARS-Coronavirus.

The expression of MXD1 is graphically depicted with mean values and p-value listed for each comparison.

Figure 2: MXD1 is transcriptionally induced in the human liver cell line Huh-7 after infection with human coronavirus HCoV 229E.

The expression of MXD1 is graphically depicted with mean values and p-value listed for each comparison.

Figure 3: MXD1 is transcriptionally induced in primary human microvascular endothelial cells after infection with Middle East respiratory syndrome coronavirus MERS-CoV.

The expression of MXD1 is graphically depicted with mean values and p-value listed for each comparison.